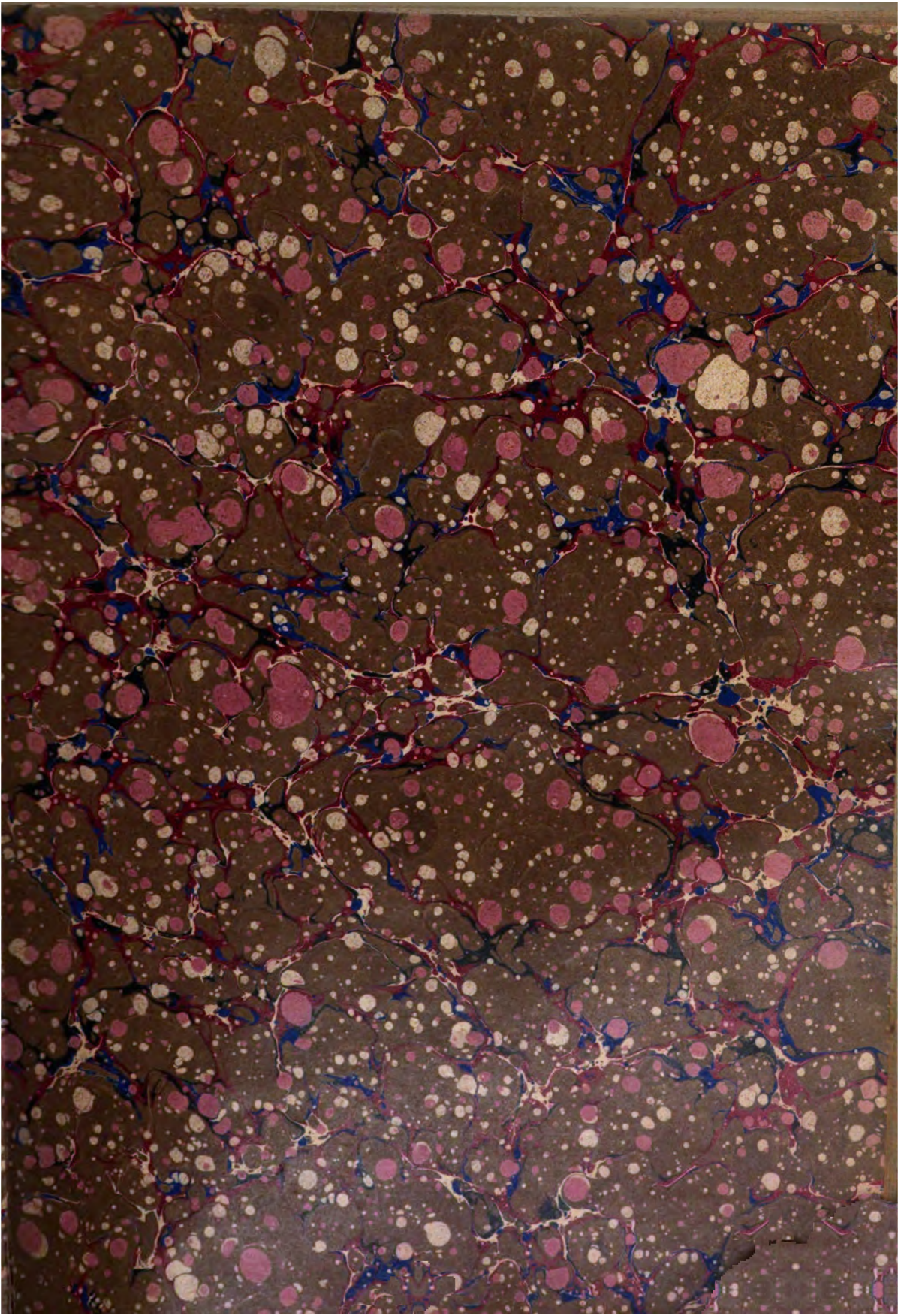

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ANNALS OF BOTANY

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ASSISTED BY OTHER BOTANISTS

VOLUME VI

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An Examination of the Species of the genus Doassansia, Cornu,

BY

WILLIAM ALBERT SETCHELL[1].

With Plates I and II.

UP to the present time there have been twelve species of *Doassansia* described. The genus was founded by Cornu in 1883 (Ann. Sci. Nat., sér. 6, T. 15, p. 285), to receive the *Perisporium alismatis* of the Systema Mycologica of Fries (vol. iii, p. 252). Cornu found abundance of this species in the neighbourhood of Paris, and was able, by a careful study of its development, to establish it firmly among the Ustilagineae in close proximity to the genus *Entyloma*. He says, that the spores of this form on *Alisma* resemble closely those of *Entyloma* both in their structure and in their method of germinating, but are collected and compacted into bundles or 'sori,' which are enclosed by a coat or 'cortex' of sterile cells. It is this cortex of the sorus which Cornu considers to be characteristic of the genus, and he has been followed by later writers in so considering it. *D. alismatis*, which must be looked upon as the typical species, possesses a very distinct and well-developed cortex.

In the same paper Cornu describes a second species, sent by Farlow from North America, which causes marked distortions of the ovaries of species of *Potamogeton*. He states that this

[1] Contributions from the Cryptogamic Laboratory of Harvard University, No. XVIII. Prepared under the direction of Prof. W. G. Farlow.

species agrees so well in its essential characteristics with *D. alismatis*, that he was encouraged to propose the new genus for their reception. He named this species *D. Farlowii*, but notes at the end that De Bary considered it identical with the *Sclerotium occultum*, Hoffm. (Ic. Anal. Fung., p. 67, Taf. 16, Fig. 3).

Farlow, also, has published notes on this species (Bot. Gaz., vol. viii, pp. 276 and 318; Trans. Ottawa Field Nat. Club, vol. ii, p. 127), and has described a new species from the White Mountains of New Hampshire, on *Epilobium alpinum* (Bot. Gaz., vol. viii, p. 277; Appalachia, vol. iii, p. 239). Fisch gave a detailed account of the structure and development of *Protomyces sagittariae*, Fuck. in 1884 (Ber. Deutsch. Bot. Gesell., Bd. II, p. 405), and demonstrated that it is very nearly related to *D. alismatis*.

Since then a number of old species have been referred to the genus, and several new ones described. Schroeter, in 1887 (Pilzfl. Schles., pp. 286, 287), added three species from the old genus *Protomyces*. Winter, in 1885 (Journ. Myc., vol. i, p. 102), described a new species from the United States, and in 1886 (Rev. Myc., vol. viii, p. 207) another new one from Australia. De Toni revised the genus in 1888 (Journ. Myc. vol. iv, p. 13), and brought in two additional species. The last addition is a species from Portugal (*D. lythropsidis*, Lag.).

These several species differ from one another, according to Schroeter (l. c., p. 286) and De Toni (l. c., p. 13), chiefly in the character of the host. The writer has been able to examine nine species in greater or less detail, and has designed to study the structural details, and where possible the development, in order to determine more accurately, if possible, the relations of these species both to each other and to the species of nearly related genera. He has also been able to study the two species described by Cornu, viz. *D. alismatis* and *D. occulta*, from living material. In the search for members of this genus four new species have been discovered, and Prof. Burrill has kindly sent a fifth. These new forms add many interesting facts to those afforded by the old ones.

I wish here to express my indebtedness to my instructor, Prof. W. G. Farlow of Harvard University, for access to the literature bearing on the subject, as well as for assistance at every point.

Doassansia alismatis (*Nees*), *Cornu.*

This is the species which Cornu studied in detail, and which must be considered as the type of the genus. The structure of the sorus of *D. alismatis* is very distinct, and, as a result of the study of it in connection with the other forms referred to *Doassansia*, it has become necessary to separate them into several groups.

D. alismatis is found in the leaves of *Alisma natans* and *A. Plantago* in Europe, Asia, and America. Harkness (Proc. Cal. Acad., ser. 2, vol. ii) has mentioned it as occurring upon *Echinodorus rostratus*. Kellerman (Trans. Kans. Acad., vol. ix and vol. x) and Galloway (Bull. Agr. Bot. Dept., viii) give *Sagittaria variabilis* as a host. These references refer to other species than *D. alismatis*; the last two to *D. sagittariae*, while the first is an undescribed species of *Entyloma* with a compact sorus, related to *D. decipiens* (cf. p. 42).

The first indication of the presence of *D. alismatis* is the appearance of a small circular spot, of a pale yellowish green, on the upper surface of the leaf. This spot increases in size and soon becomes lead-coloured on account of the developing sori which begin to form at the centre, and extend toward the periphery of the spot. At this stage, the spot is about a centimeter in diameter. As it grows larger, the edges become wavy and irregular, the interior becomes more yellow and brownish-yellow as the exhausted tissues of the leaf collapse, and finally the brown and dead centre crumbles away and leaves a hole in the leaf surrounded by a discoloured border, at the periphery of which the formation of new sori may still be proceeding. The sori are not confined exclusively to these spots, but often occur singly in other portions of the leaf remote from any spot.

The leaf of *Alisma Plantago* shows in cross-section a single layer of palisade-parenchyma, the walls of whose cells touch

one another only at the places where the pits are situated, thus leaving a vast network of small connected intercellular spaces throughout this layer, in direct communication with the series of spaces in the layers of spongy parenchyma below. The mycelium of the fungus is found traversing these spaces in all parts of the leaf, but is especially abundant in the regions where the spots occur. The mycelium is composed of slender hyphae, 2 μ to 3 μ thick, branched at short intervals, and septate. The septa are not readily seen until the contents are removed by some clearing reagent (Fig. 70). The hyphae are full of very small oil-globules. They apply themselves closely to the cells along their course, but a careful search failed to detect haustoria of any kind.

The sori are situated in both layers of the leaf, but occur most frequently in the palisade-layer. They occupy the large dome-shaped cavities in this layer just under the stomata (Fig. 68). They are nearly globose as a usual thing, vary from 120 μ to 180 μ in diameter, and are of a light brown colour. The spores are rather loosely packed together, globose, ellipsoidal, or somewhat polyhedral in shape, and possess rather thick, very light-coloured walls. They are from 8 μ to 10 μ in diameter, and their light, shining, granular contents include from one to four or five large drops of oil. The cortical layer is readily seen even with a simple lens. It is composed of radially elongated cells which measure 12 μ to 20 μ by 4 μ to 10 μ. They are comparatively uniform in shape and size, forming a contrast to the two following species in this respect. They are light brown and destitute of solid contents (Fig. 69).

The development of the sorus, as far as it has been studied, seems to agree in its details with that of *D. sagittariae* as described by Fisch (Ber. Deutsch. Bot. Gesell., Bd. II, p. 412). The hyphae collect in a loose strand in the air-space beneath a stoma, and, by means of numerous interlacing short branches, are soon formed into an irregular bunch nearly filling the cavity. The cells of the hyphae of the interior of the bunch now become swollen, filling out the spaces between the strands and causing the bunch to increase in diameter. A cross-section

of the sorus at this stage shows the central portion to be filled with a mass of parenchymatous tissue, composed of rather large, thin-walled, polygonal cells, while the outside is covered by several compact layers of fine, almost unaltered hyphae. The large cells of the interior form the spores, and the process is accompanied by a gelatinization of the walls. The spores are nearly ripened before the cortical cells are indicated as such. The latter appear just under the now somewhat diminished layer of hyphae, and seem to arise by the transformation of an outer layer of cells in all respects similar to those from which the spores are formed. They gradually become elongated radially, lose their granular contents, and take on the brown colour of the wall characteristic of maturity.

When the sorus is mature, the spores separate readily from one another and are ready to germinate. Many of them germinate while still in the leaf, especially if the leaf happens to be submerged. If the spores are freed from the sorus in a little water on a slide, and set aside in a moist chamber, the details of germination may easily be followed. This was the method employed in obtaining the account given below. The first germinations were obtained from the fresh material at Sharon Springs, N. Y.; the later ones from dried material from the same place in the laboratory at Cambridge, Mass.

The spores swell slightly on being placed in water, and the oil-globules become very conspicuous. In most cases there is only one (Figs. 1 and 2), but not infrequently there are three (Fig. 3) or four. After a few hours, the spore-coat bursts and a small germ-tube begins to protrude (Figs. 4 and 5). Usually the oil-globule or globules begin to approach the entrance to the tube at this time (Figs. 4 and 5). The germ-tube, or promycelial tube, elongates until it attains a length of 40 μ to 50 μ. The contents of the spore have gradually been withdrawn to fill the tube, and with them the oil-globules (Figs. 6 to 9); the latter have split up into smaller and smaller globules until they are finally much reduced in size (Fig. 9). When the promycelial tube or promycelium has reached its full length, it begins to appear notched at the tip (Figs. 9 and 10). The

notched appearance becomes more and more pronounced, until there are formed several distinct branches, all attached in a bunch at the tip. These are the sporidia (Figs. 11 and 12). The sporidia, when mature, separate, spreading apart from one another, and radiating out from the tip of the now fully developed promycelium (Figs. 13 and 14).

The promycelium in *D. alismatis* is long and slender, 40 μ to 50 μ long, 3 μ to 4 μ in diameter, and blunt at the tip. The sporidia vary from five to seven, are more or less fusiform, and measure 20 μ to 28 μ in length by about 2 μ in breadth at the middle. They possess very granular contents, with several large globular oil-drops regularly distributed (Fig. 14).

As the sporidia are developed, the contents of the promycelium are gradually withdrawn and septa are formed as this takes place (Figs. 12 to 14). These septa are of common occurrence in other species of the Ustilagineae, and are especially mentioned by De Bary for species of *Entyloma* (Bot. Zeit., Bd. 32, p. 89, 1874), and by Woronin for *Tuburcinia*, *Entyloma*, and others (Beitr. z. Kennt. d. Ust., 1882). Usually the contents of the promycelium are not entirely withdrawn, but a short stump is left at the top still filled with protoplasm even after the sporidia are finally separated from it. At this stage the slightest agitation of the water in which the germination is going on, is sufficient to detach these stumps with their crowns of sporidia from the emptied portion of the promycelium. The same thing happens in *Tuburcinia* according to Woronin (l. c., p. 11, Taf. II, Figs. 2 to 6), who calls this stump 'the basidial cell,' and says that, as far as he knows, it is unknown in other species with the *Tilletia*-type of germination.

The sporidia begin to germinate in many cases while still attached to the basidial cell (Figs. 15 to 18). A view where the sporidia have fallen from the basidial cell will show the details of the process more plainly (Figs. 19 to 21). It will be seen that the sporidia have conjugated at the base in pairs. Where there are six sporidia, there are three pairs, and all the sporidia have conjugated (Fig. 19); but where there are five

or seven sporidia, the odd sporidium is found unconjugated, but as having put forth the conjugating tube (Figs. 20 and 21).

The results of conjugation are best seen in the pairs of sporidia which have fallen from the basidial cell and are floating free (Figs. 22 to 31). As far as I have been able to determine, a germ-tube is uniformly produced, and it may start from the tip of one sporidium (Figs. 23 and 24), from the tips of both, or from the base (Figs. 25 to 27). Various combinations of these various methods are common (Figs. 28 to 31). As the germ-tube grows, the protoplasm is withdrawn from the sporidia, and septa are formed as occurred in the promycelium. Occasionally the promycelium does not produce a crown of sporidia but developes at once into a germ-tube (Fig. 32). Cornu did not note the conjugation of the sporidia in *D. alismatis*; and Fisch, finding that the sporidia of *D. sagittariae* did not conjugate, says that the germination of the spores in the genus *Doassansia* is like that of the spores of *Tilletia* and *Entyloma*, but lacks the conjugation (Ber. Deutsch. Bot. Gesell., Bd. II, p. 415). He also seems to regard the lack of conjugation in *D. sagittariae* as a case of apogamy (l. c., pp. 409 and 410).

The first germinations, as mentioned above, were obtained from fresh material at Sharon Springs, N. Y., and took place in the months of July and August, 1889. The later germinations from dried material were obtained in October and November, 1889, and March, 1890. The dried material was soaked over night in water, and the following morning some sori were crushed on a slide, a little water added, and the slide then placed in a moist chamber. In all the sowings thus made, mature sporidia were produced after twenty-four hours. Nearly all of the variations figured were obtained in each sowing. In no case did I observe the production of secondary sporidia, but the sporidia conjugated in pairs and produced germ-tubes. The odd sporidium was not seen to germinate in any case.

D. alismatis differs from *D. hottoniae* both in habit and in the structure of the sorus, as noted under that species. It re-

sembles *D. sagittariae* in habit, but the sori are more regular in shape and structure, and the cortical cells more uniform and more radially elongated, than in *D. sagittariae*. The germination also seems to differ in the two species.

Season. This species appears to range from June to September.

Distribution. In Europe this species has been found in France, *Cornu*; common in Germany, *Lasch! et al.*; Italy, *Spegazzini! Saccardo, Mori*; Sweden, *Johanson!* Finland, *Karsten!* Scotland, *Traill*; England, *Berkeley! Currey.* In Asia, in Western Siberia, *Martianoff!* In the United States, Iowa, *Holway!* Wisconsin, *Trelease!* Minnesota, *Farlow!* Nebraska, *Pond*; New York, *Setchell!*

Literature[1].

Sclerotium alismatis, Nees, in Fr., Syst. Myc., Vol. II, p. 257. 1822.
Perisporium alismatis, Fr., Syst. Myc., Vol. III, p. 252. 1829.
———— Wallroth, Comp. Fl. Germ., II, p. 747. 1833.
———— Rab., Kryptfl., Bd. I, p. 230. 1844.
———— Kirchner, Lotos, Bd. VI, p. 244. 1856.
Dothidea alismatis, Kirchner, Lotos, Bd. VI, p. 205. 1856.
Sphaeria (*Depazea*) *alismatis*[2], Currey, Trans. Linn. Soc., Vol. XXV, p. 334, Pl. LIX, f. 152. 1865.
Uredo alismacearum, Crouan, Fl. Finist., p. 8. 1867.
Sphaeropsis alismatis, Cooke, Handbook, p. 429. 1871.
Aecidium incarceratum[3], B. & Br., Ann. & Mag. Nat. Hist., 4 ser., Vol. XV, p. 36, No. 1469. 1875.
Protomyces macularis, Thuem., Bull. Imp. Soc. Nat. Moscow, p. 130. 1877 (not Fuckel).

[1] The literature is given as fully as possible. The local North American literature is complete, thanks to the valuable index prepared by Prof. Farlow and Mr. Seymour.

[2] Although I have seen no authentic specimen of this form of Currey's, I have little hesitation in referring it to *D. alismatis*. Cf. also Passerini under 1093 Erb. Critt. Ital.

[3] Berkeley says that this species occurs on *Sagittaria sagittifolia*, and De Toni and Plowright consider it to be identical with *D. sagittariae*. The specimens distributed by Berkeley in Fung. Eur. No. 1492, on an unnamed host, seem to me to be on a leaf of *Alisma Plantago*, and the characters of the sori are clearly those of *D. alismatis*.

Phyllosticta alismatis, Sacc. et Speg., Mich., Vol. I, p. 144. Jan. 1878.
Entyloma alismacearum, Sacc., Mich., Vol. II, p. 44. Apr. 1880.
Perisporium alismatis, Sacc., Syll. Fung., Vol. I, p. 59. June, 1882.
Doassansia alismatis, Cornu, Ann. Sci. Nat., sér. 6, T. 15, pp. 280–285, Pl. XVI, f. 1–4. June, 1883.
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——— Traill, Scot. Nat., p. 124. Jan. 1884.
——— Trelease[1], Par. Fung. Wisc., p. 28. [Extr. Trans. Wisc. Acad., Vol. VI, p. 264.] Nov. 1884 (in part).
——— Fisch, Ber. Deutsch. Bot. Gesell., Bd. II, pp. 406 and 415. Nov. 1884.
——— Arthur, Bull. Iowa Ag. Coll. Expt. Sta., I, p. 174. Nov. 1884.
Phyllosticta Curreyi, Sacc., Syll. Fung., Vol. III, p. 60. Dec. 1884.
—— *alismatis*, Sacc., Syll. Fung., Vol. III, p. 60. Dec. 1884.
Doassansia alismatis[2], Hohenb.-Heufl., Ber. Deutsch. Bot. Gesell., Bd. II, p. 458. Jan. 1885.
Entyloma alismacearum, Mori, Funghi di Modena, No. 14 (Nuov. Giorn. Bot. Ital., Vol XVIII). 1886.
Perisporium alismatis, Winter, Pilze, Abth. II, p. 69. 1887.
Doassansia alismatis, Schroeter, Pilzfl. Schles., p. 286. 1887.
——— De Toni, Journ. Myc., Vol. IV, p. 14. Mar. 1888.
——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 503. Oct. 1888.
Aecidium incarceratum, De Toni, in Sacc., Syll. Fung., Vol. VII, p. 831. Oct. 1888.
——— Plowright, Brit. Ured. and Ust., p. 267. 1889.
Doassansia alismatis, Plowright, Brit. Ured. and Ust., p. 294. 1889.
Doassansia alismatis, Webber, Cat. Fl. Neb., p. 75. 1890.
——— Peck, 43rd. Rept., p. 28. 1890.

Exsiccati.

Dothidea alismatis, Lasch., in Rab., Kl. Herb. Viv. Myc., No. 553. 1844!
——— Lasch., in Rab., Herb. Myc. (*ed. nov.*), No. 162. 1855!

[1] The conidial form on *Sagittaria* mentioned under this reference will be referred to later under *Burrillia pustulata*.
[2] Contains an account of the earlier synonymy of the species.

Physoderma maculare, Karst., Fung. Fenniae Exs., No. 1. 1861! (not Wallroth.)
Aecidium incarceratum, Berk., in Rab., Fung. Eur., No. 1492. 1871!
Protomyces macularis, Thuem., Myc. Univ., No. 1417. 1879!
Phyllosticta alismatis, Pass., Erb. Critt. Ital., Ser. II, No. 1093. 1881!
Doassansia alismatis, Holway, in Ellis, N. A. Fungi, No. 1485. 1885!
——— Johanson, in Eriks., Fung. Par. Scand., No. 263. 1888!
——— Johanson, in Pazschke, Fung. Eur., No. 3601. 1890!

Doassansia sagittariae, (*Westend.*), *Fisch.*

D. sagittariae is found in Europe and in both Americas, inhabiting the leaves of species of *Sagittaria*. In Europe the host-plant is the common *S. sagittifolia*, in North America it is found upon the varieties of the wide-spread *S. variabilis* and also upon *S. heterophylla* according to Fisch (l. c., p. 407); but this last may be a mistake, for Fisch quotes Demetrio's specimens from Missouri (Fung. Eur., No. 2902 *a*), which are upon *S. variabilis*, but does not mention *S. variabilis* among the hosts for this species. There is no reason why *D. sagittariae* should not occur upon *S. heterophylla*. Mr. G. P. Clinton has collected it at Dixon, Ill., on *S. graminea*. In South America it is found upon *S. montevidensis*.

Upon the leaves of all these species the *Doassansia* forms circular spots of a light yellow colour, which soon becomes inclined to brownish and punctate with the rather dark sori. There seems to be no swelling of the leaf, and the general appearance is very much like that of *D. alismatis*. The spots grow to be of considerable size by the continued formation of new sori at the periphery.

The sori are situated either in the single layer of palisade-parenchyma in the spaces just under the stomata, or in the spongy parenchyma in the spaces just over the stomata, or in the same tissue midway between the two surfaces in the ordinary intercellular spaces. There are about equal numbers of sori under each leaf-surface, so that the fungus can scarcely be said to inhabit the upper surface of the leaf (see Fisch, l. c., pp. 407 and 415).

The mycelium is abundant and closely resembles that of *D. alismatis* in all respects.

The sori are nearly globular, rather dark brown bodies from 100 μ to 125 μ in diameter. The spores are rather loosely compacted together, nearly spherical, and from 8 μ to 10 μ across. The outer coat is moderately thick and light-coloured, and the granular contents usually possess one large globule of oil. The cortical cells are rather short and broad with rather thin, light-brown walls. They closely cover the sorus, but are rather irregular (Fig. 71).

The germination has been given in some detail by Fisch (l. c., pp. 408 et seq.). The promycelium, according to him, has a markedly conical tip ; the sporidia are inserted at unequal distances on this tip ; they produce secondary sporidia without conjugating ; and these secondary sporidia conjugate in rare instances. The characters of the shape of the tip of the promycelium and the unequal insertion of the sporidia are not found in any other species of *Doassansia* of which the germination has been obtained, and are in striking contrast with those of *D. alismatis*, as may be seen above. Fisch also says (l. c.) that *D. sagittariae* will germinate only in spring and early summer ; while *D. alismatis*, as shown above, will germinate readily at almost any time of the year.

D. sagittariae differs from *D. alismatis* by the uniformly smaller size of the sori, by the arrangement and shape of the cortical cells, and by the characters of the promycelium and sporidia. It is near *D. alismatis*, but yet, as it seems to me, perfectly distinct from it. From *D. hottoniae* it is distinguished by its habit, and by its smaller and more globular sori.

Season. From May until September.

Distribution. *D. sagittariae* is fully as widely distributed as was the preceding species. In Europe it has been found in Italy, *Bizzozero!* France, *Briard!* Germany, *Fuckel! Magnus! Winter! Hening! Schroeter*; England, *Vize!* Belgium, *Westendorp!* in South America, in the Argentine Republic, *Spegazzini*; in North America, in Missouri, *De-*

metrio! Galloway! in Canada, *Fletcher!* in Illinois, *Clinton!* in Kansas, *Kellerman!*

Literature.

Uredo sagittariae, Westend. ined., Herb. Crypt. Belge, No. 1177. 1857!

Protomyces sagittariae, Fuck., Symb. Myc., p. 75. 1869.

—— *Bizzozerianus*, Sacc., Fung. Ital., f. 103. May, 1877.

———— Sacc., Mich., Vol. I, p. 97. June, 1877.

—— *sagittariae*, Cooke, Int. Study Mic. Fung., 4th edit., p. 227. 1878.

Entyloma Bizzozerianum, Sacc., Mich., Vol. II, p. 135. Apr. 1880.

———— Speg., Fung. Arg., Pug. IV, p. 21, No. 55. [Extr. Ann. Soc. Cientif. Arg., Vol. XII, p. 66.] Aug. 1881.

Doassansia sagittariae, Fisch., Ber. Deutsch. Bot. Gesell., Bd. II, pp. 405–416, Taf. x. Nov. 1884.

———— Winter und Demetrio, Hedw., Bd. XXIV, p. 178. 1885.

Doassansia alismatis Kellerman, Bull. Washb. Lab., I, p. 73. Feb. 1885.

———— Kellerman, Trans. Kans. Acad., Vol. IX, p. 80. 1885.

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———— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 503. Oct. 1888.

———— Galloway, Bull. Agr. Dept., Bot. Div., VIII, p. 59. 1889.

———— Plowright, Brit. Ured. and Ust., p. 295. 1889.

Exsiccati[1].

Uredo sagittariae, Westend., Herb. Crypt. Belge, No. 1177. 1857!

Physoderma sagittariae, Fuck., Fung. Rhen., No. 1549. 1865!

Protomyces sagittariae, Vize, Microfung. Britt., No. 50. 1873!

—— *Bizzozerianus*, Sacc., Myc. Venet., No. 889. 1876!

—— *sagittariae*, Winter, Fung. Eur., No. 2902 *a.* and *b.* 1883!

[1] Specimens of *D. sagittariae* occur with *Cercospora sagittariae* E. and K. No. 1502, Ellis, N. A. Fungi (1886), in the copy in the Cryptogamic Herbarium of Harvard University. Also said by De Toni to be No. 744, Gandoger, Fl. Gall. Exs., which is not accessible to me.

Doassansia sagittariae, Hening, in Sydow., Myc. March., No. 994. 1886!

———— Briard, in Roumeguère, Fung. Gall. Exs., No. 3642. 1886!

Doassansia opaca, *sp. n.*

In the Botanical Gazette for August, 1883 (Vol. VIII, p. 276), Farlow mentions that he had found *Protomyces sagittariae*, Fuckel, at Newton, Mass. He has kindly allowed me to examine these specimens, which agree with my own collected in Eastern Massachusetts and Connecticut. They have been carefully compared with authentic specimens of Fuckel's (Fungi Rhenani, No. 1549), and found to differ so decidedly in habit and structure that it seems necessary to consider them as specifically distinct.

This species also inhabits the leaves of *Sagittaria variabilis*, apparently preferring the form called var. *latifolia*. It forms spots which are at first lemon-yellow, then lead-coloured, and finally brown. They are circular in shape and somewhat swollen on both surfaces of the leaf, like blisters. The leaf is two to three times its normal thickness in the region of a spot. The individual sori cannot be distinguished, even on holding the leaf up to the light and looking through it. In this, it differs from all of the species which are described above. The spots never grow very large, seldom being over 1 cm. in diameter, and they very rarely coalesce.

The mycelium is perhaps a little coarser than that of the preceding species, but does not differ in any other respect.

The cross-section through the centre of a spot is very characteristic (Fig. 72). The sori are situated just between the palisade and spongy layers, and, by forcing them apart, cause the distortion of the leaf. This position of the sori is very uniform in all the numerous specimens examined from the various localities. It differs decidedly from the characteristic position of the sori in the other species.

The sori themselves are large (200 μ to 300 μ by 80 μ to 100 μ), globular-oblong, or almost cuboidal in shape, and

of a light-brown colour. The spores are loosely packed together, nearly spherical, and 10 μ to 15 μ wide. The walls are thick and light-brown, and the contents bright and possessing one moderately large oil-globule. The cortical cells vary greatly in shape. On the free sides of the sorus, e. g. the upper and lower sides, they are brick-shaped (Fig. 74), reminding one very much of those of *D. alismatis*; but where the sorus is crowded against another, they are more nearly cuboidal or nearly wanting (Fig. 73).

The sori develope much as in *D. alismatis*, but the gelatinization which precedes the ripening of the spores is more pronounced than in that species.

The germination of this species has not yet been obtained. Sowings have been made in nine months of the year, but with no result. Different material from different localities and of different ages has been tried, but without success. Cultures in a decoction of horse-dung have been tried, both with fresh and with dried material, but the spores have obstinately refused to germinate. It is hoped that the germination may be obtained at some future time, and then the species can be compared more thoroughly with its neighbours.

Although found on the same host as *D. sagittariae*, there are so many points of difference between them that they cannot be considered as the same. In the first place there is a difference in habit which is of some importance even in species with such feeble morphological characteristics as those of *Doassansia*. This difference in habit is constant. In the second place, the sori of *D. opaca* are two to three times the size of the sori of *D. sagittariae*. Thirdly, the spores are larger; and finally, the cortical cells are different in the two species.

Season. The pale-yellow spots that mark the first appearance of the fungus begin to show themselves about the end of the month of July, and from that time on, until the frosts destroy the leaves of the *Sagittaria*, they continue to form.

Distribution. At Newton, Mass., *Farlow!* and at Medford, Mass., and Norwich, Conn., *Setchell!* In both of the last-

mentioned localities the plants of the *Sagittaria* were situated in the water and not on the bank, as often happens.

Literature.

Protomyces sagittariae, Farlow, Bot. Gaz., Vol. VIII, p. 276. Aug. 1883. (Not Fuckel.)

Doassansia hottoniae (*Rostr.*), *De Toni.*

In the thin, slender, much-dissected leaves of *Hottonia palustris*, the sori of this species cause neither deformity nor discolouration. They are light-brown in colour, ellipsoidal in shape, and have their long axes parallel to the long axis of the leaf. The tissues of the leaf are rather spongy, and the sori are situated just beneath the epidermis of both surfaces. They are circular in cross-section (120 μ to 160 μ in diameter), and elongated elliptical in longitudinal section (220 μ to 240 μ by 120 μ to 160 μ).

The spores are polygonal, rather closely compacted, with rather thin walls, and pale contents, with usually a single large oil-drop. They are from 8 μ to 10 μ across. The cortical layer is much better developed than in *D. epilobii* but not as strongly as in *D. sagittariae* or *D. alismatis*. It consists of a close layer of cells, which are more or less polygonal in tangential section and nearly square in radial section, being 8 μ to 12 μ by 8 μ to 10 μ. They are light-brown, hyaline, and show no evidence of possessing solid contents. The walls are slightly thickened (Fig. 67). The germination has not been described.

D. hottoniae, as shown by the specimens distributed by Rostrup and Johanson in the various *exsiccati*, is a true *Doassansia*, distinguished from *D. epilobii* by its larger cortical cells. From *D. alismatis* and *D. sagittariae* it differs both in the shape of the sori and in habit. The cortical cells too are decidedly different from those of *D. alismatis* and to a less degree from those of *D. sagittariae*. (Cf. Figs. 67, 69, and 71).

Season. From June to October, as shown by the specimens distributed.

Distribution. Denmark, *Rostrup and Johanson!* Berlin, Germany, *Sydow!* France, *E. Cosson!*

Literature.

Entyloma hottoniae, Rostrup, in Thuem., Myc. Univ., No. 2222. 1884.

Doassansia hottoniae, De Toni, Journ. Myc., Vol. IV, p. 18. Mar. 1888.

——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 506. Oct. 1888.

Exsiccati.

Entyloma hottoniae, Rostrup, in Thuem., Myc. Univ., No. 2222. 1884!

——— Rostrup, in Winter, Fung. Eur., No. 3403. 1886!

——— Rostrup, in Roumeguère, Fung. Gallici Exs., No. 4727. 1888!

——— Sydow, Myc. March., No. 2322. 1888!

Doassansia epilobii, *Farlow.*

The present species, as far as is known, is strictly alpine, inhabiting the leaves of *Epilobium alpinum* in the White Mountains of New Hampshire. The tips of the leaves are the first portions affected, and the fungus is detected early by the dark sori showing through the tissues of the thin leaf. As the infected area spreads toward the base of the leaf, the older portions become at first a very pale yellow and then brownish, as the tissues are exhausted by the demands of the parasite. There is no distortion at all. The dark sori, visible through the very delicate leaf, have even more of the appearance of a *Puccinia* than have the rest of the species of *Doassansia*, and Farlow (Bot. Gaz., Vol. VIII, p. 276; Appalachia, Vol. III, p. 239) mistook it, when collecting it, for *Puccinia epilobii.* They are grouped together in small bunches of threes, fours, or fives, the larger, blacker, older ones nearer the tip, the smaller, light-brown, younger ones nearer the petiole.

The mycelium spreads through the tissues of the diseased area of the leaf, being especially abundant near the sori. The

hyphae are about 2 μ in diameter, rather infrequently branched, except in the neighbourhood of the larger air-spaces, and septate at short intervals.

The palisade-parenchyma of the leaf comprises only one row of rather short cells, while two-thirds of the thickness of the leaf is taken up with the rather loose spongy layers. The sori are situated in the larger air-spaces of the latter, just over the stomata. They are irregularly spherical or ellipsoidal in shape, 120 μ to 220 μ by 80 μ to 120 μ large, dark-brown to black on the outside, and light-brown on the inside. The spores are polygonal to almost ellipsoidal, with moderately thick brown walls, and light, oily contents. They measure 8 μ to 12 μ by 6 μ to 8 μ. The cortical cells are small, irregularly polygonal in shape, much flattened radially, with thick, dark-brown walls. They are 8 μ to 10 μ in diameter in tangential sections and 4 μ to 6 μ in radial section (Fig. 66). Outside of this layer is a more or less complete covering of indurated hyphae. The germination has never been seen.

Doassansia epilobii seems to be a true *Doassansia*, although it bears a striking resemblance in structure to the form described as *D. decipiens*. It seems to possess a true cortex of sterile parenchymatous cells, which more or less covers the sorus, but these cells are small and difficult to demonstrate, and do not differ in any greater degree from the hardened hyphal cells which cover the sori of *D. decipiens* than they do from the cortical cells of the preceding species or of *D. alismatis*. But, as far as dried material shows, it is to be put rather with the species of *Doassansia*, at least until a more careful study from fresh material is possible. Fisch suggests (Ber. Deutsch. Bot. Gesell., Bd. II, p. 407) that this is a species of *Synchytrium*. But the presence of a mycelium as well as the structure of the spores shows that it is not of that genus.

Season. Found in August, 1882.

Distribution. Occurring thus far only at King's Ravine, White Mountains, N. H., *Farlow!*

Literature.

Doassansia epilobii, Farlow ad int., Bot. Gaz., Vol. VIII, p. 277. Aug. 1883.

——— Farlow ad int., Appalachia, Vol. III, p. 239. Jan. 1884.

——— Fisch, Ber. Deutsch. Bot. Gesell., Bd. II, pp. 407 and 416. Nov. 1884.

——— De Toni, Journ. Myc., Vol. IV, p. 18. Mar. 1888.

——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 506. Oct. 1888.

——— Farlow and Seymour, Host Index, p. 46. Aug. 1888.

Exsiccati.

Doassansia epilobii, Farlow, in Ellis, N. A. Fungi, No. 1486. 1885!

Doassansia punctiformis, *Winter*.

This Australian form is known to me only from the description. It is said to have a cortex composed of one layer of polygonal cells, with thick, minutely granulated walls, which, providing the species be a true *Doassansia*, would be enough to distinguish it.

It inhabits living leaves of *Lythrum hyssopifolium*.

Distribution. Melbourne, Australia.

Literature.

Doassansia punctiformis, Winter, Rev. Myc., Vol. VIII, p. 207. 1886. (Not Schroeter.)

——— De Toni, Journ. Myc., Vol. IV, p. 17. Mar. 1888.

——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 505. Oct. 1888.

Doassansia comari, (*Berk. and Br.*), De Toni et Massee.

This species is known to me only from the descriptions. From them it seems to be a *Doassansia* with enormous sori (1 mm. to 1·5 mm. in diameter), and a distinct cortex.

Distribution. Great Britain.

Literature.

Protomyces comari, Berk. and Br., Ann. and Mag. Nat. Hist., 5 ser., Vol. I, p. 27, No. 1708. 1878.

Protomyces comari, Berl. et De Toni, in Sacc., Syll. Fung., Vol. VII, p. 321. Mar. 1888.

Doassansia comari, De Toni et Massee, Journ. Myc., Vol. IV, p. 18. Mar. 1888.

———. De Toni, in Sacc., Syll. Fung., Vol. VII, p. 506. Oct. 1888.

Doassansia obscura, *sp. n.*

While searching for *D. opaca*, I found growing upon the petioles and peduncles of the same host (*Sagittaria variabilis*, var. *latifolia*) an inconspicuous form which is clearly different from any other form either described or distributed. It lives in the petioles and peduncles, generally somewhere in the lowest third. Occasionally some sori are found situated half or two-thirds of the way up, but it is at the very base, in the parts protected by the bases of the dead or living leaves and destitute of chlorophyll, that it is most abundant. It is most frequently found also in the petioles of the outer and older leaves of the plant. When found in the petioles of the inner leaves of the plant or in the peduncles, it occupies, as a usual thing, a higher position than it does when found in the outer petioles.

When it occurs in parts possessing chlorophyll, it produces a very pale yellow indefinite spot, which is scarcely noticeable except when closely examined. But in the bases of the petioles, where there is no chlorophyll, its presence may be detected by the dark lines where the brown sori show through the more or less transparent outer tissue. It produces no distortion at all, and is therefore likely to escape the notice of the collector. The easiest way to detect it is to pull off the outer leaves of the *Sagittaria* and examine the bases of their petioles.

The inner portion of the petiole of *Sagittaria variabilis* contains large intercellular spaces shaped like the interior of a closed cylinder, with the long axis parallel to the long axis of the petiole. The *Doassansia* prefers the outer cylinders; the mycelium forms a cobweb-like network in each space

running through it in all directions. The hyphae are like the hyphae of the other species.

The large sori (150 μ to 300 μ in diameter) are arranged in a single row in each cylindrical chamber, the diameter of the sorus and the diameter of the chamber being very nearly equal. Sometimes the whole row of sori (8 or 10 in number) coalesce into one long gigantic sorus. The sori are attached to the sides of the chamber and are, as it were, suspended in it, by loose strands of hyphae, radiating from all portions of the periphery (Fig. 75).

The structure of the sorus is entirely different from that of any other species which I have had the opportunity of examining. The central portion is filled with a dense mass of very fine hyphae, closely intertwined. Around this is a variable number (3 to 8) of layers of spores loosely placed. Finally on the outside there is a conspicuous cortex of large, dark-brown cells (Fig. 75).

The hyphae of the central part are only 1 μ to 2 μ in diameter, very closely and intricately interwoven, and fill up one-third to nearly one-half of the interior of the sorus. They are indistinct, as if their walls were slightly gelatinized.

The spores are very loosely compacted together, spherical in shape, and 8 μ to 12 μ in diameter. They are light-brown, thin-walled, with the usual contents.

The cortical cells are different in shape from those of any other species. In a cross-section of the sorus they are generally heart-shaped (Fig. 76). As solid bodies they resemble the crowns and upper parts of molar teeth. A tangential view is shown in Fig. 77. The dimensions in cross-section are 12 μ to 16 μ by 8 μ to 12 μ.

The details of the development of the sorus have not been carefully studied. The hyphae form first a loose ball, as in all the other species. The outer hyphae seem to have a slight radial arrangement, and their cells become swollen. The outermost cell is always the largest and finally becomes a cortical cell; those farther in become spores, and the central confused mass of hyphae remains almost unchanged. There

seems to be no distinct layer of fine hyphae about the outside, as in the preceding species.

Spores from freshly gathered specimens germinated on being sown in water in October, 1889, and in the same month in 1890. Sowings made from fresh material in September, 1889, and in November, 1890, yielded no result. Neither did sowings made from dried material at other seasons. It seems as if October were the month in which this species germinates in nature.

The spores germinate in from 36 to 48 hours after sowing. The spores swell slightly, a split occurs on one side of the exospore, and the endospore protrudes in the form of a tube (Figs. 33 and 34). When the promycelium has reached a length of about 20 μ, the small protuberances that are destined to become the sporidia appear at the tip (Fig. 35). They gradually become elongated (Figs. 36 and 37) until the sporidia are fully formed. The sporidia are from five to seven in number, 16 μ to 17 μ long and 1·5 μ to 2 μ thick, tapering to both ends, and borne in a whorl at the blunt tip of the promycelium. As they mature, the contents are withdrawn from the promycelium, and septa are formed as in *D. alismatis*. There is, however, no basidial cell left as in that species, but the whole of the contents of the promycelium finally pass into the sporidia. The latter germinate while still attached to the promycelium, and each sporidium produces secondary sporidia. Occasionally one secondary sporidium is produced from a primary sporidium (Fig. 38), but more often the secondary sporidia occur in bunches of three to seven (Figs. 39 to 42). Other sporidia are in turn produced from the secondary sporidia (Fig. 41), and at times still others from these (Figs. 41 and 42). The contents are withdrawn from the primary sporidia by the formation of the secondary sporidia, and septa are formed, as in the case of the promycelium (Figs. 39 to 42). The secondary sporidia drop off and multiply on the slide, which soon becomes full of them. They do not appear to conjugate.

As remarked above, this species is in every way distinct.

The general habit is not striking; but is, in a certain way at least, distinctive. The size and structure of the sorus is found in no other species. The central portion of compact, fine hyphae, and the peculiar shape of the cortical cells would distinguish it at once from any other known form. Finally, the germination is different in its details from any other which has yet been obtained. It is considered as the type of a subgenus, *Pseudodoassansia.*

Season. *D. obscura* seems to be an autumnal species. It has been collected in Connecticut in the early part of September, apparently young, and, in the latter part of September and in October, it has been found mature in Massachusetts.

Distribution. Probably a wide-spread species in the United States, but escaping detection on account of being so inconspicuous. I have found it at Norwich, Conn., and at Medford and Cambridge, Mass.

Doassansia occulta (*Hoffm.*) *Cornu.*

What appears to be the *Sclerotium occultum* of Hoffmann's Icones has been re-discovered in America. Professor Farlow has kindly allowed me to examine the original specimens of *D. Farlowii*, Cornu, which were collected by James Fletcher at Ottawa, Canada, and portions of which were sent to Woronin and to Cornu. A form inhabiting the ovaries of *Potamogeton Claytonii* (*P. Pennsylvanicus, Cham.*) has been collected by myself at Norwich, Conn., in some abundance. The distortions produced by these two sets of specimens appear very much alike and agree well enough with Hoffmann's figure (Ic. Anal. Fung. Taf. 16, f. 3); but there are some differences in the spores between the two sets of forms, and therefore it has seemed better to describe them separately. My own specimens agree best with Hoffmann's figure of a cross-section of a sorus (l. c.), and therefore, for the present, they will be regarded as identical with the type of the species, while Fletcher's specimens will be described as Var. *Farlowii.*

Type. This form affects the ovaries of *Potamogeton Clay-*

tonii, the most common species of pondweed in Eastern Connecticut. It has been looked for on several other species, but never detected, while it has been found on *P. Claytonii* in a number of localities. The fungus reaches its full development at about the time when the fruits of the pondweed are fully ripened. The affected ovaries may be told from the normal fruits of the same raceme by being swollen to from 5 to 6 times the normal size and by being a dark olive-green, while the ripened fruits are more inclined to a yellowish brown. The leaves of infected plants have been examined in almost every case, but no sign of sori has been detected in them.

The mycelium is not very abundant and possesses no characters particularly distinctive of the species. It is found in the neighbourhood of the sori and to some extent also in the peduncle of the raceme. It has not been looked for in other parts of the host, but probably occurs throughout the length of the stem at least.

The topography of a cross-section of the ripe drupe-like fruit is somewhat as follows: the section itself is ovate; the cross-section of the embryo is circular and is a little to one side of the centre towards the broader, rounded end of the section; the section of the endocarp comes next, surrounding the embryo-cavity; it is also ovate, but the narrow pointed end is toward the broader end of the general section. When ripe the endocarp, or nutlet, is composed of thickened, sclerotic cells, but when young it consists of large, thin-walled, parenchymatous cells filled with starch. The epicarp is a soft layer, of about equal thickness in all parts of the section. It possesses conspicuous intercellular spaces, which are largest and loosest at the broad end of the section and on the sides. The epidermis forms the outermost layer of the epicarp.

The sori are confined to the endocarp, and are formed among its cells in the greatest abundance, forcing them apart until the endocarp becomes several times as thick as it normally would be. The cells of the endocarp retain their thin walls and starchy contents, and never assume, when

infested by the fungus, the sclerotic character of the testaceous nutlet of the ripened fruit. The epicarp retains its normal character, but soon macerates away, as it does also in the ripened fruit.

The sori are nearly globose or slightly ellipsoidal, but often very irregular, and vary from 120 μ to 140 μ by 100 μ to 160 μ. They are of a light-brown colour. They are very firmly constructed and can be crushed with difficulty. When crushed they do not separate into spores, but show peculiarities of structure which can be understood only by the study of thin sections.

The central part of the sorus is found to be made up of a mass of large polygonal cells which have the appearance of parenchymatous tissue (Fig. 79). They appear to be destitute of solid contents. About this mass of parenchymatoid tissue there is a single layer of spores, and outside of this a well-developed cortex. The spores are about 12 μ by 10 μ, sometimes slightly elongated radially, with thin walls, granular contents, and closely connected with the layers on either side of them. The cortical cells are polygonal in tangential section and decidedly flattened radially, measuring in cross-section 8 μ to 10 μ in the tangential direction and only 1·5 μ to 3 μ in the radial direction. Outside the cortex is a thin layer of hyphae (Fig. 79).

The development of the sori presents several deviations from the course of development either of *D. alismatis* or of *D. obscura*. The hyphae collect into a loose ball as in those species, but soon the inner hyphae, which are more closely compacted in this species, appear to radiate in all directions from the centre, and are surrounded by a thick coat of concentric hyphae. Fig. 80 represents this stage in *D. Martianoffiana*, a nearly related species. The radiating structure becomes more and more indistinct as the cells begin to appear in the interior of the forming sorus, until finally the central portion assumes the characteristics of parenchyma, and there are formed, just beneath the outer coat of hyphae, more or less radially elongated cells, filled with a highly refractive, gra-

nular protoplasm, destined to form spores. The cortical cells appear to be divided off from the end of the spore-cells, but the details were not clearly seen. As the sorus matures, there is little trace of gelatinization, but the outer coat of hyphae becomes thinner and thinner until at maturity there is only a scanty covering left.

When a mass of the endocarp containing sori is crushed on a slide in water, either the sori remain unbroken or else are broken into comparatively large fragments. The spores germinate in position, and the sori bristle on all sides with promycelia and sporidia. The promycelium reaches a very variable length, some beginning to form sporidia when only 20 μ long, others reaching a length of 400 μ to 450 μ before doing so. The promycelia are 4 μ to 8 μ in diameter at the periphery of the sorus, but 8 μ to 10 μ in diameter at the tip. This slightly swollen tip is very noticeable (Figs. 43 to 46).

The sori, on account of their weight, sink to the bottom of the water and the promycelia grow up more or less obliquely toward the surface of the drop until the tip is at the surface or projects slightly beyond it. There now appears a circle of 5 to 10 small protuberances on the broad, flat tip of the promycelium (Fig. 43), which proceed to grow out into long slender sporidia (Figs. 44 to 46). The sporidia are from 20 μ to 40 μ long by 2 μ to 3 μ thick, slightly bowed and tapering to each end. They present a curious dark appearance as they project wholly or partially above the surface of the water.

This stage of the germination has very much the appearance of the promycelium and sporidia of *Tilletia foetens*, which I have germinated in the laboratory from material kindly sent by Prof. J. C. Arthur. The large broad promycelium, seeking the air to form the sporidia, which are so long and narrow, are features common to both. But any close resemblance stops at this point, for the sporidia of *D. occulta* do not conjugate. They drop from the tip of the promycelium as soon as they are ripe, and either sink to the bottom or float just beneath the surface. Soon the sporidium begins to germinate

at one end (Fig. 47), or more often at both ends (Figs. 48 and 49). Chains of secondary sporidia, at length more or less branched, are formed and grow upward to the surface of the water and project above it. They fall in pieces finally, but not until after some time.

The spores germinated freely from the end of May until the middle of July. Sowings made from fresh material produced no result, nor did sowings from dried material made in October, November, and March. The period, then, for the germination of this species seems to be in the spring and early summer.

D. occulta differs in almost every respect from the species thus far enumerated, and seems to be best regarded as the type of a new section of the genus entirely different from those represented by *D. alismatis* and *D. obscura*. In all outward appearances the sori resemble those of *D. alismatis*; but the thin sections, as described above, show an entirely different structure. This same type of sorus is found in the two following species, and warrants placing these three species in a subgenus of their own, for which the name *Doassansiopsis* seems appropriate. It may be that this type of sorus is worthy of generic rank, but it seems best for the present to consider it as belonging to a subgenus.

The distinctions between *D. occulta* and the two following species will be discussed in connection with them.

Season. Occurs from August until October.

Distribution. The present species has been found in several localities near Norwich, Conn., *Setchell!* and in Germany, *Irmisch.*

Literature.

? *Sclerotium occultum*, Hoffmann, Ic. Anal. Fung., pp. 67 to 68, Taf. 16, f. 1 to 9. 1863.

Doassansia occulta, De Toni, Journ. Myc., Vol. IV, p. 16. Mar. 1888 (in part).

——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 504. Oct. 1888 (in part).

Var. Farlowii (*Cornu*).

This form was collected by James Fletcher in Ottawa, Canada, and grew on several species of *Potamogeton*. The habit is probably identical with that of the form here described as the type. Fletcher, however, says, in a letter to Prof. Farlow, that the ovaries are swollen to three or four times their normal size, and are of a greenish white spotted with reddish brown. He also says that these distorted fruits often cracked and fell to pieces, developing a white mouldy growth. I certainly found no such behaviour in my specimens. The embryo of the pondweed is often found matured in specimens of the variety, but is always aborted in my own specimens. The alcoholic material of the variety crumbles easily, and consists almost entirely of a mass of sori, while that of the type form is decidedly firmer. But all these may be due to the age of the specimens or to the difference in the grades of alcohol used in preserving them, or to something of that nature.

The structure of the sorus is essentially the same in both the type and the variety, with the exception that the spores of the latter are much elongated radially (Fig. 78) and much narrower. The larger spores of the variety measure about 16 μ by 3 μ to 4 μ. The cortical cells are rather smaller than in the type. The elongated spores are shown in Cornu's figures (Ann. Sci. Nat. 5, sér. 6, T. 15, Pl. 16, f. 5 to 6), where the elongated spores are supposed to correspond with the cortical cells of *D. alismatis*, and the sterile cells of the centre of the sorus to the spores of that species.

It may be that this striking variation in the shape of the spores is due to Fletcher's specimens being immature, as Cornu supposed them to be. I have been able to examine specimens of the original collection made in August, 1882, and alcoholic material sent to Prof. Farlow, collected in June, 1884. By tracing the development of the sori, which corresponds exactly with that described for the type, I have found the same type of spore in sori that seemed to be mature. It seems impossible to consider these two forms identical, for they differ

more in the structure of the sori than the type does from *D. deformans*, and at the same time it is not safe to separate into two species two forms which agree so closely in habit and host until further evidence can be obtained from the development and germination.

Season. Specimens apparently mature were collected by Fletcher in June and others in August. This is earlier than I have been able to detect the form found about Norwich.

Distribution. Ottawa, Canada, *James Fletcher!*

Literature.

Doassansia Farlowii, Cornu, Ann. Sci. Nat., sér. 6, T. 15, p. 287, Pl. 16, f. 5–6. June, 1883.
——— Cornu, Bull. Soc. Bot. France, Vol. XXX, p. 133. Aug. 1883.
——— Farlow, Bot. Gaz., Vol. VIII, p. 276. Aug. 1883.
——— Farlow, Bot. Gaz., Vol. VIII, p. 318. Oct. 1883.
—— *occulta*, Cornu, in Farlow, Trans. Ottawa Field Nat. Club, Vol. II, p. 127. 1884.
—— *Farlowii*, Fisch, Ber. Deutsch Bot. Gesell., Bd. II, pp. 406 and 415. Nov. 1884.
—— *Martianoffiana*, Schroeter, Pilzfl. Schles., p. 287. 1887 (as to syn. *D. Farlowii*).
—— *occulta*, De Toni, Journ. Myc., Vol. IV, p. 16. Mar. 1888 (in part).
——— De Toni in Sacc., Syll. Fung., Vol. VII, p. 504. Oct. 1888 (in part).

Doassansia Martianoffiana (*Thuem.*), *Schroeter.*

The only accessible specimens were those from Sweden, collected by Johanson, and distributed in the *exsiccati* (see below). The fungus inhabits the floating leaves of various species of *Potamogeton*, on which it forms nearly circular spots of a very pale yellow. The sori do not show as prominently as in the species of *Eudoassansia*, and there is no distortion of the leaf at all. But there appear in each of Johanson's specimens little white tufts scattered about on the

upper surface of the spot, which call to mind the appearance of the conidia in certain species of *Entyloma*.

The sori are situated just above the lower epidermis in the large air-cavities which abound in the spongy parenchyma. They are nearly globular, and are from 100 μ to 160 μ in diameter. In structure they are almost identical with *D. occulta* (type), as described above. The centre is composed of parenchymatous cells; around this is a single layer of spores, which are slightly elongated radially, and which measure 12 μ by 6 μ to 8 μ; a cortex of brown cells outside the spores; and around the whole a covering of scanty hyphae.

The development of the sorus agrees perfectly with that of *D. occulta*. At first the ball of hyphae shows a concentric arrangement; soon there appears at the centre a small darker portion, which, as it increases in size, takes on a radiating structure, and appears more of a yellowish brown than the coat of concentric hyphae, which gradually grows thinner (Fig. 80). The steps by which the spores, cortex, and central cells appear could not be detected in the dried material.

The mycelium is abundant in the intercellular spaces of the infected portion of the leaf, and forms tangled masses in the dome-shaped cavities under the stomata of the upper surface. Bunches of short unbranched hyphae extend up through the stomata, and end somewhat bluntly. But at the tips of some, long slender spores were attached, much resembling the conidial spores of *Entyloma*. These spores are about 30 μ long and 1·5 μ wide. They apparently germinate in position, and thus give rise to small bunches of tangled hyphae. There can be little doubt that they belong to the *Doassansia*, but further study from the living material is highly desirable.

Schroeter (Pilzfl. Schles. p. 287) seems inclined to consider that these two species, the one inhabiting the ovaries and the other the leaves of species of *Potamogeton*, are identical. There is little difference between the sori of the two species: the chief differences are in habit. *D. occulta* causes a considerable distortion in the species which it inhabits; *D. Martianoffiana* none at all. But the fact that D. *occulta* causes a distortion

may be due to the more abundant nourishment which is supplied by the young starch-laden cells of the endocarp. The presence of conidia seems to be characteristic of *D. Martianoffiana.* They have not been found in *D. occulta*, even after careful search. In view of the absence of satisfactory information on these points, as well as on the subject of the germination of *D. Martianoffiana*, it seems best to keep the two forms distinct for the present.

Season. Johanson's specimens were collected in the month of September. Schroeter also gives this month.

Distribution. Siberia, *Martianoff*; Germany, *Schroeter*; Sweden, *Johanson!* What appear to be young specimens of the same species have been sent to Prof. Farlow by *James Fletcher* from Ottawa, Canada!

Literature.

Protomyces Martianoffianus, Thuem., Beitr. z. Pilzfl. Sibir. II, No. 123 [Extr. Bull. Imp. Soc. Nat. d. Moscow, T. 53, 1, p. 207]. 1878.

Doassansia Martianoffiana, Schroeter, Pilzfl. Schles., p. 287. 1887 (in part).

Protomyces Martianoffianus, Berl. et De Toni, in Sacc., Syll. Fung., Vol. VII, p. 320. Mar. 1888.

Doassansia Martianoffiana, De Toni, Journ. Myc., Vol. IV, p. 16. Mar. 1888.

——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 504. Oct. 1888.

Exsiccati.

Doassansia Martianoffiana, Johanson, in Eriksson, Fung. Par. Scand., No. 264. 1888!

——— Johanson, in Pazschke, Fung. Eur. No. 3602. 1890!

Doassansia deformans, *sp. n.*

Sagittaria variabilis has proved a most fruitful host for species of *Doassansia* in the United States. Three species have already been enumerated as occurring upon it; and in the present species, the fourth and most conspicuous of all is added. It is, in all respects, abundantly distinct from any of the other species already described upon the same host.

D. deformans occurs in all the green portions of the plant; in the petioles and ribs of the leaves; in the peduncles and pedicels of the flower-stalks; and in the walls of the ovary. It produces distortions of all of these parts, some of them of very large size.

When the blade of the leaf is affected, the fungus is confined to the veins, and the leaf is curled and twisted as if suffering from the attacks of some insect; the petioles are swollen at various points and usually bent at the swollen area into a sort of knee. The same thing happens in the case of the peduncle. The pedicels enlarge and their natural purple colour is often much increased under its influence. The affected ovaries may be told by their being swollen and projecting two to three times as far from the head of ripe fruits as their healthy neighbours.

A petiole may be swollen, for a length of two or three inches, to a diameter of an inch or more. A whole raceme may be affected, and one such specimen was 2½ inches high and 1½ inches in diameter, while the peduncle just below was only ¼ inch in diameter. The bases of all the leaves of a plant are at times swollen to a mass 6 to 8 inches across. So large are most of the distortions caused by this species that it seems strange that it has not been reported before; but the fact is that, in spite of the deformities produced by it, it is not very noticeable, and resembles rather the work of insects than of a fungus.

The structure of the petioles and peduncles of *Sagittaria variabilis* has been noticed before in connection with the description of *D. obscura.* From top to bottom of these portions run cylindrical spaces which are divided into short cylinders by frequent partitions at regular intervals. It is in these short spaces that the slender mycelium of the fungus, clinging closely to the bounding cell, forms a dense network. The sori fill the spaces in crowds, and forcing apart the walls, cause each space to enlarge to several times its normal diameter. It is in this way that the distortion is produced.

In the leaves and ovaries, the spaces are smaller and the

distortions are less. On cutting across a distortion, the sori become visible to the naked eye as small brown particles, present in such numbers as to give the cross-section a mealy appearance.

The sori are globular light-brown bodies, from 100 μ to 140 μ in diameter. In structure they resemble those of the form described as the type of *D. occulta* so nearly that it is hard to find any satisfactory difference (cf. Figs. 79 and 81). The sori, on the average, are slightly smaller than those of *D. occulta*; the spores are a trifle smaller (8 μ to 10 μ by 4 μ to 8 μ); and the cells of the cortex rather larger in proportion to the spores (being 8 μ to 12 μ tangentially and 4 μ to 6 μ radially).

But the germination is entirely different from that of *D. occulta*. The spores are a fixed part of the sorus, as in *D. occulta*, and germinate in position. So we have the sori surrounded by a dense covering of promycelia and sporidia, bristling with them like a chestnut-bur with its spines. The promycelium is obconical in shape, and reaches a length of about 12 μ. At the distal end it is 6 μ in diameter and at the proximal end only 4 μ.

In *D. occulta* also the promycelium is somewhat broader at the tip, and it may be a characteristic of this section of the genus. The sporidia are usually 5 or 6 in number, and are inserted in a whorl on the blunt tip of the promycelium (Figs. 51 to 53). They are about 12 μ long and 4 μ to 5 μ thick. They are thickest at the middle and taper to both ends when mature (Fig. 52). At maturity they conjugate in pairs, either at the base (Fig. 57) or at the apex (Fig. 58). The conjugated pairs germinate by means of a germ-tube. Soon after leaving the sporidium, the germ-tube increases in diameter (Figs. 54 to 57). It is soon constricted again, and so on at regular intervals (Fig. 55). This gives the germ-tube a curious appearance, as if it were going to break up into secondary sporidia. They reach a length of about 150 μ or 160 μ in water-cultures and then die.

The odd sporidium soon drops from the tip of the promycelium and apparently dies. The sporidia which have conjugated

cling to the top of the promycelium, and this breaks away from the emptied portion of the promycelium, forming a basidial cell as in *D. alismatis* (Figs. 56 to 58). When the basidial cell germinates, as it often does, the germ-tube has the same characters as that produced from a sporidium.

The spores when set free in water, from dried material, produce promycelia and crowns of sporidia in less than twenty-four hours. Germinations were obtained in the latter part of October, 1889, and in May and July, 1890. Sowings made in August and September, 1889, and in March, 1890, were unsuccessful, although the material for the sowing was taken from the same distortion from which the materials for the successful sowings were taken. There cannot be said to be any particular time for the germination of this species. The spores seemed to be very capricious about germinating. Different lots of sori would germinate for a week readily, then for several weeks none would germinate. This may have been due to different degrees of ripeness in the sori but as they all came from the same swelling it does not seem probable.

At first view *D. deformans* seems to be a very distinct species, but when the structure of the sorus is compared with the structure of the sorus of *D. occulta*, it comes very near to that species. It suggests that the difference in habit is due to the difference in the host-plants. But the difference in the modes of germinating is striking ; and this, if constant, is sufficient reason for keeping them apart. There was no variation in my sowings, and the two species were sown on slides at the same time and kept side by side, exposed as far as possible to the same conditions. There are no satisfactory differences in the structure of the sori of the species of this group, but they are to be distinguished from one another by the habit, host, and method of germination.

Season. The first specimens are found in the last days of July and it is in its prime at about the middle of August.

Distribution. *D. deformans* was first discovered growing

upon *Sagittaria* in abundance at Norwich, Conn., in the same locality with *D. opaca.* The *Sagittariae* were growing in about six inches of water. In August, 1890, it was found in Cambridge, Mass., in a ditch full of water near some brickyards.

Cornuella lemnae, *sp. et gen. n.*

This form inhabits the older fronds of *Lemna* (*Spirodela*) *polyrrhiza,* giving no sign of its presence except as the dying fronds, becoming more transparent, show the dark sori scattered through them.

The mycelium is not very abundant and has nothing particularly characteristic about it. The sori are found in the large intercellular chambers of the spongy parenchyma just above the lower epidermis. They have all the appearance of a *Doassansia* until examined in thin sections. They are rather small, being from 50 μ to 70 μ in diameter, but occasionally one is found measuring 100 μ across. They are nearly globular or decidedly ellipsoidal and are nearly black when ripe.

The structure of the sorus forms the characteristic of the genus. The interior is composed of loosely interwoven fine hyphae (Fig. 82), which appear to be hardened and of a brownish colour. Surrounding this mass of hyphae is a compact layer of spores, which have the same general structure as the *Doassansia*-spores. They are more or less oblong in cross-section, often somewhat elongated radially, and 10 μ to 12 μ long by 6 μ to 10 μ broad. There is no trace of a cortex, nor are there any external layers of hyphae as in most of the species described above.

The development of the sorus resembles that of the rest of the *Doassansia*-group in its earliest stages. The hyphae form an irregular, tangled ball in the air-space at first. The hyphae, however, soon seem to radiate in all directions from the centre, and the free tips appear swollen (Fig. 83). The spores are formed from these swollen ends and are separate when young, but as they mature they become compacted

together into a hollow sphere, enclosing the hyphae at whose tips they are borne. Thin sections of the mature sori show that the ripened spores still remain attached to the hyphae from which they have arisen (Fig. 82).

The spores do not separate from one another when the sorus is crushed, but germinate in position. From ten to eighteen hours after being sown in water the sorus is covered with a bristly mass of promycelia and sporidia. The promycelium sometimes reaches a length of 16 μ, and is somewhat broader at the tip than it is where it arises from the spore. The sporidia are in whorls, from five to seven, on the broad blunt tip of the promycelium (Figs. 59 to 61), which is emptied of its contents and has septa (one or two), as mentioned above for other species. They are long and slender (26 μ long by about 2 μ thick), pointed at both ends, and soon drop off. They are then found to be united in pairs at the base, sometimes with a conspicuous conjugating tube (Fig. 62), but more often with almost none (Fig. 63). The germ-tube comes from the base of the pair of sporidia, as far as observed, but no germ-tubes of any length were seen. Deformities like the one shown in Fig. 64 were seen several times.

The type of germination described above was found to be constant in numerous sowings made from fresh material in the months of June and July, both in 1889 and 1890. In these months the spores germinated freely a few hours after sowing. In August of both years the spores from fresh material germinated only scantily, and many of the promycelia failed to produce sporidia. Sowings made in September of both years failed to yield any results at all.

Little need be said about the distinctness of this form. The structure of the sorus is entirely unlike anything described for any other member of the Ustilagineae. It seems necessary, therefore, to consider this species as the type of a new genus, and I take pleasure in dedicating it to Dr. Maxime Cornu, of Paris, who has so ably described and illustrated so many rare and curious Ustilagineae for us.

Season. *Lemna polyrrhiza* begins to appear on the surface

of the pools in the neighbourhood of Cambridge about the first of May. By the beginning of the last week in May the fungus is not uncommon, and is to be found from that time on until the *Lemnae* disappear in October or November.

Distribution. A pool known as 'Glacialis,' near Cambridge, Mass., was the first locality, and was discovered in June, 1889. I have also found it in Newton, Mass. Prof. J. E. Humphrey, of Amherst, Mass., has kindly sent material collected by him at Belchertown, Mass.

Burrillia pustulata, *sp. et gen. n.*

In the present form, only very recently discovered, we have a species which is still another addition to the curious forms already described. It inhabits the leaves of *Sagittaria variabilis*, forming upon them yellow spots of circular shape and small size. On the under side is seen what in dried specimens looks like a *Cystopus*. The epidermis is raised in a small blister-like swelling which finally ruptures, when a sort of powdery appearance shows beneath.

On cutting a section through an infected spot it is seen that the sori are situated just over the epidermis of the lower side. They are ellipsoidal bodies of a white or light-brown colour, being very different in general appearance from the brown sori of most of the species of the *Doassansia*-group. They measure from 200 μ to 350 μ by 150 μ to 180 μ. Often several sori have grown together into one long sorus.

The structure is different from that of any of the other sori here described. The central portion is composed of parenchymatous cells with watery contents and moderately thick walls. When the sorus is approaching maturity these cells have conspicuous globules of oil in them, but they lack the definitely globular or ellipsoidal shape of the spores, as well as the dense, granular, highly refractive contents. Outside of this mass of cells follow several layers of spores rather closely compacted together (Fig. 84). They are slightly polyhedral, 4 μ to 6$\frac{1}{2}$ μ in diameter, and resemble in all respects the spores of *Doassansia*. There is no cortex of parenchymatous cells,

but the sorus is surrounded by a close coat of hyphae. This coat is often six to eight hyphae thick, and some sections show only cross-sections of them, while others show a confused mass (Fig. 84).

The spores germinate while the sori are still in the leaf. The spores do not separate from the sorus, and therefore the promycelia and sporidia radiate out on all sides from the sorus. The promycelia of the inner spores force themselves out between the outer spores to the surface. The promycelia of the outer spores are about 15 μ long; they bear, in whorls at their tips, four to five slightly bent sporidia, which measure about 16 μ in length and 3 μ in diameter. I have not been able to learn anything farther about the development either of the sporidia or of the sori.

The relationships of this species are very perplexing. It is in no danger of being confused with any other. From the species of the subgenus *Doassansiopsis* it is distinguished by the absence of a cortex, and also by having more than one layer of spores. From all other species it is distinguished by the central mass of parenchymatous cells. It certainly seems to belong to a distinct genus which I desire to dedicate to Prof. T. J. Burrill, of the State University of Illinois, who has done so much to advance our knowledge of American parasitic fungi.

Season. The specimens were collected the last of July, 1889.

Distribution. Dixon, Illinois, *G. P. Clinton!* The specimens were sent to Prof. Farlow by Prof. T. J. Burrill.

Literature.

Prof. Trelease says, on p. 36 of his Parasitic Fungi of Wisconsin (Trans. Wisc. Acad., p. 264, Nov. 1884), that the conidia of *D. alismatis* occur upon *Sagittaria*. Specimens kindly sent by Prof. Trelease to Prof. Farlow show that the form on *Sagittaria* referred to is the present species. The 'conidia' were probably the sporidia of the sori which had germinated in position.

Doassansia Niesslii, *De Toni.*

This species inhabits the leaves and peduncles of *Butomus umbellatus*, on which it may be detected by the very pale yellow, rather elliptical spots, dotted with the dark-brown sori. The only accessible specimens were those distributed by Sydow (Myc. March., No. 2206), from the Botanic Gardens at Berlin.

Careful sections show that the sori are situated in the cortical layers just beneath the epidermis. Each sorus lies in the chamber immediately under a large stoma. Thin sections show at once that this species is not a *Doassansia*, at least as founded by Cornu. The sorus is a rather irregularly ellipsoidal body, with its long axis parallel to the long axis of the leaf or peduncle which it inhabits. The cross-section is nearly circular, and about 50 μ in diameter. In longitudinal section it is narrowly elliptical, being about 160 μ long and 50 μ across. The spores are closely packed together with very few interspaces, and the exospore, which is moderately thickened, is of a light brown colour. The spores have highly refractive light-coloured contents, with one or two rather large oil-drops in each. They are from 6 μ to 8 μ in diameter. The outer spores have darker coloured exospores than the inner ones. There is no layer of cortical cells at all. Abundant hyphae are found in the immediate vicinity of the sorus, and occasionally a few strands form a sort of partial covering for the sorus, but this is not as complete as in the two following species.

The spores seem to germinate in the sorus at maturity as they do in *D. decipiens*. The promycelia are distinct, and the sporidia long and slender, but I could not determine from the dried specimens either how they were borne or how they behaved.

Season. August and September.

Distribution. Austria, *Niessl*; Germany, *Schroeter*; *Sydow!*

Literature.

Protomyces punctiformis, Niessl, Beitr. z. Kennt. d. Pilze, p. 16. [Verhandl. d. Naturf. Ver. i. Brünn, Bd. X.] 1872.

Protomyces punctiformis, Berl. et De Toni, in Sacc., Syll. Fung., Vol. VII, p. 321. Mar. 1888.

Doassansia punctiformis, Schroeter, Pilzfl. Schles., p. 287. 1887. (Not Winter.)

—— *Niesslii*, De Toni, Journ. Myc., Vol. IV, p. 17. Mar. 1888.

———— in Sacc., Syll. Fung., Vol. VII, p. 505. Oct. 1888.

Exsiccati.

Doassansia punctiformis, Sydow, Myc. March., No. 2206. 1888! (Not Winter.)

Doassansia limosellae (*Kunze*), *Schroeter.*

Kunze discovered this species and distributed it under the name of *Protomyces limosellae.* Schroeter was the first to refer it to the genus *Doassansia.* A careful examination of Kunze's specimens shows that this species also does not possess the distinctive characters of a *Doassansia.* The sori occur in the rather thick and fleshy leaves of *Limosella aquatica*, and are said to inhabit circular brownish spots, but it is impossible to detect this in the dried specimens at my disposal.

In cross-section the leaf does not show distinct palisade and spongy layers, but is rather thick and of similar structure on both sides of the median line drawn through the row of vascular bundles. The sori are situated, on both sides, in the large intercellular spaces just under the stomata. Occasionally a sorus is situated in the tissues of the leaf midway between the two surfaces. The sori themselves are from 60 μ to 100 μ in diameter, and are of a decidedly brown colour. They are somewhat irregular in shape, generally globose, but often elongated. The spores are 9 μ to 14 μ in diameter, nearly spherical in shape, and closely packed together, yet with small spaces between them. The outer coat is thin and brownish, while the contents are pale and highly refractive, with a few, rather large oil-globules. About each sorus is a covering of hyphae, which in some places is several layers thick and in others almost wanting. The hyphae are closely applied to the mass of spores and their walls are much thickened and very brown.

Season. Kunze's specimens were collected in the autumn. Schroeter further says (l. c.) July to September.

Distribution. Known only from Germany. Saxony, *Kunze!* several localities in Silesia, *Schroeter.*

Literature.

Protomyces limosellae, Kunze, in Rab., Fung. Eur., No. 1694. 1873.
Entyloma limosellae, Winter, Pilze, Abth. I, p. 115. 1884.
Doassansia limosellae, Schroeter, Pilzfl. Schles., p. 287. 1887.
——— De Toni, Journ. Myc., Vol. IV, p. 17. Mar. 1888.
——— — De Toni, in Sacc., Syll. Fung., Vol. VII, p. 505. Oct. 1888.

Exsiccati.

Protomyces limosellae, Kunze, in Rab., Fung. Eur., No. 1694. 1873!

Doassansia decipiens, *Winter.*

I am indebted to Mr. E. A. Rau, the discoverer of this 'interesting but doubtful species' as Winter calls it, for excellent dried material of the original collection from New Jersey. Although it has been searched for carefully in Eastern Massachusetts and Connecticut on the same host, no trace of it has been seen. Consequently no fresh material has been available for study.

Doassansia decipiens is parasitic in the leaves of *Limnanthemum lacunosum,* and produces on them circular spots of a dull yellow colour. In dried specimens the sori appear very distinct in the form of small, dark-brown warts scattered irregularly through the spot. There is no distortion of the leaf at all.

The cross-section of a leaf shows that there are three distinct rows of palisade-cells, and below these, several layers of very loose spongy parenchyma. In the region of the spot all of these layers abound with the hyphae of the fungus. They are very slender (2 μ to 3 μ thick), moderately branching, and very much intertwined in places. Under the stomata one generally finds bunches of entangled hyphae, the early stages in the development of the sori.

The sori are situated for the most part in the uppermost layer of the palisade-parenchyma just under the stomata. A cross-section through the centre of a spot shows 15 to 20 of them closely packed together. They are nearly globular, often somewhat flattened vertically, and measure 60 μ to 100 μ by 75 μ to 140 μ. They appear of a very dark brown at the periphery, and of a much lighter colour in the interior. The spores are very firmly compacted together, yet with numerous though very small spaces between them. They are irregularly polygonal in shape, 6 μ to 12 μ in diameter, with moderately thick, light-brown outer walls, and pale contents with a single large or a few small oil-globules. The sori are invested with a compact layer of hyphae. Over some portions this layer is several hyphae thick, in other places a single layer only is present. The hyphae have thickened walls and are very dark brown (Figs. 65 *a* and 65 *b*). This cortex is more pronounced than in either of the two preceding species, and in some sections (cf. Fig. 65 *b* with Fig. 66) appears to be almost as true a cortex as is present in *D. epilobii*. But the hyphal character generally shows (as in Fig. 65 *a*), and this is not the case in *D. epilobii*.

Owing to the lack of fresh material, or even of material comparatively recently collected, the germination of the spores could not be obtained. Cross-sections through the older spots showed that nearly all of the spores of the older, more central sori had germinated in position. The upper part of the sorus, as well as the epidermis of the leaf, had been ruptured, and each sorus had a bunch of long filaments, the promycelia and sporidia, projecting from it and extending beyond the surface of the leaf. The promycelia could be seen distinctly and resembled those of *D. alismatis*. The sporidia were very long and slender (70 μ by 1 μ), and appeared to germinate by tubes, but both the arrangement of the sporidia on the promycelium and the details of their germination were not satisfactorily determined.

Season. Mr. Rau's specimens were collected in the early

part of August and show many sori already long past maturity.

Distribution. Known only from the original locality at Green Pond, Morris Co., New Jersey!

Literature.

Doassansia decipiens, Winter, Journ. Myc., Vol. I, p. 102. Aug. 1885.
——— De Toni, Journ. Myc., Vol. IV, p. 17. Mar. 1888.
——— De Toni, in Sacc., Syll. Fung., Vol. VII, p. 505. Oct. 1888.
——— Farlow and Seymour, Host Index, p. 79. Sept. 1890.

The three species just described are not readily distinguished from one another by the characters of the sori, and need to be carefully studied from living material. They certainly form a group distinct from that which clusters about *D. alismatis* as a type. They all have the spores compacted into sori, and readily separating at maturity. They have, however, no cortex of sterile parenchymatous cells, as the species of *Doassansia* should have. On the other hand, they are to be distinguished from the species of *Entyloma* as described by De Bary (Bot. Zeit. Bd. 32, p. 107, 1874), and as found also in *E. compositarum*, *E. lobeliae*, *E. menispermi*, and *E. physalidis*, by the possession of distinct sori. But there are species still included under *Entyloma*, such as *E. crastophilum*, which approach them in this respect. Where this group of species is to be referred ultimately can only be settled by a careful study of the greater part or all of the species of *Entyloma*. They do not belong to the genus *Doassansia*, Cornu, but are to be included for the present at least under *Entyloma*.

SYSTEMATIC ARRANGEMENT.

Genus I. **Doassansia**, Cornu. Sorus, a mass of spores surrounded by a distinct cortex of parenchymatous cells (*Eudoassansia*); or with the central portion composed of fine hyphae (*Pseudodoassansia*); or of a mass of cellular tissue (*Doassansiopsis*).

Subgenus I. *Eudoassansia.* The body of the sorus consisting entirely of spores which at maturity are readily separable from one another. Cortex well developed.

1. *D. epilobii*, Farlow.
2. *D. hottoniae* (Rostr.), De Toni.
3. *D. sagittariae* (Westend.), Fisch.
4. *D. opaca*, sp. n. Spot circular, slightly swollen on both surfaces, lemon-yellow. Sori indistinct, more or less cubical, crowded together, 200–300 μ by 80–100 μ. Spores nearly spherical, 10–15 μ in diameter. Cells of the cortex irregular in shape, form nearly cubical to brick-shaped.

   The leaves of *Sagittaria variabilis.*

   Newton, Mass., *Prof. W. G. Farlow!* Medford, Mass.! Norwich, Conn.!
5. *D. alismatis* (Nees), Cornu.

Subgenus 2. *Pseudodoassansia.* Central portion of the sorus composed of fine hyphae. Spores in irregular layers separable at maturity. Cortex very distinct.

6. *D. obscura*, sp. n. Spot light-yellow and indistinct, or none. Sori in vertical lines in the larger intercellular spaces, 150–300 μ in diameter, nearly globular. Spores loosely packed together, 8–12 μ in diameter. Cells of the cortex obconical, with the outer, broad end more or less deeply lobed; light-brown. Promycelium cylindrical, about 20 μ long. Sporidia in a whorl of 5–7, 16–17 μ long by 1·5–2 μ thick, producing secondary sporidia without conjugation.

   On petioles and peduncles of *Sagittaria variabilis.*

   Norwich, Conn.! Medford and Cambridge, Mass.!

Subgenus 3. *Doassansiopsis.* Central portion of the sorus consisting of a mass of parenchymatous cells. Spores in a single layer, not separable at maturity. Cortex distinct.

7. *D. occulta* (Hoffm.), Cornu.
   D. occulta var. *Farlowii* (Cornu.).
8. *D. Martianoffiana* (Thuem.), Schroeter.
9. *D. deformans*, sp. n. Species often forming large distortions. Sori nearly globular, 100–140 μ in diameter, pale. Spores 8–12 μ by 4–6 μ. Cells of the cortex flat, distinct. Promycelium obconical, about 12 μ long. Sporidia in a whorl of 5 to 6, short, broadly fusiform, conjugating either at the base or at the tip and producing an irregular germ-tube.

   On petioles and ribs of the leaf, on peduncles, pedicels and ovaries of *Sagittaria variabilis.*

   Norwich, Conn.! Cambridge, Mass.!

Species known to me only from description :—

10. *D. comari* (B. & Br.), De Toni et Massee.
11. *D. punctiformis*, Winter.
12. *D. lythropsidis*, Lag.

Species to be excluded from *Doassansia*:—

D. Niesslii, De Toni.
D. limosellae (Kunze), Schroeter.
D. decipiens, Winter.

Genus II. **Burrillia**, gen. n. Central portion of the sorus consisting of a mass of parenchymatous cells. Spores in several irregular, compact layers. Cortex absent.

B. pustulata, sp. n. Spot none or very indistinct. Sori at length causing small pustules on the lower surface of the leaf and finally rupturing the epidermis. Ellipsoidal, 200–300 μ by 150–180 μ. Central portion a mass of polyhedral cells. Spores in two to six rows, polyhedral, 4–6 μ in diameter. Germination occurring in the leaf. Promycelium cylindrical. Sporidia in whorls of 4 to 5. About 16 μ long by 3 μ wide.

The leaves of *Sagittaria variabilis.*

Dixon, Ill., *Mr. G. P. Clinton!* Madison, Wisc., *Prof. W. Trelease!*

Genus III. **Cornuella**, gen. n. Sorus consisting of a firm layer of spores on the outside, and of loose hyphae on the inside. Cortex absent.

C. lemnae, sp. n. Spot none. Sori globular to ellipsoidal, 50–70 μ in diameter, brownish black. Hyphae of the central part of the sorus brown. Spores borne at the tips of hyphae, inseparable at maturity, 10–12 μ by 6–10 μ. Promycelium obconical, about 16 μ long. Sporidia in whorls of from 5 to 7, about 26 μ long by 2 μ broad.

On *Lemna* (*Spirodela*) *polyrrhiza*.

Belchertown, Mass., *Prof. J. E. Humphrey!* Newton and Cambridge, Mass.!

RELATIONSHIPS.

About the genus *Tilletia* are clustered a number of distinct genera, closely related to it both in type of spore and in type of germination, but differing widely from it in the complexity of the structure of their compound sporophores. Such are the genera *Urocystis* and *Tuburcinia*, and perhaps also *Sorosporium*, *Cintractia*, and *Testicularia*. When De Bary in 1874 (Bot. Zeit. p. 81) established the genus *Entyloma*, he recognised its near relationship to *Tilletia* as well as its distinctness from it. Since then, numerous additions have been made to the genus, until at present it contains about 40 species of very diverse structure and habit. Some of these, as e. g. *E. crastophilum*, Sacc., are of much more complex structure than the simpler typical species, such as *E. microsporum* (Ung.), Schroeter, *E. lobeliae*, Farlow, &c. When, in 1883, Cornu proposed the genus *Doassansia*, it seemed probable that there might be a series of generic types clustered about *Entyloma* as well as about *Tilletia*. The present paper has endeavoured to describe a number of these types, several of which bear a striking likeness to generic types of the *Tilletia*-group.

The spores of the simple species of *Entyloma* are more or less scattered through the substance of the part of the leaf

which they inhabit. In more complex species they occur in groups or heaps somewhat contracted together. The step from such a condition to that shown in *D. Niesslii*, *D. limosellae*, or even in *D. decipiens*, is a gradual and easy one. The need for a covering of some kind for the sorus in the species with aquatic hosts is shown in *D. limosellae*, and to a much greater extent in *D. decipiens*, by the dense coat of browned and hardened hyphae. We find this covering supplied in *D. hottoniae* and species of true *Doassansiae*, by the transformation of the outermost spores to form the cells of the cortex. Otherwise the development of the sorus seems to be the same as in the preceding group. There are two sets of variations from the type of the group of the *Eudoassansiae*; in *D. obscura*, the central hyphae of the sorus remain undeveloped, while the cells of the outer hyphae are changed into the spores and the sterile cells of the cortex; whereas in the species of the subgenus *Doassansiopsis*, the cells of the central hyphae have become swollen, but instead of developing into spores, have remained with their walls, have lost their solid contents, and so have become changed into a mass of parenchymatous tissue.

The genus *Burrillia* stands in about the same relation to the group of species represented by *D. decipiens*, that *Doassansiopsis* does to *Eudoassansia*. Both groups lack the cortex; and while the sorus of *D. decipiens* is readily separable into its component spores on crushing, the sorus of the genus *Burrillia* possesses a central mass of parenchymatous cells about which the spores are compacted so firmly as to be practically inseparable either from it or from each other.

It is a difficult matter to determine the relation in which *Cornuella lemnae* stands to the other types. It may, perhaps, bear much the same relation to the *D. decipiens* group that *Pseudodoassansia* does to *Eudoassansia*. But the divergence between *C. lemnae* and *D. decipiens* certainly seems greater than, for instance, does that which exists between *D. obscura* and *D. sagittariae*. We may perhaps hope for some new form to throw more light upon the affinities in this case.

The views sketched above may be roughly expressed in the following diagram :—

Entyloma

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Cornuella ?—D. decipiens group—Burrillia

|

Pseudodoassansia—Eudoassansia—Doassansiopsis.

Cryptogamic Laboratory of Harvard University, Feb. 7, 1891.

EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Mr. Setchell's paper on *Doassansia*.

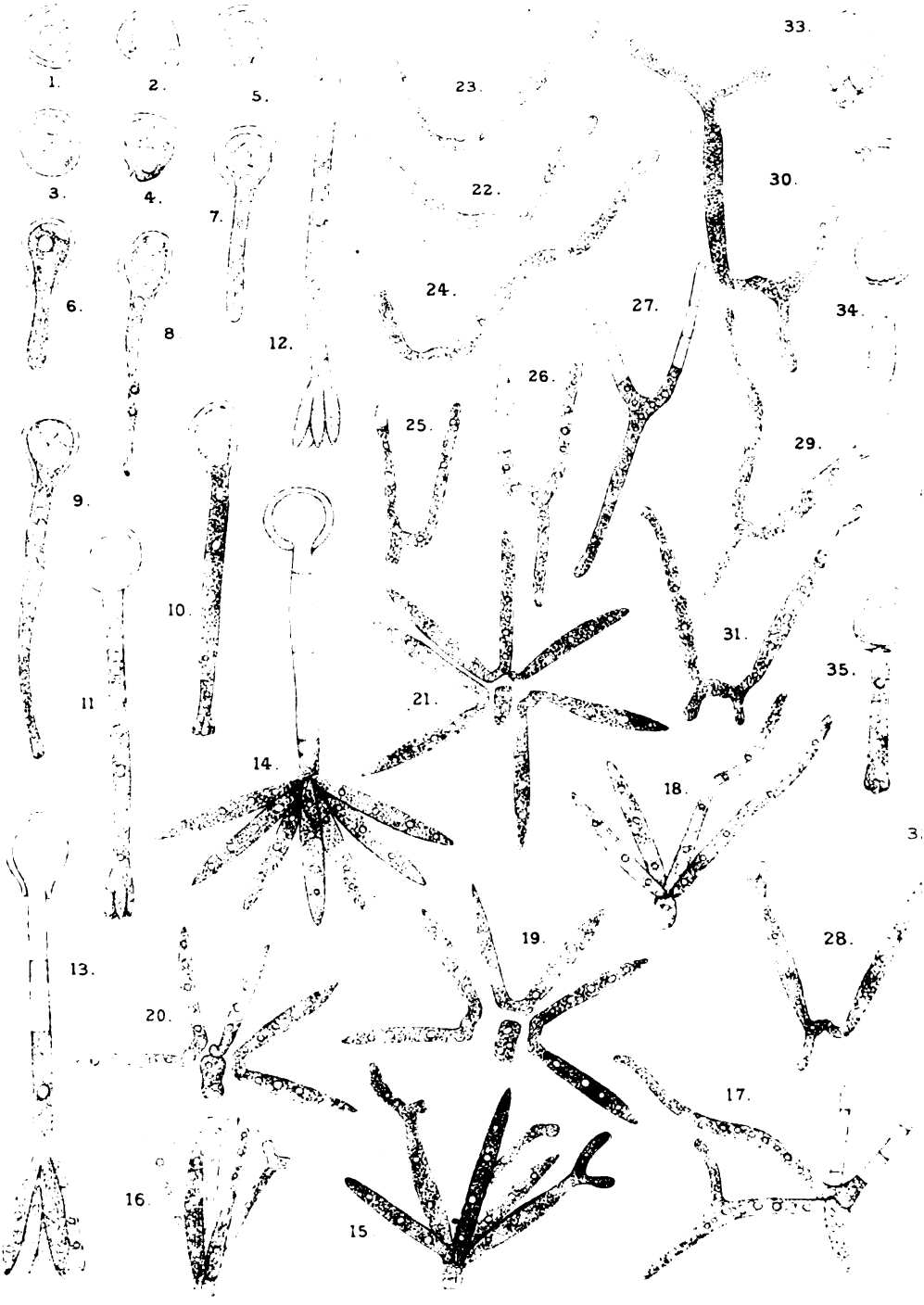
PLATE I.

Figs. 1 to 32. Germination of *D. alismatis*.
Figs. 33 ,, 42. ,, ,, *D. obscura*.
Figs. 43 ,, 50. ,, ,, *D. occulta*.
Figs. 51 ,, 58. ,, ,, *D. deformans*.
Figs. 59 ,, 64. ,, ,, *Cornuella lemnae*.
All the figures × 1000.

PLATE II.

Figs. 65*a* and 65*b*. Portion of cross-section of the sorus of *D. decipiens*. × 650.
Fig. 66. Portion of cross-section of the sorus of *D. epilobii*. × 650.
Fig 67. ,, ,, ,, ,, ,, *D. hottoniae*. × 650.
Fig. 68. ,, ,, ,, of a leaf of *Alisma Plantago*, showing two sori of *D. alismatis*. × 150.
Fig. 69. Portion of cross-section of the sorus of *D. alismatis*. × 650.
Fig. 70. Tip of hypha of *D. alismatis*. × 1000.
Fig. 71. Portion of cross-section of the sorus of *D. sagittariae*. × 650.
Fig. 72. Cross-section through a spot of *D. opaca* on the leaf of *Sagittaria variabilis*. × 87.
Fig. 73. Portion of side of cross-section of the sorus of *D. opaca*. × 650.
Fig. 74. Portion of upper part of cross-section of the sorus of *D. opaca*. × 650.
Fig. 75. Portion of cross-section of petiole of *Sagittaria variabilis*, showing cross-section of a sorus of *D. obscura*. × 87.
Fig. 76. Portion of cross-section of sorus of *D. obscura*. × 375.
Fig. 77. Portion of tangential section of the cortex of the sorus of *D. obscura*. × 650.

Fig. 78. Portion of cross-section of the sorus of *D. occulta*, var. *Farlowii*. × 650.
Fig. 79. Portion of cross-section of *D. occulta* (type). × 650.
Fig. 80. Median optical section of developing sorus of *D. Martianoffiana*. × 650.
Fig. 81. Portion of cross-section of the sorus of *D. deformans*. × 650.
Fig. 82. „ „ „ „ „ *Cornuella lemnae*. × 650.
Fig. 83. Developing sorus of *C. lemnae*. × 650.
Fig. 84. Portion of cross-section of the sorus of *Burrillia pustulata*. × 650.
All the figures were drawn from nature by the author.



SETCHELL.— ON DOASSANSIA.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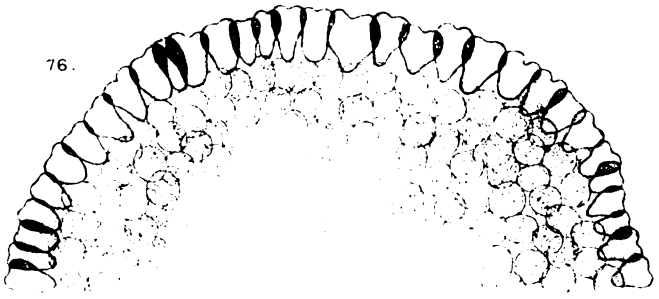
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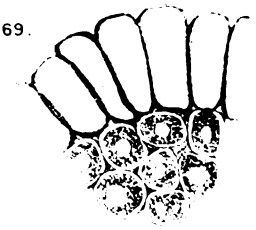
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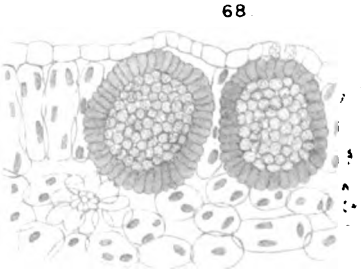
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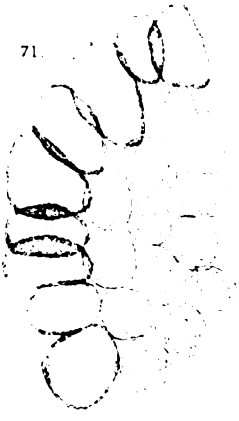
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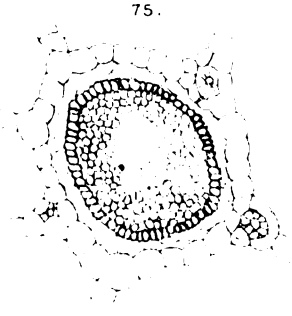
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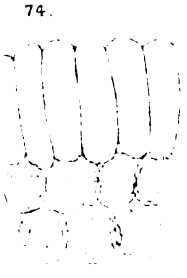
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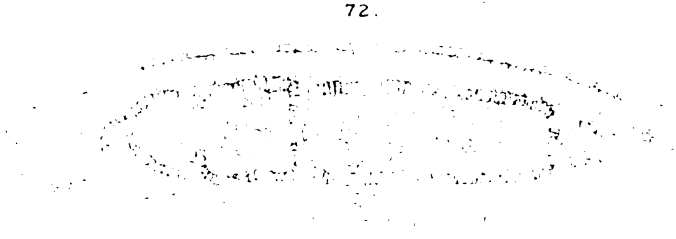
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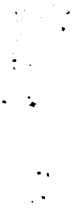
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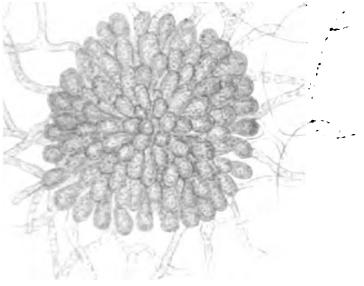
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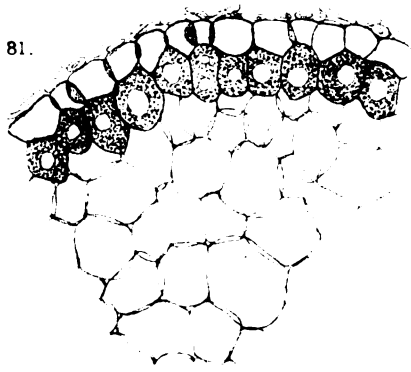
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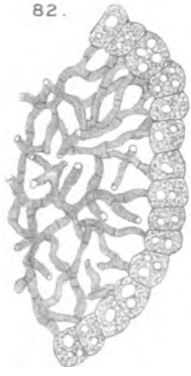
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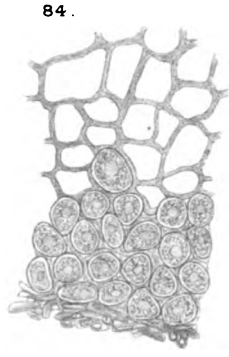
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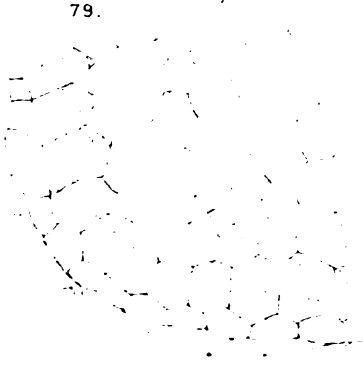
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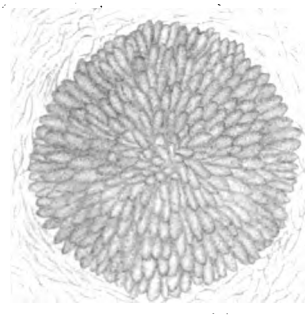
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On the Prothallium and Embryo of Osmunda claytoniana, L., and O. cinnamomea, L.

BY

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With Plates III, IV, V, and VI.

AMONG the various Pteridophytes that have been recently the subjects of investigation, the Osmundaceae have perhaps hardly received the attention they deserve, and this is especially the case in regard to the embryo.

Finding that apparently no researches had been made upon the development of the embryo of *Osmunda*, the present work was undertaken in order to make a comparative study of this in two of the common American species, *O. claytoniana* and *O. cinnamomea*. During the course of the work, however, finding that many points in the development of the prothallium and sexual organs were only imperfectly described, and that the species under consideration showed numerous peculiarities, it was decided to study critically these points in both species, as well as the embryogeny.

Both species are widely distributed throughout the northeastern United States, and differ very much in appearance from the cosmopolitan *O. regalis*, which has been the species hitherto principally studied. While the sterile leaves of *O. cinnamomea* and *O. claytoniana* are much alike, the fertile leaves differ widely. In the former all the pinnae are fertile, while

[Annals of Botany, Vol. VI. No. XXI. April, 1892.]

in the latter only a few pairs of the middle ones bear spores. A study of the histology, especially of the root[1], shows marked differences in the two species, and this is true also of the prothallium.

The spores from which the prothallia here described were grown, were sown at intervals from the 3rd to the 27th May, 1890, and the observations were continued until about the 1st June, 1891. The spores of *O. claytoniana* were gathered near Bloomington, Indiana, in part, and partly near Greencastle, Indiana. In the present year (1891) the spores ripened the last week in April, about a week earlier than in 1891. I am indebted to Prof. Bastin of Chicago for abundant material of *O. cinnamomea*, gathered near that city about May 27.

The ripe spores of both species (Fig. 1) resemble each other closely. They are nearly globular in form, and the colourless exospore sufficiently transparent to show clearly the character of the contents. The exospore is covered with numerous small protuberances, and on the upper surface are the three radiating ridges that show where the spore was in contact with the sister-spores in the mother-cell. These ridges are very conspicuous, and indicate where the exospore is ruptured when the spore germinates. The endospore is thin, though sufficiently evident, but does not show the cellulose-reaction before germination; and it is probable that here, as in *Gleichenia*[2], there is a new cellulose membrane formed inside the endospore, as the first sign of germination. The large nucleus, containing a conspicuous nucleolus, occupies the centre of the spore, but is somewhat obscured by the dense mass of chloroplasts that surrounds it. Although these are so crowded as to render the individual ones indistinct, there is no question that even in the ungerminated spore separate chloroplasts exist; and that the chlorophyll is not present in an amorphous condition as described by Kny[3] and others.

[1] Campbell, On the apical growth of the roots of *Osmunda* and *Botrychium*; Bot. Gazette, Feb. 1891.

[2] Rauwenhoff, La Génération sexuée des Gleicheniacées; Archives Néerlandaises, T. XXI. p. 157-231.

[3] Kny, Entwicklung des Vorkeimes von *O. regalis*; Pringsh. Jahrb. VIII. p. 8.

On crushing the spore, drops of a glistening substance, probably a fatty oil, may often be seen, and small starch-granules are sometimes to be detected, but in small numbers. The chlorophyll is confined mainly to the central part of the spore, but a few chloroplasts extend to the periphery.

By fixing the nearly ripe sporangia with a one per cent. solution of chromic acid, and imbedding in paraffin, the spores are easily sectioned, and the minute structure may then be readily studied (Fig. 2). The nucleus is rich in chromatin, and contains a single large nucleolus. The two spore-membranes are readily seen in such preparations.

The Germination of the Spores.

The spores germinate promptly if placed in water or on damp soil, this taking from 24–48 hours, depending upon the temperature. The exospore bursts along the three ridges, and the spore-contents, now invested with an evident cellulose membrane, protrude through the aperture (Figs. 1 *c*, 3).

The first division takes place after the spore has elongated slightly, and is usually at right angles to its longer axis. The resulting cells are of very unequal size, the larger becoming the mother-cell of the prothallium, the other simply elongating and forming the first root-hair. Unlike most ferns, the root-hair here contains considerable chlorophyll, although less than is found in the prothallium mother-cell. The first cell-wall in the latter is usually parallel with that that separates the root-hair; but not infrequently, especially in *O. claytoniana* (Figs. 4 *a*, 7), it makes a considerable angle, even a right angle in some cases, with the first wall, so that the axes of growth of the first root-hair and prothallium make an angle of 90° sometimes, instead of being the same. Kny[1] lays considerable stress upon the bipolar germination of the prothallium of *Osmunda*, as distinguished from that of the Polypodiaceae, where he regards the first root-hair as lateral; but my observations have shown that, especially in *O. claytoniana*, the

[1] Kny, l. c. p. 12.

first root-hair may have precisely the same position as in the Polypodiaceae.

Kny[1] and Luerssen[2] also emphasize the point that in *O. regalis*, and usually in *Todea*, no protonemal filament is formed, but that the first divisions form at once a cell-surface. While in the species here considered this was frequently met with in *O. cinnamomea* (Figs. 12, 13), it was very rarely the case in *O. claytoniana*, which in a large majority of instances formed a row of two or three cells before any longitudinal walls appeared, although such walls may subsequently form in the lower cells.

In case the germination is truly bipolar, the exospore is pushed up with the growing prothallium, and forms a cap at its apex (Fig. 6), but if the root-hair is lateral, the exospore remains at the base of the prothallium (Fig. 7).

While in *O. claytoniana* there are, as stated, several transverse walls formed before any longitudinal ones appear, in *O. cinnamomea* it is quite common, as in *O. regalis*, to have after the first transverse wall a longitudinal wall formed in each cell, so that the four resultant cells are arranged like quadrants of a circle (Fig. 12 *c*). One of the upper cells, as described by Kny[3] for *O. regalis*, becomes the apical cell of the young prothallium. When a protonema is formed, as usually occurs in *O. claytoniana*, the apical cell is formed as in the Polypodiaceae. After one or more transverse walls have been formed, an oblique wall is formed in the terminal cell, and immediately after a second one striking it at an angle of about 90°. The cell included between these walls is the apical cell, from which are formed two sets of segments as is usual in most fern-prothallia (Figs. 4, 10 *x*). From the time it is first formed, this cell continues to function as the apical cell until the prothallium reaches a considerable size.

Very rarely the first wall in the prothallium-mother-cell is longitudinal as is often the case in *Equisetum* (Fig. 5), and sometimes the first divisions are in three planes so that a cell-mass is formed at once (Figs. 15, 19).

[1] L. c. p. 12. [2] See Schenk's Handbuch, I. p. 171. [3] L. c. p. 5.

As germination proceeds, the chloroplasts separate and increase in size and distinctness. They are often arranged in lines extending from the nucleus to the periphery of the cell. The nuclei are large and distinct, and in the rapidly growing young prothallia are often met with in division (Fig. 4 *a*). No especial observations were made upon them, but they do not seem to offer any noteworthy peculiarities.

Growth of the Prothallium.

As soon as the apical cell is established, the divisions occur with considerable regularity. Segments are cut off alternately from the sides, and the limits of the segments can usually be traced for some time. The first wall in the young segment usually divides it into a marginal cell and an inner one. The former undergoes division mainly by longitudinal walls, and from it arise the marginal cells of the prothallium. The divisions of the inner cell are both longitudinal and transverse, and very early nearly horizontal walls are formed in the innermost cells so that the axial part of the prothallium, even in its earlier stages, is several cells thick, and the beginning of the midrib, so conspicuous in the older prothallia, is thus formed.

In *O. cinnamomea*, when a cell-surface is formed at once, it sometimes happens that for a short time both of the upper cells divide alike, and it is impossible to say which is to be the permanent apical cell (Fig. 16 *x*, *x'*), but usually this is established while the prothallium is still very small.

In the earliest stages the apical cell is comparatively broad, and the segments remain undivided, or divide slowly, so that the outer wall of the apical cell projects beyond them; soon, however, the young segments grow more rapidly, and cell-division proceeds faster, so that the outer cells are pushed forward and soon reach the level of the apical cell (Fig. 11), whose outer wall then becomes nearly straight, and the prothallium has the form of a narrow wedge, with the apical cell occupying the middle of the base. A continuance of the

growth in the segments causes them to extend more and more beyond the apical cell, which thus comes to lie in a depression, and the familiar heart-shape of other fern-prothallia is produced.

From the first the prothallium is more elongated than is common in Polypodiaceae or in *O. regalis*[1]. The very broad form of prothallium, figured for the latter by Kny[2], I have failed to find in either species examined by me.

The single two-sided apical cell persists for a long time, but is finally replaced by one of different form, or by several similar initial cells. In a number of cases observed (Fig. 23), the triangular initial had been replaced by a four-sided one, a set of basal segments being formed as well as lateral ones, as in *Pellia*[3]. Whether this condition is ever permanent, is difficult to say positively. Usually the single four-sided initial is divided into equal parts by a longitudinal wall (Fig. 27), and the resultant cells may divide again in the same way so as to form a row of similar marginal cells as in other ferns.

Attention has already been called[4] to the remarkable resemblance between the apical growth of fern-prothallia and that of certain liverworts, and these resemblances are especially marked in *Osmunda*.

For a clear comprehension of these points, careful longitudinal sections are necessary, in addition to the study of surface views of the prothallium. Longitudinal sections (Plate IV, Figs. 25, 28) show that the apical cells are much larger than appears from surface views, being very deep. Each is in fact a semi-disc, whose edge only is seen from above, and its volume is much greater than that of the adjacent cells. On comparing the two species it is found that *O. claytoniana* has the apical cells larger and less convex than *O. cinnamomea*, in both respects coming nearer the Polypodiaceae. As in the latter, the inner wall is nearly flat,

[1] L. c. Pl. I. figs. 16, 17.
[2] L. c. Pl. II. fig. 1.
[3] Leitgeb, Untersuchungen über die Lebermoose, Heft III. p. 7.
[4] Campbell, A Study of the Apical Growth of the Prothallium of Ferns; Bull. of Torrey Botanical Club, Vol. XVIII. no. 3.

and the basal segment extends from the upper to the lower surface of the prothallium. It is first divided by a transverse wall into a dorsal and ventral cell, the latter being usually the larger, and sometimes divided again by a second transverse wall before any vertical walls are formed. The subsequent divisions are more or less irregular, but the limits of the segments can usually be followed without difficulty for some distance back of the growing point.

The lateral segments seem to divide only by walls in two planes, and contribute to the formation of the marginal parts of the prothallium.

It is almost impossible to determine with certainty the number of cells that are properly to be regarded as initials in the older prothallia, as the young lateral segments are often indistinguishable from the real initials, and the central ones are from time to time divided into apparently equal cells that push aside the outer cells that have apparently been of equal rank with them, but which now cease to divide with the same regularity as before. Vertical sections rarely show more than two or three cells that can properly be called apical cells—that is, that show the characteristic form and regular method of division.

On comparing the method of growth here described with the liverworts, the closest resemblance is found in *Dendroceros*, a genus allied to *Anthoceros*, but having a thallus of almost precisely the same structure as the prothallium of *Osmunda*, except that it branches much more freely, and, according to Leitgeb [1], the form of the apical cells and their method of division is the same. This in connection with the fact that *Dendroceros*, like *Anthoceros*, has the most highly differentiated sporogonium of all the Hepaticae, confirms the view already expressed that the Anthoceroteae are the most nearly allied, among known Bryophytes, to the primitive Filicineae.

As the prothallia grow, new root-hairs arise, at first only from the marginal cells, but later also from the lower cells of the midrib. They may be formed either by the simple

[1] L. c. Heft V. p. 30.

elongation of the cell, or, as is commonly the case in the Polypodiaceae, from a papilla which is cut off from the mother-cell. Kny does not seem to have seen this latter form in *O. regalis*, but it is by no means rare in both species considered here. In *O. cinnamomea* the walls of the root-hair become dark-brown, as in *O. regalis*, but in *O. claytoniana* this is much less marked.

As the prothallia become older, their general appearance in the two species becomes quite different. In *O. claytoniana* the margins are more irregular (Fig. 22), and the colour much lighter, being sometimes almost yellowish-green, whereas in *O. cinnamomea* the colour is dark-green, approaching that of *Anthoceros*, for example. This dark colour, together with the smoother margin, and dark-brown root-hairs, gives the prothallium quite a different appearance from that of the former species.

As the prothallia grow older, the midrib becomes more conspicuous and projects strongly from the lower surface. In *O. cinnamomea*, even at maturity, it does not broaden much, even where the archegonia are borne; but in *O. claytoniana* it suddenly becomes much broader and thicker when the archegonia begin to form, and projects very strongly, just back of the growing-point, as a hemispherical cushion, very like that found in the Marattiaceae and older prothallia of the Polypodiaceae, and in this respect, as well as the form of the apical cells, seems to approach the latter.

In studying the development of the prothallium in both species, numerous irregularities were noted. Frequently, as in other Osmundaceae, a cell-mass is formed as the first result of germination (Figs. 15, 19), but this condition is only temporary, a single apical cell being early formed which then gives rise to a cell-surface as in the usual forms. Occasionally no root-hair is formed at first (Fig. 8); or prothallia may be met with composed of two parallel rows of cells without any definite apical cell (Fig. 18), recalling strongly a certain type found in *Equisetum*.

Not infrequently, especially in *O. claytoniana*, the young

prothallia branch irregularly, or in some cases there seems to be a true dichotomy (Fig. 21); but in the latter case one of the branches finally grows faster than the other which is suppressed, and the resultant prothallium does not differ much in appearance from the ordinary type. In this species, too, filamentous prothallia are common, especially when the young prothallia are crowded. These filamentous forms are much rarer in *O. cinnamomea* even when growing very close together, and are never developed to the same extent. These filamentous forms recall very strongly the similar ones found in *Trichomanes*, and taken in connection with the similarity of the sexual organs, suggest a not very remote relationship between the Osmundaceae and Hymenophyllaceae. On the other hand, some of the more massive branched forms recall those of *Equisetum*.

Kny[1] and others have called attention to the marked tendency to multiply by adventitious buds shown by the prothallia of the Osmundaceae. These secondary prothallia (Plate IV, Fig. 33 *pr*) arise from the margin usually, but may be formed from the lower surface. An apical cell is usually early established, and the subsequent growth is essentially the same as in the primary ones. As they often form near the base of the old prothallia, they may become entirely independent by the dying away of the cells of the primary prothallium, and sometimes whole groups of these secondary prothallia are found growing from a single primary one. They grow rapidly after they are formed, and produce perfectly normal sexual organs, and presumably embryos.

The chloroplasts, which in the ungerminated spore are small and crowded, separate as the cells expand, and increase rapidly in size, and by repeated divisions give rise to new ones. They are thin flattened plates, lying close to the wall of the cell, and from mutual pressure usually somewhat polygonal in outline (Fig. 32). As usual, after exposure to light, small starch-granules are evident in them. Several times very large chloroplasts of peculiar form were seen in prothallia of *O. cin-*

[1] L. c. p. 7.

namomea, otherwise perfectly normal (Fig. 31). They were thin irregular plates, many times larger than the normal ones, in some cases so large as to cover completely one side of the cell. Whether these were the result of simple arrest in the normal division, due to some unexplained reason, or, as is less probable, were reversions to an earlier type, such as *Anthoceros*, where there is normally but one chloroplast in a cell, it is impossible to say.

Goebel[1] states that unfertilized prothallia of *O. regalis* may live for several years and reach a length of 4 cm. In my cultures of *O. cinnamomea* and *O. claytoniana*, the prothallia were still growing vigorously more than a year after the spores were sown, and probably would have continued to do so had they been preserved.

Goebel[2] also describes a dichotomy of the older prothallia of *O. regalis*, but I failed to find any cases among the prothallia of the species examined by me; but it is not at all unlikely that it may occur. The formation of lobes close to the growing point, which is very common in *O. cinnamomea*, may possibly be a case of suppressed dichotomy, as in an undetermined species of Polypodiaceae examined by me, in which a true dichotomy occurred, this was brought about by the central part of the apical region growing out into a lobe dividing the growing point into two as in various liverworts. The formation of these lobes in the Osmundaceae gives the older prothallia their peculiar wavy outline. (See Goebel, Outlines, p. 199. Fig. 148.)

The Antheridium.

Under favourable circumstances the antheridia appear in *O. claytoniana* a little more than a month from the time of germination, and continue to form for at least a year. How much longer they might continue to develop I cannot say. In *O. cinnamomea* the first antheridia appeared about two weeks

[1] Goebel, Outlines of Morphology and Classification, p. 199.
[2] L. c. p. 200.

later than in *O. claytoniana*, and continued to form as long as the prothallia were kept. While they are always present in the large archegonium-bearing prothallia, numerous prothallia bearing antheridia exclusively are formed, as is common among the Polypodiaceae. These male prothallia are often irregular in form, and in *O. claytoniana* are frequently filamentous, especially when they are much crowded (Figs. 29, 30). Upon the latter the antheridia may be either terminal or marginal: in the flattened prothallia they are borne either upon the margin or lower surface, especially of the wings, being of rare occurrence upon the midrib. They are relatively large and easily seen with the naked eye as little glistening specks studding the surface of the prothallia. They arise mainly in acropetal order, but new ones may also arise among the older ones. In their development the two species agree closely. The antheridia are sufficiently transparent to study most of the points in the living state, but it was found desirable to section them in studying the details of the division of the central cell, and the development of the spermatozoids.

Kny[1] and Sadebeck[2] have described the structure of the antheridium in *O. regalis*, but not very much in detail, and Sadebeck's statement that the only difference between the Osmundaceae and Polypodiaceae is, that the two ring-shaped cells in the latter are in the former divided into two cells each, is not borne out, either by Kny's account or my own investigations.

In studying the divisions in the living antheridium, those antheridia should be selected that are on the margin. A cell projects slightly above the level of its neighbours, and in it is formed a wall cutting off the projecting part. This is quickly followed by a second wall that divides the smaller cell into two; an outer cell (Fig. 34 *n*), nearly triangular in outline, and an inner four-sided cell (*m*). In the former a varying number of divisions occur, cutting off from its inner faces a series of more or less tabular cells, very much as is the case in the segmentation of an ordinary three-sided apical cell.

[1] L. c. p. 9. [2] Sadebeck, in Schenk's Handbuch, Vol. I. p. 182.

The cells thus cut off form the basal part of the antheridium. Sometimes when the number is large, a pedicel is formed, and the antheridium projects strongly from the prothallium. (Fig. 60 shows an extreme case.) The upper segments are shallower, and when the full number is formed, a dome-shaped wall (Fig. 36) arises in the upper cell, which meets the basal cells and encloses a nearly tetrahedral cell from which the sperm-cells are formed. (This cell is the shaded cell in Fig. 36.)

The central cell is quite destitute of chlorophyll, and has a large distinct nucleus imbedded in dense highly refractive protoplasm.

The subsequent divisions of the outer dome-shaped cell are not always exactly the same, but the differences are not important. In it are first formed two or three walls running more or less obliquely over the apex. Either at the top, or at one side, the last-formed walls meet so as to enclose a small cell (Figs. 39, 41 *o*), that marks the point where the ripe antheridium opens. This cell is often triangular in shape, and in form and position very much resembles the opercular cell of the Marattiaceae[1]. About the time that the first division in the peripheral cell occurs, begins the division of the central cell This corresponds closely with that in other ferns. The first wall is nearly vertical, and is followed by a second at right angles to it, so that the central cell, seen from above, is divided into four nearly equal cells (Fig. 42). A formation of nearly regular octants is often perceptible, and in these anticlinal walls are formed; but soon no regular arrangement can be traced, and a mass of polyhedral cells with dense contents fills the central part of the antheridium. Chlorophyll is present in the peripheral cells, but the granules are small and scattered, so that the cells appear almost colourless. The nuclei are distinct and the division-walls conspicuous. In the earlier stages, the division-walls between the central cells are thin and not very evident, but about the

[1] Jonkman, La Génération sexuée des Marattiacées; Archives Néerlandaises, T. XV. Pl. VII, figs. 65-72.

time that the spermatozoids begin to form, they become much thicker and very conspicuous, probably at this time undergoing a chemical change by which a portion of the cellulose becomes converted into mucilage which swells up on the application of water. In microtome-sections, stained with alcoholic Bismarck brown, these walls then stain very strongly (Fig. 59), while in the earlier stages this is not the case.

When ripe, or nearly so, the application of water causes the antheridium to open, the mechanism being the same as in other Archegoniatae. The sperm-cells become isolated, and on the rupture of the wall of the antheridium are forced out. The opening is effected by the forcing off of the opercular cell (*o*), and through the opening thus formed the sperm-cells are discharged. *Osmunda* thus differs from the Polypodiaceae in this respect and approaches the Marattiaceae and Bryophytes. If the antheridium is perfectly ripe, the walls of the ejected sperm-cells are almost immediately dissolved and the enclosed spermatozoids liberated; but the antheridium will often open before it is ripe, and in such cases the spermatozoids do not escape at once, or if too immature, they may not be set free at all.

Both species proved to be admirably adapted to the study of the development of the spermatozoids, and this point was critically studied. For the earlier stages of the sperm-cells and the changes in the nucleus prior to the formation of the spermatozoids, material fixed with a one per cent. aqueous solution of chromic acid was employed, and after washing and staining with alum-cochineal, this was sectioned with a Minot-microtome.

If the sperm-cells are examined before the final divisions have taken place, the nucleus is seen to be large, and provided with a conspicuous nucleolus (Fig. 47). It stains deeply while the cytoplasm remains almost perfectly colourless. In the later stages the nucleolus becomes less conspicuous; and after the final division, preliminary to the formation of the spermatozoids, can no longer be certainly seen.

Nothing noteworthy was observed in the division of the

nuclei, and owing to their small size it was not possible to decide the number of the nuclear segments. At all stages the nucleus stained deeply with the alum-cochineal.

After the final divisions, the nuclei remain slightly flattened in the plane of division, recalling somewhat the same stage in *Pellia*[1].

After the final division is completed, the nuclei gradually assume the form of resting nuclei before the differentiation of the spermatozoid begins (Figs. 48, 49). The nuclear segments become less distinct, and the chromatin more uniformly distributed. About the same time, the walls of the sperm-cells begin to acquire their mucilaginous consistence, and readily separate.

The development of the spermatozoids may be readily followed, and corresponds essentially to that of the other Pteridophytes and Bryophytes. The first sign of the formation of the body of the spermatozoid consists in the appearance of a cleft or depression on one side of the somewhat flattened nucleus (Fig. 50). This increases in depth, the nucleus in the meantime contracting, until, seen from the side, it appears crescent-shaped, and is really a short, thickish band. The two ends of the band now lengthen, the whole becoming narrower and thinner, and the nucleus gradually assumes the form of a flattened spiral, with one end tapering to a fine point and more closely coiled than the other end. The young spermatozoid lies perfectly free in the sperm-cell, and is nowhere in contact with its wall (Figs. 51–53).

In the full-grown spermatozoid there are about two complete coils which form a much flatter spiral than is common among ferns. This flattening is due to the flattened form of the nucleus of the sperm-cell, and recalls the form of the spermatozoid in some of the Bryophytes and in *Equisetum* rather than those of the Polypodiaceae, where, even in the sperm-cell, the spermatozoid has the elongated cork-screw

[1] Campbell, Zur Entwickelungsgeschichte der Spermatozoiden; Ber. der Deutschen Botanischen Gesellschaft, 1887.

form characteristic of the free-swimming condition, and the nucleus of the sperm-cell is globular.

Owing to the readiness with which the sperm-cells separate, even in the younger stages of the spermatozoids, the main points in the development of the latter may be very satisfactorily studied by allowing the prothallia to remain in water until the older sperm-cells are spontaneously discharged, and then carefully crushing the younger antheridia so as to liberate the younger ones. By treating these with a drop of dilute acetic (or better, osmic) acid and then with gentian or methyl-violet, as is done in studying the nuclear division in pollen, the nuclei are strongly coloured without staining the protoplasm. For permanent preparations, however, the whole prothallium should be fixed with one per cent. chromic acid, and, after staining with alum-cochineal, sections can be made with a microtome.

From a careful study of material treated by these methods, there seems no room for doubt that the whole body of the spermatozoid, with the possible exception of a thin film of cytoplasm upon the surface, is derived from the nucleus, and is due to a direct transformation by the nucleus into the body of the spermatozoid. The body of the spermatozoid stains uniformly, in all stages, with the various nuclear stains, and in no case is it in contact with the wall of the sperm-cell, but lies free in its cavity. Not the slightest trace of a special nucleus within the body, as claimed by Belajeff[1], was to be seen. The cilia arise late in the process of development, and are derived from a zone of cytoplasm that surrounds the upper coil of the spermatozoid (Fig. 54 *c*). This was most clearly seen when the nearly ripe sperm-cells were treated with very dilute osmic acid and stained with methyl-violet. This shows plainly the young cilia, which are then seen to arise from the zone of cytoplasm by its splitting into extremely fine filaments, one end of which remains attached to the upper coils of the spermatozoid, while the other is free.

When the ripe sperm-cells are discharged from the

[1] Berichte der Deutschen Botanischen Gesellschaft, Dec. 1889.

antheridium, the wall is still very evident, but soon is completely dissolved and the spermatozoid escapes. The latter resembles more nearly the spermatozoid of *Equisetum* than that of the other ferns. There are but two complete coils, usually, and the hinder one is relatively larger than in the Polypodiaceae (compare Pl. V, Figs. 57, 58). In swimming there is a peculiar undulating movement of the large hinder coil that recalls strongly the movement seen in the spermatozoids of *Equisetum*. Attached to the hinder coil, and often adherent to it, is the usual vesicle, the remains of the central part of the sperm-cell. It quickly takes up water and becomes much enlarged, and when the spermatozoid is killed with iodine in iodide of potassium, it swells up to several times its original size. This reagent also swells the body of the spermatozoid. To study the free spermatozoids most satisfactorily, osmic acid is the best fixing agent. A drop of the dilute acid, followed by a drop of weak alcoholic methyl-violet, stains both the body of the spermatozoid and the cilia, and is especially useful in the study of the latter. They are attached, not directly to the pointed end, but to the coil below, and cover considerably more space than described by Buchtien [1], who limits them to a very narrow area.

The final divisions of the sperm-cells are simultaneous, so that the spermatozoids all mature about the same time. Their number is large, usually 100 or more.

On comparing the structure of the antheridium and spermatozoids with those of other Pteridophytes, we find that in the form of the antheridium and the arrangement of the peripheral cells, the Hymenophyllaceae [2] and Gleicheniaceae [3] are the nearest among the ferns to the Osmundaceae, and that the Gleicheniaceae seem to stand between them and the Polypodiaceae. There are, however, points of resemblance to the Equisetaceae, in the earlier divisions of the antheridium, and its large size, and to the Marattiaceae in the arrangement of

[1] Buchtien, Entwickelungsgeschichte des Prothallium von *Equisetum*, p. 38.
[2] Bower, The Oophyte of *Trichomanes*; Annals of Botany, Vol. I.
[3] Rauwenhoff, l. c. p. 42.

the opercular cells and method of dehiscence. The spermatozoids closely resemble those of *Equisetum* both in form and movement; and in the smaller number of coils suggest an approach to the simpler form found in the liverworts and most mosses.

The Archegonium.

The prothallia that bear archegonia are larger than the males and always heart-shaped. They are more elongated, especially at the base, than is common in Polypodiaceae. Almost from the first, the midrib, several cells in thickness, is present, passing abruptly into the wings of the prothallium, which are but one cell thick. As we have seen, growth is at first due to a single triangular apical cell, which is replaced by a four-sided one, as in *Pellia*, and then, in most cases at least, by several similar initials, as in other ferns. These large prothallia produce antheridia first, but in smaller numbers than the strictly male plants: nevertheless new ones appear after the formation of archegonia begins, and self-fertilization must frequently occur.

The midrib, which is at first only four or five cells thick, becomes later three or four times as thick, and in *O. claytoniana* very much broader after the archegonia begin to form. In this species, too, the formation of lateral lobes in the sinus at the front of the prothallium is less marked than in the other species, although the young prothallium is much more irregular in outline.

Owing to unfavourable circumstances, the archegonia were late in developing, and I cannot say how soon they are formed ordinarily. The first full-grown archegonia were not formed in my cultures until more than four months after the spores germinated, but from that time they continued to form as long as the plants were kept. During the late autumn and winter growth was slow, but about the end of January it began again vigorously and so continued.

As in *O. regalis*[1], the archegonia do not cover the whole

[1] Kny, l. c. p. 10.

surface of the cushion back of the apex, but form a row on either side of the midrib, while from the central part root-hairs alone are produced. *O. cinnamomea* corresponds almost exactly in this respect to *O. regalis*; but in *O. claytoniana* the archegonia are formed in greater numbers and extend further toward the centre of the midrib, so that in their distribution, as well as in the broad forward part of the midrib, this species resembles the Polypodiaceae more than do the others.

In studying the development of the archegonium, the prothallia were imbedded in paraffin and sections made at right angles to the midrib. In this way many of the archegonia are cut vertically, and no difficulty was experienced in finding all stages.

No correspondence could be detected in their origin with the early divisions of the segments of the apical cells, as is the case in some Polypodiaceae, and the youngest are formed some distance back of the apex. While formed in approximately acropetal succession, new ones arise also among the older ones.

The mother-cell of the archegonium (Fig. 61 *m*) is scarcely distinguishable either in form or contents from its neighbours, and it is not always easy to decide whether a special cell will develop into an archegonium or not. Sometimes it is deeper than the secondary cells, and the nucleus somewhat larger; but its small size and indifferent character offer a strong contrast to such specialized forms as *Isoëtes* for instance, where from the first the archegonium-mother-cells are immediately recognizable.

The first division wall is at right angles to the axis of the archegonium, and divides the mother-cell into an inner and an outer cell (Fig. 68). The next division is usually parallel with the first, and divides the inner cell into two, so that as in other ferns the young archegonium consists of three superposed cells (Fig. 62). In some cases, however, the division of the inner cell does not take place, or it may occur simultaneously with the first division in the outer cell. Fig. 68 shows

a young archegonium of *O. cinnamomea* in which the nucleus of the outer cell is in process of division, while that of the still undivided inner cell is just preparing for division.

By the time the young archegonium has reached this three-celled stage, it is easily recognized, but still very small, and the contents of the cells differ somewhat from that of the adjacent cells. The nuclei are larger and the protoplasm, especially of the middle cell, denser. Of the three cells usually present, the outer is the mother-cell of the neck, the second the mother-cell of the egg and canal-cells, and the lower simply divides once or twice, and contributes to the formation of the venter of the archegonium.

The neck-mother-cell divides by a vertical wall into equal parts which are almost immediately divided again by others at right angles to the first, and these four cells, as usual, give rise to four rows of neck-cells (see Figs. 63, 64 *h*). Before any further divisions occur, the neck-cells begin to project above the surface of the prothallium, and the central cell enlarges and its upper wall becomes strongly convex (Fig. 64). The primary neck-cells now elongate rapidly, and divide repeatedly by transverse walls, until the neck consists of four rows of from 5 to 7 cells each (Figs. 66, 67). Some further elongation takes place after the divisions are completed, but this is due in part to mere mechanical stretching caused by the absorption of water. As the neck elongates, the upper part of the central cell grows up with it and is soon separated by a wall from the lower part (Plate V, Fig. 65 *c*). This is the primary canal-cell, and soon after a second cell, the ventral canal-cell, is also separated from the central cell (Fig. 66). It has been generally supposed that the ventral canal-cell is the equivalent of one of the polar bodies in the animal ovum, and that it is cut off shortly before the egg matures; but in both species of *Osmunda* examined by me, it is formed long before the archegonium is ripe. After the separation of the ventral canal-cell, a further division of the primary canal-cell occurs. Usually this seems to be confined to the nucleus, but in *O. cinnamomea* (Fig. 69) two cases were seen where a very evident

wall divided the canal-cell into two complete cells. It is possible that this may occur oftener than it seems to, and that in some cases the wall may be absorbed after its formation, but in most cases it seems more likely that no such division wall is formed. The occasional formation of such a wall however, regularly the case in the Marattiaceae[1], indicates a probable reversion to the type always found in the Bryophytes.

A longitudinal section of the nearly full-grown archegonium (Fig. 67) shows a straight neck consisting of about six tiers of cells, and having its central part occupied by a row of three (or four) cells, of which the lowest is the egg (*o*). The neck-cells have small nuclei, and in the living state appear almost transparent and with little chlorophyll, but sometimes numerous colourless, glistening granules that seem to be of albuminous nature, and which are very conspicuous in material fixed with chromic acid (Fig. 69), are present. The central row of cells contains finely granular protoplasm, but not as dense as is usually the case in these cells. Almost no starch is present in these cells in either *O. cinnamomea* or *O. claytoniana*, which is somewhat remarkable, as both Kny[2] and Luerssen speak of the large amount of starch in the canal-cells and egg of *O. regalis*.

As the egg-cell approaches maturity its nucleus becomes very large and distinct (Fig. 70). One and sometimes two nucleoli are present, but it is not very rich in chromatin, a condition that is not unusual in the egg-nucleus.

In several instances, when the archegonium seemed about ready to open, an appearance was noticed that looked very much like the formation of a true polar body, and was not to be confounded with the ventral canal-cell. In these cases (Fig. 73), in the upper part of the egg was a not very clearly defined body that coloured strongly with alum-cochineal, and was apparently nuclear in its nature. Where this was observed, the nucleus of the egg seemed to have lost part of its chromatin, and in what seemed to be later stages (Fig. 71) the

[1] Jonkman, l. c. p. 216. [2] L. c. p. 11.

nucleus had contracted and was noticeably smaller than in the younger egg-cell. This 'polar body' was noticed several times, and in these cases the ventral canal-cell partially disorganized, and the two nuclei of the other neck-cells, were unmistakeable. As the actual division of the nucleus of the egg was not seen, of course it cannot be stated positively that the body in question is a true polar body; but the apparent diminution of the nucleus of the ripe egg, and the behaviour of the body toward staining agents, make it extremely probable that this is the case. Admitting that we have to do here with a true polar body, of course it is an open question whether the ventral canal-cell is also to be so regarded, or if it is merely the physiological equivalent of the other neck-cells; i. e. as simply concerned in the opening of the archegonium, and helping to form the channel down to the egg.

At maturity the division-walls of the central row of cells, and probably also the inner walls, in part, of the neck-cells, become mucilaginous, and the opening of the archegonium follows very quickly on being put into water, especially if the prothallia have been kept rather dry for a few days. The neck-cells become very turgid, and as they separate the four rows diverge widely, and usually some of the upper cells become entirely detached. At the same time the remains of the canal-cells are forced out (Fig. 72).

Owing to the overlying cells, the egg is only vaguely seen, and in order to study it satisfactorily, sections must be made. In sections of the fresh archegonium, the egg appears colourless, and shows the receptive spot and nucleus. In microtome-sections of chromic acid material, it is seen that the contents are not uniform, but that the lower part of the egg shows a reticulate arrangement of the granular protoplasm, as if there were vacuoles present. The upper part, about one-third, is almost entirely free from granules, and constitutes the receptive spot. The nucleus is smaller than in the younger egg, and contains usually a single nucleolus, and is tolerably rich in chromatin.

Fertilization.

The entrance of the spermatozoids may be readily seen, but owing to the number of cells surrounding the venter of the archegonium, it is difficult to see the penetration into the egg in the living archegonium. This is more easily seen in *O. claytoniana* than *O. cinnamomea*. To see the process, a number of male prothallia that had been kept rather dry for a few days, were teased out in a drop of water, and after lying in this for a few minutes, or until a sufficient number of spermatozoids had escaped, were transferred to a slide, upon which a female prothallium with ripe archegonia was placed with the lower surface uppermost, and covered with water. The horizontal position of the archegonia makes it easier to note the entrance of the spermatozoids, and to follow them down to the egg, than is the case in most ferns; but as before stated, it is not possible to follow certainly the further history of the spermatozoid in this way.

Within a few minutes the ripe archegonia open, and the spermatozoids, attracted by the substance discharged from the archegonium, collect about its open mouth, and very soon one finds its way in. With the ciliated end down it revolves rapidly, not seeming to be much impeded in its movements by the mucilaginous matter thrown out by the archegonium, as is usually the case. Suddenly, with a quick movement, unlike the slow, worm-like movement observed in most ferns, it slips through the neck down to the central cell, where its rotary movement is resumed. After about three or four minutes it can no longer be seen, and presumably is within that time taken up by the egg. Other spermatozoids usually make their way into the central cell, but so far as observed only one ever penetrates the egg. After fertilization is effected, the lower neck-cells approach, but not enough to prevent the passage of other spermatozoids. Within a few hours the inner walls of the upper neck-cells begin to show the brown colour always seen in the fertilized archegonium.

To study the changes that take place after the entrance of

the spermatozoid, the prothallia were treated as in studying the development of the spermatozoids. As the entrance of the spermatozoid can be seen, it is only necessary to allow them to enter, and then, after waiting as long as may be desired, to plunge the specimen into the fixing fluid. On examining specimens so treated it is found that the nucleus of the egg moves toward the receptive spot at the time of fertilization, and that the spermatozoid, immediately after its entrance, has undergone but little change (Fig. 76). The spermatozoid, almost at once, comes into contact with the female nucleus, and with it moves toward the centre of the egg. Here the spermatozoid gradually loses its coiled form, contracting until it forms an oblong nucleus, in close contact, apparently, with the female nucleus, although it was not always easy to tell whether it was simply in contact with it, or actually within. The process is a slow one, as in one case, twenty-four hours after the entrance of the spermatozoids the two nuclei were still distinguishable (Fig. 78). Finally, the two nuclei are completely fused, and a single nucleus is seen, sometimes larger than the nucleus of the unfertilized egg, and containing usually, if not always, two nucleoli (Fig. 79). The fertilized egg is now surrounded by a membrane that probably begins to form almost immediately on the entrance of the first spermatozoid, and prevents the penetration of others. This seems probably from the fact that although numerous spermatozoids often penetrate to the central cell (Fig. 74), only one, so far as observed, ever enters it. No sign of a separation of the nuclear segments of the copulating nuclei was seen. Although numerous sections of the freshly fertilized egg were made, I was not fortunate enough to find any in which the nucleus was undergoing division.

As the archegonium matures, a single layer of narrow cells is formed around the central cell. These are especially distinct when the archegonium is cut transversely. After fertilization these cells begin to divide actively in all directions. Their contents show more abundant protoplasm and larger nuclei, and, indeed, all the evidences of actively growing cells.

The Embryo.

The first division of the fertilized egg is the same, with respect to the archegonium, as that of the Polypodiaceae, i. e. the basal wall is parallel with the axis of the archegonium; but the second or quadrant wall is also parallel with the axis of the archegonium, instead of transverse, as in the other Filices, although its position with reference to the prothallium is the same; that is, parallel with its surface. The position of the quadrants, therefore, and later the primary organs of the embryo developed from them, is the same with reference to the prothallium as in the other ferns, but not as regards the archegonium.

The lateral more or less varying position of the archegonium causes more or less diversity in the direction of growth in the primary organs of the embryo, and a corresponding difficulty in orienting the prothallia in making sections. The results given here were obtained from a comparative study of a large number of series of sections, cut in three directions—longitudinal, horizontal, and transverse.

The primary organs—stem, leaf, root, and foot—are established as soon as the two first walls are formed in the young embryo. These walls, lying at right angles to each other, divide the embryo into four nearly equal cells, two epibasal and two hypobasal, i.e. two turned toward the front of the prothallium, and the others toward the back. In each of these cells are next formed the octant-walls (Fig. 81), and the embryo now consists of eight tetrahedral cells, as in other ferns, and, as is probably the case in all of these, one of each pair of octant cells becomes the apical cell of the organ arising from the quadrant. In the foot-quadrant, it is true, the divisions are quite irregular, but even here, the first divisions correspond to those in the other quadrants, although very early all trace of an apical cell is lost. In all the organs there is considerably less regularity than obtains in the true leptosporangiates, both in regard to the position of the earlier walls and in the subsequent differentiation of the tissues.

Of the four quadrants the two epibasal form the stem and cotyledon; the hypobasal the root and foot. At this stage the cells of the young embryo (Figs. 80, 81) are nearly transparent, and the granular contents comparatively scanty and confined to the periphery of the cells and the vicinity of the nucleus, from whence extend threads and thin plates of granular protoplasm. The nuclei are large, and have a well-marked nucleolus. As the embryo grows larger the contents of the cells become denser.

The first divisions in all the octants are the same, so that it is not possible to say which one in each quadrant is destined to be the future apical cell; but soon the divisions become more irregular in one of each pair of octants, and its apical cell ceases to be recognizable as such. The first division wall in each octant (Figs. 82, 83, 84) runs in a curved direction, so that it strikes the basal and quadrant walls, and divides the octant into a tetrahedral cell, bounded on its other faces by the basal, octant, and outer walls of the embryo; and a second cell bearing the form of the segment of an ordinary tetrahedral apical cell, except that it is deeper than usual. Sometimes the first wall in the octant, instead of cutting the basal wall, strikes the median and octant walls. In either case, the embryo at this stage (Figs. 82, 83) consists of sixteen cells, all of which have one free outer wall.

Owing to the difference in the position of the first walls in the octants in different instances, the relative position of the apical cells varies a good deal, and the position of the organs in the older embryo is, of course, influenced by the direction of growth in the axis of the organ due to the position of the primary apical cells.

Before the second segment is cut off from the apical cell of each octant, the first segment divides by a periclinal wall into an inner and outer cell (Fig. 85, 2). Apparently in the octants that are not to form the growing-points of the organs of the embryo, no more segments are cut off from the apical cell, and the latter is obliterated by the formation of a periclinal wall, after which no regular succession of cells can be made

out. In the foot this is probably the case in both octants, as it was not possible to detect any regularity in the divisions after the very first one. In the other quadrants, however, the apical cell of one octant persists as the permanent growing point.

The embryo retains, for a longer time than is usual its original nearly globular form, all the organs growing about equally, and some of the organs projecting beyond the others, as is common in most Pteridophytes, this being especially true of the cotyledon.

The Cotyledon.—The cotyledon arises from the lower epibasal quadrant of the embryo. Its direction of growth is determined in part by the first walls in the octants that compose it. The outer octant, usually at least, becomes the apical cell of the cotyledon. If the first segment of the apical cell is formed in contact with the octant wall, this throws the apical cell very much to one side of the median line of the embryo, and the axis of the leaf may in consequence be nearly at right angles to this. If, however, the first segment is nearly parallel with the basal wall, the apical cell will lie nearly in the centre, and the growth of the cotyledon corresponds, for a time at least, with the axis of the embryo. Even in the latter case, however, owing to considerable growth in the inner octant, the growing-point is pushed to one side of the median line, and the cotyledon seems to grow out laterally from the embryo. Whether seen from the side or from above, the apical cell is triangular, and is really a tetrahedron, from whose lateral faces successive segments are cut off (Figs. 88–94 *L*). Sometimes, especially in the earlier stages, the divisions in the segments present a good deal of regularity. Each segment is first divided into an inner and outer cell, and the latter then divides into two by a wall parallel with the side of the apical cell. Both inner and outer cells undergo further divisions, and it is not until a late stage that the primary tissues are clearly distinguishable. The young cotyledon has the form of a cone, but as growth upon the lower and inner surfaces is stronger than upon the upper, it soon begins to curve upward and outward. This is most plainly seen in

sections cut parallel with the surface of the prothallium. As the cotyledon lengthens, it flattens out somewhat, and the three-sided apical cell is probably replaced by a two-sided one, as in the Polypodiaceae, but this point was not positively proven.

As it becomes older, the primary tissue-systems become differentiated, but are not as prominent as is usually the case. The plerome-cylinder seems to arise exclusively from the innermost of the two cells into which each segment of the apical cell is at first divided ; while the outer and larger one gives rise to dermatogen and periblem. The former consists of a single layer of pretty well-marked, nearly iso-diametric cells, which undergo no further periclinal division. The periblem consists of about three layers of cells that increase a good deal in length as the cotyledon lengthens. The plerome, as usual, forms a central cylinder of cells, in which the longitudinal divisions predominate, so that they are much elongated. The nuclei of these cells are also elongated, instead of round, as in the dermatogen and periblem.

As the leaf continues to grow, it curves strongly backward, and on account of its position grows out laterally, lying close against the under side of the prothallium. Owing to its varying position, however, it is difficult to get perfectly straight sections, and in consequence it is not easy to determine positively the exact form and divisions of the apical cell. There is probably here, as in Polypodiaceae, a true dichotomy of the apical cell, giving rise to the dichotomous branching of the veins, but owing to lack of material this point was not investigated. But as the appearance and venation of the full-grown cotyledon corresponds closely with that of the Polypodiaceae, there is no reason for doubting that this is the case.

As the cotyledon begins to lengthen, short glandular hairs develop near the apex (Fig. 100), and probably serve for protection, as is so often the case in the older sporophyte of most ferns, including *Osmunda*. These hairs are short, usually but two cells long, the upper somewhat enlarged and secreting probably a mucilaginous matter. These cells stain very strongly with Bismarck-brown.

The cotyledon does not break through the calyptra until a late stage, and in this respect, as well as others, shows its primitive character. After the cotyledon has broken through, it lengthens rapidly, but the increase in length, as well as the subsequent increase in thickness, is due largely to the simple enlargement of the cells. The petiole grows out toward the edge of the prothallium until it reaches it, and then grows upward, and then the lamina, which has been sharply bent backward against it, with the inner (upper) surfaces of the two lobes in contact, gradually flattens out. It, as well as the petiole, is almost perfectly smooth, the glandular hairs seen in the young stages having pretty much disappeared. The lamina is obscurely two-lobed. The single fibro-vascular bundle of the petiole forks at the base of the lamina, and each of the two branches divides again, and sometimes these branches divide once more (Fig. 98). Corresponding to the division, the margin of the primary lobes is indistinctly lobed.

A cross-section of the petiole of the full-grown cotyledon (Fig. 101) is strongly convex upon the outer side, and nearly flat upon the inner surface. The epidermis is composed of strongly convex cells, not noticeably different from those of the ground-tissue below it. The latter are somewhat irregular in outline (in the figure this is somewhat exaggerated through slight shrinkage of the inner ground-tissue in the process of imbedding), and become smaller as they approach the single fibro-vascular bundle in the centre. This is nearly circular in outline, and lies nearly in the centre, but slightly nearer the inner side. It is pretty clearly defined, but a true bundle-sheath is hardly to be distinguished. It is composed of nearly uniform thin-walled cells, some of which are probably to be regarded as sieve-tubes, but not clearly recognizable as such. Toward the inner side, and separated from it only by one or two rows of cells, is a group of small tracheides (*xy*), which, like all of the earliest ones, are marked with close annular or reticulate thickenings. The bundle, while perhaps to be regarded as concentric, approaches closely, in the arrangement of its elements, the collateral bundle of *Ophio-*

glossum, *Equisetum*, and *Isoëtes*, and strengthens the view that the concentric bundle is a secondary and not the primary form among the Filicineae.

Cross-sections of the young lamina show a better marked epidermis on both sides than is found in the petiole. These are separated usually by two layers of mesophyll-cells. Toward the margin these are in close contact, as they are throughout in the very young leaf, but as the lamina expands, large intercellular spaces arise between them. Stomata are present on both surfaces, but their development was not followed. The fibro-vascular bundles are of the same type as those of the petiole, but are smaller.

The main difference observed in the cotyledons of the two species examined was the colour. This in *O. claytoniana* is a very light, yellowish green, while in *O. cinnamomea* it was much darker. This difference also exists, as before noted, in the prothallium.

The Stem.—The stem arises from the upper epibasal quadrant, as usual, and its early divisions correspond closely to those in the leaf-quadrant. The first segment in the octant that becomes the apical cell, seems to be always turned toward the basal wall, and growth in this octant is stronger, so that the apical cell very soon comes to lie almost exactly in the axis of the embryo, and its direction of growth is coincident with it. As the growth of the cotyledon tends more and more upward, it soon forms almost a right angle with the stem.

At first the four faces of the tetrahedral apical cell are almost equal, but in the older embryo, the lateral faces are deeper (Fig. 99). The segments are cut off slowly and the apex of the stem projects but slightly. As in the cotyledon, the first wall in each segment divides it into an inner and an outer cell: from the former the plerome arises, from the latter dermatogen and periblem. Whether each segment gives rise to a leaf, I cannot state, as the development of the embryo was not followed beyond the second leaf which arises, in part at least, from the inner stem-octant.

Owing to the extreme shortness of the stem, it is difficult to determine whether it possesses a fibro-vascular bundle distinct from that of the leaves: but before the second leaf is clearly evident, procambium-cells are formed which seem to belong properly to the stem, and to arise from the inner cells of the segments of the apical cell.

The Root.—The root of the mature sporophyte of *Osmunda*[1] differs much from that of the true Leptosporangiatae, and it was an interesting question whether a study of the root of the embryo would throw any light upon the significance of these differences. In both species considered here, the later roots have usually a four-sided apical cell which shows less regularity in its divisions than is the case in ferns with the ordinary three-sided cell. (In *O. regalis*, according to Bower[2], there may be either a single three- or four-sided cell, or a group of initials. *Todea* according to Douliot and Van Tieghem[3] has a three-sided apical cell.) The root originates from the lower hypobasal quadrant, as in other Filicineae.

As with the leaf, the direction of growth varies a good deal, and is dependent upon the same causes. If the octant wall is oblique (which happens regularly in some ferns, e.g. *Pilularia*), the larger of the resultant octants becomes at once the apical cell; but if the two octants are of equal size, it is not possible to determine at first which is to be the apical cell.

Most frequently the first segments are formed parallel with the quadrant wall and the axis of growth becomes very soon almost vertical, but it is sometimes for a short time horizontal. Owing to this variation, sections were usually somewhat oblique, and it was often difficult to see clearly either the form of the apical cell or its divisions.

When, however, the sections passed straight through it, the appearance was the same, both in longitudinal and transverse section, and it appeared triangular. That is, in the embryo

[1] Campbell, Notes on the roots of *Osmunda* and *Botrychium*; Bot. Gazette, March 1891.

[2] Bower, The Meristems of Ferns; Annals of Botany, Vol. III. no. xi. pp. 310, 311.

[3] Bower, l. c. p. 388.

of *Osmunda,* the apical cell of the root has regularly the tetrahedral form found in the Leptosporangiatae and Ophioglosseae.

Occasionally it is quite large (Fig. 90 *A*) and conspicuous, but usually it is smaller. At first, of course, its outer face is free (Pl. VI, Fig. 91 *r*), and segments are cut off only from the lateral faces. As the apical cell at this stage is unmistakeable, the primary root must be regarded as originating simultaneously with the other organs of the embryo, and morphologically equal to them, and of course, of exogenous origin; very soon, however, a periclinal wall arises, cutting off the first cell of the root-cap, and from this time, with every set of lateral segments, a basal segment also is formed. When fairly established, it is found that the division of the segments corresponds in the main with that found in the other Filicineae having a single tetrahedral apical cell. Each segment divides first into two parts, by a vertical wall; and each semi-segment then divides into two cells, a small inner one which is the initial for the plerome, and a larger outer one, which by further division gives rise to periblem and dermatogen, and in part, also, to the root-cap.

When compared with the other Filicineae, it resembles most nearly *Botrychium*[1], with which it agrees in the small size of the apical cell, and the large size of the segments and their slow division at first, as well as the greater irregularity in the divisions and the bulky character of the whole root.

Series of cross-sections through the apex and below it show much less regularity than is the case in the Polypodiaceae, and at a very short distance from the apex all trace of the limits of the segments is lost. Immediately below the apex, their boundaries can usually be made out, their zig-zag lines meeting at the centre. The sextant walls can also usually be made out, striking these at varying angles. Beyond this it is not possible to trace any invariable arrangement of the cell-walls. The majority of the walls are periclinal, and the result is a more or less perfect concentric arrangement of the cells. The limits

[1] Campbell, l. c.

of the plerome are not nearly so well marked as in most ferns, and a distinct bundle-sheath is not present. At first all the cells of the plerome contain protoplasm and a distinct nucleus, but later the tracheids, as usual, lose their protoplasmic contents. There are usually two groups of tracheids formed, and from these points, as usual, the formation of others proceeds toward the centre of the bundle. The rest of the bundle is composed of nearly similar elongated cells, but no true sieve-tubes were noted.

The root-cap is not as regularly stratified as in the Polypodiaceae, and in this also *Osmunda* comes nearer *Botrychium*. It was not examined in the full-grown root, but probably, as in the mature plant, it is derived in part from the lateral segments of the apical cell.

A comparison of the first root of the two species shows no very noticeable differences. The divisions are perhaps a little more regular in *O. claytoniana*, and the segments rather smaller as compared with the apical cell; and the divisions in the young segments seemed to be rather more rapid, so that it approaches more nearly the type of the Polypodiaceae.

The Foot.—The foot is formed mainly from the upper hypobasal quadrant, but encroaches more or less upon all the others. Very early its cells cease to show any definite order of division, and as they divide more slowly than those of the other organs of the embryo, while at the same time the whole foot enlarges, they soon become noticeably larger than the actively dividing cells of the other organs. The exact limits of the foot cannot be defined, as it merges insensibly into the other parts of the embryo, and in the later stages before the embryo breaks through the calyptra, occupies nearly or quite half of the entire embryo. This increase in size is due almost exclusively to simple expansion of the cells which become very large and nearly colourless. They lose most of their protoplasmic contents, and serve simply as absorbent organs. They are in close contact with the cells of the prothallium, and encroach upon them until the foot penetrates deep into the prothallium (Figs. 95, 96), partially destroying the cells. The

cells in immediate contact with the prothallium-cells sometimes grow out into short, root-like processes, that recall the similar organs in the foot of the sporogonium of the Anthoceroteae, and of course serve the same purpose. On account of the great development of the foot, the embryo grows for a long time at the expense of the prothallium, and is late in breaking through the calyptra.

When the embryo is about to break through the calyptra, the first tracheary tissue can be detected, first forming in the axis of the embryo and proceeding from this point into the organs of the embryo. The tracheids are short and marked with close annular or reticulate thickening.

The calyptra is very large (Fig. 96 *cal*). After the archegonium is fertilized, active growth begins in the cells of the venter, which keeps pace with the growth of the embryo and forms a covering entirely around it, two cells thick in most places, but sometimes more. It is not until the embryo is far advanced that this is finally ruptured. The cotyledon usually breaks through first, and then the root, which being usually vertical soon penetrates the ground and fastens the young plant to it.

Both the very large foot and the very large calyptra recall strongly the Bryophytes in which the sporophyte prominently derives part of its nourishment from the oophyte.

Soon after an archegonium is fertilized, the regular apical growth of the prothallium ceases, and soon none but vertical walls are formed in the apical cells, so that the forward part of the prothallium, like the rest of the margin, is but one cell in thickness. Finally all growth ceases and the prothallium dies.

Frequently more than one archegonium is fertilized as in the Gleicheniaceae[1], but as a rule only one embryo develops, although it is not at all uncommon to find several archegonia where the egg has evidently been fertilized, as is shown by its enlargement and investment with a cell-wall. Only one case was met with when two embryos were present, but one of

[1] Rauwenhoff, l. c. p. 53.

these was very much in advance of the other, and it is probable that the larger one would have ultimately starved out the other.

Comparison with the Embryo of other Pteridophytes.—Comparing the embryo of *Osmunda* with that of other Pteridophytes, it seems to approximate most nearly, as might have been expected, that of the Polypodiaceae, but differs in several particulars from them, i.e. the very large foot, the position of the quadrants with reference to the archegonium; the greater irregularity in the divisions of the quadrants; the small size of the apical cell of the root and the greater size of the root itself; and the imperfect differentiation of the primary tissues. In all these respects it departs furthest from the Marsiliaceae, the most specialized of the Leptosporangiatae, and probably approaches the Ophioglosseae; but as the embryology of the Ophioglosseae is entirely unknown, and that of the Marattiaceae almost equally so, a comparison with these is at present impossible. The Gleicheniaceae, to judge from Rauwenhoff's[1] imperfect account of the embryo, are nearer the Polypodiaceae than the Osmundaceae in this respect. With *Equisetum*[2] and *Isoëtes*[3] there is little in common beyond the first divisions of the embryo.

Summary and Conclusion.

The principal points brought out in the foregoing pages may be summarized as follows:—

1. The spores of *Osmunda claytoniana* and *O. cinnamomea* germinate immediately and may form a protonema as in the Polypodiaceae, this being especially noticeable in the former species, where the formation of a cell-surface at once is unusual; or a cell-surface, or even a cell-mass, may be the first product of germination.

[1] L. c. p. 52.

[2] See Sadebeck, 'Die Gefässcryptogamen;' in Schenk's Handbuch.

[3] Campbell, Contributions to the Life-history of Isoëtes; Annals of Botany, Vol. V. no. xx.

2. A single two-sided apical cell is early established which gives place to a single nearly square one, and ultimately to a row of marginal initials. No cases were observed where marginal growth was at once established.

3. The prothallium is traversed by a thickened midrib of nearly uniform diameter in *O. cinnamomea*, but broader in front in *O. claytoniana*.

4. Branching of the young prothallium is especially marked in *O. claytoniana*. Adventitious prothallia are formed later in both species, but especially in *O. cinnamomea*.

5. The chloroplasts are sometimes of extraordinary size in *O. cinnamomea*.

6. The antheridia differ in structure from those of other ferns. They approach most nearly those of the Hymenophyllaceae and Gleicheniaceae. The spermatozoids resemble most nearly those of *Equisetum*. They arise by direct transformation of the nucleus. The cilia and vesicle are of cytoplasmic origin.

7. The archegonium sometimes has the neck-canal-cell divided. Little or no starch is present in the canal-cells or the egg. A polar body, distinct from the ventral canal-cell, may be present.

8. Several spermatozoids usually penetrate to the central cell of the archegonium, but only one enters the egg, which then secretes a wall that prevents the entrance of others.

9. The first division in the embryo is parallel with the axis of the archegonium, as is also the second; but the position of the quadrants with respect to the prothallium is the same as in the other ferns.

10. The primary organs are determined by the formation of the quadrant walls. Leaf and stem arise from the epibasal half of the embryo; root and foot from the hypobasal.

11. Stem, leaf, and root grow from a tetrahedral apical cell, which is one of the original octants of the embryo.

12. The foot is very large, and the embryo for a long time dependent upon the prothallium. The calyptra is also large, and these points, together with the late differentiation of the

tissue-systems, are to be regarded as evidences of the primitive character of the Osmundaceae.

13. The embryogeny approaches most nearly, among the forms investigated, to the less specialized leptosporangiate ferns.

14. More than one embryo may begin to form, but probably only one reaches maturity.

From a study of the points just recapitulated, and a comparison of the two species studied with each other and other selected forms, some light, it is hoped, may be thrown upon the systematic position of the Osmundaceae.

O. claytoniana in several particulars seems to connect the other Osmundaceae with the true leptosporangiate ferns. The usual formation of a protonemal filament in germination, and the rare occurrence of a cell-surface at first, as well as the method of the establishment of the apical cell, is the same as in these forms, while in *O. regalis*[1], and frequently in *O. cinnamomea*, the formation of a cell-surface begins at once. In *Todea*[2], it is true, a cell-row may be first formed. On the other hand, the strong tendency to branch, shown in the young prothallia of *O. claytoniana*, recalls those of *Equisetum*. The differentiation of the forward part of the midrib, where the archegonia are borne, is probably the beginning of the cushion of tissue found in this position in most Leptosporangiatae and Marattiaceae.

The frequent formation of a filamentous, protonema-like prothallium in this species, so different from the ordinary form, is noticeable, and has its nearest approach among the Hymenophyllaceae. When we look further, other points of resemblance are noted. The large green spores, the horizontal annulus, the structure of the sexual organs in the Hymenophyllaceae, are quite as much or more like the corresponding parts in the Osmundaceae than like these points in the Polypodiaceae.

O. claytoniana agrees with the Marattiaceae in the strongly

[1] Kny, l. c. p. 5. [2] Luerssen, in Schenk's Handbuch, I. p. 171.

developed archegonial cushion and the dehiscence of the antheridia.

As has already been pointed out in a former paper [1], the correspondence in the development of the prothallium of ferns, especially the Osmundaceae, with the thallus of many Hepaticae, is too obvious to be overlooked. If the sexual organs of the two groups are compared, we find here, too, that the correspondences are greatest in the Osmundaceae. The antheridia are larger than in any of the Leptosporangiatae, and often distinctly stalked, and their method of dehiscence is more like that of the liverworts. The somewhat simpler form of the spermatozoids may be cited also, especially when contrasted with the many coiled spermatozoids of such specialized forms of *Marsilia*.

The straight neck of the archegonium, while also probably a primitive structure, cannot be certainly considered as such, since the curved neck of the archegonium, found in most other ferns, is also found in certain liverworts, e.g. *Asterella*, where this condition is obviously due to the conditions under which fertilization is effected.

The occasional presence of a division-wall in the neck-canal-cell, however, is in all probability a character inherited from an ancestral form, in which, as in all Bryophytes, the neck-canal-cell is regularly divided by transverse walls. Of the other Pteridophytes the Marattiaceae and some species of *Lycopodium* show the same thing.

Several peculiarities in the embryo also indicate the primitive nature of the Osmundaceae. The large size of the foot, and the consequent long dependence of the embryo upon the prothallium, and the late differentiation of the organs and tissue-systems, are all evidences of this. Accompanying this is the very large calyptra.

We have seen that the root of the embryo has a single tetrahedral cell, as in all the other Filicineae except the Marattiaceae and *Isoëtes*, if we regard the latter as belonging

[1] Campbell, On the Affinities of the Filicineae; Bot. Gazette, Jan. 1890.

here. As the root of *Equisetum* also has this form of apical cell, it seems probable, as Bower[1] suggests, that this is the primitive form from which the single four-sided cell found in the later roots of the species of *Osmunda* under consideration, and the group of initial cells in the roots of the Marattiaceae, have been derived. It would be interesting to know whether in the first root of the embryo of the latter, anything approaching a single apical cell of the ordinary type is to be found.

The points of resemblance between the Osmundaceae and so many other groups, shown especially in *O. claytoniana*, indicate that we have to do with a primitive, undifferentiated group, standing near the junction of several others. Next to them, and probably connecting them with the Bryophytes, are the Ophioglosseae, with *Ophioglossum* as the most primitive form. Through *Botrychium*, *Ophioglossum* is connected directly to the Osmundaceae, and through them to the whole leptosporangiate group of ferns.

The position of the Marattiaceae is difficult to determine on account of our imperfect knowledge of the embryo, and almost complete ignorance of both prothallium and embryo in the Ophioglosseae. While showing some resemblances to the Osmundaceae, I am rather inclined to look for an origin of the group lower down, perhaps directly from the Ophioglosseae, although the structure of the later roots of the Osmundaceae does show points of resemblance to the Marattiaceae, and the absence of a midrib in the prothallium of the latter recalls the prothallium of the higher Leptosporangiatae.

As the living Marattiaceae, however, are but a remnant of a once predominant group, it is not safe to assume that the prothallium of the living forms represents necessarily its primitive character. It may perhaps bear the same relation to the primitive forms that the prothallium of say an *Aspidium* does to hat of *Osmunda*.

The Equisetineae have usually been regarded as holding a position entirely apart from the other Pteridophytes, but

[1] Bower, Meristems of Ferns, p. 318.

a careful study of the development of the prothallium shows so many points of resemblance to that of the Filicineae, that a distinct relationship, remote it is true, is hardly to be questioned. The early stages of the prothallium, development of the sexual organs, structure and development of the spermatozoids, and early divisions of the embryo—all of these correspond closely with the Filicineae, especially *Osmunda*. Whether they correspond equally with the same points in the Ophioglosseae, where the nearest affinities would be looked for, remains to be seen. The latest researches of Buchtien [1], show quite close resemblances, too, in the growth of the female prothallium, which are, however, disguised by the formation of lobes, at the base of which the archegonia are formed, and the subsequent shifting of the archegonia to the upper surface of the prothallium, although they are originally formed upon the lower side.

Van Tieghem has also called attention to certain resemblances in the sporophytes of *Equisetum* and *Ophioglossum*, and it is highly probable that when the embryogeny of the latter is known that the resemblances will be still more marked.

It is well known that in *Equisetum* the fibro-vascular bundles are collateral, while in most of the Filicineae they are concentric; but in *Ophioglossum* the bundles are collateral, and this is true of the stem, at least, in *Botrychium* and *Osmunda*; and as we have seen, a trace of this structure is visible in the bundle of the petioles of the cotyledon of *Osmunda*, as well as the smaller veins of the leaves of the ferns.

Just as the primitive type of sporangium derived from the eusporangiate ferns has persisted in the Spermaphytes, so we may assume has the collateral fibro-vascular bundle; and the concentric bundle, characterizing the more specialized ferns, is a derivative of this. Really the two forms are not very different, and may merge almost insensibly into each other, as is clearly shown by comparing the bundles in the petiole of different species of *Botrychium*.

The impossibility of making a fair estimate of the relation-

[1] L. c. p. 23.

ship existing between the Equisetineae and Filicineae from a study of the one surviving genus of the former class is of course plain, especially as there is every reason to look upon this genus as a degenerate one. Nevertheless, the evidence seems strong enough to warrant the assumption of a closer relationship between the two groups than is usually admitted, even perhaps to unite the two groups into a single one opposed to the Lycopodineae.

In the course of these investigations I have seen no reason to change the opinion already expressed [1], that the eusporangiate, and not the leptosporangiate ferns, are the primitive forms. Since this was written, Prof. Bower [2] has admitted the force of the arguments then brought forward, and has himself added a very weighty argument in favour of this view based upon geological evidence. The great majority of carboniferous and pre-carboniferous ferns [3] appear to have been allied to the Marattiaceae, and there is evidence that the Ophioglosseae also existed; but if the latter were like their living descendants, the soft nature of their tissues must have prevented their preservation in a fossil state in any but the most exceptional conditions. Sporangia referable to the Osmundaceae also occur; but, according to Solms-Laubach [4], no true leptosporangiates occur before the Mesozoic.

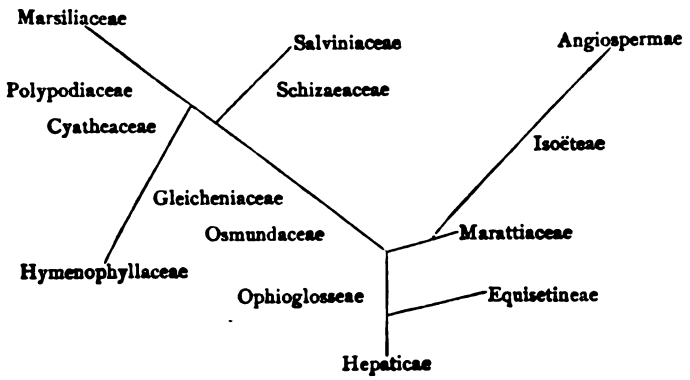
The simpler living Ophioglosseae probably resemble the primitive ancestral forms from which the other ferns have sprung. From this primitive stock we may assume that very early the Equisetineae arose, and later the Marattiaceae, both forms culminating in the carboniferous. The latter group probably gave rise to forms like *Isoëtes* through which the Angiosperms later developed. A third group, the Leptosporangiatae, derived from the Ophioglosseae through forms like *Botrychium* and *Osmunda*, are the prevailing ferns of modern times, and at present constitute a vast majority of living

[1] The Affinities of the Filicineae, p. 5.
[2] Annals of Botany, Vol. V. no. xix.
[3] Solms-Laubach, Palaeophylotogie, p. 146.
[4] L. c. p. 157.

Filicineae. The end-branches of this series are seen in the heterosporous Marsiliaceae and Salviniaceae. Of the homosporous forms, the Gleicheniaceae connect the Osmundaceae with the Cyatheaceae and Polypodiaceae. The Schizaeaceae probably form a distinct branch, as do the Hymenophyllaceae. The last probably originated early in the development of the series, and their peculiarities are probably due to the nature of their environment.

Whether or not the Lycopodineae form a branch of the original pteridophyte stock, or whether they have an entirely distinct origin directly from the Bryophytes, must remain for the present an open question.

The relationships of the groups assumed here may be graphically represented by the accompanying diagram:



EXPLANATION OF FIGURES IN PLATES III, IV, V, AND VI.

Illustrating Professor Campbell's paper on *Osmunda*.

PLATE III.

Fig. 1. Fresh spore of *Osmunda claytoniana*. *a*, surface view from above; *b*, optical section; *c*, a similar spore beginning to germinate. × 350.

Fig. 2. Microtome-section of a similar spore, fixed with chromic acid and stained with haematoxylin. × 650.

Fig. 3. A germinating spore of the same. *a*, in optical section; *b*, surface view; *r*, first root-hair; *sp*, spore-membrane. × 350.

Fig. 4. Three very young prothallia of *O. claytoniana*. × 300. In *a*, the nucleus of the end-cell is in process of division; in *b*, the apical cell, *x*, is already established; *r*, the root-hair.

Fig. 5. A young prothallium of *O. claytoniana*, in which the first wall in the prothallium is at right angles to the wall that cuts off the root-hair. × 300.

Fig. 6. A young prothallium of *O. claytoniana*, of the 'bipolar' type. *sp*, exospore. × 300.

Fig. 7. A similar prothallium with the root-hair-lateral. × 300.

Fig. 8. Prothallium of *O. claytoniana*, in which no root-hair has been formed. × 350.

Fig. 9. Filamentous prothallium of *O. claytoniana*. × 250.

Fig. 10. Young prothallium of *O. claytoniana*, showing the apical cell, *x*. × 300.

Fig. 11. An older prothallium of the same species. × 300.

Fig. 12. Three very young prothallia of *O. cinnamomea*. The first two walls in the prothallium-mother-cell are numbered. × 250.

Fig. 13. A prothallium of *O. cinnamomea*, the same age as in Fig. 12, but the basal cell undivided. × 250.

Fig. 14. Prothallium of *O. cinnamomea* with apical cell, *x*. × 250.

Fig. 15. Young prothallium of *O. cinnamomea*, which has formed a cell-mass at once. × 350.

Fig. 16. Prothallium of *O. cinnamomea* of about the same age as 15, with two apical cells, *x*, x^1. × 350.

Fig. 17. Prothallium of *O. claytoniana*, two weeks old. × 350.

Fig. 18. Young prothallium of *O. claytoniana*, composed of two parallel cell-rows. × 350.

Fig. 19. Prothallium of the same, in which cell-division has taken place in three planes. × 350.

Fig. 20. Male prothallium, about four months old, bearing antheridia. × 100.

Fig. 21. Prothallium of *O. claytoniana*, two weeks old, showing dichotomous branching; x, x^1, apical cells. × 300.

Fig. 22. Outline of a prothallium of about two months, of *O. claytoniana*. The midrib is indicated by the dotted lines. × 45.

Fig. 23. Microtome-section of an older prothallium, parallel with the surface, showing the single, four-sided apical cell, x. Chromic acid—Bismarck-brown. × 350.

PLATE IV.

Fig. 24. Apex of a prothallium of *O. claytoniana*, of about three weeks. × 350.

Fig. 25. Vertical section of a full-grown prothallium of *O. claytoniana*. Chromic acid—Bismarck-brown. × 300.

Fig. 26. Prothallium of *O. cinnamomea*, of about six weeks. × 100.

Fig. 27. Microtome-section, parallel to the surface, of an older prothallium of the same. Chromic acid—Bismarck-brown; x, x^1, two apical cells (?). × 350.

Fig. 28. Vertical section through a prothallium of about the same age as 27. Chromic acid—Bismarck-brown. × 300.

Figs. 29, 30. Two male prothallia of *O. claytoniana*, of the filamentous type. *an*, antheridia. × 100.

Figs. 31, 32. Two cells from the prothallium of *O. cinnamomea*, showing the form of the chloroplasts: 31, large abnormal ones; 32, the ordinary form. × 600.

Fig. 33. Base of a prothallium (*Pr*) of *O. claytoniana*, from which is growing a secondary prothallium (Pr^1) with antheridia (*an*). × 110.

Figs. 34–38. Successive stages in the development of the antheridium of *O. claytoniana*, seen in optical section. *m*, basal-cell; *n*, mother-cell of antheridium. The contents of the central cells are shaded. × 600.

Fig. 39. An older antheridium of *O. claytoniana*. *a*, upper view of surface; *b*, optical section; *c*, lower surface; *o*, operculum. × 600.

Fig. 40. Superficial view of one of about the same age as 39, but turned at right angles to it. × 600.

Fig. 41. Upper surface of a full grown antheridium of *O. claytoniana*; *o*, opercular cell. × 600.

Figs. 42, 43. Transverse optical sections of young antheridia of *O. claytoniana*. × 600.

PLATE V.

Fig. 44. Vertical microtome-section of a full-grown antheridium of *O. claytoniana*. Chromic acid—alum-cochineal. × 650.

Fig. 45. Similar section of a younger antheridium of *O. cinnamomea*. Chromic acid—alum-cochineal—Bismarck-brown. × 650.

Fig. 46. Similar section of an antheridium of *O. cinnamomea*. The central cells are undergoing the last division previous to the formation of the spermatozoids. Chromic acid—alum-cochineal—Bismarck-brown. × 650.

Fig. 47. Two of the central cells from a young antheridium of *O. claytoniana*. Chromic acid—alum-cochineal. × 1200.

Figs. 48–56. Development of the spermatozoids of *O. claytoniana*. × 1200. Figs. 52–55, from acetic acid—gentian-violet preparations; 54, Osmic acid—methyl-violet; the others chromic acid—alum-cochineal preparations.

Fig. 57. Free spermatozoid of *O. claytoniana*, killed with osmic acid, and stained with methyl-violet. *v*, the vesicle. × 1200.

Fig. 58. Spermatozoid of *Onoclea struthiopteris*, treated in the same way. × 1200.

Fig. 59. Vertical microtome-section of a nearly ripe antheridium of *O. cinnamomea*. Chromic acid—alum-cochineal—Bismarck-brown. × 350.

Fig. 60. Young antheridium of *O. claytoniana*, with unusually developed pedicel. × 600.

Figs. 61, 62. Development of the archegonium of *O. claytoniana*; *o*, central-cell; *h*, neck-canal-cell; *b*, ventral canal-cell; *L*, basal cell; *n*, *n'*, nuclei of neck-canal-cell. All longitudinal microtome-sections, of chromic acid material, stained with alum-cochineal and Bismarck-brown. × 600.

Fig. 68. Very young archegonium of *O. cinnamomea*, showing the first division of the neck-cell. The central cell is also preparing for division. × 650.

Fig. 69. Section of older archegonium of *O. cinnamomea*, with the neck-canal-cell divided into two, *c*, *c'*. Chromic acid—alum-cochineal—Bismarck-brown. × 600.

Fig. 70. Vertical section of the base of the archegonium, just before the disintegration of the canal-cells. *b*, ventral canal-cell; *o*, egg. Chromic acid—alum-cochineal. × 650.

Fig. 71. Ripe egg of *O. cinnamomea*, just before the archegonium opens. Chromic acid—alum-cochineal. × 650.

Fig. 72. Vertical section of a freshly opened archegonium (living). *o*, egg; *h*, remains of canal-cells. × 350.

Fig. 73. Section through the base of a nearly ripe archegonium, at the time of the separation of the polar body (?) *p*. *b*, ventral canal-cell. Chromic acid—alum-cochineal. × 650.

Fig. 74. Vertical section of a recently fertilized archegonium, showing the spermatozoids within the central cell. Chromic-acid—alum-cochineal—Bismarck-brown. × 350.

Fig. 75. The basal part of the archegonium shown in Fig. 74, more highly magnified. The egg is somewhat shrunken, but a spermatozoid (*sp*) is clearly seen in contact with the nucleus of the egg.

Fig. 76–78. Fertilization of the egg of *O. claytoniana*. Chromic acid—alum-cochineal preparations. *o*, the female pronucleus; *sp*, sperm-nucleus. × 650.

Fig. 79. Fertilized egg of *O. claytoniana*, shortly before its first division. Two nucleoli are visible in the nucleus; *ar*, mouth of archegonium. × 650.

Fig. 80. Transverse section of a four-celled embryo of *O. claytoniana, in situ.* ×350. (The terms, transverse, vertical, and horizontal are used with reference to the prothallium. Unless otherwise specified, the drawings are from microtome-sections of material fixed with chromic acid, stained *in toto* with alum-cochineal, and on the slide with alcoholic Bismarck-brown.) The basal, median, and octant-walls are numbered respectively I, II, III; *L.* is the cotyledon; *st*, the stem; *r*, the root; and *F*, the foot; where not specified, the figures are magnified 350 diameters. The arrow in drawings indicates the axis of the embryo.

Fig. 81. Transverse section of an eight-celled embryo, *em.*

PLATE VI.

Figs. 82, 83. Median longitudinal sections of two young embryos of *O. claytoniana.*

Fig. 84. *A.B.* Two nearly median sections of a similar embryo of *O. cinnamomea.*

Fig. 85. (1, 2, 3, 4). Four horizontal sections of a somewhat older embryo of *O. claytoniana.*

Figs. 86, 87. Median horizontal sections of two similar embryos of *O. cinnamomea.*

Fig. 88. Vertical, and

Fig. 89. Transverse median sections of older embryos of *O. claytoniana.*

Fig. 90. *A.B.C.* Three sections of an embryo of *O. cinnamomea* in which the apical cell (*r*) of the root, was especially well marked.

Fig. 91. (1, 2). Two vertical sections of a somewhat younger embryo before the first segment of the root-cap has been formed.

Fig. 92. Horizontal section of the cotyledon and foot of an old embryo of *O. claytoniana.* × 300.

Fig. 93. The apex of the root of the same embryo. × 300.

Fig. 94. Horizontal section passing through the cotyledon and root of an advanced embryo of *O. cinnamomea.* The root is cut somewhat obliquely.

Fig. 95. Transverse section of the prothallium of *O. claytoniana*, showing the lateral position of the embryo (*em*). ×50.

Fig. 96. Similar section of an embryo of *O. cinnamomea* showing the calyptra (*cal*). ×100.

Fig. 97. Transverse section of an embryo of *O. cinnamomea*, passing through the cotyledon *L* and the foot *F.* The axis of the leaf is here almost at right angles to that of the prothallium.

Fig. 98. Young sporophyte of *O. claytoniana*, still attached to the prothallium. ×6.

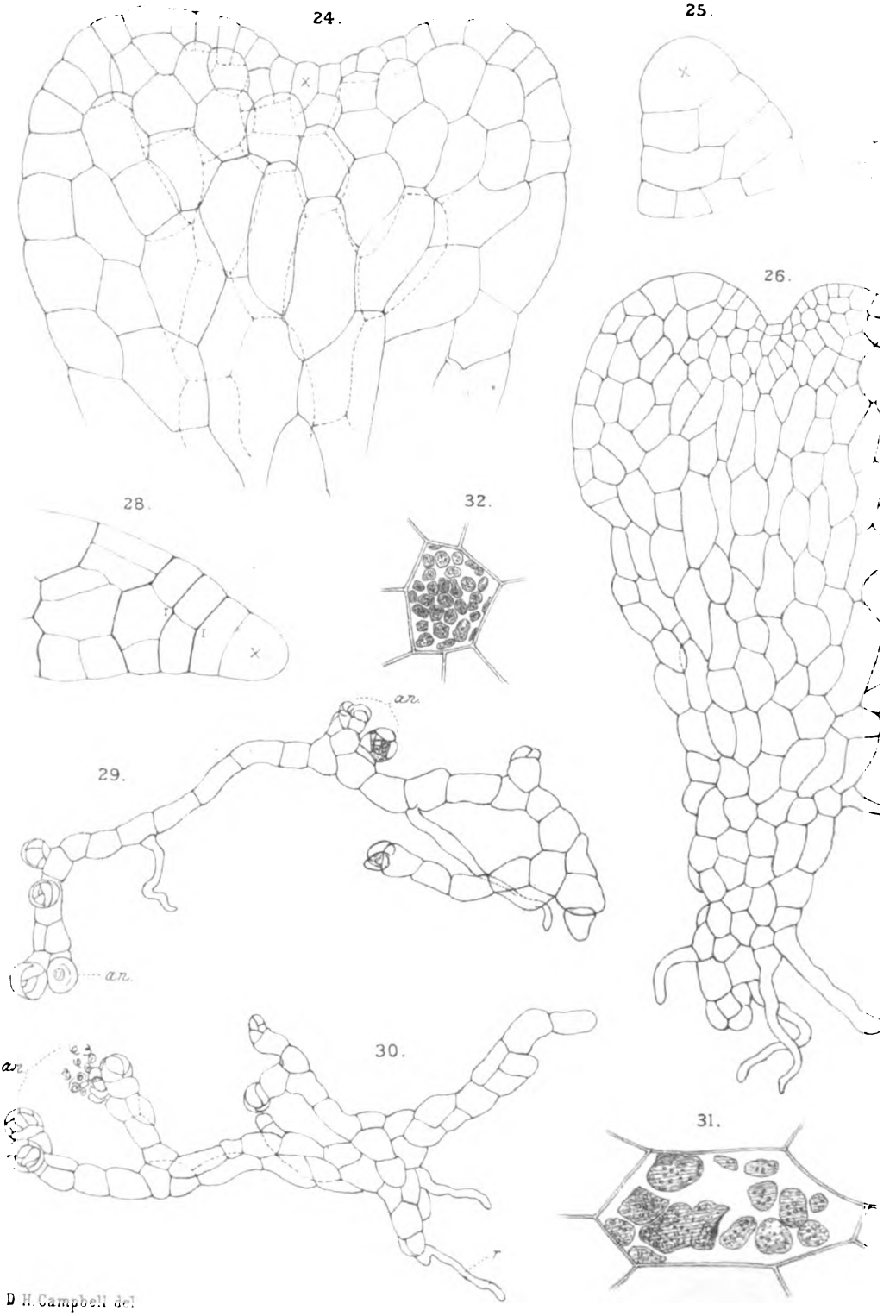
Fig. 99. Horizontal section of an advanced embryo of *O. cinnamomea* passing through the stem-apex; *x*, the apical cell of the stem. Alcohol—alum-cochineal—Bismarck-brown.

Fig. 100. Section of young cotyledon of *O. cinnamomea*. *g*, glandular hair.

Fig. 101. Cross-section of petiole of nearly full-grown cotyledon of *O. claytoniana*. *xy*, xylem of vascular bundle.

Fig. 102. Cross-section of the apical cell of the first root of *O. cinnamomea*. Alcohol—alum-cochineal—Bismarck-brown.

Fig. 103. Longitudinal section of the young primary root of *O. claytoniana*; *Pl.* plerome.



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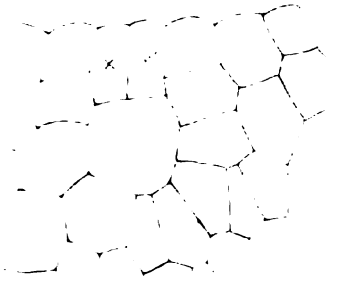

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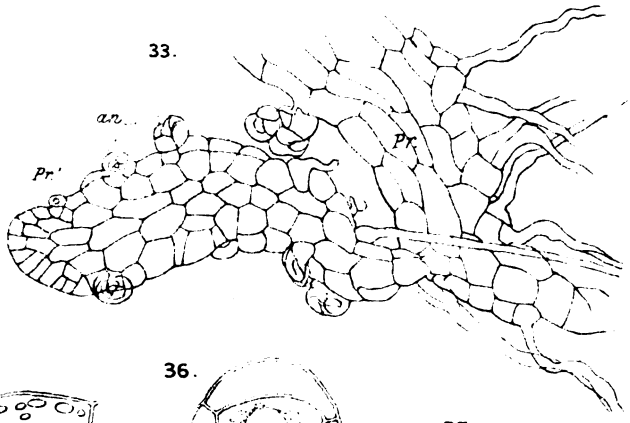

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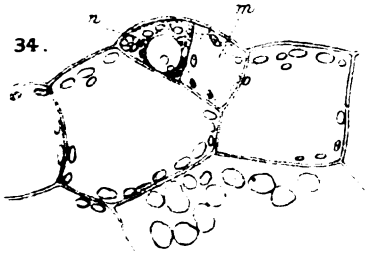

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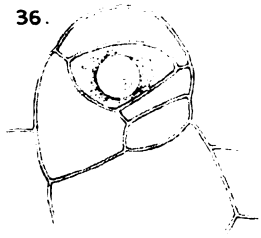

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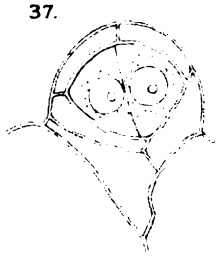

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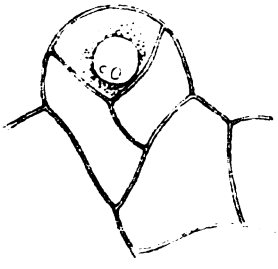

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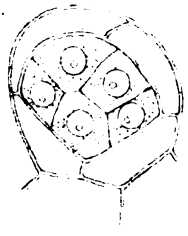

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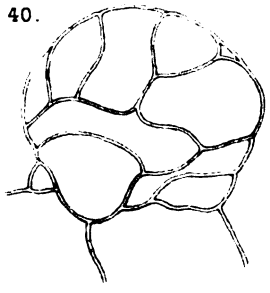

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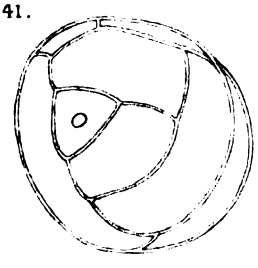

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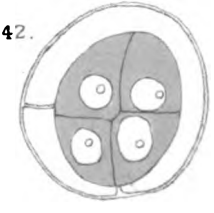

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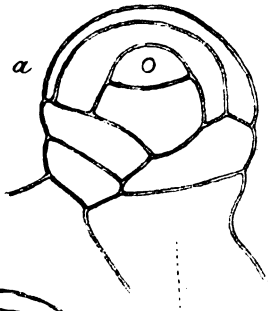

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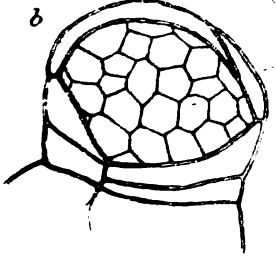

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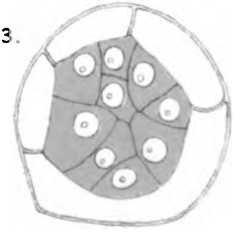

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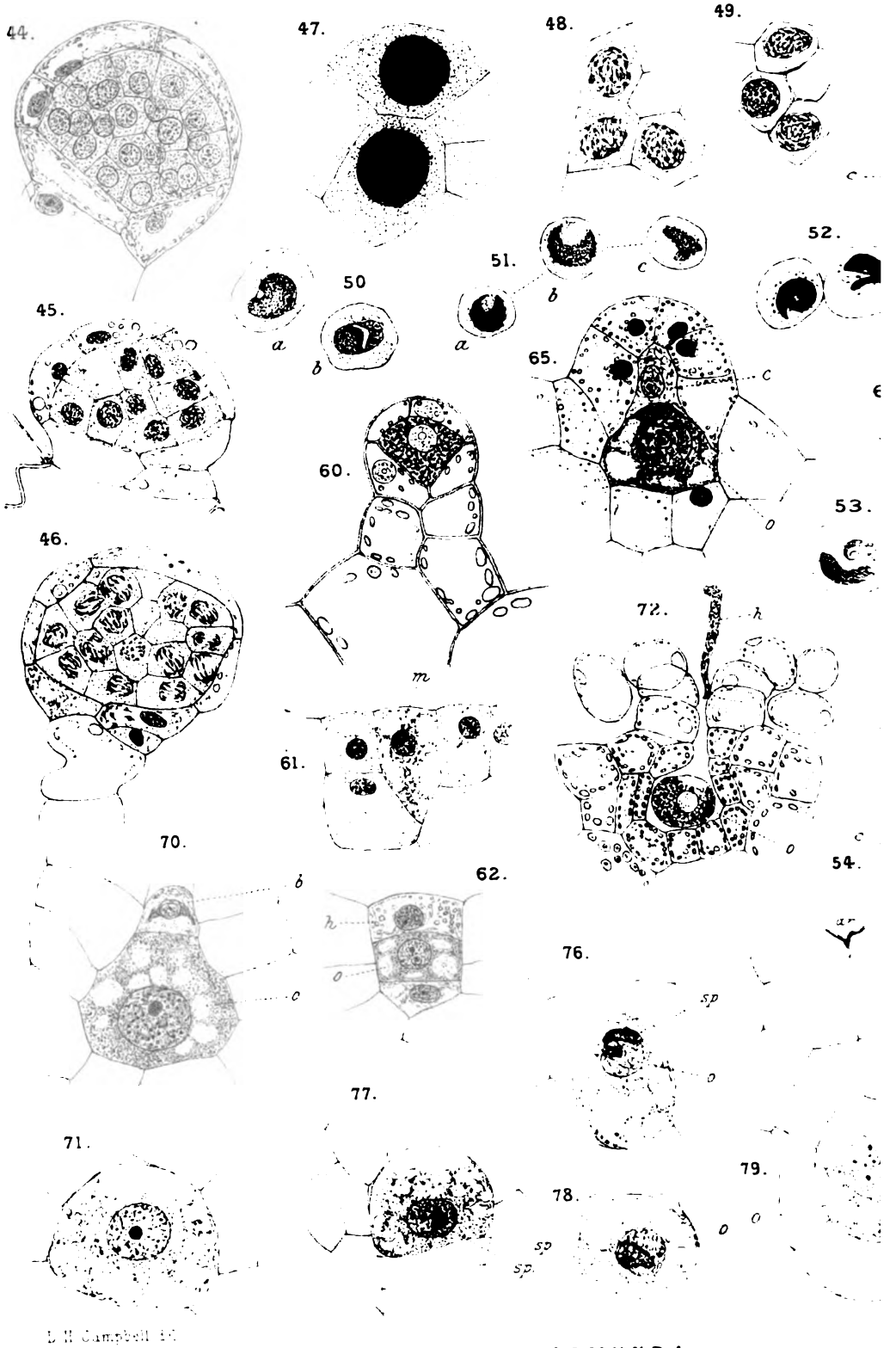

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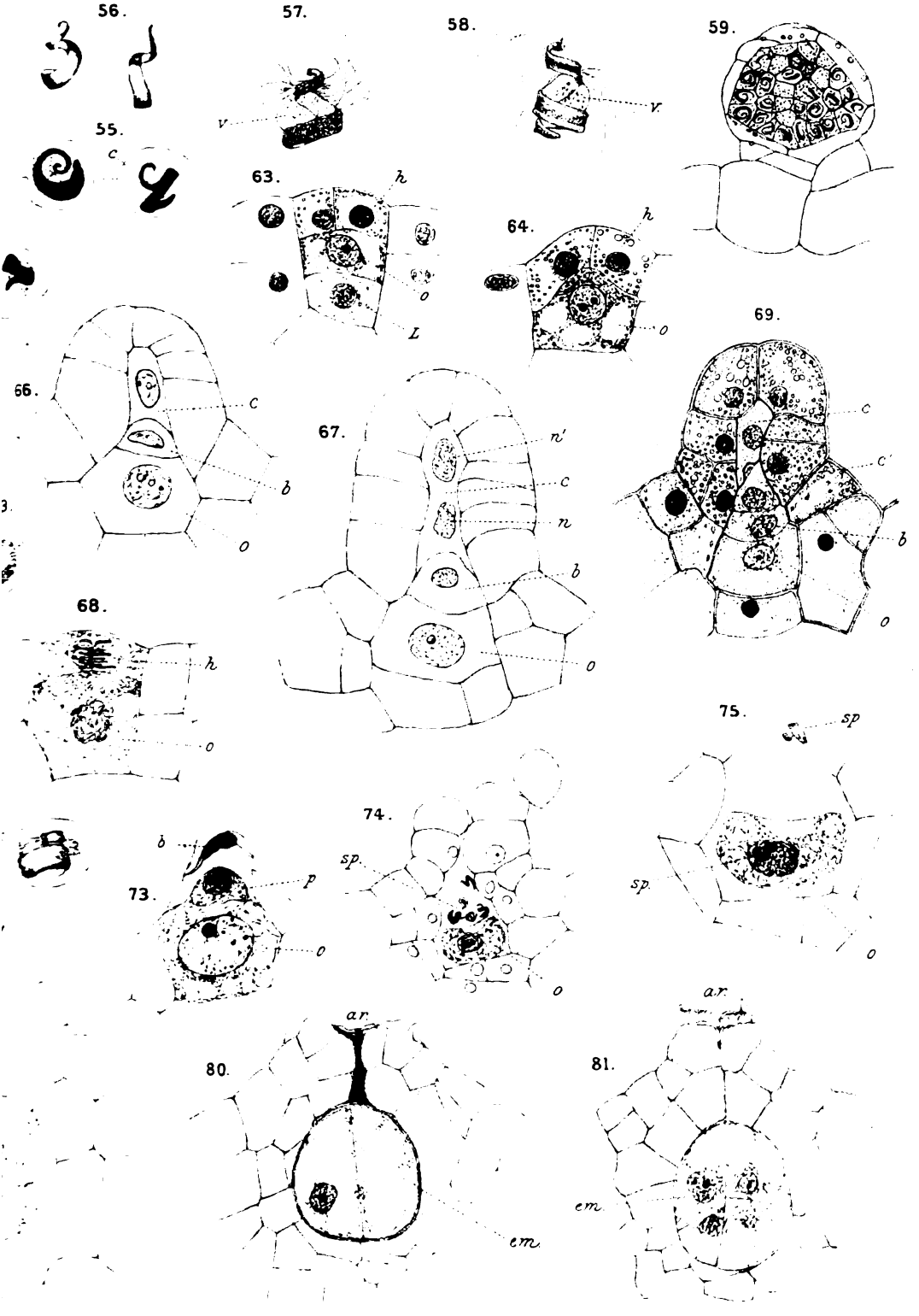

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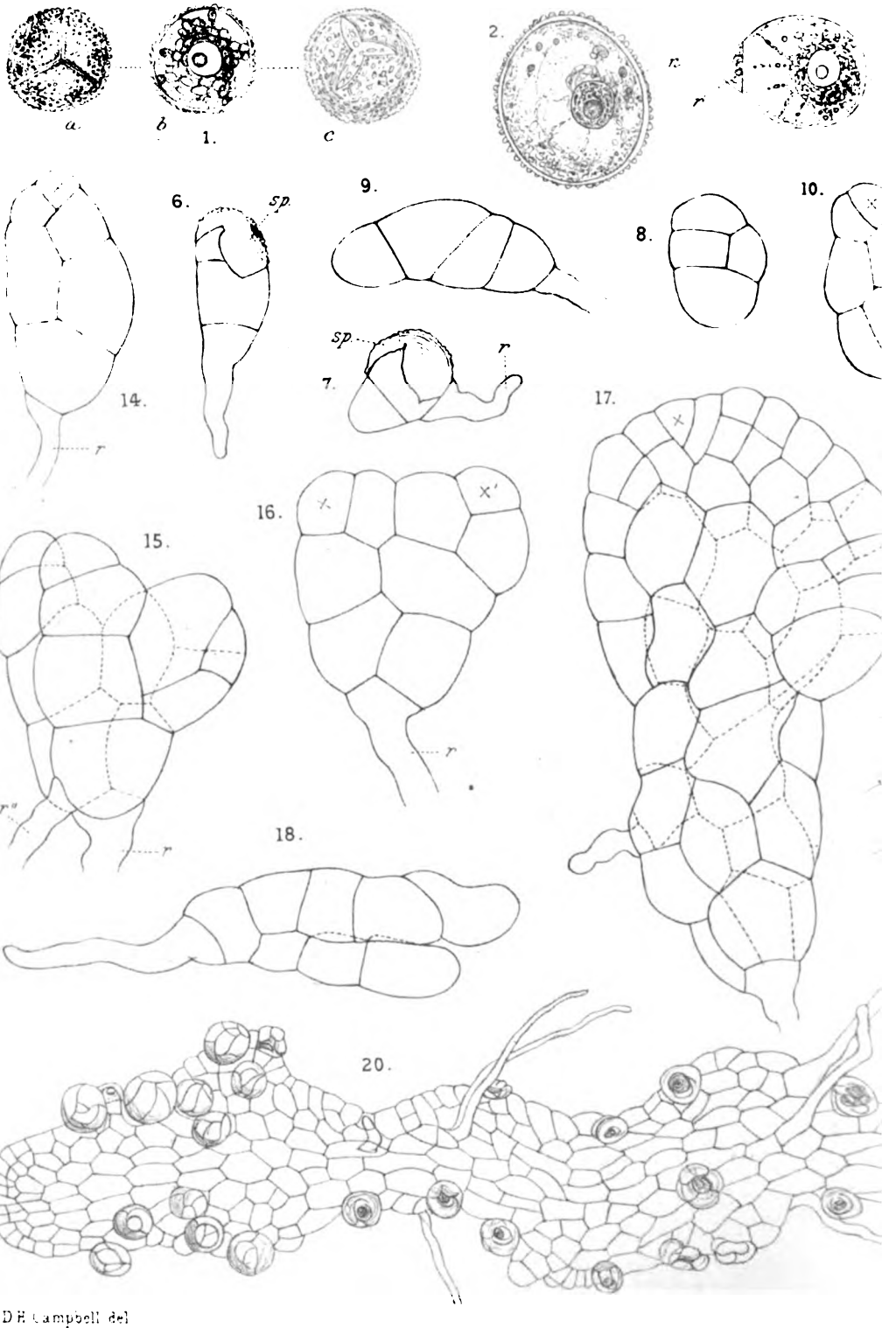

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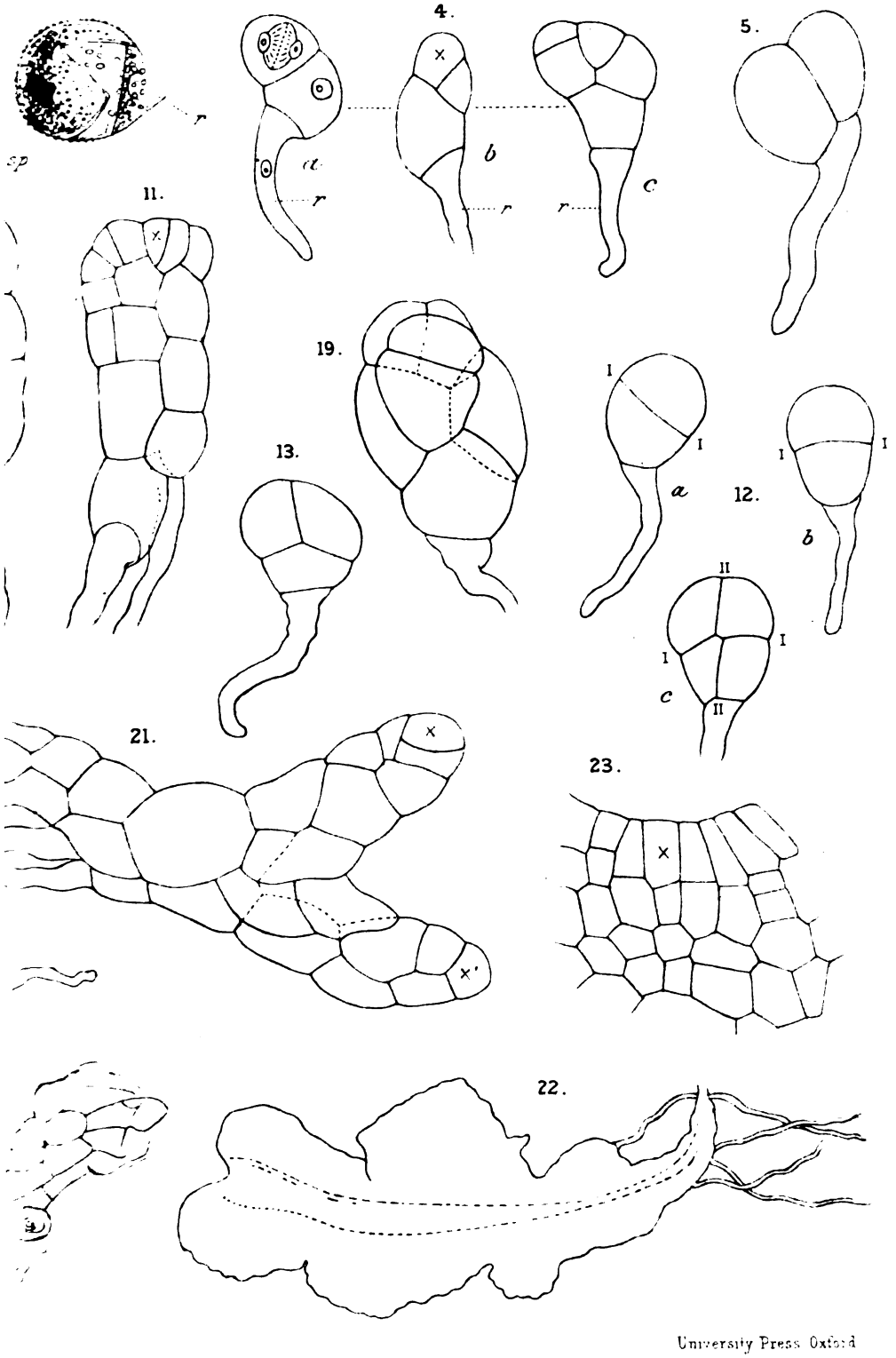

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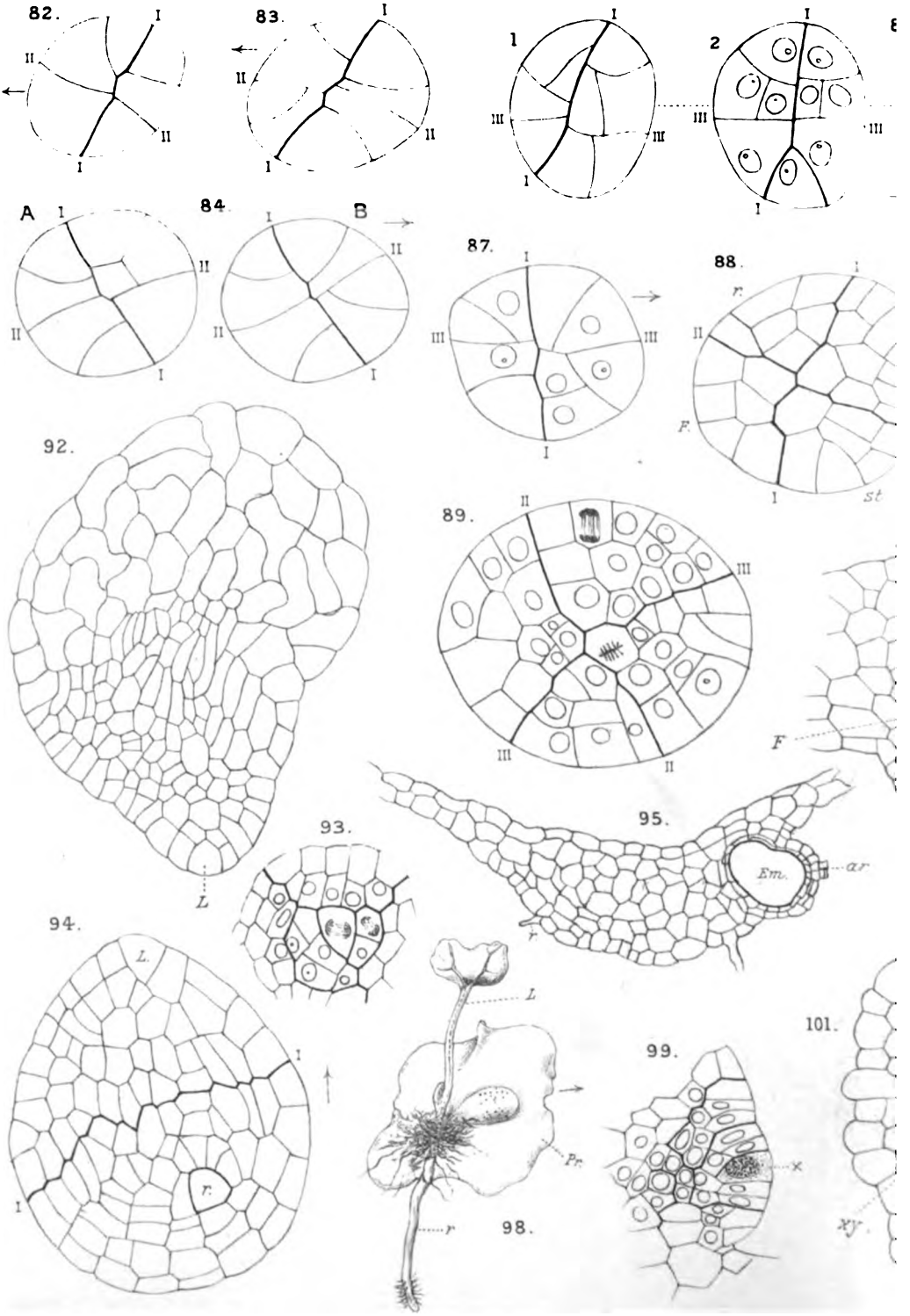

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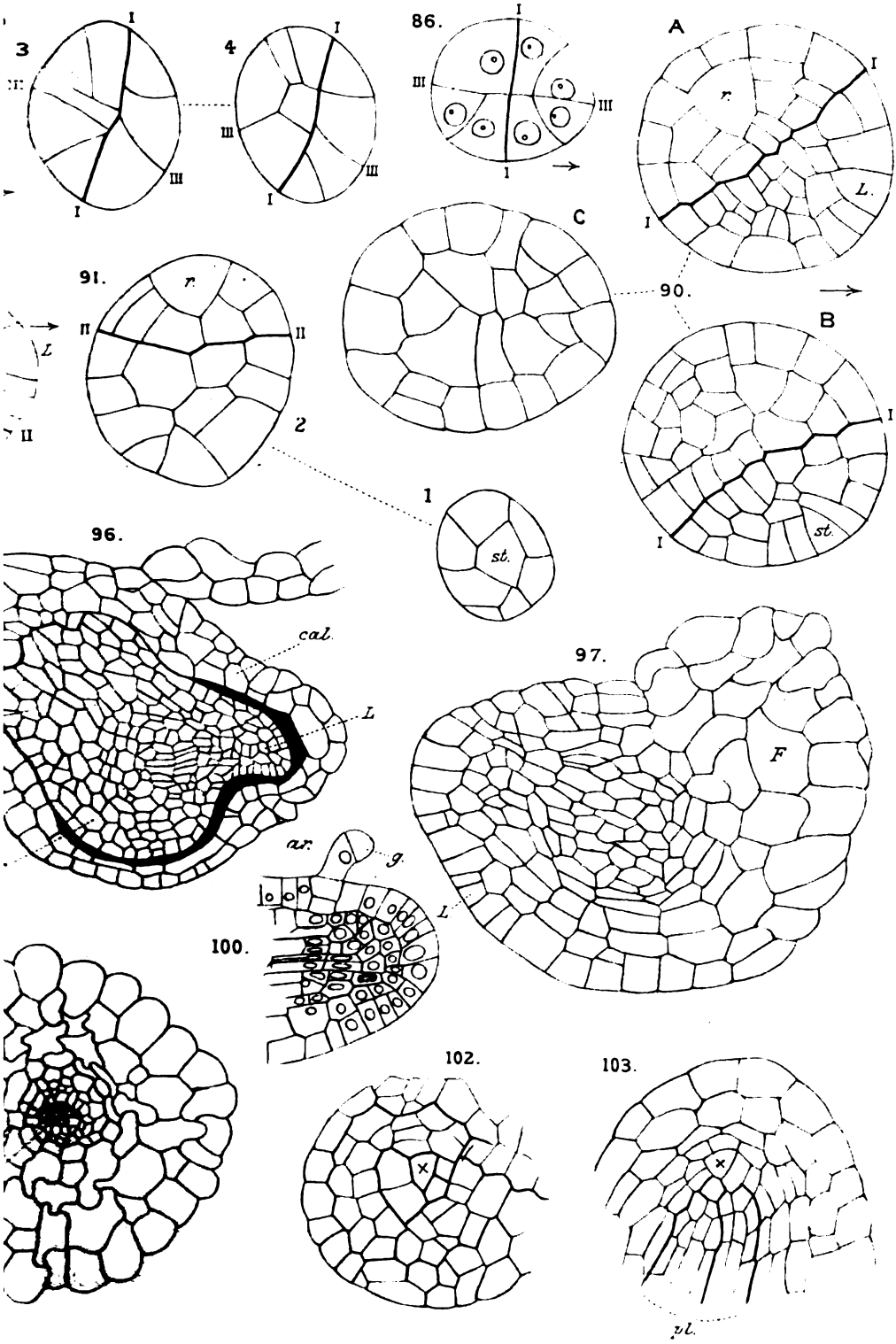

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On the Vascular Cryptogamia of the Island of Grenada.

BY

J. G. BAKER, F.R.S.

Keeper of the Herbarium, Royal Gardens, Kew.

THE following is a list of the Vascular Cryptogams collected in the Island of Grenada (Windward Group) of the West Indies, by Mr. R. V. Sherring, F.L.S., during the winter of 1890–91. Mr. Sherring visited the island under the direction of the Joint Committee of the Government Grant Committee of the Royal Society and of the British Association for the Advancement of Science, appointed to investigate the fauna and flora of the Lesser Antilles.

The island is situated between 11° 58′ and 12° 80′ N. lat., and in 61° 40′ W. longitude, and is about twenty-one miles long by twelve miles in breadth, with an area of 125 square miles. It is about eighty miles due north of Trinidad. The centre of the island is entirely occupied by mountain ranges, which rise in one place to a height of 2,749 feet. Their upper slopes are clothed with forests, and the luxuriance of the general vegetation shows that the rainfall must be large. One of the most remarkable natural features is the crater-lake, called the Grand Étang, which is situated on a mountain ridge at an elevation of 1,740 feet above sea-level. The hollow which surrounds this lake contains the finest forests and most luxuriant vegetation in the whole island. When Mr. Morris, during his late mission to the West Indies, paid a hurried visit to the

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island in January, Mr. Sherring was able to show him in this district alone about sixty species of ferns in a single day.

GLEICHENIACEÆ.

Gleichenia pubescens, H. B. K.

G. pectinata, Presl. Common and very variable.

CYATHEACEÆ.

Cyathea arborea, Sm. The common tree-fern of the island; the trunk reaching a height of 20–30 feet.

C. muricata, Kaulf. A large number of Mr. Sherring's specimens appear to belong to this little-known species. He distinguishes three forms, one softer in texture, which grows on damp ridges at Bellevue and Grand Étang; a second with a taller trunk and more coriaceous frond; and a third form still firmer in texture, which grows in swamps. The trunk varies in length from 7 to 20 feet.

Hemitelia grandifolia, Spreng. The commonest of all the ferns of the island, and very variable. A form not uncommon in open ground, fully fertile, with frond not more than a foot long and pinnae only half an inch broad. Trunk always short, at most 1–1½ ft. long.

H. horrida, R. Br.

Alsophila aspera, R. Br. Very local.

A. Elliottii, n. sp. Trunk very short. Stipe a foot long, not paleaceous, armed with strong, spreading prickles. Frond sub-deltoid, bipinnate, 2–3 ft. long, moderately firm in texture, green and glabrous on both surfaces; pinnae lanceolate, 2 in. broad, the lowest a little dwarfed; pinnules linear-oblong, sessile, ½ in. broad, obtuse or subacute, conspicuously crenate. Veins in pinnate groups opposite the final lobes: veinlets 5–6-jugate, simple, ascending. Sori medial.—Nearest the Brazilian *A. atrovirens*, Presl. First noted in a sterile state by Mr. W. R. Elliott at Antoine, Bellevue; afterwards by Mr. Sherring in sparing fructification at Pyrenees, on the southern slope of St. Catherine's peak.

HYMENOPHYLLACEÆ.

Hymenophyllum polyanthos, Sw. Both type and var. *H. andinum*, V. D. B. Not common.

H. hirsutum, Sw. Both type and var. *H. lanatum*.
H. lineare, Sw. Only seen in one locality.
H. ciliatum, Sw. Common and very variable.
H. fucoides, Sw. Common on Feddon's Camp Mountain.
Trichomanes spicatum, Hedw. One of the commonest ferns in the southern half of the island.
T. membranaceum, L. Four localities.
T. reptans, Sw. Very common on trees.
T. muscoides, Sw. One locality only, on trees in a swamp.
T. pusillum, Sw. The only form seen was *Didymoglossum angustifrons*, Fée, in two localities.
T. Krausii, H. & G. Common on trees, chiefly at Birch Hill.
T. sinuosum, Rich. Common on trunks of tree-ferns. Very fine at the Grand Étang.
T. Bancroftii, H. & G.
T. Kaulfussii, H. & G. Very common and very fine.
T. alatum, Sw. Two localities, on rocks and trees.
T. crispum, L. Very common, the typical form, on trees.
T. crinitum, Sw. Very rare on trees, 2000–2700 feet. Very fine.
T. pyxidiferum, L. Common on trees in the high woods.
T. radicans, Sw. Very rare. Only on one rock in the south of the island.
T. pinnatum, Hedw.
T. rigidum, Sw. Very common and fine.

POLYPODIACEAE.

Dicksonia cicutaria, Sw. The var. *D. incisa*, Fée, more common than the type.
Hypoderris Brownii, J. Sm. Wet, rocky gully in Feddon Mountain, alt. 1800–2400 feet. Only known before in Trinadad and Porto Rico.
Lindsaya trapeziformis, Dryand. The type common. Var. *L. falcata*, Willd., not so common. Mr. G. W. Smith has also lately collected var. *arcuata*, Kunze.
L. guianensis, Dryand. Common, especially on open banks.
Adiantum Kaulfussii, Kunze. Dry banks in two localities.
A. obliquum, Willd. Type and var. *bipinnatum*, which connects *obliquum* with *intermedium*.

Adiantum intermedium, Sw. The only form seen in one locality only has rhomboid final segments half an inch long.

A. tetraphyllum, Willd.

A. villosum, L. Common on open banks at a low elevation.

A. pulverulentum, L.

A. concinnum, H. B. K. Only in a few places on the western coast.

Pteris laciniata, Willd. Common in damp forests.

P. grandifolia, L. Found in only one place in a rocky gully in Mount St. Catherine by Mr. Greaves.

P. aculeata, Sw. Common in damp forests.

Lomaria attenuata, Willd. Common on trees.

L. L'Herminieri, Bory. The commonest fern on the peak of the highest mountain, descending to 2000 feet.

L. onocleoides, Spreng. Summit of St. Catherine's peak.

L. procera, Spreng. High mountains only, on the ground.

Blechnum occidentale, L. Common from sea level up to the high peaks.

Asplenium serratum, L. Common.

A. lunulatum, Sw. Trees, not common.

A. obtusifolium, Sw. Very wet rocks.

A. cultrifolium, L. One locality only.

A. auriculatum, Sw. Common on trees.

A. laetum, Sw. Common in damp woods.

A. cuneatum, Lam. Trees, not common.

A. pumilum, Sw. One locality, in corners of dry rocks, found by Miss McServey.

A. rhizophyllum, Kunze. Local on dry cliffs.

A. Shepherdi, Kunze. Common in damp forests.

A. grandifolium, Sw. Wet gullies, local.

A. crenulatum, Baker. Local, damp forests. The allied subarborescent *A. radicans*, Schk. (*A. dubium*, Mett.), so abundant in St. Vincent, was not seen by Mr. Sherring in Grenada.

A. Godmani, Baker. In three localities, at an elevation of about 2000 feet. This was only discovered a very short time ago in St. Vincent. (See Annals Bot. V. 166. xi.) A form with deeply repand segments is commonest here.

A. marginatum, L. Very fine, the fronds reaching a length of 10 feet, with pinnae above 2 feet long.

Aspidium semicordatum, Sw. Dry rocks, local.

A. plantagineum, Griseb. Very common in watercourses.

A. trifoliatum, Sw. Common, especially var. *A. Plumieri*, Presl, on wet rocks.

Nephrodium patens, Desv. Common, the type and var. *N. macrurum*, Baker.

N. trichophorum, Baker. Common.

N. tetragonum, Hook. Two forms, one in the lowland woods, and the other high up in the mountains.

N. sanctum, Baker. Gathered only by Mr. W. R. Elliott.

N. conterminum, Desv. Common on wet banks of streams.

N. Sprengelii, Hook. Common.

N. nemorosum, Baker (*Aspidium nemorosum*, Willd. Sp. Plant. V. 83). Stipe above a foot long, densely clothed up to the top with spreading brown linear paleae. Frond ample, tripinnate, membranous, bright green, with very scaly rachides; lowest pinnae the largest, oblong-lanceolate, 6–9 in. long; final segments linear-oblong, obtuse, $\frac{1}{12}$ in. broad. Veins 3–5-jugate in the final segments; veinlets simple, erecto-patent. Sori medial on the veins. Indusium minute, fugacious. Damp forests on both the east and west sides of the island. Allied to *N. amplum*, *furcatum*, and *Grisebachii*. I am identifying this with Willdenow's plant by comparison with a specimen named by Dr. Kuhn, gathered by Sintenis in Porto Rico (No. 5885). We have not had the plant from any other island.

N. amplum, Baker. Rare.

N. villosum, Presl. Damp woods, not common.

N. effusum, Baker. Local.

N. molle, Desv. Rare.

N. brachyodon, Hook. Banks of streams.

N. macrophyllum, Baker. Fairly common.

Nephrolepis exaltata, Schott.

N. acuta, Presl. Only seen in two places.

Oleandra nodosa, Presl. On trunks in the forests.

Polypodium flavo-punctatum, Kaulf. Wet gullies.

P. decussatum, L. Fairly common.

P. crenatum, Sw. Common on banks of streams.

P. tetragonum, Sw. Lowland banks.

P. furcatum, Mett. Peak north west of the Grand Étang, alt. 2200 feet.

P. trifurcatum, L. With the last, and also on St. Catherine's peak.

Polypodium serrulatum, Mett. Common on trunks at a high level.
P. Hartii, Jenm. Rare on trees, 1700–2400 feet. Only seen before in Jamaica and Dominica.
P. trichomanoides, Sw. Rare.
P. jubaeforme, Kaulf. Common on trunks.
P. cultratum, Willd. One place on St. Catherine's peak.
P. pendulum, Sw. Frequent on trunks.
P. suspensum, L. Common on trunks.
P. taxifolium, L. Not common.
P. pectinatum, L. Common.
P. sororium, H. B. K. Common on trees.
P. vacciniifolium, L. & F. Frequent on trees at low levels.
P. loriceum, L. Frequent on trees.
P. neriifolium, Schk. Frequent on trees.
P. incanum, Sw. Rocks near the prison.
P. piloselloides, L. Common on trees.
P. aureum, L., var. *P. areolatum*, H. B. K. Common on trees.
P. repens, L. Frequent on trees.
P. Phyllitidis, L. Common on trees.
P. lycopodioides, L. Frequent.
Monogramme seminuda, Baker. Bellevue and Grand Étang districts only.
Gymnogramme calomelanos, Kaulf. Common.
G. elongata, Hook. Common on trees.
Vittaria lineata, Sw. Not common.
V. remota, Fée. Fine on trees at Birch Grove.
Antrophyum lanceolatum, Kaulf. Common.
Meniscium reticulatum, Sw. One of the commonest ferns in the island.
Hemionitis palmata, L. Very rare.
H. citrifolia, Hook. Common and very fine.
Acrostichum Sherringii, n. sp. Rootstock suberect; paleae scarcely any. Sterile frond linear, 6–9 in. long. $\frac{5}{8}$–$\frac{3}{4}$ in. broad at the middle, thin, bright green, glabrous, the short stipe distinctly but narrowly winged nearly or quite to the base; veins distant, erecto-patent, simple or forked. Fertile frond narrower, with a very long slender naked castaneous stipe. Two places only, on trees in the forests on St. Catherine's peak.—Near *A. simplex*, from which it differs by its thin texture, more distant veins, winged stipe of the sterile frond, and very long stipe of the fertile frond. Fruits in May and June.

A. conforme, Sw. Common on trees.
A. Lingua, Raddi. Common on trees. Fruits in June and July.
A. latifolium, Sw. Common and variable.
A. L'Herminieri, Bory. Very rare.
A. Aubertii, Desv. Common, the type and two varieties, one of them matching *A. mollissimum*, Fée, Fil. Bras. 7, tab. 2, fig. 3. Though so widely spread in South America, we have not had this before from the West Indies.
A. apodum, Kaulf. Trees, not common.
A. viscosum, Sw. Trees, several forms.
A. boryanum, Fée. On trees in very damp woods on Feddon's Camp Mountain, alt. 2000 feet.
A. sorbifolium, L. Frequent, both on trees and rocks, fruiting freely in one locality.
A. osmundaceum, L. Common in the forests, on trees.
A. cervinum, Sw. Not common.
A. nicotianaefolium, Sw. Common in the lower forest vallies.
A. crinitum, L. Rare, on trees in woods at 2000 feet.
A. aureum, L. In swamps, especially near the coast.

SCHIZAEACEAE.

Schizaea fluminensis, Miers. Generally on the base of palm trees, alt. 1500–2000 feet. Not known in the West Indies before, but it may be an extreme variety of *S. dichotoma*.
No *Anemia* has been seen in the island, though the genus has been specially sought for in likely places.
Lygodium venustum, Sw. Common on trees in the lowlands. *L. volubile*, so common in Jamaica, was not seen in Grenada.

MARATTIACEAE.

Danaea polymorpha, Leprieur. Cleared woods at Chantilly.
D. nodosa, Smith. Forest at Feddon Camp Mountain.
D. elliptica, Sm. Common at the south end of the island.
D. alata, Sm. Damp gully at foot of St. Catherine's peak.

OPHIOGLOSSACEAE.

Ophioglossum reticulatum, L. Open pastures, Bellevue.

LYCOPODIACEAE.

Lycopodium taxifolium, L. Common on trees in the forests.
L. cernuum, L. Very common on banks, ascending to the peaks of the highest mountains.
L. dichotomum, Jacq. Trees in damp forests.
L. verticillatum, L. Rare, on trees.
Psilotum triquetrum, Sw. On trees, not common.

SELAGINELLACEAE.

Selaginella flabellata, Spring. Everywhere common, both in the forests and open ground.
S. rotundifolia, Spring. Damp banks, rare.
S. albo-nitens, Spring. High forests only.

The total number of ferns gathered in Grenada by Mr. Sherring is 145. As far as ferns are concerned the island does not offer many salient characteristics, most of the species being universally diffused through the West Indies. The two novelties are a tree-fern and an *Acrostichum*. *Hypoderris Brownii* was known before only in Trinidad, and *Schizaea fluminensis* and *Acrostichum Aubertii* are continental types now added for the first time to the West Indian floras. The other species of the greatest interest on the score of rarity are *Cyathea muricata*, *Nephrodium nemorosum*, *Polypodium Hartii*, *Asplenium Godmani*, and *Danaea polymorpha*. The absence of two such common West Indian ferns as *Asplenium striatum* and *Lygodium volubile*, and of all the Anemias, is very remarkable.

On the Characters, or Marks, employed for classifying the Schizomycetes.

BY

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THOSE who have contributed to the bringing about of the existing state of chaos in the classifying of the Schizomycetes have much to answer for, and the task of unravelling the tangled skein of records will be no less honoured than onerous, which is saying a good deal. Without implying any competition for that honour, it may be of some little use to try and show how the chaos has come about, and to discover a way out of it, or at least to discover one or two paths which might be put together to make a way out of it.

I take it that two chief sets of causes have been at work, in different directions, to bring about the deadlock; on the one hand, the botanists of the past decade have confined their attention too exclusively to the morphological characters of the various species they have created, while, on the other, the bacteriologists—using the word simply in its technical sense—have directed their attention too exclusively to the behaviour of *their* species on or in certain media, especially on gelatine. It is not implied, intentionally at any rate, that either class of observers has wilfully neglected the observations of the other; but it needs no pointing out that each

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has unconsciously heaped up an immense store of trouble for the new type of bacteriologist which the needs of the times are bringing forward. The trouble has arisen quite naturally, owing to the two sets of observers having had their backs turned to one another, and their attention concentrated along different avenues of research. Let us look for a moment at the kinds of characters that each has brought into the foreground, and then try to make out from the work of the few who are now turning round as they work (so to speak) and looking at each other's efforts with scientifically sympathetic eyes. Since it is not my object to write a history of bacteriology, I pass over the work of the earlier observers Leeuwenhoek, Will, O. F. Müller, Bory de St. Vincent, Spallanzani, Ehrenberg, Dujardin, Pertz, Hallier, Burdon-Sanderson, Pasteur, and others, simply reminding my readers that many very interesting facts had been recorded, and even classifications of micro-organisms constructed, long before Cohn's time.

The school which culminated in the brilliant efforts of Cohn was almost entirely concerned with the preparation of the ground on which the subsequent struggles were fought, and from which the new departures were taken.

Ehrenberg, Kützing, Rabenhorst, Schröter, Warming, Cohn, and others had recorded, prior to 1880, a considerable number of forms of Bacteria of various kinds. For the most part these records were records of 'finds': that is to say, each observer overhauled the contents of the ponds, aquaria, macerating-troughs, and so on, at his disposal, and faithfully delineated the forms of the organisms found therein, named them, and added the habitat, &c. The method was the usual one of an exploring botanist in a new country, and quite properly so. The results began to take a modern shape under the hands of Cohn, who, in 1872 and 1875, brought forward his long celebrated system of classification of these organisms, based almost entirely on their forms as found and recorded; though, at the same time, I think Cohn was more alive to the imperfections and tentative character of his

proposed system than is always admitted. Cohn's great merit, in fact, was in pointing out that there is a *relative* consistency in the recurring forms met with, sufficient to enable us to describe them more or less definitely: he did not insist on the absolute persistence of these forms to the extent he has been supposed to have done.

Two double sets of dissentients to the Cohn-Ehrenberg school, of the decade prior to 1881, seem to have arisen about this period, and I shall briefly sketch the peculiarities (as I understand them) of each of these camps, or schools, or whatever we choose to term them, merely reminding the reader that each touches the period just referred to in very different ways and at different points.

First, there was a double set of botanists. One of these sets may be best referred to as the systematists, who seem to have directed their attentions almost entirely to the getting hold of every new form of Schizomycete, as soon as it was published, and no matter by whom, and giving it a name, implying that the form recorded is a species. This set of workers, of very unequal merit, has culminated in the unquestionably brilliant leaders, Winter, the deplored compiler of the celebrated Pilz-Flora of Winter and Rabenhorst, and Trevisan and De-Toni, the splendidly talented and industrious compilers of the volume on Schizomycetes of Saccardo's monumental Sylloge Fungorum, and now *the* authority on European systematic mycology.

The second of this set may be termed the morphologists, and their distinguishing feature—the one which binds them together as a band of workers—has been the investigation of the *development* as well as the forms of the Schizomycetes. Influenced throughout more or less by the two masters of microscopic methods, De Bary and Brefeld, and also, it should be mentioned, by Cohn himself, who was an exceedingly able investigator, quite alive to the morphology of the subject he tried to set in order, this assiduous band of observers, comprising Cienkowski, Prazmowsky, Billroth, Lankester, Dallinger, Eidam, Hueppe, Klebs, Klein, Kurth,

Van Tieghem, and others, has culminated in the modern partial school of Zopf. It should be noted, however, that this series of observers has already undergone an important differentiation along two more or less diverging lines of thought: some of them, influenced by the writings of Naegeli, Billroth, and Zopf especially, have declared emphatically against the systematists, in so far that they have either denied the existence of species altogether among Schizomycetes, or have at least claimed that as the polymorphy of these organisms is now shown to be so marked—and it must be admitted that polymorphy, as measured by the Cohn-Ehrenberg standard, *is* very marked—species cannot be defined by the morphological and developmental characters of the Schizomycetes.

The others, comprising Beyerinck, Kurth, Cienkowski, Winogradsky, Klein, &c., remaining more faithful to the cautious utterances of Cohn and De Bary on this point of polymorphy, have satisfied themselves with declaring, or implying, more or less clearly, that, while polymorphy cannot be denied and should not be under-estimated, the difficulty of finding diagnostic morphological characters is after all a relative one, largely due to the minuteness of the organisms, and the few and simple differential features that they possess; or at least that they exhibit under our microscopes.

This double set, constituting a school, if we so choose to put it, of botanists, have undoubtedly done wonders during the last decade. It is only necessary to mention De Bary's study of *Bacillus Megaterium*, Prazmowsky's and Van Tieghem's of *Clostridium butyricum* and *Leuconostoc*, Klein's on the spores of Bacilli, Brefeld's work on *Bacillus subtilis*, Kurth's on *Bacterium Zopfii*, and *Photobacteria*, Winogradsky's on Sulphur-bacteria and *Nitromonas*, Lankester's and Zopf's on *Cohnia* (*Clathrocystis*) *roseo-persicina*, Billet's on *Cladothrix*, Beyerinck's on *Bacillus cyano-fuscus*, and very many others, to see what enormous strides have been made in our knowledge of the forms and evolution of Schizomycetes during the period referred to, and what a weighty mass

of evidence is accumulating which must have, and is having, its effect in checking mere recording of forms, on the one hand, and wild speculations on the other.

The second double set of observers, which we may call the non-botanists—without implying the slightest want of respect for the magnificent edifice of knowledge which they have erected—may, it seems to me, be said to have taken their origin from two sources. One of these sets, which has culminated in the grand school now centred in the Pasteur Institute in Paris, sprung quite naturally from the epoch-making work of Pasteur on fermentations[1], and its leading characteristics are unquestionably derived from the teachings and writings of the illustrious master who still directs the school. We may, I think fitly, denominate it the school of Pasteur and Duclaux. Its leading feature, and the one which binds it together as a very compact body, is the concentrated attention to the processes of zymotic energy displayed by micro-organisms. It has concerned itself very little with questions of morphology, and still less with the interests of the systematists, towards whom, in fact, its attitude seems occasionally somewhat supercilious.

Since I am committed to the invidious task of reminding my readers of some of the leading work of each set of investigators, it is only necessary to point to the following as examples of the magnificent achievements of the Pasteur-Duclaux school: the works of Downes and Blunt, Arloing, Duclaux and Roux on the action of light on the spores of *Bacillus anthracis*; Schloesing and Müntz, Warrington, Frankland and Winogradsky on nitrification; Metschnikoff on phagocytes; Tyndall on dust; Hansen on yeasts; and many others influenced by the author of the classical works, Études sur le Vin, Études sur la Bière, and the director of the great experiments on hydrophobia now being carried out. It is unnecessary to go into the details of Pasteur's labours on anthrax, fowl-cholera, vaccination, immunity,

[1] It is therefore pre-Cohnian in many respects, though it touches Cohn's work, and that of his contemporaries, at many important points.

&c.; they are known to all. The great link between this school and the one to be taken next is the specialisation of the labours of the Pasteur-Duclaux school in the direction of pathology, and the ferment-theory of disease.

The other branch of the non-botanical workers started more directly from the Cohn-Ehrenberg school of bacteriologists, under the direct leadership of Koch. It originated distinctly, I think, with Koch's path-breaking work on *Bacillus anthracis*, and the foundation of the Mittheilungen aus dem kaiserlichen Gesundheitsamte in 1881, and has been carried on ever since, under the banner of the great German doctor, by men like Flügge, Fraenkel, Gaffky, Eberth, Briegen, Pfeiffer, Woodhead, and a whole army of pathologists. On the whole it has kept more in touch with the systematists, especially of the Cohn-Ehrenberg school as shown by the works of Flügge, Migula, Cornil, and Babès, &c., though its special work is marked throughout as pathological in nature.

The contributions to our knowledge of anthrax, cholera, and tuberculosis made by Koch, Gaffky, Klein, Hankin, and others, suffice to show this; and it may be remarked in passing that such work on the part of the pathologists of the German and French schools at once explains their departure from the older traditions of bacteriology. Another remarkable feature of the Koch-Flügge school, if we may thus term it, has been their extraordinary fertility in the devising of methods of culture and of staining. The same has been true of the Pasteur-Duclaux school, and it is indeed a very invidious task to compare and contrast the two in respect of their achievements in any part of the general domain they have opened up; but I am not attempting to detract in the slightest from the high honours of either by trying to select what seem to be the distinguishing peculiarities in their special lines of development of the science.

It seems to me that while the German school has paid particular attention to the methods of gelatine-culture and of staining by means of aniline-dyes, the French school has rather developed the methods of culture in liquid media, and

the examination of the products of action of the Schizomycetes. This would seem to explain why the Koch-Flügge school has given us several special modes of staining:—e.g. Kühne's methylene-blue method, the Ziehl-Neelson carbol-fuchsin method, the Gram-Weigert, and Koch's various ingenious methods of preparing, staining, and mounting Bacteria; why the various developments of culture on solid media have come from Germany; and why the characteristic forms, colours, and liquefying powers of the colonies of Schizomycetes have received so much attention at the hands of the Koch-Flügge school.

The above way of looking at the history would also seem to explain some peculiarities of the Paris school. The perfection to which they have carried the method of dilution-cultures, as exemplified by Miquel's results at the Montsouris laboratories; their recent triumph, the Chamberland filter; the successful pursuit of anaërobic Bacteria at a time when such anomalous organisms were looked at with suspicion; and last, but by no means least, their remarkable persistence and success in the employment of virus-material which they treat as if it contained Schizomycetes, although no one can demonstrate the presence of organisms in it—I refer of course to the hydrophobia-virus—reminds one of the methods of the chemist and physicist with their assumptions of atoms and molecules which no man has ever seen.

Each of these two schools has imparted much information to the other, and, naturally, their mutual reactions tend to eliminate their differences as schools in some respects, and to emphasize them in others. I think, however, that, taken as a whole, each has its special peculiarities much on the lines sketched above. Each of the schools, moreover, has given evidence of its fertility in the branching out of more special little bands of workers, whose particular object is to apply the results of bacteriology in certain directions. The hygienic institutions of various countries may be cited as examples, and nothing better illustrates the truth of the preceding remarks than the persistent difference in methods of culture

between the Montsouris Observatory in Paris, and the various German institutes of hygiene: the former severely criticises the gelatine-plate method as untrustworthy, the latter employ it almost exclusively.

Enough has been said to show how it has come about that various bands of observers have been traversing and mapping out the enormous domain of bacteriology, each with little or no regard for the presence or work of the other. The result may be compared to a number of maps, begun by various parties of surveyors, each starting along a different route and with no pre-arranged plans as to scales, comparative surveys, or intercommunication of any particular kind. Moreover, one set of explorers has confined its attention chiefly to contours, while another has recorded climate, and another artistic features, and so on, whence the difficulties of comparing the results and compiling a map up to date are very great.

All are more or less conscious of the need of a good systematic account of these organisms, however; and I now propose to try and set forth in some detail what kinds of characters are being used by those who wish to inform others how given 'species' may be distinguished. I shall of course confine my remarks entirely to modern work.

In order to remind the reader of the scheme propounded by Cohn, I append his system in a tabular form (Table I) as put forward in 1875.

TABLE I.—*Cohn*, 1875.

Tribe I. GLOEOGENAE.

Cells free, or connected by intercellular substance into slimy colonies (zoogloeae).

A. Cells free, or grouped in pairs or fours.

Chroococcus (Naeg.). Cells globular.

Synechococcus (Naeg.). Cells cylindrical.

B. Cells, in the resting state, gathered into amorphous zoogloea-masses.

(a) Cell-membrane passing imperceptibly into the intercellular substance.

(i) Cells devoid of phycochrome, very small.
Micrococcus (Hall. emend.). Cells globular.
Bacterium (Duj.). Cells cylindrical.
(ii) Cells containing phycochrome, larger.
Aphanocapsa (Naeg.). Cells globular.
Aphanothece (Naeg.). Cells cylindrical.

(β) Intercellular substance stratified concentrically into shells.
Gloeocapsa (Kg., Naeg.). Cells globular.
Gloeothece (Naeg.). Cells cylindrical.

C. Cells united into definitely circumscribed zoogloea-masses.

(α) Families in flat layers, arranged in one plane.
(i) Cells in fours, arranged in one plane.
Merismopedia (Meyen).
(ii) Cells irregularly arranged on the periphery of a sphere.
Clathrocystis (Henfr.). Families clathrate. Cells spherical.
Coelosphaerium (Naeg.). Cells cylindroid-wedge-shaped: families forked.

(β) Colonies aggregated into spheroidal, many-layered masses.
(i) Numbers of cells definite.
Sarcina (Goods.). Cells in fours, globoid, colourless.
Gomphosphaeria (Kg.). Cells cylindroid-wedge-shaped, irregularly disposed, containing phycochrome.
(ii) Numbers of cells large and indefinite.
Ascococcus (Billr. emend.). Cells colourless, very small.
Polycystis (Kg.).
Coccochloris (Spr.).
Polycoccus (Kg.), &c. } Cells larger, and containing phycochrome.

Tribe II. NEMATOGENAE (Rab.).

Cells arranged in filaments.

A. Filaments always unbranched.

(α) Filaments free or matted together.
(i) Filaments cylindrical, colourless, and obscurely segmented.
Bacillus (Cohn). Filaments very thin and short.

Leptothrix (Kg. emend.). Filaments very thin and long.

Beggiatoa (Trev.). Filaments thicker, and long.

(ii) Filaments cylindrical, containing phycochrome, evidently segmented. Reproductive cells unknown.

Hypheothrix (Kg.).

Oscillaria (Bosc.), &c.

(iii) Filaments cylindrical, segmented, and forming gonidia.

Crenothrix (Cohn). Colourless.

Chamaesiphon, &c. Containing phycochrome.

(iv) Filaments spirally twisted

* Devoid of phycochrome.

Vibrio (Ehr. emend.). Filaments short, slightly undulated.

Spirillum (Ehr.). Filaments short, spiral, rigid.

Spirochaete (Ehr.). Filaments long, spiral, flexile.

** Containing phycochrome.

Spirulina (Link). Filaments long, spiral, flexile.

(v) Filaments moniliform.

Streptococcus (Billr.). Without phycochrome.

Anabaena (Bory). *Spermosira* (Kg.), &c. } Containing phycochrome.

(vi) Filaments tapering to the apex, like riding-whips.

Mastigothrix, &c.

(β) Filaments joined into zoogloea-masses by intercellular substance.

Myconostoc (Cohn). Filaments cylindrical, colourless.

Chthonoblastus (Kg.), &c. *Limnochlide* } Filaments cylindrical, and containing phycochrome.

Nostoc, *Hormosiphon*, &c. Filaments moniliform, and with phycochrome.

Rivularia (Roth). *Zonotrichia* (Ag.), &c. } Filaments tapering, like riding-whips, and containing phycochrome.

B. Filaments branched falsely.

(α) Without phycochrome.

Cladothrix (Cohn). *Streptothrix* (Cohn). } Filaments cylindrical.

(β) Containing phycochrome.

Calothrix (Ag.). *Scytonema* (Ag.), &c.	Filaments cylindrical.
Merizomyria (Kg.). *Mastigocladus* (Cohn).	Filaments moniliform.
Schizosiphon (Kg.). *Geocyclus* (Kg.), &c.	Filaments tapering, like riding-whips.

It will be remembered that Cohn was attempting a scheme to embrace the whole of the Schizophyta, and not merely the Schizomycetes; and although we now exclude the forms containing 'phycochrome,' as relegated to their proper position among the lower Algae, it seemed advisable to retain them in the above scheme, as Cohn did in his classical memoir. It will be obvious to all who are acquainted with the subject that Cohn's chief divisions have always afforded important bases for subsequent systems of classification of these organisms; though it was soon shown, by Koch, Prazmowsky, and others, that the zoogloea cannot be employed as a distinguishing mark in the sense Cohn employed it, and other characters had to be sought for the primary divisions. I now propose to set forth some of the best known of these systems, which will at the same time mark the main points of progress attained since Cohn's time.

In 1881, Winter published his system, designed for his edition of Rabenhorst, and I append his tabular *résumé*—or key—in its original form, as it best illustrates the author's attempt to make a definite Flora for the group.

TABLE II.—*Winter*, 1881.

1.	Cells spherical or ovoid	2
	Cells cylindrical—short or long	5
	Cells lanceolate, ribbon-like, spirally coiled .	*Spiromonas.*
2.	Cells isolated, or in chains, or grouped in amorphous slime	*Micrococcus.*
	Cells in large numbers, united into colonies with definite contour	3

3.	Colonies hollow, the cells in a single peripheral layer	*Cohnia.*
	Colonies solid throughout, and filled with cells	4
4.	Cells few, and joined in regular families, each with a definite number . . .	*Sarcina.*
	Cells in larger numbers, aggregated in irregular colonies, each with an indefinite number	*Ascococcus.*
5.	Cells shortly cylindrical, isolated, or two or more loosely joined	*Bacterium.*
	Cells as long cylinders, united into filaments	6
6.	Filaments isolated or matted together .	7
	Filaments in rounded gelatinous matrix .	*Myconostoc.*
7.	Filaments unbranched	8
	Filaments with false-branching . . .	*Cladothrix.*
8.	Filaments rectilinear	9
	Filaments spiral or curved	11
9.	Filaments short and distinctly segmented .	*Bacillus.*
	Filaments long, segments usually obscure .	10
10.	Filaments very slender	*Leptothrix.*
	Filaments thicker	*Beggiatoa.*
11.	Filaments short, spiral with a few turns, or merely curved: stiff	*Spirillum.*
	Filaments longer, and with numerous spiral turns, and flexile	*Spirochaeta.*

[Appendix—*Sphaerotilus* and *Crenothrix*.]

In the meantime the controversy had begun as to the meaning of 'species' among the Schizomycetes. Billroth, in 1874, had stated his conviction that all the forms are mere varieties of one fundamental species, and some experiments of Buchner's (1882) with *Bacillus anthracis* and *B. subtilis* (which Buchner thought he had proved to be convertible one with the other) seemed to support the idea. Naegeli, with whom Buchner was associated, took up a similar view, and thus arose the split, already referred to, between the extremists who regarded the polymorphism of the Schizomy-

cetes as universal, and those who committed the error of paying too little attention to the existence of polymorphism in the group.

As usually happens in such cases, the truth lies somewhere between the extremes, and the reader unacquainted with the literature cannot do better than consult De Bary's beautiful fourth lecture on this subject, where the evidence for and against is weighed with the fairness and thoroughness so characteristic of that gifted master of morphology.

Van Tieghem, in 1884, proposed to take into account the planes of division of the cells as furnishing the chief bases for dividing the Schizomycetes into three primary groups, thus :—

TABLE III.—*Van Tieghem*, 1884.

I. Divisions in one plane only. Thallus filamentous, or forming aggregates of segments.
  A. Simple forms.
    (a) Non-sheathed.
      (i) Of minute spheroidal cells, in gelatinous matrix or free. May be more or less seriate.
        Micrococcus.
      (ii) Elongated in one plane, and free.
        * Rodlets short and at once free.
          Bacterium.
        * * Rodlets longer, and may remain for a time in series.
          Bacillus.
        * * * Filaments.
          Leptothrix.
      (iii) Elongated in spiral form.
        * Short comma-like twisted rodlets.
          Vibrio.
        * * Longer and helicoid.
          Spirillum.
        * * * Longer still, and with numerous turns.
          Spirochaete.

(β) Sheathed forms.
(i) Unbranched.
Crenothrix.
(ii) With false ramifications.
Cladothrix.

B. Colonial or aggregated forms.
(α) Non-sheathed.
(i) Micrococcus-like cells.
Punctula.
(ii) Rod-like cells.
Polybacteria.
(β) Sheathed.
(i) Micrococcus-like cells.
Ascococcus.
(ii) Rod-like cells.
Ascobacteria.
(iii) With spiral segments.
Myconostoc.

II. The planes of division run in two directions, and the membrane-like surfaces break up into groups of quadrates.
Merista.

III. There are three planes of division, resulting in the development of solid cuboidal masses.
Sarcina.

It may be regarded as an objection to Van Tieghem's system that the three chief divisions are so very unequal, and that some of the characters employed for subdividing the first primary group, which contains nearly all the forms, are of more importance than those used for separating the three main divisions. This criticism seems well-founded if we remember that planes of division only affect the vegetative stages. Van Tieghem himself points out that the division-planes in the second and third groups do not always follow equally rapidly, and in their proper order: a young *Merista* may be uniseriate, and a young *Sarcina* meristate.

It should be stated that Van Tieghem does not himself draw up a detailed table, possibly because he recognised how

difficult it was to put these three groups on the same footing.

The further subdivision of the larger genera was based on the behaviour of the Schizomycetes towards the substratum—chromogenes, zymogenes, and pathogenes respectively—an idea started by Schröter and Cohn, and already partly employed by Winter and others, and one which has gained ground since.

Van Tieghem seems to have relegated the characters derived from the method of spore-formation to quite a subordinate position, whereas De Bary, it will be remembered, elevates this into a diagnostic character of the highest importance.

On the whole, we may regard Van Tieghem's contributions to the classification of the Schizomycetes as consisting in the recognition of the importance of the mode of division and the behaviour towards the substratum. In no other way can it be considered as an advance on Cohn and Ehrenberg's system.

Flügge, who has exerted considerable influence on the pathologists, especially in Germany, arranged the Schizomycetes in groups, much after the method of Cohn. I give his system in Table IV.

TABLE IV.—*Flügge*, 1886.

I. Cells spherical or ovoid.
  A. Cells isolated, or merely seriate, or in amorphous aggregates.
    Micrococcus.
  B. Cells forming colonies more or less definitely circumscribed.
    (*a*) Colonies solid and entirely filled with the cells.
      (i) Colonies large, irregular, and numbers indefinite.
        Ascococcus.
      (ii) Colonies small, regular, and numbers definite.
        Sarcina.
    (*β*) Colonies excavated, with simple layers of cells at the periphery.
      Cohnia.

II. Cells cylindrical.

A. Cells as short rodlets; isolated, or aggregated into loosely united or gelatinous families.

Bacterium.

B. Cells several or many times longer than broad, and united into filaments.

(α) Filaments isolated, or matted together, or in fasciculi.

(i) Filaments not branching.

* Filaments straight.

† Filaments short and distinctly segmented.

Bacillus.

†† Filaments long and segments indistinct.

Very thin.

Leptothrix.

Thicker.

Beggiatoa.

** Filaments undulate or spiral.

† Short and rigid.

Spirillum (*Vibrio*).

†† Long and flexile.

Spirochaete.

(ii) Filaments with false ramification.

Cladothrix (*Streptothrix*).

(β) Filaments enveloped in rounded gelatinous matrix.

Myconostoc.

The chief advance here, in addition to the expurgation of certain genera no longer admitted as Schizomycetes, is the greater clearness in definition of the forms, gained partly by the fusion of trivial genera, and partly by the expression of the diagnostic characters. Nevertheless, Flügge's modification of Cohn's system suffers from the same defects as Van Tieghem's and the other older schemes, namely, that the forms selected as types are often only form-genera, and we undoubtedly meet with transient phases of one and the same filamentous genus which would be placed in two or more genera if such a system were rigidly followed.

Hueppe, whose book on methods, especially, has deservedly attained a world-wide reputation, has proposed a scheme

which brings into the foreground De Bary's suggestion that the distinction between endosporous and arthrosporous forms is a real one, and should be insisted upon: Hueppe, however, does not make the distinction so fundamental as De Bary proposed, but employs it as a subsidiary character, as Table V will show.

TABLE V.—*Hueppe*, 1886, and modified later.

I. Vegetative stage Coccoid.
  A. Cocci seriate in single chains.
    (α) In zoogloea-masses of medium size.
      (i) With endospores.
        Endo-streptococcus.
      (ii) Without endospores.
        Arthro-streptococcus.
    (β) In pronounced zoogloea-masses.
      Leuconostoc.
  B. Cocci in fours, or in short chains.
    Devoid of endospores (?), arthrospores only (?).
      Merista.
  C. Cocci in fours or eights, but not in chains. Endospores or not.
      Sarcina.
  D. Cocci in irregular masses of various kinds.
    (α) No definite arrangement.
      Micrococcus.
    (β) Grouped like bunches of grapes.
      Staphylococcus.
    (γ) In rounded zoogloea-masses.
      Ascococcus.

II. Vegetative stage rod-like.
  A. Forming filaments or single cells, flexile or rigid, more or less segmented or not, and with no distinction into base and apex.
    (α) Filaments straight or undulate, arthrosporous. No endospores.
      Bacterium.
    (β) Filaments straight or undulate or spiral. Arthrosporous only.
      Spirulina (*Proteus*).

(γ) Filaments straight or undulate, and with endospores.
(i) Rodlets not altered in shape during sporification.
Bacillus.
(ii) Rodlets fusiform, or undergo some changes in shape as spores form.
Clostridium.

B. No filaments, but spindle-shaped rods which undergo division in the *longitudinal* direction, and develope endospores.
Pasteuria.

C. Filaments, differentiated into base (usually fixed) and apex.
(α) Filaments not distinctly septate or divided, and without sheath.
(i) Devoid of sulphur granules.
Leptothrix.
(ii) Containing sulphur granules.
Beggiatoa.
(β) Filaments segmented and sheathed.
(i) Unbranched.
Crenothrix.
(ii) Branched (false branches).
Cladothrix.

III. Vegetative stage consisting of spiral filaments or segments, flexile or rigid.
(α) Arthrosporous only.
Spirochaete.
(β) Endosporous.
(i) No alteration in form of the sporogenous cells.
Spirillum.
(ii) The cell changes in shape as the spores are developed.
Vibrio.

The main advances in the De Bary-Hueppe scheme are, besides the distinct one of the employment of the spore-characters, the much clearer rendering of the diagnoses derived from the vegetative forms, and the embracing of the new types *Clostridium* of Prazmowsky, and (subsequently) *Pasteuria* of Metschnikoff. There is also a much more thorough analysis of the various forms allied to *Micrococcus*, though the diffi-

culties of this type are by no means overcome. Hueppe, among his numerous other contributions to bacteriology, has shown clearly how much the formation of zoogloea depends on circumstances, and is therefore a character to be employed very cautiously in distinguishing genera and species.

Zopf, in 1885, devised a scheme of classification which, in spite of the admitted difficulties in practical application, has the merit of being a very praiseworthy attempt at a scientific summary of our knowledge. It differs from the preceding especially in that the author tries to bring out the polymorphy of the Schizomycetes. Zopf divides these organisms into four main groups, as shown in Table VI.

TABLE VI.—*Zopf*, 1885.

I. Coccaceae.

Only cocci or serial chains or groups of cocci, so far as is known. No spores known. Divisions in 1, 2, or 3 planes.

A. Divisions in one plane only; the cocci in moniliform series, but separating later.

Streptococcus.

B. Divisions in two planes at right angles, leading to the formation of plates; the cells separating eventually.

Merismopedia.

C. Divisions in three planes, and therefore leading to the formation of packet-like colonies; the cells separating later.

Sarcina.

D. Divisions in one plane only, and the cocci separate at once, forming irregular or botryoid groups.

Micrococcus (with *Staphylococcus*).

E. Like *Micrococcus*, but the colonies immersed in dense gelatinous investment.

Ascococcus.

II. Bacteriaceae.

Usually presenting coccus—(may be absent),—rodlet,—and filamentous forms; the rodlets and filaments being spirally curved or straight, and presenting no difference between base and apex. Divisions in one plane only, so far as known. Spore-formation known in some: in others unknown and perhaps absent.

A. Cocci and rodlets, or only rodlets known, and arranged in linear series or filaments. No spores known.

Bacterium.

B. Filaments spiral: segmented into rodlets (long or short), or into rodlets and cocci. No spores known.

Spirillum.

C. Filaments spiral, and forming spores in the long or short segments.

Vibrio.

D. Develope cocci and rodlets, the former containing spores.

Leuconostoc.

E. Rodlets, or rodlets and cocci, in linear or spiral filaments. Spores developed in the rodlets or in cocci.

Bacillus.

F. As *Bacillus*, excepting that the sporogenous rodlets are peculiarly swollen.

Clostridium.

III. Leptotricheae.

Filaments, which are differentiated into base and apex, and may be linear or spirally curved, segmented into rodlets and cocci. Spores unknown.

A. Filaments sheathed. Cells without sulphur-granules. Aquatic forms.

Crenothrix.

B. Filaments not sheathed. The segments with sulphur-granules. Aquatic.

Beggiatoa.

C. Filaments not sheathed, and much divided up by numerous successive septa. No sulphur granules. Aquatic.

Phragmidothrix.

D. Filaments sheathed or not; segmentation not remarkable. Cells devoid of sulphur granules.

Leptothrix.

IV. Cladotricheae.

Filaments falsely branched. Breaking up into cocci, rodlets, and straight and coiled filamentous segments. No spores[1] known.

Cladothrix.

[1] This is no longer the case according to Billet.

Zopf's classification, admirable as it is in many respects, is difficult to work in practice, because it is necessary to have all the stages of development before we can decide on the position of a species: at the same time it should be noted, that in this very respect it is as far ahead of the merely tabular classifications, used for hurriedly determining the name of a form, as a good Flora is ahead of a mere museum catalogue of plants.

It is, in fact, just in respect of this particular attention to all the facts in the *development* of the species that Zopf's classification is scientifically so far in advance of his predecessors. Unquestionably it renders the problems more difficult, because it insists on the working out of all the phenomena before a species is accepted; but, since such a scheme must embrace all the merely diagnostic *form-characters* used by the Cohn-school, it must be admitted to be superior to their system. The matter of difficulty of application, in such a connection, cannot be urged as a reason for desisting from obtaining and recording all that can be discovered regarding an organism.

The only really valid objection to a purely scientific classification is the old objection of the purely utilitarian 'practical man,' and even then the validity of the objection is relative. This leads me to bring out the point that the bacteriologists, in the widest sense of the word, are really looking at the question of classification from at least two very different points of view. On the one hand we have the botanists, who direct their attention to the organism, the Schizomycete, itself, as a biological phenomenon to be examined and reported upon as thoroughly as possible: for them, no classification is complete which does not record, or (what amounts to the same thing) imply in its records, all the life-phenomena of the organism, including its pedigree.

On the other hand we have the pathologists, hygienists, brewers, chemists, &c., who regard the organism simply as an object to be named for convenience in reference, because it brings about certain changes in the tissues, waters, and other

media which they are more especially concerned with. They do not care, and naturally so, what vagaries the organism exhibits, so long as they can recognise it when they meet with it.

As matter of experience, however, it is just these vagaries that bring about the sources of error which beset them on all hands, and hence they are equally interested with the botanist in having them cleared up, and explained.

It must not be overlooked, moreover, that many of them are alive to the dangers referred to, as witness Cassededebat's industrious and able investigation of the differences between the true typhoid bacillus, and the various false ones which simulate it: also the numerous researches which have been made on the distinguishing characters between *Bacillus subtilis* and *B. anthracis*, and so on.

Whence we come to the conclusion that, whatever may be believed to the contrary, the real interests of 'bacteriologists' of all kinds are identical. Exactly the same kind of discussions, and apparent difference of interests, arise in the relations of Forestry, Agriculture, Horticulture, &c., to Botany; but in these cases also the broadest thinkers all recognise the true state of affairs.

At the same time, botanists must concede that the big special problem of working out these life-histories, and of compiling the ideal classification, still a long way ahead of us, devolves upon themselves. It is useless to merely criticise the imperfect tabular classifications of the pathologists and hygienists and others: the only thing to do is to take the organisms in hand and expose their vagaries by cultivating them under the microscope, and subjecting them to the tests devised by modern morphologists and physiologists.

The most recent and the most thorough classification of the Schizomycetes extant, is that of De-Toni and Trevisan, published in 1889 in Saccardo's 'Sylloge Fungorum.' It embraces the description of more than 650 species, and may be taken as the most complete account of the Schizomycetes, from the systematists' point of view, that has ever been

attempted. When we reflect that Winter, even so lately as 1881, only described sixty-nine species, we obtain some idea of the extraordinary activity which has been displayed within the last ten years. I append Trevisan and De-Toni's scheme in tabular form.

TABLE VII.—*De-Toni and Trevisan*, 1889.

I. TRICHOGENAE.

Presenting three vegetative stages—filaments, rodlets and cocci. The filament is the typical individual, sheathed or not, and is usually differentiated into apex and base, the plants being fixed by the latter and radiating from a central point. Some have no distinction between base and apex. Rodlets and cocci enclosed in the filaments.

A. Spores (arthrospores) developed in special sections of filaments (pseudo-sporangia) (**Crenotricheae**).

Crenothrix. Filaments simple, sheathed.

B. Spores (arthrospores) in the normal filaments.

(*a*) Filaments falsely branched (**Cladotricheae**).

(i) Sheathed.

Sphaerotilus. Filaments uniform in diameter from base to apex. Arthrospores very numerous. Divisions in three planes.

Cladothrix. Filaments widening upwards. Arthrospores developed in pairs in individual rodlets.

(ii) Filaments devoid of sheaths.

Nocardia. Arthrospores produced by the transformation of cocci.

(*β*) Filaments simple (**Kurthieae**).

(i) Arthrospores 4–5 in individual rodlets.

Detoniella.

(ii) Arthrospores consisting of transformed cocci.

Rasmussenia. Filaments fixed below.

Kurthia. Filaments equal throughout and free.

C. Spores absent, or unknown. Filaments simple (**Leptotricheae**).

(*a*) Filaments sheathed and differentiated into base and broader apex, fixed.

Leptotrichia. Reproduced by rod-shaped gonidia.

(β) No sheaths, equal in diameter throughout. No rod-like gonidia.

Phragmidothrix. Fixed. Reproduced by numerous cell-divisions in two planes, longitudinal and transverse.

Beggiatoa. Free. Divisions transverse only.

[Appendix *Agonium.*]

II. Baculogenae.

Presenting three stages, as before, filaments, rodlets, and cocci; but here the rodlet is the typical individual, and gives rise to the filaments and cocci. Filaments transitory, free, not sheathed, and with no distinction into base and apex: merely due to the prolongation of rodlets as yet imperfectly segmented.

A. Rodlets and cocci nude—i.e. with no special investment or 'capsule' (**Bacilleae**).

(α) Endosporous.

(i) Rodlets dividing by repeated *longitudinal* divisions (**Pasteurieae**).

Pasteuria. Rodlets inaequipolar. Spores.

(ii) Rodlets dividing by repeated *transverse* divisions.

* Rodlets connected into a network (**Thiodictyeae**).

Thiodictyon. Rodlets aequipolar.

* * No reticulated coenobium.

† Rodlets straight or curved, but never *spirally* twisted (**Eubacilleae**).

§ Spores not larger than the major transverse diameter of their mother-cells.

P Spores developed in normal and unaltered rodlets.

1. Contents of rodlets homogeneously diffused.

Mantegazzaea. Rodlets fusiform.

Bacillus. Rodlets cylindrical.

2. Contents bipolar.

Pasteurella.

P P Spores developed in specially swollen ellipsoidal or fusiform rodlets.

Clostridium. Contents uniform.

§ § Spores with diameter greater than the transverse diameter of mother-cells.

Cornilia. Spores in normal rodlets of which the median part swells.

Vibrio. Spores in special rodlets with a swollen apex.

† † Rodlets spirally coiled (**Spirilleae**).

Spirillum. Rodlets cylindrical. Spores smaller than mother-cells.

Spiromonas. Rodlets compressed. Spores unknown.

(β) Arthrosporous.

Pacinia. Rods cylindrical, straight or curved. Filaments often undulous-flexuose or with irregular false spirals.

Bacterium. Rodlets ellipsoid, straight. Filaments never with false spirals.

B. Rodlets and cocci invested with a special membrano-gelatinous 'capsule' (**Klebsielleae**).

(α) Rodlets straight or curved, never spirally twisted (**Eu-Klebsielleae**).

(i) Capsule repeatedly branched.

Winogradskya.

(ii) Capsule simple, never branched.

Klebsiella. Contents uniformly diffused in rodlets.

Dicoccia. Contents of rodlets bipolar.

(β) Rodlets spirally twisted.

Myconostoc.

[Appendix *Cystobacter*—see *Winogradskya* (?).]

III. Coccogenae.

Exhibiting one condition only—i. e. cocci.

A. **Ascococceae.**

Cocci associated in colonies and surrounded by a firm gelatinous investment, or cyst.

(α) Cocci segregated in the mucous matrix.

(i) Cocci destitute of special cysts, but gathered together in families invested by the universal cyst (**Eu-Ascococceae**).

* Cocci very numerous and grouped in large families.

† Cysts homogeneous, not lamellated.

Lamprocystis. Families solid, and then hollow, and eventually irregularly clathrate. Cocci dividing at first in three, and then in two planes.

Ascococcus. Families solid at all ages. Cocci dividing in one plane.

†† Cysts lamellated.

Bollingera. Families solid in all stages. Cocci dividing in three planes.

* * Cocci not very numerous. Families small.

† Cysts pluri-lamellose.

Leucocystis. Cocci dividing in three planes.

†† Cysts homogeneous, not lamellated.

Cenomesia. Cysts very large and dense. Cocci grouped at the periphery, in families which eventually become hollow. Divisions at first in all planes, then two only.

Thiothece. Cysts rather large and dense, persistent. Cocci sparse and remote. Divisions in one plane.

Thiocystis. Cysts large, subdeliquescent. Cocci in small crowded families. Divisions in three planes.

(ii) Cocci surrounded by special cysts: no universal cysts. (**Gaffkyeae**).

Chlamydatomus. Cysts firm, persistent, numerous, in dense groups, solid throughout.

Gaffkyea. Cysts tenuous, eventually diffluent, solitary, never in dense groups.

(β) Cocci joined loosely into filamentous series in the mucous matrix. Universal cysts tenuous, and soon deliquescing. No special cysts. (**Amoebacterieae**).

Amoebobacter. Cocci dividing in one plane.

B. **Sarcineae**.

Cocci in strata, one or more deep, and surrounded by more or less evident mucous matrix. No cysts. Endospores smaller than the mother-cells—cocci—which produce them.

(α) Cocci closely packed in firm cartilaginous-mucous matrix.

Thiopolycoccus. Cocci densely grouped without order, in irregular compact families. Divisions in one plane.

Sarcina. Cocci in regular, cubical, closely packed families of eight. Divisions in three planes.

(β) Cocci loosely aggregated in a flattened mucous matrix.

Lampropedia. Cocci loosely grouped in fours, regularly distributed in a firm mucus in flat tables, like parallelograms. Cocci dividing in two planes.

Thiocapsa. Cocci few, in irregular families, in a firm flat mucous membrane, without definite outlines, loosely associated without order. Divisions in three planes.

Pediococcus. Cocci in fours, the small regular families loosely connected in one stratum, enveloped by an amorphous, thin, hardly conspicuous deliquescent mucus. Divisions in two planes.

C. **Streptococcaceae.**

Cocci in moniliform chains. Arthrospores, larger than the mother-cells, developed in or at end of chains.

(α) Filaments (chains) in membranous gelatinous capsules.

Leuconostoc. Capsule large, dense, lamellose.

Schuetzia. Capsules compressed, thin, not lamellated.

(β) Filaments (chains) in cylindrical sheaths.

Perronciloa. Sheath membrano-gelatinous.

(γ) Filaments (chains) devoid of capsule or sheath.

Babesia. Filaments falsely dichotomous, with arthrospores at apex.

Streptococcus. Filaments simple, with scattered arthrospores.

D. **Micrococceae.**

Cocci devoid of either cysts, capsules, or definite sheaths of

any kind, and not arranged in chains. Endospores in cocci, and smaller than they are.

Neisseria. Cocci paired.

Staphylococcus. Cocci in botryoidal groups.

Micrococcus. Cocci solitary, or scattered without order in amorphous zoogloea-masses.

Unquestionably a large number of the species are 'bad'; that is to say they are so imperfectly described that one cannot forthwith recognise a given form as belonging to a species recorded in the monumental volume under review; but it is by no means the least valuable function of a work like this to show in what directions more remains to be done, and this alone would have justified the publication of the one hundred and sixty odd pages of closely packed, and industriously compiled, information in this book.

But the treatise in question does much more than that. It shows what great advances are being made in the discovery of new *types* of Schizomycetes, as a glance at the table will show, and how (as a natural consequence) new ideas as to the relative value of characters have to be entertained.

This brings me to another phase of the subject in general. The real difficulty in classifying organisms like Schizomycetes is not so much that they are so small, especially in these days of homogeneous immersions and improved staining and illuminating methods, as that (largely consequent on their minuteness, it may be admitted) they exhibit so few morphological characters. A Fungus, like *Mucor* or *Penicillium*, has organs and differentiated parts which can be described very definitely; but when one deals with minute structures like *Micrococcus* or *Bacterium* the case is different.

Now the researches of the last fifteen years or so have brought to light numerous points which can be made use of in classifying these tiny specks of living matter, quite apart from their shapes and sizes, and those of their spores, capsules, zoogloeae, &c., and Trevisan and De-Toni have made considerable use of these accessory characters, which, by the bye, we

owe very largely to the efforts of the non-botanists as well as to those of the botanists.

Some of these characters had already been drawn into use, e.g. the chromogenic, zymogenic, or pathogenic powers, but there are others which are coming more and more into use as the subject progresses. Such are the shapes, colours, and mode of extension of the colonies in the mass, when grown on certain solid media, and especially gelatine and agar-agar: the powers of the colonies to liquefy the gelatine, by peptonising it, and the shapes and mode of progress of the excavations made. We owe nearly all these characters, and especially the systematisation of them, to the non-botanists of the Koch-Flügge school.

Then, again, more attention is being paid to the temperatures at which the cultures flourish—the optimum-temperatures as Sachs has it. There are forms which will grow at temperatures as low as 0° C., and there are others which will grow, not merely live but *grow*, at 60° to 70° C. and even slightly beyond, e.g. Miquel's *Bacillus thermophilus*; and a whole host of species are known which flourish below 20° C., as contrasted with species which require 30° or 40° C., and something has been done towards utilising these characters for classifying the Schizomycetes.

Every one now knows that, as Pasteur first discovered, some Bacteria are anaerobic, while others are aerobic, facultative or obligate in each case as may be, and these peculiarities have been pressed into the service.

Miquel has, only this last year, proposed to employ such characters as the above for drawing up a 'bacterial flora,' for the use of specialists who are engaged in the analysis of water. As it is both interesting and instructive—I shall criticise some of the points later on—I have appended the outline in Table VIII.

TABLE VIII.—*Miquel*, 1891.

Miquel first separates the aerobian from the anaerobian forms, subdividing according to the temperatures, as follows:—

I.		II.	
Aerobian, growing		*Anaerobian*, growing	
at 20° C. . .	= Section A	at 20° C. . .	= Section D
only above 20° C.	= „ B	only above 20° C.	= „ E
only above 40° C.	= „ C	only above 40° C.	= „ F

He then proposes to break up the 'Sections' into 'Tribes,' as follows:—

Section A—i. e. Aerobian species which grow at 20° C.

			TRIBE
(α) Cells = *Cocci*	Pathogenous . . .	=	I
	Zymogenous . . .	=	II
	Saprogenous . . .	=	III
(β) Cells in *Filaments*	Pathogenous . . .	=	IV
	Zymogenous . . .	=	V
	Saprogenous . . .	=	VI
(γ) Cells = *Spirilla*	Pathogenous . . .	=	VII
	Zymogenous . . .	=	VIII
	Saprogenous . . .	=	IX
(δ) Cells of other forms	Pathogenous . . .	=	X
	Zymogenous . . .	=	XI
	Saprogenous . . .	=	XII

Section B is then divided up in similar fashion.

The following tabular statement shows how Miquel then proceeds to further subdivide each 'Tribe' of each 'Section' into 'Groups,' according as the forms will or will not grow on nutrient gelatine, the colour and other peculiarities of the colonies, and so on.

Aerobian forms.

Developing at 20° C.

As cocci, which are pathogenous = Tribe I.

* Growing on ordinary nutrient gelatine.

† Colonies white or grey.

§ Liquefying the gelatine . . = Group 1

§§ Non-liquefying . . . = „ 2

† † Colonies yellow or yellow-greenish.
§ Liquefying . . . . = Group 3
§ § Non-liquefying . . . = „ 4
† † † Colonies red or reddish.
§ Liquefying . . . . = „ 5
§ § Non-liquefying . . . = „ 6
* * Will not grow on ordinary nutrient gelatine.
† Grow in alkaline gelatine.
§ Colonies whitish, &c.
(i) Liquefying . . . = „ 7
(ii) Non-liquefying . . = „ 8
§ § Colonies yellowish, &c.
(i) Liquefying . . . = „ 9
(ii) Non-liquefying . . = „ 10
§ § § Colonies reddish, &c.
(i) Liquefying . . . = „ 11
(ii) Non-liquefying . . = „ 12
† † Grow in acid gelatine.
§ Colonies whitish, &c.
(i) Liquefying . . . = „ 13
(ii) Non-liquefying . . = „ 14
§ § Colonies yellowish, &c.
(i) Liquefying . . . = „ 15
(ii) Non-liquefying . . = „ 16
§ § § Colonies reddish, &c.
(i) Liquefying . . . = „ 17
(ii) Non-liquefying . . = „ 18
† † † Grow on blood-serum . . . = „ 19
† † † † Grow in broth.
§ Producing turbidity . . . = „ 20
§ § Forming deposits . . . = „ 21
§ § § „ films at surface . . = „ 22
† † † † † Grow in *animal* juices sterilised *without heating*.
§ Producing turbidity . . . = „ 23
§ § Forming deposits . . . = „ 24
§ § § „ films at surface . . = „ 25
† † † † † † Grow in *vegetable* juices sterilised *without heating*.
§ Producing turbidity . . . = „ 26

§ § Forming deposits . . . = Group 27
§ § § „ films at surface . . = „ 28
† † † † † † † Grow in *mineral* solutions.
§ Producing turbidity . . . = „ 29
§ § Forming deposits . . . = „ 30
§ § § „ films at surface . . = „ 31

Tribes II, III, &c. to XII are then broken up in the same way, and then each group—1 to 31—is subdivided according to its microscopic characters as follows. I have put it into tabular form.

I. Aerobian forms.
Section A, growing at 20° Centigrade.
(*a*) The organism is a coccus.
Tribe I. Pathogenous forms.
* Capable of growth on ordinary nutritive gelatine.
† Colonies white or grey.
§ Liquefying the medium.
P Monococcus.
Colonies white.
Colonies grey.
Colonies iridescent.
P P Diplococcus.
Colonies spherical.
Colonies discoid.
Colonies lamelliform.
P P P Streptococcus.
Colonies mamillated.
Colonies with prolongations.
Colonies irregular.
P P P P Tetracoccus.
Colonies radiating.
Colonies motile.
Colonies amoeboid.
P P P P P Sarcinae.
Colonies very opaque.
Colonies translucent.
Colonies concentrically zoned.

And so on with each of the other groups.

Miquel's scheme, by no means the only one of the kind, it

should be stated, is only suggested as a possible way out of a well-known and much felt difficulty, namely, the very natural one that obtrudes itself on the non-botanical bacteriologists, who meet with numerous forms of Schizomycetes in their records, of rapidly identifying these forms and learning whether the same have been met with before. I am scarcely concerned here with the question whether such knowledge is worth anything or nothing: personally, I feel that all conscientious comparative records are valuable, however much we may deplore the fact that these forms are usually merely recorded, and not studied further.

The first character employed by Miquel is that of aerobism. Now it is in some cases extremely difficult to determine whether a Schizomycete is aerobian or not, but of course the question is more easily answered if the organisms are always cultivated on or in the same medium. There is evidence to show, however, that an organism may be anaerobian in saccharine solutions, but aerobian on gelatine, whence difficulties may arise to those who neglect such facts.

Miquel's second character is the temperature. This is a relatively easy point to make out in some cases, but it presents undoubted difficulties where the optimum-temperature lies close to the demarcation point (20° C.) selected, and it is by no means clear how we are to get over these difficulties. In any case the character ceases to be useful where the optimum-temperature is 18–22° C.

Miquel's third diagnostic character is the form of the organism. Obviously this is subject to all the criticism that has been accorded to the morphological systems referred to; but I may now point out a truth which is frequently overlooked by those who criticise too severely the attempts of the systematists, namely, that if we have two aerobian Schizomycetes, capable of growth at the *same* temperature on the *same* medium, then if one of them persists in developing as a *Micrococcus* and the other as a *Bacillus*, we are justified in regarding them as distinct species. True, the converse does not follow, if both grow as *Micrococci* or as *Bacilli* they may

or may not be distinct; but we must be thankful for small mercies where Schizomycetes are concerned, and a great point is gained when we have, as here, good grounds for a safe conclusion. It does not affect the truth of the above statement if the *Micrococcus*-like form gives rise to a *Bacillus*-like form, or the *Bacilli* to *Micrococci* on *different media*, or under different conditions: the only fairly comparable cases are those where the forms are growing under like circumstances. This has now been recognised for some time by many of the workers in both the French and German schools of bacteriology, as reference to the works of Flügge, Hueppe, Eisenberg, Miquel, Macé, and others abundantly testify.

Miquel then proceeds to employ a character very difficult of application in this country, because the question whether a Schizomycete is pathogenic, zymogenic, or chromogenic is not answered forthwith by the circumstance of finding the given form in the tissues, or in a fermenting medium, and so on. It can only be determined by experiment, and I need not refer to the difficulties set up in this country owing to the clamour and activity of a possibly well-meaning, but certainly ill-informed, faction of sentimentalists.

The next character employed by Miquel is an exceedingly useful one in general. If we take a sample of water, containing several forms of Schizomycetes, as almost all waters do, and distribute it equally in nutrient gelatine, in beef-broth, and in solutions containing sugar, the resulting growths are certain to differ, and often differ enormously. I assume that the conditions as to temperature, access of air and light, &c. are the same.

The question then arises, are the differences due to the fact that the initial sample of water contained a number of aerobian species, capable of growing at the chosen temperature, equal to the aggregate number of forms found in the three media? This question is a perfectly pertinent one, and we could put another, namely, are the different forms met with in the three different media mere adaptation-forms to these media?

The fact which militates most distinctly against the latter view is that there is no evident correspondence between the numbers of the forms in the three different media. But, on the other hand, there is experimental evidence to show that a form which grows like an ordinary *Bacillus* in a saccharine medium may look very different if cultivated in beef-broth, and so on. Such facts should make us very circumspect in dealing with such cases as mixed cultures.

The case is different, however, when we are deciding as to the identity or distinctness of two pure cultures. If we find that, other circumstances being equal, one of the forms will grow readily on gelatine, but the other will not, then the conclusion is justified that they are distinct: the converse is not true, however. It may be remarked here that a close examination of the literature shows abundantly that many bad records are due to negligence of these, now obvious, precautions that all the circumstances of comparison should be equal, including even the apparently trivial, but really important one, that the nutritive gelatine, broth, or other medium, should be of the same stock and make.

Having once 'run a form down' to this point, it is pretty clear that Miquel's further characters—the importance of which has been recognised more and more since cultivation on solid media was introduced by Brefeld and Koch—are both distinctive and, on the whole, easy of application. The colour and shape of the colonies, the liquefaction of the gelatine, the formation of scums, production of pigments, the shape of the cells and their mode of aggregation, and so forth, are all points comparatively easy to observe, and their utility needs no comment.

It is pretty clear then that a scheme like this of Miquel's, if properly and consistently applied, is calculated to perform two great functions in advancing our knowledge of Schizomycetes.

In the first place, it satisfies the subsidiary requirements of the specialist who merely wants to 'spot' a given form, and, as said, we are not concerned in criticising the desirability of that object.

In the second place, it records and classifies a number of facts of great value to the systematist and to the physiologist. True, it leaves him the trouble of putting the facts into his schemes, but I see no valid objection to that, as it is naturally part of his work.

The only objection to such schemes as the one just criticised seems to be that they obviously lead to the creation of 'multiple species'; because, since the pathologist tabulates one set of forms, the water-analyst another, the sewage-examiner another, the agricultural expert another, and so on, we have the difficulty of unravelling these various records.

Unfortunately this last criticism is at present the more cogent because no one scheme has as yet been decided upon, and every book on the subject propounds a different scheme.

I will simply illustrate the last remark by the following table taken, in outline, from Woodhead's recent little book, Bacteria and their Products, since it shows the application of a similar scheme to pathological forms—not entirely, but chiefly. I only select a few of the species to illustrate each group.

TABLE IX.—*Woodhead*, 1891.

1. The organism is a **Micrococcus**.
   - I. Grows on gelatine, but does not liquefy it.
     - A. The colonies are white.
       - (*a*) Colonies small, not confluent, slow-growing.
         - *Streptococcus pyogenes.*
         - *S. erysipelatosus.*
         - *S. pyogenes malignus*, &c., &c.
       - (*β*) Colonies confluent, and grow luxuriantly.
         - (i) Cocci arranged irregularly.
           - *Micrococcus candicans.*
           - *M. ureae.*
           - *Staphylococcus cereus albus.*
         - (ii) Cocci arranged like a dumb-bell—diplococci.
           - *Diplococcus lacteus faviformis.*
           - *D. albicans amplus*, &c.
         - (iii) Cocci arranged as *Sarcinae.*
           - *Micrococcus tetragenus.*

B. The colonies are yellow.

(α) The colonies form raised drops.

Staphylococcus cereus flavus.

Sarcina lutea, &c.

(β) The colonies form flat deposit-like masses.

Micrococcus versicolor.

C. The colonies are red.

Micrococcus cinnabareus.

M. roseus, &c.

D. The colonies are black.

Black 'torula' (not a Schizomycete).

II. The gelatine is liquefied.

A. The colonies are white.

Staphylococcus pyogenes albus.

Micrococcus ureae liquefaciens.

Sarcina alba, &c.

B. The colonies are yellow.

(α) The liquefaction proceeds slowly and imperfectly.

Micrococcus flavus desidens, &c.

(β) The gelatine becomes completely fluid.

(i) Colonies confined to the centre of the liquefying area.

Staphylococcus pyogenes aureus.

(ii) Colonies both in centre and at periphery of liquefying area.

Micrococcus radiatus.

M. flavus liquefaciens, &c.

III. There is no obvious growth on gelatine at 22° C.

Diplococcus intracellularis meningitidis.

Micrococcus pyogenes tenuis, &c.

2. The organism is a **Bacillus**.

I. The nutrient gelatine is not liquefied.

A. Colonies white, no staining of the gelatine near the growth.

(α) Colonies as minute translucent drops on plates—as delicate growths in streak- or puncture-cultures.

Bacillus cholerae-gallinarum.

B. septicus agrigenus, &c.

(β) Colonies colourless, forming thin films on plates, &c.

(i) Odourless.

Bacillus acidi-lactici.

B. typhosus (Eberth).

Bacterium coli commune, &c.

(ii) Distinctly odorous.

Bacillus ureae.

B. pyogenes foetidus, &c.

(γ) Colonies form white 'nail-head projections' on plates, &c.

(i) Colonies microscopically small, with granular margins.

Bacterium pneumoniae, &c.

(ii) Colonies with smooth borders.

Bacterium lactis aerogenes, &c.

(δ) Colonies branched irregularly, not circumscribed.

Bacterium Zopfii.

B. Colonies colourless, but the gelatine near is stained.

(α) Staining greenish.

Bacillus erythrosporus, &c.

(β) Staining blue or greyish brown.

Bacillus cyanogenus.

(γ) Staining violet.

Bacillus janthinus.

C. Colonies cream-coloured.

Bacillus of septic pneumonia.

D. Colonies yellow.

Bacillus luteus.

B. fuscus, &c.

II. The nutrient gelatine is liquefied.

A. Colonies white; nutrient substratum not coloured.

(α) Colonies branched, or with processes.

(i) Colonies not motile.

Bacillus anthracis.

B. ramosus liquefaciens.

B. subtilis, &c.

(ii) Colonies motile and swarming, rapidly liquefying the gelatine.

Proteus vulgaris, &c.

(β) Colonies circumscribed, without branches.

(i) Bacilli large—2·5 μ broad.

Bacillus megaterium.

(ii) Bacilli not more than 1 μ broad.

* Developing *Clostridium* forms before sporification.

Clostridium butyricum, &c.

* No *Clostridium* forms.

Bacillus mesentericus vulgatus, &c.

B. Colonies or substratum coloured.

(α) Colouring-matter red.

Bacillus prodigiosus, &c.

(β) Colouring-matter green.

Bacillus fluorescens-liquefaciens, &c.

(γ) Colouring-matter violet.

Bacillus violaceus.

III. The organisms will not grow on nutrient gelatine, and only on other media at higher temperatures, and in the presence of air.

Bacillus tuberculosis.

B. mallei, &c.

IV. Organisms anaerobic—i. e. will not grow in presence of air.

Bacillus tetani.

B. butyricus, &c.

V. Organisms described in the tissues, but will not grow under ordinary conditions in cultures outside the body.

Bacillus Leprae, &c.

3. The organism is a **Spirillum.**

(i) Gelatine liquefied.

Spirillum cholerae-asiaticae, &c.

(ii) Gelatine not liquefied.

Spirillum rubrum, &c.

(iii) Not yet cultivated on artificial media.

Spirillum Obermeieri, &c.

This leads me to the enunciation of a suggestion which I think might occupy the attention of experts at the next Hygienic Congress, and might, it seems to me, guide us to a

path out of the profound wilderness now obscurely darkening our maps under the name of bacteriology. The suggestion is that botanical bacteriologists and the bacteriologists engaged in pathological, hygienic, and other departments of science, meet and attempt to determine some international scheme for recording the peculiarities of the Schizomycetes they meet with, and see if some common ground of agreement cannot be attained.

In conclusion, it is important that all who are interested in the study of Bacteria should try to obtain answers to as many as possible of the following questions before they publish a 'new species.' These questions have been formulated gradually from the experience of numerous workers since Cohn's time, and I have already shown how the answers to them lend themselves to what systems of classification we possess. Obviously a complete description of a species requires an answer to all of them, and possibly others.

1. *Habitat*:—

This should be carefully recorded, under such headings as Air, Soil, Water (Fresh, Stagnant, Sea, Thermal, Mineral, &c.), Milk, Food, Faeces, Dead or living Animals, Plants, &c.

2. *Nutrient medium*:—

The best pabulum should then be sought for—the organism having of course been separated by suitable methods, and obtained as pure cultures. It should be stated clearly whether it will grow on gelatine, agar, or potatoes, or in broth, saccharine liquids, mineral solutions, &c., and its further behaviour traced on or in that which suits it best. In deciding this point it should be clearly observed whether the medium serves best when neutral, or slightly acid or alkaline.

3. *Gaseous environment*:—

It is important to determine whether the Schizomycete is aerobian or anaerobian, as many forms which will not grow on or in the above or other media in air, will do so when the free access of oxygen is suppressed, partially or entirely. It should also be noted whether carbon-dioxide, hydrogen,

or nitrogen affect this matter; and experiments *in vacuo*, or under pressure may give further information.

4. *Temperature*:—

The range of temperature within which growth and other functions are carried on should be clearly recorded; and the *optimum-temperature* is even more important than the *maximum* and *minimum* cardinal points. It is best to determine these most in detail with the organism growing on or in the best nutrient medium; and it must be remembered that the cardinal points are not necessarily the same for all media.

5. *Morphology* and *life-history*:—

It seems advisable to defer the working out of the biological details until the best conditions of growth, &c. have been determined on pure mass-cultures. The prevailing forms of cells will of course be recorded, and cultures (examined from time to time, or, better, continuously observed under the microscope), must be made to determine the morphological changes. The shapes, sizes, mode of union, and sequence of division in the growth-forms; development of zoogloeae; aggregation into colonies, presence of sheaths, capsules, cysts, matrix, &c.; the development of spores—endospores or arthrospores: motile forms, cilia; flexibility or rigidity of filaments; involution forms, &c. all come under this head.

6. *Special behaviour*:—

If spores are obtainable, the further peculiarities should present fewer difficulties, except in abnormal cases—which exist, however. The growth on gelatine should give characters of the following kind; but it must be noted that these characters may vary if the conditions are varied, and precautions taken accordingly. Answers should be obtained to at least the following questions:—Does the organism peptonise and liquefy the gelatine? If so, is the liquefaction complete? If incomplete, what is the shape and course of the liquefying area, funnel-shaped, tunnelled, general, &c.? What are the sizes, colours, and shapes—lumpy or flat, circular, radiately branched, &c.—of the colonies?

If it only grows in fluids, are skin-like pellicles formed, or

precipitates, or merely a turbidity? What colour-changes, if any?

In all cases, the development of gas-bubbles, odours, and so on should be carefully noted. The products of fermentation or putrefaction may be left for special enquiry; a remark which is by no means to be taken as undervaluing the enormous and ever-growing importance of such enquiry, but simply because the subject lies outside my present theme, and we must put a limit to the discussion.

7. Finally, wherever possible it should be determined clearly whether the Schizomycete is pathogenic or not; whether it induces special fermentations, or nitrification, or reductions; whether sulphur-granules are deposited in its cells, or compounds of iron in its walls; whether it can alter starch, cellulose, &c.; and whether it can live in ordinary waters and so on. The resistance of its spores to desiccation, high temperatures, isolation, the action of anti-septics, and so on, may also be mentioned as subjects for investigation.

If we had answers to all these questions, with respect to the 650 odd 'species' of Saccardo's Sylloge, it is pretty certain that some changes of importance would result, for no one can doubt that *the* great cause of multiple species has been growth under different conditions. If we could have *every* 'species' that will grow on a normal gelatine at 20° C., compared on that medium and at that temperature, under like conditions, the advantage would be enormous; and similarly with all 'species' which will only flourish in bouillon at 35° C., and so on.

Bacteriology is, after all, a sort of microscopic horticulture; and what we want is a kind of bacteriological congress to decide on the best standard methods of comparison and growth. When a form is once isolated, and growing under the best conditions, the morphologist can then take it in hand and work out the details. I see no other way of emerging from the chaos the subject is now in.

NOTES.

THE GENUS MELANANTHUS, Walpers.—In 1850 Dr. J. Walpers described (Botanische Zeitung, viii. p. 788) a Brazilian plant under the name of *Melananthus dipyrenoides*, which he designated 'novum genus ex ordine Phrymacearum.' Bentham and Hooker (Genera Plantarum, ii. p. 1137) refer to it among the 'genera dubia' of the Verbenaceae, suggesting that it might be a species of *Lippia*. Recently Dr. P. Taubert described and figured (Engler's Botanische Jahrbücher, xii. Beiblatt 1, p. 15, t. 1 A, fig. 2, *a–c*) a plant which he identified with Walpers's *Melananthus*, and no doubt correctly, I should say, judging from the very full description referred to above. On seeing the figure, I was at once struck by its very strong resemblance to *Microschwenkia* of Bentham, published in the Botany of the Biologia Centrali-Americana (ii. p. 438, t. 67, A. f. 1–5), and on comparing the figures and descriptions I was convinced that the two plants were of the same genus, and in all probability the same species, though the one was from Brazil and the other from Guatemala. Mr. Bentham had placed *Microschwenkia* in Solanaceae, next to *Schwenkia*, doubtless on account of its being so very much like his *Schwenkia fasciculata*, described in De Candolle's Prodromus (x. p. 195), where, however, neither the ovary nor the fruit is mentioned. But the strangest thing of all is, that *Schwenkia fasciculata* is the very same plant. Of course, if Bentham had examined the ovary he would never have placed this plant in *Schwenkia*, because it has a one-celled ovary with one erect ovule, and a one-seeded fruit dehiscing in two valves; whereas in *Schwenkia* the fruit is two-celled with several or many seeds in each cell.

The synonomy runs thus:—*Melananthus dipyrenoides*, Walpers, syn. *Schwenkia fasciculata*, Bentham, and *Microschwenkia guatemalensis*, Bentham. It inhabits Brazil and Guatemala, and probably the intervening country, for as it is a very inconspicuous plant, it may have been overlooked. Vauthier, Gardner (5567), and Glaziou (5856 and 8349) collected it in Brazil, and Bernoulli in Guatemala. The specimen collected in the latter country is a poor starved one.

With regard to the affinities of this singular plant, I think it is better placed in Verbenaceae than Solanaceae.

W. BOTTING HEMSLEY, Kew.

[While the foregoing paragraph was in the printer's hands, the March number of the Berichte der deutschen botanischen Gesellschaft was received; and it contains an article of twenty pages by Dr. Solereder, 'Ueber die Versetzung der Gattung Melananthus, Walp. von den Phrymaceen zu den Solanaceen.' Dr. Solereder has independently recognised the generic identity of *Melananthus* and *Microschwenkia*, but in the absence of material, he has retained the Guatemalan plant as distinct. Further, as the result of a very full and minute investigation, he comes to the conclusion that *Melananthus* is a genuine Solanacea, and most nearly allied to *Schwenkia*.

W. B. H.]

ON THE NUCLEI OF THE HYMENOMYCETES[1].—The following is a preliminary account of some observations upon the structure of, and changes which take place in, the nuclei of the basidia of *Agaricus* (*Stropharia*) *stercorarius*.

The young basidia of *A. stercorarius*, and of other species of Agarici which I have examined, contain two nuclei together with a small quantity of protoplasm enclosing one or two vacuoles. These nuclei appear to pass into the basidium from the hymenial hyphae. At a very early stage the two nuclei fuse together to form a single large nucleus, which is placed near the centre of the basidium.

The basidium increases in size, the vacuoles disappear, and it becomes filled with a dense granular protoplasm which is stained deeply by the ordinary stain, haematoxylin, carmine, &c., the nucleus increases in size with the basidium and finally takes up a position near the apex of the basidium.

The structure of the nucleus is similar to that of the higher plants. It consists of a nuclear membrane enclosing a dense nucleolus, and a threadlike network. The nucleolus stains very deeply, the threads slightly, and by the ordinary methods of staining are difficult to distinguish from the protoplasm.

The nuclei vary in size, generally speaking they are from 3·5 to

[1] Abstract of paper read at the Cardiff meeting of the British Association, 1891.

4 μ in diameter, but many of them have a diameter of 4·5 to 5 μ. The nucleoli are about 1·9 to 2 μ in diameter.

Soon after the nucleus has taken up its position near the apex of the basidium, it begins to divide, first of all into two, then into four. The division is an indirect one and takes place before the appearance of the sterigmata. Previously to the division, the nucleus elongates slightly in the direction of the long axis of the basidium; its outline becomes somewhat irregular; the threadlike network accumulates at the apex, and the nucleolus takes up its position at the opposite end of the nucleus. The nucleolus gradually disappears, and at the same time a group of deeply staining short threads or granules appears in place of the threadlike network at the upper end of the nucleus.

The nuclear membrane now seems to disappear, but an irregular and somewhat clear space surrounds both the nucleolus and the deeply stained chromatic elements. These latter separate into two groups which pass to either side of the basidium. In this way two new nuclei are formed, which are small at first, but gradually increase in size, and have a structure similar to that of the parent nucleus. The two nucleoli appear to be formed from the chromatic elements. Each of the two daughter nuclei now elongates and divides in the same manner as the primary nucleus. The four nuclei thus formed have a structure similar to the parent nucleus, but are much smaller.

The four nuclei now pass, previously to the development of the sterigmata, to the lower end of the basidium, where they come into such close contact with each other as to appear as if fused together; it is not quite certain whether fusion does or does not take place; in any case the nuclei undergo certain changes resulting in the accumulation of a more or less irregular mass of chromatin on their walls. This chromatin presents the appearance of a very loose network surrounding the four nucleoli.

While these changes are taking place, the four sterigmata appear at the apex of the basidium. At the apex of each sterigma a spore is produced, and protoplasm from the basidium passes into the spores.

The nuclei at the base of the basidium now separate and pass to the apex. Each nucleus takes up a position at the base of one of the sterigmata, and this position is retained for some time. The protoplasm of the basidium passes into the spores which are increasing in size. Vacuoles appear in the protoplasm of the basidium, and finally nearly the whole of it is transferred to the spores.

The outline of each nucleus gradually disappears. The nucleolus becomes smaller—small enough to pass without difficulty into the spore, but whether such a passage takes place or not I have not been able to determine. The spores certainly do not contain a nucleus until a very late stage, e. g. after the formation of the thick spore-membrane.

When the spores are ripe they are seen to contain two nuclei, which may be derived from the single nucleus which passes into them in some way or other from the basidium.

HAROLD WAGER, Leeds.

NOTE SUR L'ECTOCARPUS FENESTRATUS, Berk.— Sous le nom d'*Ectocarpus fenestratus*, Berk. in herb. Griffiths MSS., Harvey a figuré, dans le Phycologia britannica, Pl. CCLVII, une espèce qui n'a jamais été retrouvée depuis que Mme Wyatt l'a découverte à Salcombe, en mai 1843. Dans leur Revised List of the British Marine Algae (Ann. of Bot., V, p. 79), MM. Holmes et Batters ne citent, pour cette espèce, qu'une seule localité, la localité originale. Les frères Crouan ont indiqué à Brest un *Ectocarpus fenestratus* (Florule, p. 161), mais il est permis de douter qu'il s'agisse effectivement de la plante de Harvey, les auteurs ayant auparavant donné ce même nom à une espèce tout à fait différente. Cette rareté, le mode de ramification attribué à l'*E. fenestratus*, la forme insolite des sporanges, la gracilité du pédicelle qui les porte, faisaient de cette Algue une sorte de rébus dont la solution ne pouvait être trouvée que par l'examen des échantillons mêmes sur lesquels elle a été fondée.

Il existe actuellement deux échantillons connus, ou plus exactement deux demi échantillons, car tous deux sont la moitié gauche d'une touffe coupée en deux. Ce sont vraisemblablement des parties d'une même plante dont Mme Wyatt a préparé deux exemplaires, mais ce ne sont pas les deux moitiés d'un même exemplaire. L'un de ces échantillons se trouve dans l'herbier de Kew ; c'est celui qui a été nommé par Berkeley ; l'autre est conservé dans l'herbier de Harvey, à Trinity College, Dublin. Grâce à la bienveillante amitié de M. W. Thiselton-Dyer et de M. E. Perceval Wright, il m'a été donné d'examiner ces précieux échantillons[1].

Par le port et par la ténuité de leurs filaments, les deux exemplaires rappellent l'*E. Crouani*, Thur. ; l'un et l'autre sont dépourvus de point

[1] See Annals of Botany, V. p. 227.

d'attache. Leur extrême fragilité et l'obligation de les ménager le plus possible ne permettaient pas d'en faire une étude complète ; je crois toutefois avoir obtenu des données assez précises pour qu'il soit possible de déterminer assez exactement les affinités de cette espèce.

On remarque tout d'abord que les sporanges qui garnissent la partie inférieure des filaments sont insérés sur un article plus court que les articles végétatifs ordinaires (Fig. 1, *A*). Or, parmi les *Ectocarpus* de nos côtes, ce caractère ne se rencontre guère que dans les *E. Lebelii* (Fig. 1, *C*), *cespitulus*, J. Ag., *irregularis*, et un petit nombre d'autres espèces. De ces espèces, les unes ont les sporanges sessiles,

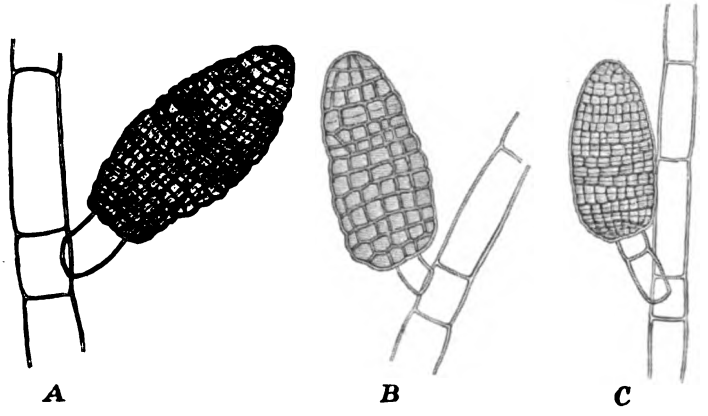

Fig. 1.—*A*. Reproductive organ (antheridium ?) of *Ectocarpus fenestratus* (Berkeley's specimen at Kew). *B*. Plurilocular sporangium of *Ectocarpus Lebelii*, Crouan. *C*. Antheridium of *E. Lebelii*. (All figs. × 250.)

comme l'*E. irregularis*, les autres ont les sporanges pédicellés. C'est parmi celles-ci que se place l'*E. fenestratus*. Si maintenant on compare l'*E. fenestratus* aux *E. cespitulus* et *Lebelii*, on trouve qu'il se rapproche davantage de ce dernier par la forme des sporanges et la dimension des filaments. Les croquis ci-joints montrent jusqu'où va cette ressemblance.

Le rapprochement avec l'*E. Lebelii* est encore fortifié par la circonstance suivante. J'ai indiqué, il y a quelques années (Thuret, Études phycol. p. 24), qu'on trouvait, dans l'*E. Lebelii*, outre les sporanges pluriloculaires ordinaires (Fig. 1, *B*), des organes reproducteurs contenant des anthérozoïdes semblables à ceux des *Fucus* ou des *Cutleria*. Ces

organes se distinguent des sporanges, sur les échantillons desséchés, par la dimension beaucoup moindre des corps qu'ils renferment (Fig. 1, *C*). Or, dans l'*E. fenestratus*, la grandeur des logettes que présentent les organes reproducteurs (Fig. 1, *A*) est précisément la même que dans les anthéridies de l'*E. Lebelii*.

Convaincu, pour ma part, de l'étroite affinité, sinon de l'identité des *E. fenestratus* et *Lebelii*, je reconnais toutefois, qu'il serait prématuré de les réunir dès à présent. En effet, je n'ai jamais vu les filaments de l'*E. Lebelii* ni aussi allongés, ni aussi rameux vers le haut que ceux du *fenestratus*. La fructification est aussi plus localisée vers la base. Quand les sporanges de l'*E. Lebelii* se développent à une plus grande hauteur que d'habitude, les articles qui les portent ne se distinguent plus par leur brièveté; c'est aussi le cas de l'*E. fenestratus*.

Pour conclure, il me semble très vraisemblable que les *E. fenestratus* et *Lebelii* ne sont que deux formes d'une même espèce; ce dernier en représentant la forme ordinaire, l'autre un état accidentel. Ainsi s'expliquerait que, depuis un demi siècle, il n'ait plus été rencontré. À présent que l'on a des indications plus précises sur une partie de ses caractères, on ne tardera pas à le retrouver soit en Angleterre, soit ailleurs, et alors on pourra juger définitivement s'il constitue ou non une espèce distincte. Enfin, il y a lieu d'espérer que les deux moitiés complémentaires des échantillons de Kew et de Dublin existent dans les collections de Mme Griffiths.

ED. BORNET, Paris.

ALGOLOGICAL NOTES. NO. 3[1]**; SPORE-LIKE BODIES IN CLOSTERIUM.**—While examining the algal flora of that district of Surrey which lies between Haslemere and Farnham[2], and especially the neighbourhood of that elevated hollow so familiar to botanists, the 'Devil's Punch-bowl,' during the summer of 1891, I met with a remarkable appearance in two species of *Closterium*, which does not correspond to anything which I find recorded in botanical literature. The examples were obtained from different gatherings, extending over several days, during the first half of August, but all from a very limited area, a corner of the 'Punch-bowl' itself. The

[1] Continued from Vol. IV. p. 172.

[2] See Journal R. Microscopical Society, 1892, p. 4.

species in which these peculiarities occurred were *Closterium lanceolatum*, Ktz., and *C. striolatum*, Ehrb., both fairly common species.

A represents the appearance of one of the abnormal specimens of *C. lanceolatum*. Towards the centre of the frond was an oval deep-green body, about 40μ by 30μ, enclosed in a well-marked coat of cellulose. In the rest of the frond the green contents appeared to have undergone complete disorganisation; the central row of chomatophores had entirely disappeared, though the protoplasm was still tinged with green in places. Around the oval body it was quite colourless. Several examples of this structure were observed; in some of which there was only one of these bodies, in others two, and they were then situated nearly symmetrically, one in each half of the frond. They corresponded almost precisely to sexually formed zygosperms of the species in form and size.

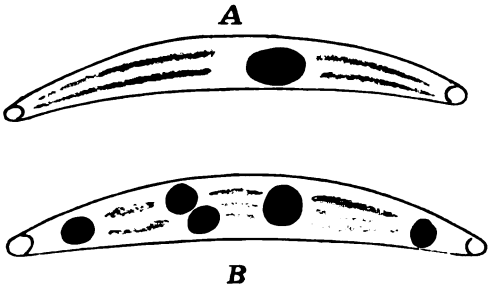

Fig. 2.

A similar appearance was represented by numerous specimens of *C. striolatum*; only that here the numbers of the spore-like bodies varied from only one to four or five in the same frond, as represented in *B*. They were in this species quite spherical (as also are the zygosperms), their diameter varying between 20 and 40 μ. Here also the chromatophores had completely disappeared. In both species the hyaline vesicles at the extremities of the frond were unchanged, and displayed the usual Brownian movement of the particles contained in them.

The suggestion naturally presents itself that these spore-like bodies are the resting or encysted condition of a parasite. The lowest organisms seem as liable as the higher ones to the attacks of parasitic fungi. These belong mostly to the orders Chytridiaceae and Monadineae, and have been observed in the Diatomaceae, Nostocaceae,

Desmidiaceae, Zygnemaceae, Vaucheriaceae, Cladophoraceae, Oedogoniaceae, and other green Algae; and, singularly enough, these two same species of *Closterium*, *C. lanceolatum* and *striolatum*, have both been described as infested by a parasitic *Chytridium*. After a careful comparison with such descriptions and figures as are accessible to me, I am quite unable to identify these structures with the resting-condition of any known parasitic fungus; while their very regular form, their thick coat of cellulose, and their bright green contents, suggest a totally different nature.

The following are the principal memoirs consulted, though this is by no means a complete bibliography of the fungus-parasites of Algae :—

BRAUN, A.: Ueber Chytridium (Monatsber. Berl. Akad., 1856).

REINSCH: Beobachtungen über die Parasiten in Desmidienzellen (Pringsheim's Jahrbücher, Vol. XI. 1876).

SOROKINE: Ueber Olpidiopsis (Arch. Bot. Nord de France, 1883). I know this only by quotation.

FISCH: New Chytridiaceae (Sitzber. Phys.-med. Gesell. Erlangen, Vol. XVI, 1884); und Beiträge zur Kenntniss der Chytridiaceen, 1884.

MAGNUS: New Chytridiaceae (Verhandl. Bot. Vereins Prov. Brandenburg, Vol. XXV, 1884).

ZOPF: Ancylisteae et Chytridiaceae (Verhandl. k. Leop.-Car. Akad. Naturf. Vol. XLVII, 1885); Die Pilzthiere oder Schleimpilze, 1887; Untersuchungen über Parasiten aus der Gruppe der Monadinen, 1887.

DANGEARD: New Chytridium (Bull. Soc. Bot. France, Vol. VIII, 1886); Mém. sur les Chytridinées, 1888.

ROSEN: New Chytridium (Cohn's Beiträge, Vol. IV, 1887).

LAGERHEIM: Olpidiella (Morot's Journ. de Bot., Vol. II, 1888); New Chytridiaceae (Hedwigia, Vol. XXIX, 1890).

DE WILDEMAN: Parasitic Chytridiaceae (Ann. Soc. Belge Microscopie, Vol. XIV, 1890).

DE BRUYNE: Monadines et Chytridiacées, parasites des Algues du Golfe de Naples (Archives de Biologie, Vol. X, 1890).

No. 4; NON-SEXUAL PROPAGATION AND SEPTATION OF VAUCHERIA.—In examining some *Vaucheria* obtained from the Regent's Canal, London, in October 1891, I observed a mode of production of non-sexual spores differing somewhat from anything that I find hitherto described, or that I have myself observed. As the alga was not in fructification, I cannot be quite certain about the species, but have little doubt about its being *V. sessilis* var. *caespitosa*. In several of the filaments the extremity was open, and the green

endochrome was escaping from it by jerks and with considerable force; there was no constriction of the filament, and no formation of septum below the protoplasm which was thus ejected. The bodies thus escaping were not ciliated zoospores, but naked unciliated masses of coarsely granular protoplasm, coloured bright green by chlorophyll, and moved about in the water with a jerking motion. The escape took place in the afternoon, between noon and two P.M.; in one instance several such bodies were ejected in succession from the same filament. After a time they came entirely to rest, rounded themselves off into a perfectly spherical form, and became invested with a very thin cell-wall of cellulose. About two-thirds of the 'spores' or non-sexual propagation cells thus formed were coloured bright green by chlorophyll; the rest consisted of colourless granular protoplasm, in which a Brownian movement of the particles was clearly seen. The phenomenon here described seems to me a very interesting intermediate one between the process of formation of zoospores by expulsion of the protoplasm, and that of 'brood-cells' by the abstriction of the end of a filament. Hanstein (Einige Züge aus der Biologie des Protoplasmas: in Bot. Abhandl. Vol. IV. 1882) states that when a filament of *Vaucheria* is injured, the portion between the injury and the apex of the filament forms itself into a cell by the secretion of a cellulose-septum above the injury, dead portions of protoplasm being during the process expelled into the water. He also saw the expulsion of balls of living protoplasm into the water as the result of injury to the filaments; but did not observe that these became clothed with cellulose and assumed the function of spores and gonids. In the instances observed by me (which were rather numerous) there was nothing to show that the process was pathological. I hope, however, to be able to repeat the observations, and to trace the further history of the 'spores.'

The filaments of *Vaucheria* are usually described as unseptated, except when about to form the sexual organs of reproduction, or when in the 'gongrosira' condition; though Bates and Cooke have recorded occasional septation of the ordinary filaments. This observation I am able to confirm in specimens of *V. sessilis* var. *caespitosa* obtained in September 1891, from a mill-pond at Waddon, near Croydon, Surrey. Several of the filaments examined were observed to be divided by septa, either at considerable intervals, or sometimes two or three very near together. These septa were more often

oblique than exactly transverse, and were always thick and gelatinous, sometimes of very great thickness: the wall of the filament was never constricted, but was sometimes widened at the septum. Being towards the end of the season, many of the *Vaucheria*-filaments were more or less in a state of decay; in some instances the walls of the filaments had entirely disappeared, and the septa alone remained suspended in the water as thick discs of gelatinous cellulose.

ALFRED W. BENNETT, London.

TREMATOCARPUS.—Dr. A. Zahlbruckner[1] describes a number of new Lobeliaceae of the Vienna Herbarium, among them an assumed new genus, based upon Hooker and Arnott's *Lobelia macrostachys*, a native of the Sandwich Islands. The type being in the Kew Herbarium, I was induced to investigate the matter, and there is no doubt that Dr. Zahlbruckner has founded his genus upon a misinterpretation of the facts, or he has had a different plant before him. His name, as its composition indicates, was chosen on account of the presence of pores in the capsule, which he supposed to be the mode of dehiscence. He refers to Hooker and Arnott's description and to Hillebrand's Flora of the Hawaiian Islands, and comes to the conclusion that these botanists had not seen ripe fruit of the plant in question; but this is an error, so far as the latter are concerned, because there are excellent specimens bearing ripe fruit in the Kew Herbarium, presented by Hillebrand. Dr. Zahlbruckner, who founds his genus on the presence of pores in the capsule, describes it as follows: 'Capsula infera, lignosa, vertice clausa et umbonata, lateraliter inter costas praesertim versus basim foraminibus ovalibus aut rotundatis dehiscens.'

On examining the specimens in the Kew Herbarium I find that there are pores in a few of the capsules, but, except that they are between the ribs, they are not regularly placed, and in some of the capsules there is only one; and they are evidently, in all cases, the work of some insect. Whether Dr. Zahlbruckner has mistaken such punctures for dehiscing pores I am, of course, not prepared to say; yet I think I may safely assert that the capsules of *Lobelia macrostachys* do not dehisce by lateral pores. The proposed new genus, therefore, seems to fall to the ground.

W. B. HEMSLEY, Kew.

[1] Annalen des K. K. Naturhistorischen Hofmuseums, VI. p. 430.

On the Nature and Development of the Corky Excrescences on Stems of Zanthoxylum.

BY

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With Plates VII and VIII.

CONICAL excrescences on the trunks of trees, frequently of striking size and marvellous symmetry, have always attracted the attention of settlers and explorers in foreign lands; and these objects are consequently by no means uncommon in our museums.

Perhaps the name most frequently met with in collections is that of *Zanthoxylum Clava-Herculis*. The stems of this species vary considerably in their appearance, according to the regularity and size of the cones. Sometimes the specimens are covered by a cracked, irregular bark, but in the majority of cases the surface of the stem is covered by isolated limpet-shaped protrusions (Figs. 1, 2).

The excrescences are, it is true, of little value economically, and the trunks bearing them are usually preserved merely as curiosities. Yet the 'Ambeck' or thorny cinnamon of the Colonial Exhibition of 1886 consists of these bodies, while the cones on Araliaceous stems are said to be sold as a cosmetic in the bazaars of Burmah. Many of the smaller branches, especially of *Zanthoxylum Clava-Herculis*, are used

[Annals of Botany, Vol. VI. No. XXII. July 1892.]

in the manufacture of walking-sticks; while the hard pyramidal masses are broken off the 'knobwood' of South Africa to serve as playthings for the children [1].

These structures, although more or less characteristic of *Zanthoxylum*, are by no means confined to this genus. A glance at the list at the end of the present paper will sufficiently demonstrate this fact. Nor are the trees bearing them limited in their geographical distribution; for, while the Zanthoxylums occur in various parts of Africa, Asia, and America, the other genera are found all over the tropical and semi-tropical portions of the globe.

In spite of the frequency with which these corky excrescences are found in Museums, there does not appear to have been any attempt to describe their nature or development. My attention was first drawn to them by Mr. Walter Gardiner, who placed at my disposal material of *Zanthoxylum alatum* collected at Kew Gardens, and suggested that it might be of use to have the development of the cones carefully traced in at any rate one species. In working out the development of the cones, I have had the advantage of abundant material, generously supplied by the authorities at Kew, both of *Zanthoxylum alatum* and of *Caesalpinia Nuga*. As there appears, however, to be no considerable difference in the development in the two cases, I have contented myself with a description of the former.

In both of these plants the cones arise, in the first instance, as corky cushions beneath the thorns (Fig. 3); and there is no reason to doubt that this is their point of origin in other plants, of which old trunks alone are available for examination. In some of these cases, indeed, scars may be observed at the summits of the cones, while occasionally a minute thorn may be found still attached in this position.

The thorns of *Zanthoxylum alatum*, in spite of their non-stipulate nature, appear in pairs at the bases of the leaves with such regularity that it is easy to trace them into the young bud (Fig. 4). A series of transverse sections through

[1] Guide to North Gallery, Kew, No. 381.

the bud, cut in paraffin with the microtome, exhibits the leaves in different stages of development (see Fig. 5). In such a series the successive leaves arise as protrusions on the stem following a well-marked spiral arrangement (phyllotaxis $= \frac{2}{5}$). The thorns are found on either side of the leaf where it unites with the stem, and the determination of the earliest stages of these outgrowths is greatly assisted by the constant presence of a lysigenous gland at the base of each.

The first differentiation of the gland may be observed in about the third leaf from the apex. It consists of a small area of cells, with granular contents, staining deeply with haematoxylin, situated between the vascular ring and the epidermis (Fig. 6). Around this group the neighbouring cells are already assuming the concentric arrangement of a sheath. As, however, the gland becomes more distinct, there is an increase in the number of cells between it and the epidermis. And this increase of cells, accompanied, as it is, by the protrusion of the epidermis at this point, may be regarded as the first stage in the development of the thorn.

In the thorn at the base of the next leaf but one, the gland is already fully formed, and the surrounding flattened cells form a many-layered sheath (Figs. 7, 7 a). A mass of tissue has been intercalated between the gland which is still close to the fibro-vascular bundle, and the epidermis. The latter may be seen to divide by anticlinal walls, and its cells, at this point, remain much smaller than the epidermal cells of adjacent parts.

In a more advanced stage of development, the gland-cells have become disintegrated, and many of the sheathing-cells around have likewise disappeared (Fig. 8). The cells on each side of the gland have become collenchymatous, and are occasionally cut across by dividing walls. The thorn is prominent, and its cells have already begun to elongate in the direction of its axis. Cells in a state of division may be seen at various points, but these are now principally confined to a narrow band of tissue at the base of the thorn, just outside the collenchymatous cells surrounding the gland.

This layer of tissue forms the meristem of the thorn, and the further cell-formation becomes more and more restricted to this basal portion; while the cells nearer the apex become rapidly elongated, pitted and thick-walled (Fig. 9). In a transverse section of a thorn at this stage it will be seen that the thickening of the cell-walls takes place to a greater extent towards the surface of the thorn than at its centre; so that a mantle of hard mechanical tissue is formed around a softer core. This may take place to a much greater extent in other thorns than in those of *Zanthoxylum alatum*. Thus the hard persistent claw-like thorns of *Caesalpinia Nuga* are, in the specimens I have examined, perfectly hollow,—an arrangement quite in accordance with the laws of mechanical construction laid down by Schwendener. As illustrating the extent to which the thickening proceeds in thorn-cells, it is only necessary to macerate a soft thorn of *Zanthoxylum*. In this case it is easy to obtain cells in which thickening and pitting have rendered it impossible to determine the position of the original cell-cavity (Fig. 10). The further growth of the thorn until the autumn appears to be essentially the same in character as that already described.

In sections of some of the thorns gathered at this later period, however, the merismatic regions appear to have become much more sharply defined (Fig. 11). It is seen upon examination that, in such cases, the merismatic cells are sharply marked off from the underlying cells of the cortex. They are much shorter and more closely packed than before. The merismatic cells hitherto were isodiametrical, and the cells developing from them rapidly became irregular. The cells now being formed, on the contrary, assume and retain the brick-shaped character so frequently seen in those developed from secondary meristems. It is, furthermore, from the first, easy to distinguish the cells which owe their origin to these two kinds of merismatic tissue; and the dividing line between them becomes more readily discernible as the thorn grows older. This is due to the fact that the cells on the two sides differ considerably in shape, and also in the character and

thickness of their walls, and depending thereupon, in their hardness and general macroscopic characters.

The cells now formed rapidly assume the appearance of corky tissue: they are found in sheets, and exhibit rings of growth exactly similar in appearance and nature to the rings of growth in an ordinary *Pinus*-stem. The thorn is thus gradually elevated, and the 'corky cushion' first makes its appearance. There is a rupture of tissues around the base of the thorn. This appears, however, before the formation of cork: and is probably due to the great tension to which the epidermal cells are subjected by the rapid increase in size of the lower parts of the thorn, after the capacity for growth in the epidermal cells has become diminished.

In more advanced thorns, selected at haphazard, from the bark of older parts of the tree, the cone has already reached a considerable size (Fig. 3). In rough sections the dividing line between the thorn proper and the corky base is now readily visible with the naked eye. This line of separation, observable in the first instance because of the difference of the form and the manner of thickening of the cells on either side, is now emphasized by the appearance of a split across the base of the thorn in this region (Fig. 12).

The cells of these older thorns are softer and much more easily cut than in younger ones, and this decay, together with the split already noticed, probably leads to the later separation of the thorn from its more durable corky base. Such a separation accompanied by long continued cork-formation in these localised areas, undoubtedly leads to the formation of the accurately chiselled pyramidal excrescences of such frequent occurrence in older *Zanthoxylum*-stems.

General Notes on Cork-Formation in Thorns[1].

1. While the excrescences upon a large number of stems are regular and conical, this is not always the case. Not infrequently two or three cones may be seen to spring from a

[1] The literature of the subject is very extensive, including, as it does, in the first place, numerous works on descriptive Botany, besides special treatises on

common base, while in other cases the irregularity becomes more pronounced. Such irregularities may be readily explained on the assumption that the phellogen of adjoining cones has fused, so that the thorns, at first separate, have subsequently been raised upon a common base. The various degrees of irregularity observable in the bark of *Zanthoxylum Clava-Herculis*, for instance, are probably due to this cause. (See Figs. 1 and 2.)

2. In other cases, such as *Acacia pentaptera*, the thorns are never raised upon isolated cones, but upon ridges extending the whole length of the plant. In this *Acacia* the stem has a star-shaped transverse section. The end of each 'ray' of the star is capped by a fibrous mass, and outside this, upon the edge of the stem, is a ridge of cork with thorns at intervals.

A similar state of things is observable in *Euphorbia lactea*. The stipulary thorns appear upon corky ridges, three of which are met with in the transverse section.

3. The sharp thorns in a specimen of *Erythrina lithosperma*, brought from Ceylon by Mr. M. C. Potter, have hard, rounded, stony bases, and are readily detached with their bases from the decaying bark. The tenacity of the latter may, no doubt, be very different in the living state.

4. A number of thorns with corky bases have a further

timbers in foreign countries. On the other hand, the anatomical portion of the work requires a careful study of the literature of thorns and of cork.

Up to the year 1873, no work of great importance appeared upon the anatomy or morphology of thorns, although many writers had published short notice concerning them. In the following two years, however, a sudden interest was awakened in these structures, and half a dozen works of considerable merit and exhaustiveness appeared. Of these, Delbrouck's paper in Hanstein's Abhandlungen, II, 1875, is the most important. In it the author carefully summarised the work of some fifty previous observers, and dealt with the whole subject, with copious illustrations of the anatomy and development of thorns. Since that time no work of any completeness has appeared. In none of these various papers can I find any reference to the cones in question, although several notices appear of the cork-formation in thorns.

The literature of cork-formation is more extensive, but, with few exceptions, the papers deal with the ordinary cork of our dicotyledonous trees and shrubs. Perhaps, of recent papers, the nearest to the present subject is that of Miss Gregory on the development of corky wings in certain trees of North America, published in the Botanical Gazette, 1889–90.

peculiarity in that a layer of corky cells of some thickness is continuous over the surface of the thorn. This continuation of cork-layers over the thorn is a phenomenon readily noticeable in many plants in botanical gardens, and it is by no means confined to thorns with corky bases. In specimens of *Trevesia*, if the thorn is pressed by the finger, a cap of whitish transparent tissue becomes detached, leaving behind a conical core of bright green colour. The cap is formed by subepidermal cork-layers; and the twofold function of the thorn for purposes of assimilation and of protection is at once evident. Among the plants in which this peculiarity may be observed are *Leea horrida* (Ampelidae), *Eriodendron anfractuosum* (Sterculiaceae), *Erythrina insignis* (Leguminosae), *Aralia Maximowiczii* (Araliaceae),—plants belonging to widely different orders.

5. The development of cork in the stipulary thorns of *Euphorbia splendens* is, according to Mittmann, peculiar. He states that, at the base of the thorn, there are 4 to 8 layers of cork-cells below the epidermis. At some distance from the base, a thick-walled lignified prosenchyma pushes between the epidermis and the cork-layers. As the base is left behind, these prosenchymatous cells increase in number, as also do the cork-layers. About the middle of the thorn, the 6 to 8 cork-layers arch over and meet one another. Above this point the prosenchyma increases and the cork disappears. 'The upper part of the thorn not only becomes dried very soon, but, when fully formed, becomes separated by several layers of cork-cells, from the lower part which still continues growing[1].'

6. Professor Balfour tells me that the thorns of Apocynaceae of the section Carisseae frequently form a corky separation layer at their base. The cases of *Rosa*, *Robinia*, and Cactaceae are referred to below.

To explain the biological significance of cork-formation,

[1] Mittmann, Beitr. zur Kenntn. der Anatomie der Pflanzenstacheln, Inaug. Diss. Berlin, 1888.

beneath the thorns already described, would need a careful study of the plants in their native surroundings. It seems, however, probable that, in the majority of cases, the corky cushions have as their function the retention of the thorns—at any rate in the young plant—after secondary thickening has commenced. In such scandent thorny plants as *Caesalpinia Nuga*, where such an increase in thickness is inconsiderable, the excrescences, although of remarkable length, increase but little in thickness with age (Fig. 13). Their presence, in this case, by extending (sometimes threefold) the circle of operations of the persistent claws, probably renders the thorns both more dangerous as weapons of offence, and more powerful as organs of climbing.

Very different is the explanation offered of the presence of a corky layer at the base of the thorns in *Rosa*. Kauffmann, to whom belongs the credit of proving that the periblem takes part in the formation of Rose-prickles, writes concerning these organs: 'The cork formed beneath the prickles enables the latter to be easily pulled off, or even to fall off of their own accord[1].' An advantage to the plant, in this case, would be the prevention of a rupture of tissues on the forcible separation of the thorns. It is also conceivable that thorns, thus readily separable, may penetrate climbing animals, and be borne away by them; and in that case the contrivance may be analagous to the extreme brittleness of the spines of *Opuntia*.

Of a somewhat similar nature to that in *Rosa* would appear to be the cork formation in *Robinia Pseudacacia*. Mittmann describes it thus: 'The thorns become dried up at the end of their first period of vegetation, and become separated by a corky layer from the underlying tissues; they remain, however, attached to the tree for several years[2].'

Whatever the function of these corky layers may be, there can be no doubt as to the significance of the tough cushions at the base of each bunch of *Cactus*-spines. Delbrouck has

[1] Kauffmann, Ueber die Natur der Stacheln, Bull. Soc. imp. nat. de Moscow, 1859.

[2] Mittmann, l. c.

given these a very careful examination. After describing their mode of origin, and attempting to determine the morphological value of the thorns, he states regarding the older stages: 'These *Cactus*-spines quickly lignify; they become fixed and prevented from injuring the tissues beneath by a resistent basal tissue. Thus, at the same time as lignification takes place, a cork-cambium-like tissue arises, which, in quick succession, pushes off layers of firmer substance, by which the spines are very firmly glued together[1].'

List of Plants whose Thorns have Basal Cork-Formation.

In works on Descriptive Botany, all that is noticed on this head is whether the plant is thorny or not. And in the accompanying illustrations no notice is taken of any peculiarities of bark in the older plant, the flower and young shoot alone being figured. A study of these works has been unproductive of results, with a few exceptions.

In works devoted to the description of timbers and the bark of trees, it might be expected that the corky excrescences would be referred to; and, in compiling the following list, a good many examples have been obtained from Gamble's Manual of Indian Timbers. The majority of the cases have, however, been noted in looking over the specimens in Botanical Gardens and Museums,—in the latter case not always fully named. The list does not profess to be complete, but may serve as a basis for anyone interested in the subject, and will show sufficiently well the wide distribution of the phenomenon in question. My thanks are specially due to Mr. J. R. Jackson, the Curator of the Kew Museums, for his assistance in allowing me free access to the specimens.

Malvaceae.

Eriodendron anfractuosum. Specimen in Kew Museum with spines half an inch long, over which the thin outer bark is continued. There is probably a corky contact base.

[1] Delbrouck, Die Pflanzenstacheln, Hanst. Bot. Abh. ii, p. 74, 1875.

Bombax malabaricum. Specimen in Natural History Museum, South Kensington, of a similar character to the last-named. See also Gamble, Manual of Indian Timbers, p. 44.

Rutaceae.

Zanthoxylum acanthopodium. Gamble, l. c. viii, and specimen in Kew Museum with good cones.

Z. ailanthoïdes (?). Kew Museum: good small cones, from Nagasaki, Japan.

Z. alatum. Kew Gardens. Specimen in Kew Museum, from Forest Department of India, has merely rudiments of cones left.

Z. brachyacanthum. Kew Museum: small rubbed specimen from New South Wales.

Z. Budrunga. Gamble, l. c. ix.

Z. capense. 'Knobwood:' Miss Marianne North's picture-gallery at Kew, No. 381; specimen in Kew Museum, from Olifant's-hoek.

Z. carolinianum. Kew Museum: fine cones, whose longest diameter is transverse to length of stem: from Florida. (See Fig. 15.)

Z. Clava-Herculis. Cambridge and Kew Museums. Many specimens, some regular, some irregular; the roughness of bark in young parts is taken advantage of in the manufacture of walking-sticks. (See Figs. 1, 2, 14.)

Z. emarginatum. Kew Museum: well-marked cones: from Bahamas.

Z. finlaysonianum (?). Hooker, in Flora of British India, i. 496. Doubtful species.

Z. hamiltonianum. Gamble, l. c. ix. Kew Museum: fine round cones with thorns still at apex: from Darjeeling.

Z. ovalifolium. Kew Museum, small cones, from Darjeeling.

Z. oxyphyllum. Gamble, l. c. viii. Kew Museum: good specimen with cones longitudinally grooved, from Darjeeling.

Z. planispina. Cambridge Botanic Garden.

Z. Rhetsa. Hooker, in Flora Brit. Ind. i. 495.

Z. senegalensis. Kew Museum.

Z. (doubtful species). Kew Museum: 'Ambeck,' or thorny cinnamon, of Colonial Exhibition, 1886: bark and fine twin cones, two inches long.

Z. (doubtful species). Kew Museum: specimen from China with beautiful small thorns with corky bases.

Toddalia aculeata. Kew Museum.

Simarubeae.

Ailanthus malabarica. Photograph, purporting to be of this plant, in the possession of Mr. M. C. Potter, taken at Peradeniya.

Rhamnaceae.

Zizyphus, nov. sp. (?). Kew Museum: cones in pairs, and with remains of branch between each pair.

Leguminosae.

Erythrina caffra. Kew Museum: cones 2½ × 2 inches at base and 1½ inches high, each bearing a minute thorn at the top.

E. sp. (?). 'Capewood,' Kew Museum: fine long cones.

E. Crista-galli. Kew Museum: grown at Kew, with bark half an inch thick with thorns still attached. There are no separate cones.

E. lithosperma. Cambridge Museum: specimen brought from Ceylon by Mr. Potter. The thorns have a hard woody base, and are readily detached with their rounded base from the decaying bark.

E. indica (?).

E. stricta. Kew Museum: like *Crista-galli*, from Darjeeling.

Robinia Pseudacacia. Cork formed beneath the thorns, but no cones. (Mittmann, l. c.)

Caesalpinia japonica. Kew Museum: blunt protuberances with thorns rubbed off: from Nagasaki.

C. Nuga. Cambridge and Kew Museums. (Fig. 13.)

C. Sappan. Kew Museum: picture of plant with thorns on corky (?) bases: from India Museum.

C. sepiaria. Gamble, xvii.

Mezoneurum cucullatum. Gamble, 134.

Piptadenia macrocarpa—one of the plants known as Angico—(Kew Museum) has excrescences exactly similar to the more irregular *Zanthoxylum* ones, but whether arising from thorns or not does not appear.

Acacia pentaptera. Kew Museum: for description see above, p. 160.

Acacia (?). Kew Museum: collected by Burchell, with thorns seated at apex of very long cones, reminding one of the older stages of *Caesalpinia Nuga.*

Acacia (?). Kew Museum: collected by Sir J. D. Hooker in Khasia, with well-marked cones.

Rosaceae.

Rosa. A corky layer formed at the base, but no cushion formed (Kauffmann).

Araliaceae.

Aralia spinosa. Kew Museum: cork formed under a series of thorns; no special cones.

Cacteae. The thorns are imbedded, at their base, in a resistent tissue formed by a cork-cambium (Delbrouck): see figure of *Echinopsis oxygona* in D.'s paper, 'Die Pflanzenstacheln;' also Goebel in Schenk's Handbuch der Botanik, iii (1), p. 271.

Euphorbiaceae.

Euphorbia lactea. Kew Museum: for description see above, p. 160.

E. splendens. For Mittmann's description see above, p. 161.

EXPLANATION OF THE FIGURES IN PLATES VII AND VIII.

Illustrating Mr. Barber's Paper on Corky Excrescences on Stems of *Zanthoxylum*.

Figs. 1 and 2. Specimens of *Zanthoxylum Clava-Herculis*; (i) from Kew Museum, (ii) Cambridge Botanical Museum,—from photographs.

Fig. 3. Old thorns of *Zanthoxylum alatum*. Fig. 4. Young shoot of *Z. alatum*.

Fig. 5. Transverse section through the apex, showing the arrangement of the young leaves. The faint lines represent the vascular system.

Fig. 6. Transverse section through a young leaf-base, showing the first appearance of the gland at the base of the thorn.

Fig. 7. The first appearance of the young thorn and vascular bundle.

Fig. 7 a. The gland at its base, more highly magnified.

Fig. 8. Young thorn, longitudinal section.

Fig. 9. Elongated pitted cells of a young thorn.

Fig. 10. An isolated pitted cell from a large green thorn.

Fig. 11. Longitudinal section through the base of the thorn in autumn, showing the formation of the secondary meristem, from a photograph.

Fig. 12. Small portion of a longitudinal section through the point of junction of a thorn and its corky base. The cells on the two sides of the split differ in size and character. The larger cells are derived from the primary meristem at the base of the thorn, while the smaller brick-shaped cells are the first products of the secondary meristem which gives rise to the corky cushion below the thorn.

Fig. 13. *Caesalpinia Nuga*, from a drawing belonging to Mr. W. Gardiner.

Fig. 14. *Z. carolinianum*, from a photograph of a specimen in Kew Museum.

Fig. 15. 'Stems' in Kew Museum, brought from Khasia by Sir J. D. Hooker, from photographs: *a. Zanthoxylum*, probably *Z. hamiltonianum*. *b. Mesoneurum cucullatum*. *c. Toddalia aculeata*.

Fig. 1.

Fig. 2

Fig. 3

Fig. 9.

Fig. 7.

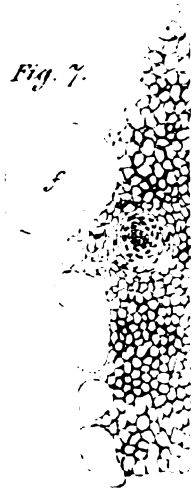


Fig. 8.

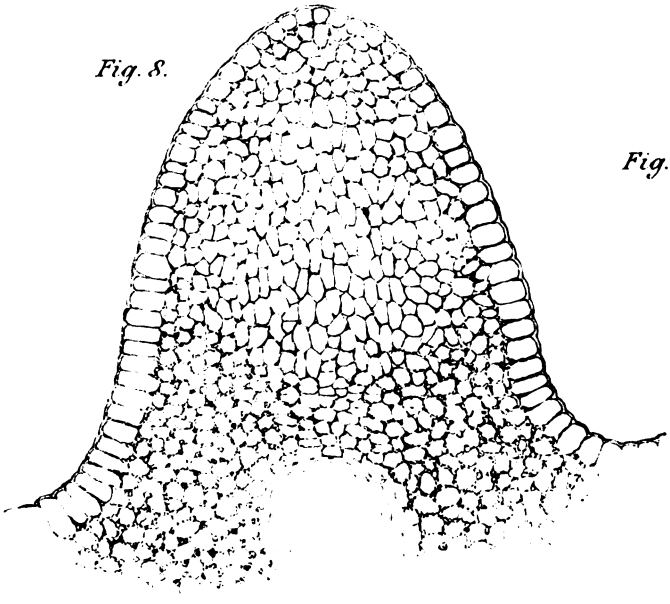


Fig. 10.

Fig. 11.

From Photo. & Drawings by C A Barber

Fig. 4.

Fig. 5.

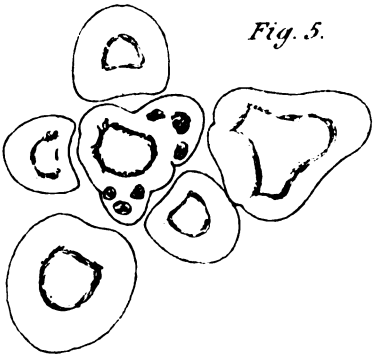


Fig. 7 a.

Fig. 6.

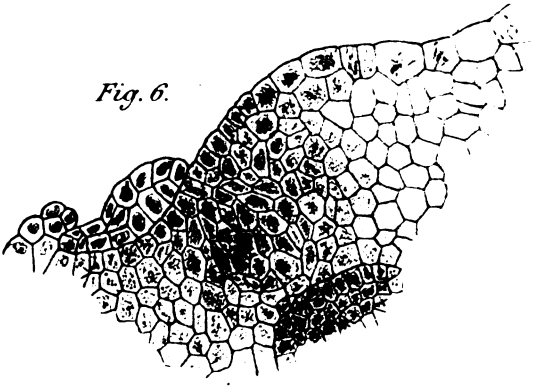
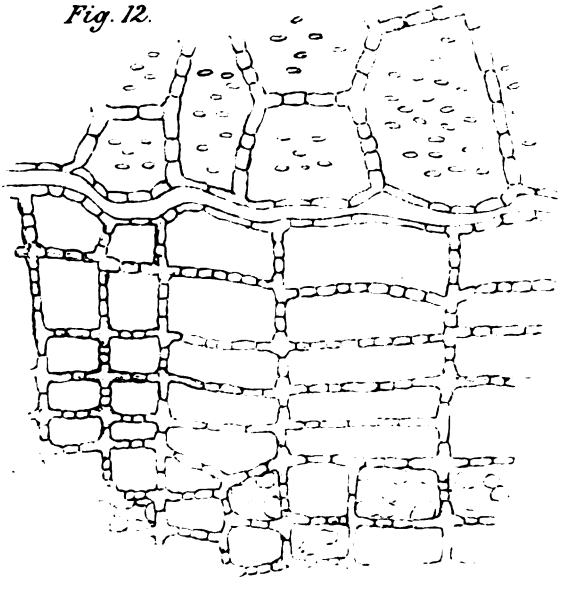


Fig. 12.



University Press, Oxford

Fig. 13.

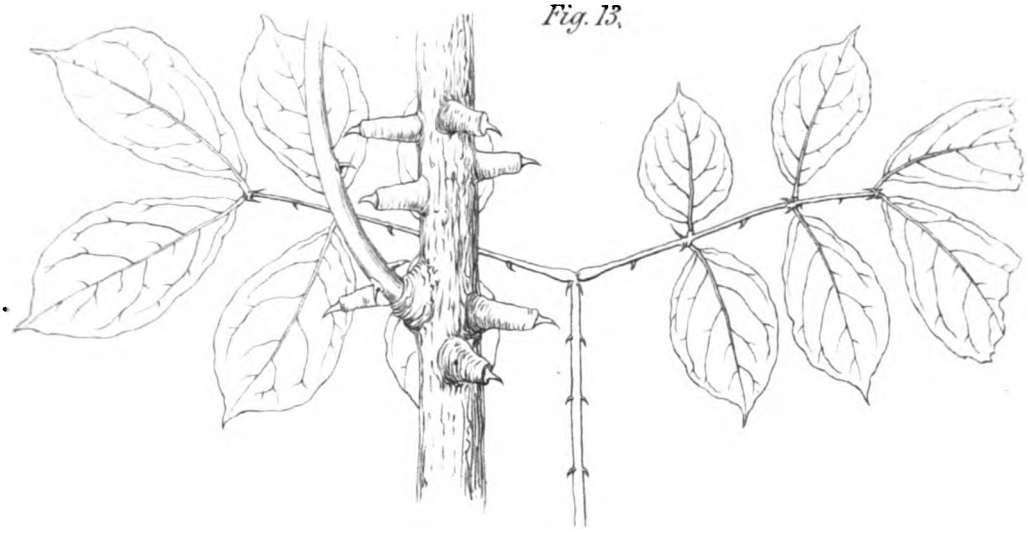
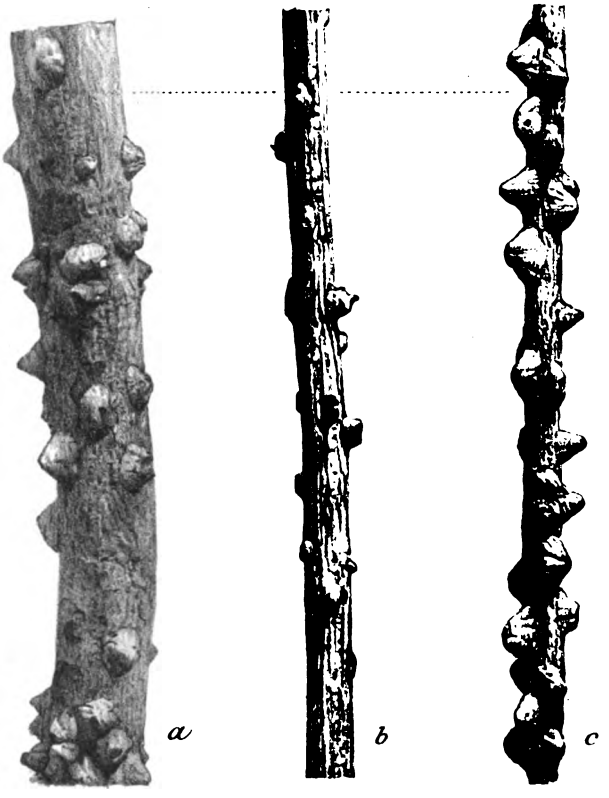


Fig. 14.

Fig. 15.



From Photo

University Press Oxford.

BARBER. — ON ZANTHOXYLON.

On the Action of Aniline on Green Leaves and other Parts of Plants.

BY

EDWARD SCHUNCK, PH.D., F.R.S.

AND

GEORGE BREBNER.

With Plate IX.

IN a paper entitled 'The Chemistry of Chlorophyll' published in these Annals[1] it was shown by one of us that on exposure to the action of aniline many green leaves undergo in a very short time a remarkable change, manifested by the disappearance of the usual green and the development of an intense brown colour, the latter being due to the formation of a peculiar, well-defined crystallisable substance, to which the name 'anilophyll' was given. The object of the present communication is to give the results of the further study of this reaction, and the conclusions to which we have thereby been led. The general result at which we have arrived is this:—that the reaction referred to is not so intimately connected with the presence of chlorophyll—if by chlorophyll we mean as stated in the paper just named 'the substance to which the pure green colour of ordinary healthy leaves and other vegetable organs is due'—as was at first supposed; that it is due in fact to a process of oxidation which the aniline employed

[1] Vol. III. pp. 65-120.

[Annals of Botany, Vol. VI. No. XXII. July 1892.]

undergoes when certain conditions prevail. Before proceeding any further, however, it may be well to give a few additional details regarding the chemical and physical properties of anilophyll, the substance to which the action of aniline on the leaves gives rise. We need not here describe the improvements effected by us in the preparation in a state of purity of the substance, since they are such as would easily suggest themselves to anyone repeating the process.

In the paper referred to, anilophyll is said to melt at 190°–192° C. On carefully purifying, however, the melting-point rises to about 200°, a lower melting-point indicating a certain amount of impurity. Anilophyll has the properties of a weak base, as its behaviour to sulphuric and hydrochloric acids proves. On being heated with concentrated hydrochloric acid in a sealed tube to 130°, it is completely decomposed, yielding carbonaceous products together with a little aniline. On treatment with potassium chlorate and hydrochloric acid it yields chloranil. On carefully dropping bromine into a weak solution of anilophyll in choloroform, there is an immediate deposit of dark bronze-coloured scales. The compound thus formed is extremely unstable; it may be filtered off and dried at the ordinary temperature without decomposition, but when heated to 50° it disengages hydrobromic acid, and it is also decomposed when an attempt is made to recrystallise it from any solvent. By the action of an excess of bromine on anilophyll a colourless crystalline substance is formed having all the properties of tribromaniline.

The composition of anilophyll corresponds to the formula C_{24} H_{19} N_3 O, which requires in 100 parts C 78·90, H 5·20, N 11·50, O 4·40. The mean of two analyses was as follows:—C 78·63, H 5·59, N 11·70, O 4·08.

The properties and reactions just described, as well as the percentage composition, being considered, it became extremely probable that the substance was in fact a derivative of aniline formed by some process in which oxygen played a part, and that it might be possible to obtain it without having recourse to vegetable organisms.

A few simple experiments sufficed to verify this supposition. The effect of strong oxidisers on aniline being well-known, and the products to which they give rise having been minutely examined by chemists, it became necessary to employ less energetic oxidisers for our purpose. On passing a current of air through a solution of aniline in acetic acid very little effect is produced. Hydrogen peroxide and ozone act, however, very differently. On adding a solution of hydrogen peroxide to a solution of aniline in acetic acid and heating, there is an immediate formation of a crystalline deposit, which when the action is completed forms a mass of garnet-coloured needles. These being filtered off and crystallised from benzol are found to have properties identical with those of anilophyll from leaves. A specimen thus prepared yielded in 100 parts C 78·84, H 5·88, N 11·37, O 3·91. On passing a current of ozonised air through a solution of aniline in acetic acid a similar effect is produced; a crystalline deposit is formed, which after being purified is found to consist of anilophyll. It is necessary that acid should be present at the same time, for neutral or alkaline solutions of aniline yield no trace of anilophyll with hydrogen peroxide. The conditions for the formation of anilophyll from aniline seem therefore to be the simultaneous presence of an excess of acid and some form of active oxygen, such as ozone or hydrogen peroxide. It is not a matter of indifference however, what particular acid is taken; with hydrochloric or nitric acid the process results in the formation of colouring matters belonging to the class of indulines[1]. In place of acetic acid any one of the following acids may be employed—carbonic, formic, propionic, butyric, valerianic, palmitic, succinic, malic, tartaric, citric, tannic, or phthalic acid. Oxalic acid, singular to say, completely hinders the reaction, possibly because it is itself more readily oxidised than aniline; binoxalates hinder it to a certain extent, but neutral oxalates do not interfere with the formation of anilophyll. It is

[1] Very dilute nitric acid does, however, produce anilophyll, and there is reason to suppose that the brown colour acquired by aniline on standing, especially on exposure to light, is due to the same substance.

difficult to say what part the acid plays in the process. From the fact that in anilophyll the number of atoms of N is to that of the C atoms as 1 : 8, not as 1 : 6 as in aniline, it might be inferred that the acid enters into the composition of the product. On the other hand, we found that in preparing anilophyll in three different ways, using acetic acid, propionic acid, and butyric acid respectively, the three products did not differ either as regards properties or composition, the analyses yielding closely concording numbers the mean of which for 100 parts was C 79·13, H 5·55, N 11·55, O 3·77. We were unable, too, to detect nitrogen in the form of ammonia or nitric acid along with the product obtained, so that if there is elimination of nitrogen it remains uncertain under what form this takes place. It is not our intention to enter on any further consideration of the chemical nature of anilophyll. The remainder of our paper will be devoted to a discussion of the bearing of our experiments on the vexed question of the presence of active oxygen in the cells of plants.

We may here intercalate a few remarks as to the identity or non-identity of anilophyll with any previously described derivative of aniline. At the time when the 'Chemistry of Chlorophyll' appeared, the substance seems to have been almost unknown. Leeds[1] appears indeed to have observed it, for by the action of hydrogen peroxide on a solution of aniline in acetic acid he obtained a brownish crystalline mass which, however, he did not further examine, the main product of the action according to him being azobenzol. A few years later Zincke and Hagen[2] described a substance obtained by the action of benzoquinone on aniline having properties very similar to those of anilophyll, which they called dianilidobenzoquinone-anilide. A similar body was obtained by Kohler[3] and named hydroxyazophenin, the formula $C_{30}H_{24}N_4O$ being assigned to it. Lastly Fischer and Hepp[4] give an account of a compound obtained by the action of o-nitrophenol on aniline which they call dianilidoquinonanil,

[1] Berichte d. deutschen Chem. Gesellschaft, 14, 1383. [2] Ib. 18, 787.
[3] Ib. 21, 910. [4] Liebig's Annalen, 262, 247.

and which according to them is identical with the products previously described by Zincke and Hagen and by Kohler. We have prepared some of the product according to the process of Zincke and Hagen, and found its properties to coincide in every respect with those of anilophyll. Taking this fact into consideration it might perhaps have been thought proper for us to have adopted one or another of the names given by the chemists referred to. We prefer, however, to retain the trivial name anilophyll bestowed on it in the first instance, so as to avoid confusion for the readers of this journal and give them an easily pronounceable word instead of one of the unwieldy terms to which its place in the system would entitle it.

Considering what has been stated by us regarding the origin and chemical properties of anilophyll it may be concluded that the formation of the substance, when green leaves are subjected to the action of aniline, must be due to the simultaneous presence of some form of active oxygen and some acid. The experiments we are about to describe leave it uncertain what particular form of active oxygen it is that produces the reaction, and whether the passive oxygen present may not possibly have become 'activated'[1] before the reaction takes place.

With regard to the reaction with aniline, green leaves may be divided into three classes. The first class comprises such as turn brown rapidly, i. e. in a few seconds, or at the longest a few minutes; to this class belong the leaves of the common ash, beech, holly, thorn, dandelion, mint, and many other plants. In the second class are found such leaves as act with fair rapidity, the leaves of *Tradescantia*, and of many other monocotyledons, affording examples. The third class comprises such leaves as are discoloured slowly or not at all; such are the leaves of the lower and some of the higher monocotyledons, as well as of certain genera of dicotyledons, such as *Ribes*, *Rumex*, and others, and even of some individual species. Leaves

[1] We employ the words 'activate' and 'activation' as equivalents of the German terms 'aktiviren' and 'Aktivirung.' Bacon uses the term 'activate' in the sense of heightening the effect of any physical agent, as e. g. the cold of snow or ice is activated by nitre or salt.

with watery cells, which contain a large amount of cell-sap with little protoplasm, of which *Echeveria* leaves afford a good example, seldom or never react well with aniline. There can be no doubt too that the thickness of the cuticle, the greater or less waxiness of its surface, and other physical conditions, affect the rapidity of the discoloration. In cases where little or no discoloration takes place, the cause must be sought in the absence of one or both of its essential conditions, these conditions being, as before stated, the simultaneous presence of some form of active oxygen and an organic acid, other than oxalic. In the case of *Rumex* it is probably the presence of oxalic acid, or rather of superoxalates, that prevents the reaction taking place.

When a leaf belonging to the first class is painted with aniline, the discoloration shows itself in a very short time, either in the shape of spots, as in the holly, or along the veins, as in the mint leaf, and then the whole leaf acquires a uniform brown colour (Plate IX, Figs. 1–3). When the cells of such a leaf are examined under the microscope, the appearance is such as depicted in Fig. 4, which represents a few cells from the mesophyll of a holly leaf near the midrib. The chlorophyll-corpuscles, which practically occupy their normal position, are of a rich brown colour, and the cell-sap is hardly, if at all, tinted; the outline between the cytoplasm and cell-sap is not well marked; a slight plasmolysis has taken place, but such is not always the case. With such leaves as are discolored very slowly, several distinct stages in the process may be traced under the microscope. The first stage is represented in Fig. 5, which shows several cells of a *Tropaeolum* leaf after being painted with aniline. Here viscid green drops have exuded from the chlorophyll-corpuscles, and run together to form larger ones, which lie scattered about in the cells of the mesophyll, the corpuscles, which are now completely colourless, occupying their usual peripheral position. In the second stage, shown in Fig. 6, the green viscid drops have turned brown; otherwise the appearances presented are much the same. In the third stage of the reaction, as shown in

Fig. 7, crystals of anilophyll are formed, and radiate from the brown masses which become somewhat paler; the other appearances as before.

In the 'Chemistry of Chlorophyll' it is stated that etiolated leaves do not yield anilophyll on treatment with aniline. This statement was not confirmed by later experiments. On taking equal quantities of normal green and of etiolated ivy leaves, and operating in the usual manner, we obtained from the latter quite as much as from the former, and the product from the etiolated leaves was moreover more easily purified than that from the green leaves, being less contaminated with amorphous matter. Having obtained so favourable a result from etiolated leaves, we were not much surprised when we found that other parts of plants, such as petals, underground stems, &c., also gave anilophyll on treatment with aniline. These other parts of plants were found to differ in this respect *inter se* just as green leaves do, i.e. some reacted rapidly and well, others more slowly, others not at all. In the case of petals, colour seemed to afford no clue to the amount of reaction; some yellow petals, such as those of the yellow chrysanthemum and yellow dahlia, reacting well, others not so well; some white petals being extremely sensitive, others not, just as some tubers give a decided reaction, others next to none. The white flowers of *Sambucus nigra*, devoid as they are of chlorophyll and other colouring matters, are rapidly turned brown by aniline, and give a good yield of anilophyll—50 grams. of these flowers giving in one experiment 0·0492 grm. of pure anilophyll, a larger amount than is obtained from some green leaves which react well with aniline. Among blue flowers we may mention those of *Aconitum Napellus*, which give a very good yield of anilophyll. The crystals of the latter formed in the cells of the sepal are very distinct, and form an interesting object under the microscope (Figs. 8, 9). The hairs of the sepal are especially active in the formation of anilophyll; in some instances they retained their purple colour, though a considerable amount of anilophyll had crystallised out, in and on the protoplasm.

That green herbaceous stems should react as green leaves do was to be expected, but that colourless rhizomes should give a still better reaction is a fact that we were not prepared for. On taking 250 grms. of the green stems of garden mint, and the same weight of colourless rhizomes of the same species, and treating with aniline in the usual manner, we obtained from the green stems 0·16 grm., from the colourless rhizomes 0·36 grm. of anilophyll. An equal quantity by weight of mint-leaves yielded 0·85 grm. of anilophyll, this comparatively large yield being easily accounted for seeing that besides the large surface exposed by the leaves, they contain per unit of area a much greater quantity of functionally very active living cells as compared with either green stems or rhizomes. The cut surface of potatoes turns brown in about an hour on being painted with aniline, but the yield of anilophyll is very poor, as might be anticipated if it be true that we have an abundant formation of anilophyll only where there is plenty of functionally active protoplasm.

Certain other dark brown to black amorphous colouring matters which are formed along with anilophyll are referred to in the 'Chemistry of Chlorophyll,' and need not be dealt with here. We may, however, remark that they are much more abundant in the product derived from green leaves than in that from other parts of plants, such as flowers and rhizomes. Occasionally we observed the formation, along with anilophyll, of a coloured crystalline substance, differing distinctly in appearance from the former, which it seemed partly to replace. It crystallised in orange to orange-red rhombs, was readily soluble in alcohol and closely resembled azobenzene, with which it may indeed be identical; that substance having been obtained by Leeds as a product of the action of hydrogen peroxide on aniline. The crystals were first observed in the thick fleshy root of *Aconitum Napellus* after treatment with aniline, and they were also obtained from dandelion leaves and roots. A body having a similar appearance is formed by the action of platinum-black on aniline in the presence of acetic acid.

Though we see no reason to suppose that the peculiar action

of aniline on leaves and other parts of plants can by any means be promoted, it is on the other hand very easy to prevent it taking place entirely. We have repeated the experiments with various gases and have come to the conclusion that, after remaining for some time in an atmosphere of carbonic acid or hydrogen gas, leaves no longer acquire a brown coloration when treated with aniline, the former gas being perhaps more efficient in this respect than the latter. After treatment with boiling alcohol, leaves no longer acquire a brown colour on treatment with aniline, nor does the extract yield any anilophyll after the addition of aniline and evaporation.

In the 'Chemistry of Chlorophyll' it is stated that by the action of boiling water leaves do not entirely lose their power of reacting on aniline, but on carefully repeating the experiment we found that after boiling with water continuously for a half to one hour the property was completely lost. In some cases, however, the power to react on aniline, though lost so far as the leaves themselves were concerned, was found to be present, though much weakened, in the watery extract. This was seen most conspicuously in the case of the leaves of the common ash (*Fraxinus excelsior*). An aqueous extract of ash-leaves was made by boiling the leaves for three-quarters of an hour; a little aniline was added, and the extract kept at a temperature of 86° C. After the lapse of about two hours crystals of anilophyll were deposited, but the reaction, it is true, was considerably slower and the yield less than if the leaves had been treated in the usual manner. An aqueous and acidulated solution of aniline forms under the same circumstances little or no anilophyll, so that the production of the latter must be due to the presence of some transforming agent in the extract itself. That the transforming agent is reproduced after the quantity originally present is spent, is evident from the fact that after filtering off the first deposit, a fresh one is quickly produced, and this may be repeated many times. Aqueous extracts of other leaves such as mint, holly, &c., react much more slowly, showing signs of a crystalline deposit only after a day or more, and the final yield is generally small. Dis-

tillates obtained from the watery extracts of ash and mint leaves showed no trace of crystals on the addition of aniline and standing, even after the lapse of a week, so that it appears that the active agent contained in the extract, whatever it be, does not pass over with the vapour of water.

It remains for us to say a few words as regards the bearing of our experiments on the vexed question of the presence of active oxygen, in some form or other, in the cells of plants. This question has been much debated, some observers maintaining that the presence of active oxygen may be easily demonstrated, while others assert that it is entirely absent. We are inclined to think that this want of accord is due to the fact that active oxygen is sometimes present, sometimes absent, owing to some cause or causes which are unknown, and are not even hinted at by those who have treated the subject. In one of his numerous memoirs on active oxygen Schoenbein states[1] that the expressed juices of many plants, especially of dandelion and lettuce, give a reaction similar to that of hydrogen peroxide with tincture of guiacum and with acidulated starch iodide solution. According to Pfeffer this reaction would not indicate the presence of hydrogen peroxide within the living cell, since expressed juices may have, and have indeed been found to have the property of activating passive oxygen. In his interesting research entitled 'Beiträge zur Kenntniss der Oxydationsvorgänge in lebenden Zellen[2],' Pfeffer proves conclusively that the cells of the plants he examined do not contain active oxygen in the form of hydrogen peroxide. We repeated two of Pfeffer's experiments and were able to confirm the results arrived at by him. In the case of the staminal hairs of *Tradescantia*, using a solution containing 0·25 per cent. of hydrogen peroxide, we observed the complete precipitation of the colouring-matter with mere retardation of the protoplasmic streaming; the latter returning in full vigour after the surplus hydrogen peroxide had been washed out. In the case of only one cell did we see any change in the colour

[1] Journ. f. prakt. Chemie, 102, 155.

[2] Abh. d. math.-phys. Cl. d. k. Sächs. Ges. d. Wiss. xv. 1889.

previously to precipitation. In the case of the *Vicia Faba* rootlets we found the reaction to take place exactly as described by Pfeffer.

Wurster [1] recommends the use of tetramethylparaphenylenediamine, which acquires a violet colour on exposure to active oxygen, whereas it remains entirely unchanged on exposure to ordinary passive oxygen. It is best used in the form of test-paper, in which form it indicates the presence of active oxygen in various substances and mixtures, more especially plant-juices. It does not, however, show what particular form of active oxygen is present, and it also becomes coloured by nitrites.

As regards plant-juices, Pfeffer objects to Wurster's experiments as being made with the contents of dead, not of living, cells. Whether the same objection would apply to one of Wurster's experiments, in which he operates on *Leontodon* leaves crushed under mercury, may be questioned. Bokorny [2] objects to Wurster's test because it shows a reaction with so many substances, including ordinary oxygen, and under so many circumstances that it cannot be considered as indicating the presence of active oxygen at all, adding that for hydrogen peroxide starch iodide solution is a far more sensitive test. To this Wurster replies [3], re-asserting the superiority of his test over any other, and again stating that it is not in the least affected by ordinary oxygen, however long applied. Referring to Pfeffer's experiments he says that the plants, or rather parts of plants, with which the latter worked do not affect his own test-paper.

Without pronouncing any opinion on the divergent views of various observers, we may state that we have tried several tests for the presence of active oxygen in the expressed juices of plants, more especially starch iodide solution and Wurster's test, the latter in the form of test-paper, called by us, following the example of the inventor, simply 'tetra-paper.' We find, as Wurster did, that the expressed juices of various plants

[1] Ber. d. d. Chem. Ges. 19, 3195. [2] Ib. 21, 1101.
[3] Ib. 21, 1525.

impart a violet colour to the test-papers, but not to the same extent in all cases. In some few instances, indeed, the test-paper gave a reaction when the leaf or other part of a plant did not show any coloration with aniline. In by far the greater number of cases, however, where the leaf or other part of a plant turns rapidly brown with aniline, its expressed juice gives an intense reaction with tetra-paper. The reactions indeed seem to run parallel, i. e. when there is an intense reaction with aniline we have an intense reaction with tetra-paper, while a medium reaction with aniline accompanies a medium reaction with tetra-paper, and a feeble or total want of coloration with aniline corresponds to a feeble reaction or want of reaction with the test-paper. Leaves may from this point of view be divided into three classes, viz. (1) such leaves as react well with aniline, and the expressed juices of which also react well with tetra-paper as well as with acidulated starch iodide solution; (2) such as react with aniline, and the expressed juices of which colour tetra-paper but not starch iodide solution; (3) such as do not react either with aniline, tetra-paper, or starch iodide solution. The leaves of dandelion and lettuce belong to the first class; their expressed juices affect Schoenbein's test, but they soon cease to do so as Schoenbein himself states. The power to react on tetra-paper is also soon lost, but when with this there is also a reaction with starch iodide solution it endures for a much longer time. Such differences may be due to the fact that these tests differ in sensitiveness, the tetra-paper being the most sensitive, the starch iodide solution the least so. We made use of another of Wurster's tests for hydrogen peroxide, viz. *a*-naphthylamine in conjunction with common salt, but without success, since the juices of such plants as react well with aniline do not affect the reagent named.

The general conclusion to which our experiments have led us is this:—that the cells of many plants, especially of the leaves, contain some form of active oxygen in immediate proximity to or associated with the protoplasm during the living state of the cell. It is necessary to justify this conclusion considering the

very decided and emphatic manner in which Pfeffer has expressed himself in opposition to the view that active oxygen —hydrogen peroxide, ozone, or atomic oxygen—is present in the cells of plants. Referring to his experiments with certain plants in relation to hydrogen peroxide, cyanine, &c., Pfeffer says[1]: 'Jedenfalls sind diese Indicatoren ein Zeugniss für den Mangel einer jedwelchen Oxydation, wie sie das immerhin verhältnissmässig schwach wirkende Wasserstoffsuperoxyd zu erzielen vermag, wie sie aber nicht durch den auch in der Zelle vorhandenen passiven Sauerstoff hervorgerufen werden. Als Ausdruck dieser Differenz soll auch in Folgendem allgemein vom Fehlen eines activirten Sauerstoffs geredet werden, wenn auch die empirischen Erfahrungen allgemein ein Beweis für das Fehlen jedwelcher entsprechenden Oxydation in der Zelle sind, gleichviel ob solche von einem einfachen Process oder einer Kette von Vorgängen abhängig ist.' With regard to a secondary formation of active oxygen, Pfeffer remarks[2]: 'Eine secundäre Betheiligung des activirten Sauerstoffs ist in den Hypothesen über den Athmungsvorgang öfters ins Auge gefasst, aber wohl wesentlich nur deshalb, weil nachweislich in manchen chemischen Oxydationsprocessen, insbesondere bei Autoxydationen, Sauerstoff activirt wird. Doch kann, wie auch schon hervorgehoben wurde, keineswegs solche Activirung für alle Oxydations-processe gefordert werden, und so am wenigsten für die physiologische Verbrennung.'

Now, as we have seen, there are two very important facts, which, contrary to Pfeffer's views, seem to make it very probable that there is some oxidising agent more active than mere molecular oxygen present in the living cells, more especially such as contain chlorophyll, of a large number of plants. These two facts are as follows:—(1) A large number of leaves turn rapidly brown on treatment with aniline, the discoloration being almost instantaneous when the aniline has not to penetrate the cuticularised epidermis and even in the latter case only occupying about ten seconds. This dis-

[1] Beiträge, p. 431. [2] Ib. p. 488.

coloration indicates the formation of a crystallisable body of well defined properties called anilophyll.

(2) The same body is formed outside the plant by the action of hydrogen peroxide or ozone on aniline in the presence of a weak acid, but not at all, or at least very slowly, by the action of ordinary oxygen. There can be no doubt, therefore, that the formation of this body in the cell is due to the presence there of some form of active oxygen.

The question that remains to be considered is whether the active oxygen is present in the cell as such, or whether it is formed from passive oxygen by some process of activation. In Schoenbein's experiments, as he himself allowed, the expressed juices of plants did not in the first instance give the reaction of hydrogen peroxide, but only after a time, in consequence of the activation of atmospheric oxygen to which they were exposed. In our experiments, however, which were conducted with mechanically uninjured cells, there could be no question of expressed juices, the reaction with aniline taking place while the sap, etc. was still contained within the cells. Moreover these expressed juices do not react with aniline, so far as our experience goes, although with very few exceptions, plants, the expressed juices of which affect Wurster's tetra-paper, also show a reaction when painted with aniline. It seems probable, indeed, that the reaction taking place within the cell and that shown by expressed juices are distinct. It is still possible, however, that the effect observed within the cell may be due to a rapid activation of the passive oxygen, which, according to Pfeffer, is diffused throughout the cell and pervades its contents. In support of this view it may be urged that the death of the cell is undoubtedly brought about by the action of the aniline and that the coloration of the corpuscles may therefore be a *post-mortem* phenomenon. On the other hand, the action of the aniline does not lead, as might have been expected, to very much disturbance in the cell-contents, only a slight amount of plasmolysis taking place, and the chlorophyll-corpuscles retaining much their original position at the periphery of the contents (see Fig. 4).

In the case of the hair of the sepal of *Aconitum*, so little disturbance had taken place in the cell-sap, that, although anilophyll had crystallised out, in and on the shrunken protoplasm, the cell-sap retained its purple colour almost unchanged. It seemed to us, that in every case examined under the microscope, it was some constituent or constituents of the protoplasm to which the reaction was due. From a chemical point of view, it is difficult to understand the rapidity with which the reaction takes place in the cell if active oxygen is not present at the same time. In the plant-cell the phenomena described may be frequently seen after a few moments and at the ordinary temperature, whereas in a laboratory-experiment, using aniline and hydrogen peroxide, the formation of anilophyll only takes place slowly, unless heat be applied at the same time. Hence it would follow, assuming that there is an activation of oxygen in the cell, that the process is more rapid when it passes through two distinct stages than when it passes through one only, which is hardly likely.

It is not at all necessary to suppose that if active oxygen be present in most cells it should exist there in the form of hydrogen peroxide or of ozone—indeed, it is highly probable that it is present in some other form. In the cases described by Pfeffer it is possible that there may have been active oxygen present, but of a kind not sufficiently potent to act on the chromogens or the colouring-matters of the cells, but still able to produce an oxidising effect on aniline. The introduction of hydrogen peroxide in the manner described by Pfeffer would then have induced changes which would not under ordinary circumstances have taken place. We may state in corroboration of this view that having treated the purple petals of *Tradescantia*—the staminal hairs with which some of Pfeffer's experiments were made being too minute for our purpose—with aniline, we obtained by this means a quantity of anilophyll. We see no objection to the assumption that there are substances in the protoplasm—probably associated with specialised microsomata, etiolin and chlorophyll-

corpuscles—which have the power of combining with oxygen to form peroxide-like substances. There are certain substances which are known to possess the property of bringing the atmospheric oxygen into a more active condition before making use of it to complete their own oxidation. Schoenbein showed this to be the case with ether, valerianic aldehyde, fatty and ethereal oils. Speaking of oil of turpentine, he says [1], 'Wie ich zu seiner Zeit gezeigt habe, ist dieser bewegliche Sauerstoff nicht an Wasser, sondern an das Terpentinöl gebunden.' Following the same line of investigation as Schoenbein and Brodie, Kingzett [2] showed that, by the action of air, the terpene of oil of turpentine is partly converted into a body having the properties of a peroxide which is able to act on starch iodide test-paper, and which we also found to affect Wurster's tetra-paper. It is the possible presence of such peroxide-like bodies in the living cell, whether formed from terpenes or not, compounds ready to part with their loosely combined oxygen, to which the reaction with aniline and the formation of anilophyll may be due.

Kingzett states that, when treated with water, his peroxidised terpene yields hydrogen peroxide along with various acids, but it is hardly necessary to suppose that this second stage of the process is passed through within the cell. We do not see why there should not be such bodies in the protoplasm, but if they are to subserve respiration they would again part with their oxygen, before themselves undergoing further oxidation, and thus be regenerated. Dr. Armstrong has recently shown [3] that, by exposing oil of turpentine together with moist oxygen to sunlight, a crystalline substance is formed which he calls 'sobrerol'; it is an oxidation-product of a terpene belonging, its discoverer thinks, to the group of the alcohols. Through the courtesy of Dr. Armstrong we obtained a specimen of his sobrerol, which, in aqueous solution, we found did not affect Wurster's reagent. Now here we have a well-

[1] Journ. f. prak. Chemie, 105, 198.
[2] Journal of the Chemical Society, new series, vol. xiii. p. 210.
[3] Ib. vol. lix. p. 315.

authenticated instance in which a body renders oxygen active previously to its being made use of to form a neutral oxidadation-product of the body itself. To sum up, therefore, the reaction with aniline in leaves, &c., coupled with the purely chemical reactions, would seem to restrict us to one of the following alternatives:—Either there is some more active form of oxygen than that in ordinary air present in many, if not in all, living green and other cells; or there is present some oxygen-carrier which is at the same time an oxygen intensifier, thus bringing about the necessary physiological respiration.

Though the conclusions at which we have arrived are somewhat indefinite, we venture to think that the experiments we have described may tend to elucidate the important subject to which they refer.

EXPLANATION OF FIGURES IN PLATE IX.

Illustrating Messrs. Schunck and Brebner's paper on Anilophyll.

Fig. 1. Leaf of common holly (*Ilex aquifolium*), half of the underside of which was painted with aniline, and was figured soon after the reaction had commenced.

Fig. 2. Holly-leaf painted on the entire under surface with aniline, and figured after the reaction was complete.

Fig. 3. Leaf of garden mint (*Mentha viridis*) painted on the under surface with aniline, and figured soon after the reaction had commenced.

Fig. 4. Cells from the mesophyll of a holly-leaf which had been treated with aniline, and was sectioned soon after treatment. × 600.

Fig. 5. Palisade-cells from a leaf of *Tropæolum majus*, which had been treated with aniline, and was sectioned 1 h. 40 m. after treatment. The chlorophyll had exuded in green viscid drops. × 600.

Fig. 6. Palisade-cells from a leaf of *Tropæolum majus* which had been treated with aniline, and was sectioned four days after treatment. The exuded chlorophyll-masses had turned brown. × 600.

Fig. 7. Palisade-cells from a leaf of *Tropæolum majus*, which had been treated with aniline. Crystals of anilophyll had developed from brown masses, such as shown in the preceding figure. The sections had lain for some days in dilute glycerine. × 600.

Fig. 8. Portion of a hair and cells of a sepal of monkshood (*Aconitum Napellus*), which had been treated with aniline. Crystals of anilophyll had formed within the cells. × 600.

Fig. 9. Cells from a sepal of monkshood which had been treated with aniline. Crystals of anilophyll had formed within the cells. × 600.

Fig. 1.

Fig. 2.

Fig. 3.

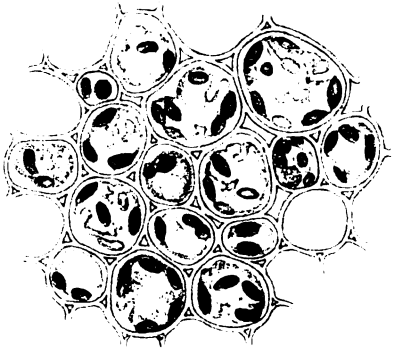


Fig. 4

Fig. 8.

G Brebner del.

SCHUNCK. — ON CHLOROPHYLL.

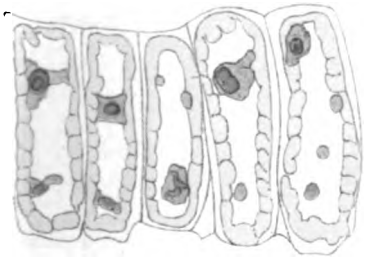


Fig. 5.

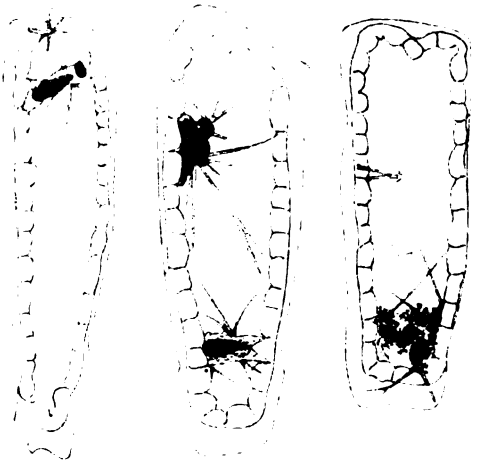


Fig. 7.

Fig. 6.

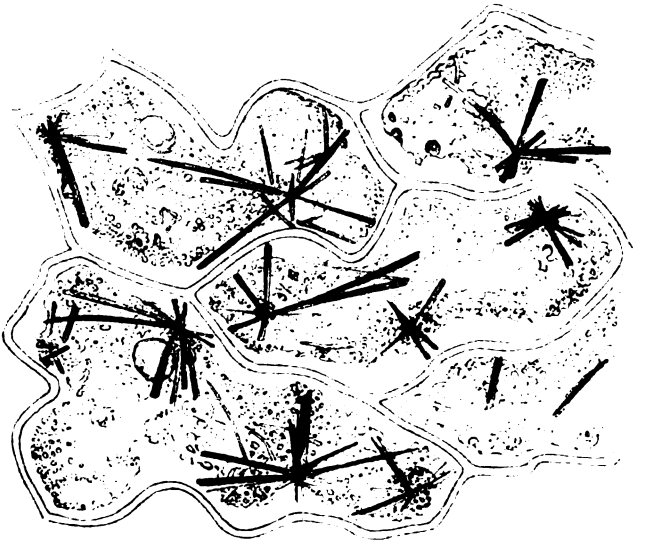


Fig. 9.

University Press, Oxford

On Schmitziella; a new Genus of Endophytic Algae, belonging to the order Corallinaceae.

BY

EDW. A. L. BATTERS, B.A., LL.B., F.L.S.

With Plate X.

THE alga which forms the subject of the present paper was discovered by Dr. Bornet in 1854 on some specimens of *Cladophora pellucida*, Kütz., from Cherbourg, but it was not till long afterwards, when he had written the note on *Melobesia* (*Choreonema*) *Thureti*, Born., which appeared in the Études Phycologiques in 1878, that he made a careful examination of it, and even then, being anxious to verify from the living plant certain details which the dried material did not show with sufficient clearness, he made no allusion to this interesting alga in the Études. Unfortunately since 1872 Dr. Bornet has not met with the plant.

In the meantime, while collecting at Torquay in 1885, I found what I believed to be an unknown alga, and, thinking it generically distinct from any described form, I placed it in my Herbarium under the name *Erythrocelis Cladophorae*, nov. gen. et sp., intending at some future date to publish an account of it; but owing to a variety of circumstances this intention was never carried into effect. Last year, while at Puffin Island, I once more found the plant, and on showing it to my friends Mr. G. Murray and Mr. R. J. Gibson, with whom I was collecting, they strongly urged me to publish the notes I had prepared so long before. *Erythrocelis Cladophorae*,

[Annals of Botany, Vol. VI. No. XXII. July, 1892.]

Batt., was consequently inserted in the Revised List of British Marine Algae[1] by Mr. Holmes and myself, and I partially rewrote my notes on the genus with a view to their publication. While the Revised List was in the press I learned for the first time that Dr. Bornet had also found the plant, and named it in honour of Dr. F. Schmitz, of Greifswald. Being anxious that the name of Prof. Schmitz should remain associated with an interesting genus, which by its affinity with the Squamariaceae recalls the work Prof. Schmitz has done on this group of the Florideae, I gladly adopted Dr. Bornet's suggestion that the genus *Schmitziella* should be published in our joint names, and accordingly made the necessary alteration in the Revised List when correcting the proofs.

The following is the diagnosis we propose for the new genus:—

SCHMITZIELLA, Bornet & Batters.

Thallus haud incrustans, endophytus, planus, membranaceus, pseudo-parenchymaticus, venosus, intra membranae laminas exteriores articulorum *Cladophorae pellucidae* extensus. Fructus sub cuticula *Cladophorae* in pustulis conceptaculiformibus hemisphaerico-depressis, apice poro pertusis, elevata evolventes, sparsi, minuti, pericarpio proprio clauso orbati, soros nematheciosos formantes.

Thalli nervis primariis e cellulis elongatis pluri-seriatis (2–8), longe excurrentibus formatis, secundariis monosiphoniis pinnatim egredientibus, alternis, una cum praecedentibus reticulum efficientibus maculae cujus cellulis (ramulis) irregularibus plus minus densis implentur. Et carposporis et sphaerosporis paraphysibus paucis immixtis. Sphaerosporis oblongis, zonatim divisis. Antheridiis ignotis.

Schmitziella endophloea, Born. & Batt.

Character idem ac generis. Sporangiis, 20 μ longis, 12 μ latis, aut binas aut quaternas sporas foventibus.

Hab. in *Cladophora pellucida*, cujus thallum haud infrequenter colore pulchre coccineo tingit.

Gallia. Cherbourg! St. Malo! Belle-Isle-en-Mer! (Gilgencrantz!) Guethary!

Anglia. Torquay! Puffin Island! Isle of Man! Anglesea!

[1] Annals of Botany, V.

Although I have examined a large number of specimens of *Cladophora pellucida*, which is by no means an uncommon alga on the shores of both France and England, only on two occasions have I met with *Schmitziella*, which I therefore conclude is a somewhat local plant. The rarity of the alga is compensated for, in a certain measure, by the fact that where it does occur it takes possession of a large portion of the host-plant; thus a single specimen of *Cladophora pellucida* which has been attacked by this endophyte often furnishes an abundant supply of material for purposes of study.

The health of the *Cladophora*, to all appearance, is not in the least affected by the presence of the endophyte which is found equally in the cell-walls of both young and old specimens, its presence being betrayed by the beautiful red colour it communicates to their stems. So far as I have observed, the straggling deep-water form of the species is the most liable to be attacked.

Both at Torquay and Puffin Island the infected plants were growing in deep rock-pools very much shaded by overhanging boulders, and owing to the depth of the water it sometimes happened that only the long secondary branches of the *Cladophora* were obtained, and as these did not always exhibit the characteristic marks of the species I was at first misled into thinking that *Schmitziella* grew on more than one species of the genus. Up to the present, however, *Schmitziella* has been found only on *C. pellucida*, although I know of no reason why it should not occur on other species, or at any rate on those with a perennial base, such as *C. catenata* or *C. prolifera*.

As so often happens with specimens of *C. pellucida*, the plants which had been attacked by *Schmitziella* were also more or less covered with encrusting Melobesiae, but the purplish, chalky fronds of the epiphytes could not for a moment be mistaken for the rose-red, delicate thallus of the endophytes. The action of light does not appear to be necessary for the healthy development of *Schmitziella*, for on removing the chalky and nearly opaque fronds of the Melo-

besiae, I found in that portion of the cell-wall of the *Cladophora* which was situated immediately beneath them, some beautiful specimens of the endophyte, sterile it is true, but more richly coloured than specimens from unencrusted portions of the host-plant.

How the spores of *Schmitziella* make their way into the cell-wall of the *Cladophora* is rather obscure, but still it is worth while taking the following circumstances into consideration. In the genus *Cladophora* the zoospores make their escape through a small aperture in the cell-membrane situated at the upper end of each cell just below the point where it is attached to the cell immediately above it. Now in *C. pellucida* the dissepiments of the cells are situated at the forkings of the branches and ramuli, and it is just at this spot that the young plants of *Schmitziella* make their appearance. It seems probable therefore that the spores of the *Schmitziella* get lodged in the axils of the branches of the host-plant—that is at a spot where either there is an aperture ready-made for them, or at any rate where, we must suppose, the membrane can be most easily pierced—and there germinate. Once having gained an entrance between the layers of the cell-wall of the host-plant, the endophyte grows very rapidly and spreads from cell to cell of it. I have traced the filaments of one *Schmitziella* plant through three of the very long cells of the *Cladophora*.

The earliest stage in the development of the frond of *Schmitziella* that I observed consisted of seven very irregularly shaped cells (Plate X, Fig. 2). The spore appears to have divided into four parts, one of which has again divided into three. In the next stage observed, growth appears to have taken place in two directions (Fig. 3), the spore as in the former case having divided into four parts, the two central of which have again divided so as to form rudimentary lateral branches. After this stage the plant developes very rapidly, and the frond soon assumes the appearance represented in Fig. 5, in which it will be seen that the cells are arranged in a more or less filamentous manner; but it is not till a later

stage in its development that the manner in which the mature frond is built up is easily observed. At first the thallus consists of rows of rose-coloured cells arranged in filaments which, by their excessive branching in one plane, form a network, and finally a more or less compact pseudo-parenchymatous membranous expansion, which being confined between the outer layers of the thick compound cell-wall of the *Cladophora*, and frequently entirely surrounding the cells of the host-plant, assumes, of course, the form of a hollow cylinder.

On examining one of these strata it is at once obvious that it is composed of cells arranged in filaments of two sorts—the primary and the secondary filaments. The primary filaments are composed of long cylindrical cells, and extend straight forward for a considerable distance either singly or in close parallel rows of from 2 to 8 (usually 3 or 4) threads: not infrequently, however, after continuing in company for some distance, the filaments of which a group is composed separate again and diverge in opposite directions, each continuing its course alone or joining with other filaments to form a new group. The very much and irregularly branched secondary filaments, on the other hand, are composed of short very variously shaped cells which arise laterally from the primary filaments. Where a number of primary threads run side by side in close proximity they form, as it were, one filament, the secondary branches arising only from those which form the border, and from that side of them which is not contiguous to the other filaments of the group: thus where five threads run in parallel rows the three middle ones are unbranched, lateral branches arising from the two outer ones on that side which is most removed from the centre (Fig. 6). As has already been stated these secondary filaments are always very much branched, and, continuing to send out branches, form, at the side of the primary filaments in the space which separates the isolated primary filaments or groups of filaments, an ever increasingly compact irregular network, and finally a more or less compact stratum of small

cells. The mature thallus of *Schmitziella* thus presents the appearance of a membranous veined expansion, the long-celled primary filaments representing the veins (Fig. 7).

At first all the branches of both primary and secondary filaments are confined to one plane, but as the formation of the thallus continues it sometimes happens that a branch is pushed out of its place and creeps over or under the already formed network of cells. These displaced filaments, in their turn, send out branches, and in this way the thallus becomes in places a two- (very rarely a three-) layered membrane. Where this occurs all the filaments of the *Schmitziella* are sometimes confined between the same two layers of the compound cell-wall of the *Cladophora*; but more frequently each layer of the thallus of the *Schmitziella* is contained between separate layers of the cell-wall of the host-plant.

The cells of which the thallus is composed are laterally united to each other in a very loose manner, and it is sometimes not very easy to determine to which branch a cell belongs, but usually it is no very difficult matter to trace the branching. The shape of the cells is most variable, oval, oblong, or sickle-shaped cells being most frequently met with, but sometimes they are so irregular that it would be useless to attempt to describe them. The cell-membrane is always thin and delicate, never chalky, as is the case in nearly all the other members of the order.

The reproductive organs are developed in nemathecial sori, which are more or less numerously scattered over the surface of the thallus, and arise on its upper surface in the form of flattened hemispherical protuberances. To form these, the oval or oblong cells of the thallus are collected together at various spots, and from them, by transverse division, smaller roundish cells are cut off either singly or by twos. These smaller cells, becoming united more or less firmly to one another, form the basal layer of the sorus. By an almost simultaneous upward growth of these smaller cells, which themselves develope into its component threads, the flattened hemispherical sorus is

formed, the exterior layer of the *Cladophora* cell-membrane being, of course, raised locally along with it.

So far as I have observed there is not much difference in the form of the tetrasporic and cystocarpic sori, and it is sometimes rather difficult to say to which class a sorus should be referred, the long terminal cells of the central group of paraphyses in the tetrasporic sori somewhat resembling trichogynes.

The tetrasporic sori are roundish in outline and are often scattered in great numbers over the surface of the thallus: the cystocarpic on the other hand are larger, more flattened, and not nearly so numerous, but I am not sure that these characters are constant. The central portion of each tetrasporic sorus is occupied by a group of sterile filaments composed of from two to four short cells and an elongated terminal cell which is often very much attenuated upwards. The upward growth of this central group of paraphyses, which are always the first to be formed, locally raises and finally ruptures the exterior layer of the cell-wall of the *Cladophora*, the edges of the torn membrane, released from pressure, are turned back on to the surrounding membrane and form as it were a ring around the opening made by the paraphyses (Figs. 9 and 13). A mature sorus consequently resembles a conceptacle with a hyaline pericarp and a more or less prominent ostiole, the enclosing cell-wall being represented by the raised portion of the cell-membrane of the *Cladophora*, and the ostiole by the hole formed by the central group of paraphyses. The remaining filaments of the same sorus, with the exception of the outermost or marginal row, are nearly all fertile. The shorter cells of the basal layer of the sorus lengthen and form short filaments, the terminal cells of which expand into at first oval then oblong sporangia, which, by transverse division of their contents, finally form two- or four-parted tetraspores—Dr. Bornet's Cherbourg plants produced tetraspores (Fig. 11); my Torquay and Puffin Island ones bispores. Here and there between the sporangia an isolated filament remains sterile, and forms a paraphysis similar to those of the central bundle. As before

stated, the marginal row of filaments, usually composed of short two-celled threads, always remains sterile, forming a ring around the empty sorus [1].

The development of the sorus proceeds from the centre outwards: first the central group of paraphyses is formed; then the sporangia surrounding it come to maturity and are discharged through the opening in the cell-wall of the *Cladophora* made for that purpose by the central paraphyses; then follows the maturing and discharge of the sporangia further removed from the centre, those nearest the edge of the sorus being the last to mature; finally only the narrow ring of sterile marginal filaments, the central group of paraphyses, and here and there an isolated paraphysis, remain.

The cystocarpic sori in the same manner as the tetrasporic are scattered over the surface of the thallus. The same almost simultaneous upward growth of the basal cells takes place, forming a compact sorus, the central threads of which are composed of from three to five cells, while those of the remainder of the sorus are much shorter. Only these central threads develope into carpogenic filaments, a very few of them continuing sterile and forming paraphyses, similar to those of the central group in the tetrasporic sori. While the apical cells of the sterile filaments remain comparatively short, those of the central group of filaments lengthen and form the carpogones, elongating upwards into long thin trichogynes, which, like the central group of paraphyses in the tetrasporic sori, locally raise and finally break through the outer layer of the cell-membrane of the *Cladophora* in united bundles which project for some distance through the opening thus made (Fig. 12). The cell-membrane of the *Cladophora* is rather tough, and although the sharp-pointed paraphyses usually pierce it without bending or distortion of any kind, the trichogynes are frequently bent when they come in contact with its under

[1] The marginal ring of short paraphyses represents in a rudimentary form the enclosing wall of the conceptacles in which the reproductive organs of the other members of the Corallinaceae are developed. In *Schmitziella*, therefore, the transition from the unenclosed sorus to the conceptacle is well-marked.

surface; and when they finally break through, and are freed from tension, they project through the opening with a slight bend in the opposite direction. The portion of the tough cell-membrane of the *Cladophora* which covers the sori of the *Schmitziella* is, of course, very much stretched locally when the tetraspores and carpospores are mature, but owing to its rigidity it does not collapse after their discharge but retains the form of the sorus.

Antheridia have not been observed.

The genus *Schmitziella* belongs without any doubt to the Order Corallinaceae, although of course it differs in some points from all the other genera. The formation of the thallus in particular differs from that of the great majority of the members of the Order, but a nearly similar formation is to be found in *Melobesia callithamnioides*, Falkbg. and *Hapalidium callithamnioides*, Crn. Its endophytic mode of life, again, finds its analogue in the parasitic genus *Choreonema*, Schmitz, with which, however, it has very little else in common. The distinguishing characteristic of the genus consists in the absence, in all but the most rudimentary form, of the enclosing wall with which the reproductive organs of all the other Corallinaceae are surrounded. The reproductive organs of *Schmitziella* are produced, as has been shown, in unenclosed nemathecial sori surrounded by a ring of very short paraphyses, while those of all the other genera are enclosed in conceptacles with but a small apical opening.

The filamentous thallus with its thin cell-walls which do not contain a vestige of chalk, the endophytic mode of life, and above all the unenclosed sori of *Schmitziella*, taken either singly or all together, separate it from all the other genera of the Order and mark it as a distinct and well-defined genus.

In conclusion, I would return my sincere thanks not only to Dr. Bornet for his advice and assistance in the preparation of this paper, but also to Prof. Schmitz, who has kindly placed his notes, based on an examination of the specimens sent to him by Dr. Bornet and myself, at my disposal.

EXPLANATION OF PLATE X.

Illustrating Mr. Batters' paper on *Schmitziella.*

Fig. 1. *Cladophora pellucida*, Kütz., and *Schmitziella endophloea*, Born. & Batt. Natural size.

Figs. 2–5. Various stages in the development of the thallus of *Schmitziella.* × 750.

Fig. 6. Marginal portion of a young specimen, showing the branching and the manner in which the pseudo-parenchymatous layer is formed. × 750.

Fig. 7. Portion of thallus more mature. × 750.

Fig. 8. Vertical section through a young branch of *Cl. pellucida*, showing section of the thallus of *Schmitziella.* × 750.

Fig. 9. Transverse section through a tetrasporic sorus. × 750.

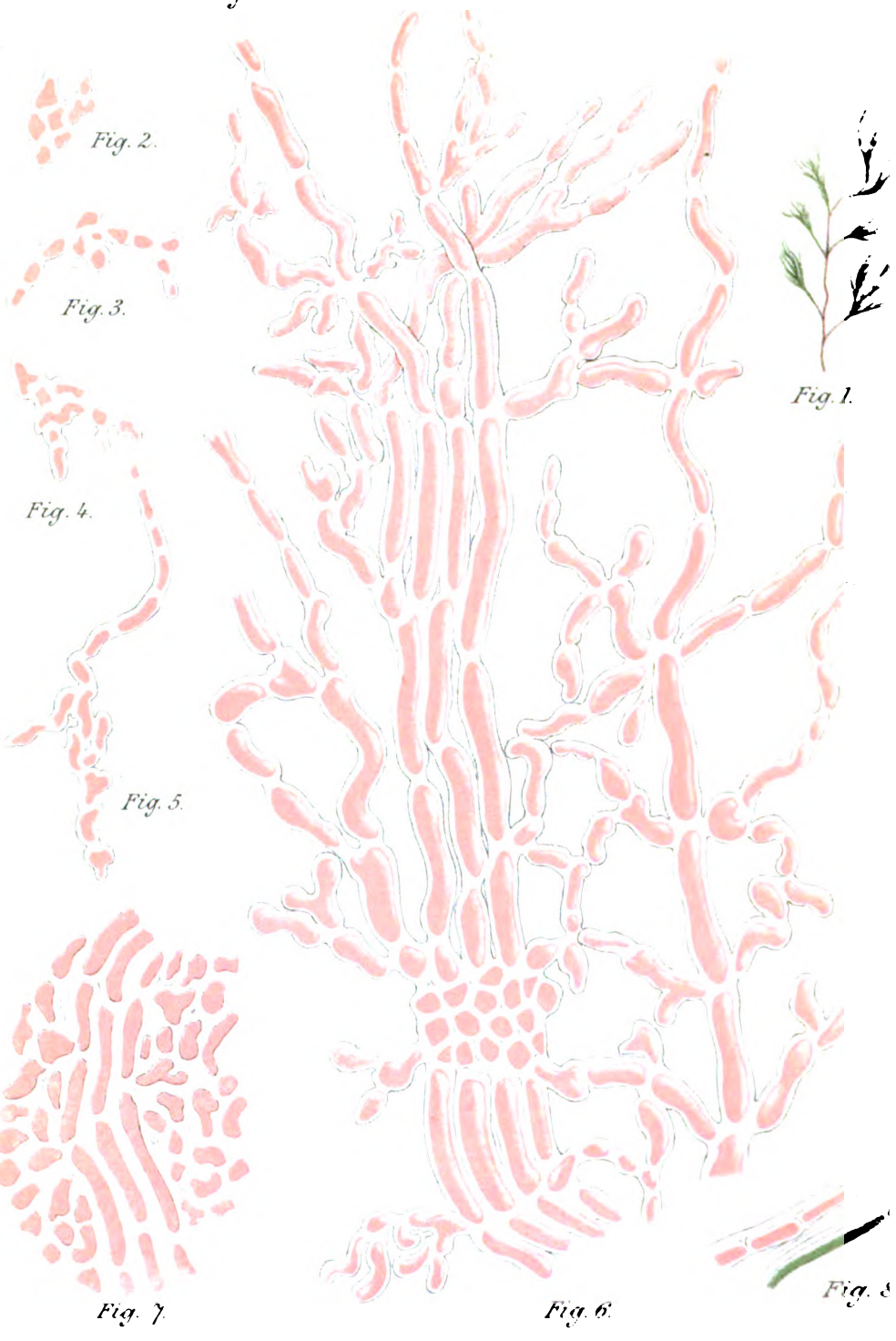
Fig. 10. Bispores from a Torquay specimen. × 1000.

Fig. 11. Tetraspores from a Cherbourg specimen. × 1000. (From a sketch by Dr. Bornet.)

Fig. 12. Transverse section through a young cystocarpic sorus showing trichogynes. × 750.

Fig. 13. Transverse section through a nearly mature cystocarpic sorus. × 750.

Fig. 14. Carpospores from same. × 1000.



F A Batters del.

BATTERS. — ON SCHMITZIELLA.

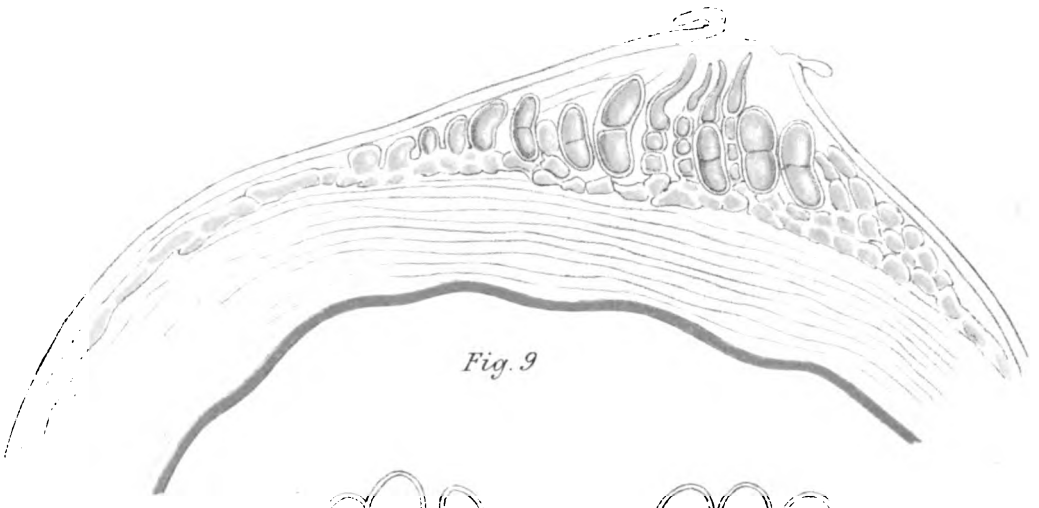


Fig. 9

Fig. 10.

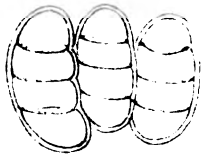


Fig. 11.

Fig. 12.

Fig. 14.

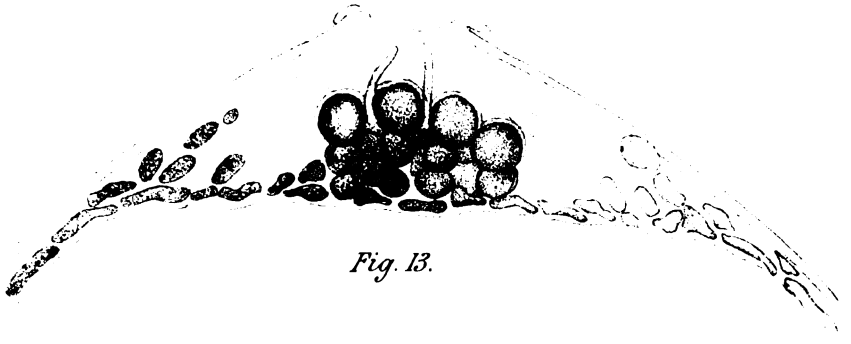


Fig. 13.

University Press, Oxford.

On the Occurrence of Vegetable Trypsin in the fruit of Cucumis utilissimus, Roxb.

BY

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FEW lines of research into the processes of vegetable physiology have led in recent years to more important results than that which has been concerned with the distribution, character, and action of the several so-called unorganised

NOTE.—In 1885–6, Brigade-Surgeon E. Bonavia, M.D., sent to Kew seeds of several Cucurbits. Of these he gave the following particulars:—

'Etawah, N.W.P., 20 Oct., 1885.

'Next mail I shall send you seeds of three varieties of wild *Cucumis*, which I firmly believe are the wild parents of the famous Lucknow melons called "Chitla Kharbooja" (Spotted Melon) and others. These wild melons are called "Kachree" by natives. In common with the *Carica Papaya* (Papīta) they soften muscular fibre, and natives cook them with tough meat to make it tender. The milk of the common fig (*Ficus Carica*) has similar properties. It is not improbable that all contain Papaine.'

Two of the varieties were grown at Kew and proved to be the *Cucumis utilissimus* of Roxburgh, who gives Kakri as the vernacular name. *C. utilissimus* in the Flora of British India is reduced to a variety of *C. Melo*, Linn. It is a variable plant; but the fruit is always elongate or cylindrical.

'Etawah, N.W.P., 9 Feb., 1886.

'This hot weather I shall try and secure for you some seed of the Lucknow spotted melon, which I think owes its origin to the Kachree wild cucurbit I sent you seeds of. Please note that the papaine-quality of the latter was told me by a very intelligent native gentleman, and he was very sure that this quality was possessed to his knowledge by only three plants:—Papau, Kachree, Anjir. Anjir is the vernacular for the common Fig. He said he often used the Kachree fruit cut

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ferments or enzymes. The action of diastase in converting starch into sugar, established by Baranetzki [1], has been shown to be intimately concerned alike with the ordinary processes of nutrition of the adult plant, and with the phenomena of the germination of seeds and tubers, and further to be the means in some cases of starting the growth of the pollen-tube [2]. Side by side with diastase other similar bodies have been identified, by whose agency cellulose [3] and inulin [4] undergo similar transformation. The nitrogenous reserve materials deposited in temporary reservoirs have also been shown to be called into active consumption by other members of the same group, while glucosides and oils have also special enzymes to attack them.

The ferments which have the property of digesting nitrogenous substances like fibrin and albumin have been less conspicuous than diastase, but their existence has been clearly established by Reess and Will [5] in the leaves of *Drosera*; by Gorup-Besanez [6] and by Vines [7] in the pitchers of various

up with tough meat and stewed together. He is clear about the fact of its making tough meat tender. I have never tried it, but have tried the milk of the fig, and it does soften tough meat.'

Dr. Bonavia also sent to Kew seeds of a very pretty gourd. He remarked:—'They are new to me, although I have been looking out for such things for the last twenty-eight years. They were sent to me by Major Buller, police officer, Gonda Oudh. He says they are largely grown in the Nepal Terai.'

They turned out to be a form of the pumpkin (*Cucurbita maxima*, Duch.).

The Kachree fruited rather sparingly at Kew, and it was some little time before fruits were available for examination. However, in 1891, all the available material was placed in the hands of Professor Green. By an unfortunate mistake Dr. Bonavia's pumpkin was sent him in the first instance instead of the Kachree. It is interesting to observe that the results of its examination were purely negative. In the Kachree, as will be seen, he had no difficulty in detecting a tryptic ferment. It is, however, clear that this is not characteristic of the Cucurbitaceae generally.

W. T. T. D.

[1] Die stärkeumbildenden Fermente in der Pflanze, 1878.

[2] Green, On the occurrence of diastase in pollen. Brit. Assoc. Reports, Cardiff, 1891.

[3] Brown and Morris: Journal of the Chem. Soc., lvii. June, 1890.

[4] Green: this Journal, vol. i. 1888.

[5] Bot. Zeit., Oct. 29, 1875.

[6] Ber. d. deutsch. Chem. Gesellsch., May, 1876.

[7] Journal Linn. Soc. Botany, vol. xv.

species of *Nepenthes*; by Wurtz[1] and later by Martin[2] in the fruit of the Papau (*Carica Papaya*); by Wittmack and by Hansen in the latex of the Fig. (*Ficus Carica*)[3]; and by the writer in the germinating seeds of the Lupin[4] and the Castor Oil plant[5]. To these another Indian plant may now be added in *Cucumis utilissimus*, Roxb., the Kachree gourd.

During the present autumn I have had, through the kindness of the Director of the Royal Gardens, Kew, the opportunity of examining the fruit of this plant, which has in India the reputation of possessing the same properties as those of the Fig and the Papau. The fruit is in appearance much like a small vegetable marrow, about six inches in length. It is yellow in colour, and when cut has an aroma similar to that of the water-melon. Its pulp is extremely succulent and the expressed juice is faintly acid in reaction.

The first series of experiments made were directed only to ascertain whether the juice has, as suggested, any action upon a proteid body. It was pressed from the central pulp, and filtered till quite clear. Two equal volumes were taken, and one of them boiled for a few minutes to destroy any enzyme that might be present. The two volumes were then put into labelled beakers with a little thymol, and to each a measured quantity of egg-albumin was added. The albumin was prepared by boiling white of egg for about five minutes and then forcing it through very small-meshed wire gauze, which reduced it to a fine state of sub-division, so that it could be accurately measured. The two beakers with their contents were then set in an incubator at a temperature of 34° C.

The action began slowly in the unboiled portion, and proceeded continuously and gradually. In two days about half the albumin had been dissolved. In the control-beaker, with the boiled extract, no change could be observed, either in the quantity or the appearance of the albumin. Microscopic

[1] Wurtz: Comptes Rendus, June, 1880.
[2] Martin: Journal of Physiology, vol. v. 1884, p. 213.
[3] Hansen: Arb. d. bot. Inst. in Würzburg, iii. 1885.
[4] Green: Phil. Trans., vol. 178 B, 1887, p. 39
[5] Green: Proc. Roy. Soc., vol. 48, 1890, p. 370.

examination of both liquids showed them to be free from micro-organisms.

The juice therefore possesses the property of dissolving coagulated egg-albumin and loses this power on being boiled.

While this experiment was proceeding, part of the pulp immediately under the rind of the fruit, was extracted with water, in which ·2 % of potassic cyanide had been dissolved as an antiseptic. The extract was filtered from the pulp after standing over it for twenty-four hours, and was alkaline in reaction, owing to the presence of the cyanide. The pulp itself, like the expressed juice, was faintly acid. With this extract a similar experiment to the first one was carried out, and similar results were obtained, showing that the power of dissolving coagulated proteid, presumably a ferment-action, extends to the pulp as well as to the juice of the fruit.

On boiling the extracts in both these experiments the liquid became opalescent. This was found to result from the presence of a proteid body coagulating by heat. Both the liquid and the granular deposit which formed gave a good xanthoproteic reaction. So many of the enzymes known to physiologists having been found to be associated with such proteids, and particularly with members of the globulin class, it seemed not unlikely that such an association might be found here. Globulins are characterised especially by their insolubility in pure water, and their ready solubility in solutions of common salt of about 3–10 % strength. A series of experiments was therefore arranged with a view to ascertaining whether the enzyme is more easily extracted by salt solution than by water or faintly alkaline fluids.

Two portions of the pulp, of equal bulk, were taken and mashed up separately in a mortar, one with 70 *cc.* of a solution containing 3 % Na. Cl. and ·2% KCN ; the other with 70 *cc.* of ·2 % KCN solution only. After twenty-four hours the two extracts, labelled for convenience of reference C and D, were filtered and carefully neutralised with very weak acid. The antiseptic action of the potassic cyanide was always found to be sufficient to prevent any contamination with micro-organisms, but as

neutralisation involved the decomposition of the cyanide, it was thought advisable to add another preservative agent. Attention has recently been directed by several observers to oil of mustard [1] as possessing strong power in this direction, and it was therefore chosen. About 30 *cc.* of each extract was put into a bottle furnished with a graduated scale, and a measured quantity of egg-albumin, prepared as before, was added. Each was then shaken up with 1 *cc.* of oil of mustard, and placed in the incubator. The oil of mustard is very slightly soluble, and a good deal of it floated on the top of the liquid. Being somewhat volatile, the air in the upper part of the bottles contained some of its vapour. As the action of the extracts proceeded the antiseptic was found to work admirably, no putrefactive changes taking place all the time it was continued—a period of several days.

The mode of observation was to shake the graduated bottles every morning, and on the albumin settling to the bottom, as it did in about 2–3 minutes, to measure the quantity remaining by the scale.

The following table will enable a comparison of the activity in the two cases to be made :—

Time of digestion.	Diminution of albumin in C.	Diminution of albumin in D.
36 hours	16%	8%
60 ,,	25%	18%
108 ,,	29%	20%

The extract C had thus evidently greater ferment-power than D, and it is fair therefore to infer that the enzyme is more easily extracted by a weak salt solution—a fact which points either to its being a globulin in nature, or more probably associated with a globulin constituent in the cells of the plant. The fact that the first watery extract made possessed ferment-power would be explained by the fact that in the plant are many inorganic salts, by whose assistance it would be dissolved on the addition of water.

[1] Among others, Cadeac et Mennier, Recherches expérimentales sur l'action antiseptique des essences, Annales de l'institut Pasteur, iii. 1889; also Roux.

The association with a globulin would also be suggested by the observation already mentioned, that on boiling, the extract became turbid from the presence of coagulated proteid.

The most powerful proteo-hydrolytic ferment that has hitherto been found in plants is the vegetable trypsin occuring in the Papau, which has been carefully worked out by Martin [1]. The gourd under examination appearing to resemble this fruit in many respects, experiments were next undertaken to see if the two ferments also are alike. The points investigated were (1) the medium in which the *Cucumis*-ferment is most active, (2) the products of the decomposition which it initiates.

An extract prepared by salt solution from the pericarp, and one obtained by similar treatment of the central pulp in which the seeds were embedded, were carefully neutralised. Three tubes of each were prepared--one contained the extract diluted with an equal volume of ·4 % H. Cl., one the same with an equal volume of water, and the third the same with an equal volume of 3 % $Na._2 Co._3$. Boiled controls of all were exposed side by side with them. To each was added a measured quantity of the egg-albumin, and the two sets with their controls were put side by side in an incubator at 34° C. They were labelled E_1 E_2 E_3 and F_1 F_2 F_3, and their controls E_{1b} F_{1b}, &c. The digestion was in this case continued for three days. During the experiment and at its conclusion the greatest activity was found to be shown by the alkaline tubes of both sets, while the acid had least power. Like papaïn, therefore, the ferment acts most advantageously in a faintly alkaline medium.

The products of the decomposition were examined in a digestion carried out in dialysing tubes, controls being used, in which the extract was boiled before adding the albumin. These may be referred to as G and H. After twenty-four hours peptone was found to be present in the dialysate of G in

[1] Op. cit.

sufficient quantity to give a good biuret reaction. After 2 days the experiment was stopped, and the dialysates compared. That of the control H gave no evidence of peptone by the biuret test. Both were then evaporated to dryness over a water bath. The residue from the dialysate of G was much the more copious of the two.

These residues were dissolved in a small quantity of distilled water, and precipitated by neutral acetate of lead, a reagent which throws down peptones, and forms an insoluble compound with leucin, an amide body which occurs in the profound decomposition of proteids brought about by tryptic ferments. The appearance of leucin in the dialysate would afford evidence that the ferment under examination is a trypsin and not a vegetable pepsin.

Comparing the two after addition of the acetate of lead it was found that there was a precipitate in both, but much the greater quantity in G. After well washing, these precipitates were suspended in water, and a stream of H_2S passed through, till the liquid was saturated with gas and all the lead thrown down as Pb. S. Most of such peptone as was present in G was by this treatment rendered insoluble and was removed by filtration with the lead sulphide. The filtrate, containing now any leucin that might have been present, and any diffusible bodies that, being present in the original extract, behaved with regard to lead acetate in the same way as leucin, was concentrated to a very small bulk, and treated with boiling alcohol, in which leucin is soluble. After filtering, this alcoholic extract was in turn concentrated, and allowed to deposit the crystalline matter which it contained. Both dialysates, treated thus, threw down crystals, G depositing the greater amount. The crystals from G were found to be of two kinds: one, not very plentiful, separating in rosettes, which were doubly refractive; the others, in much greater quantity, had not a very definite crystalline form, and were only very feebly, if at all, doubly refractive. H contained only the rosettes. The crystals were then purified by repeated crystallisation from water, till they were

nearly free from admixture, and were finally allowed to form very slowly in a watch-glass. Those from G were found, when examined under the microscope, to be of two kinds, the rosettes already spoken of, and others having the characteristic appearance of leucin crystals aggregated into rough rounded clumps. Those from H, as before, only showed the doubly refractive rosettes.

G then contained crystals of two kinds, H only one, and those absent from H were found to resemble leucin in appearance. The doubly refractive rosettes were present in the two extracts in about equal amounts. These were probably present in the fruit before extraction, while the leucin was formed during the digestion.

From the crystallised residue of G so obtained, several of the rounded clumps were separated and carefully dried. They were then put into a small hard glass tube and heated strongly in a flame. They sublimed without melting, and were deposited again in crystalline form on the upper cool portion of the tube. This is strong confirmation of their being leucin, as this is the only body derived from proteo-hydrolytic decomposition of albumin that will sublime unchanged.

I had unfortunately at this point come to the end of my material, and was therefore unable to investigate the ferment further in the direction of isolation.

To summarise my results, I find that:—

(1) The fruit of *Cucumis utilissimus*, Roxb., contains in its juice and in its pericarp a proteo-hydrolytic ferment, capable of dissolving coagulated egg-albumin.

(2) This ferment is either globulin in nature, or associated with a globulin in the cells of the plant.

(3) Like papaïn, it works best in a slightly alkaline medium; less readily in a neutral one, and least of all in the presence of acid.

(4) Like papaïn, again, it effects a very complete decomposition of the albumin, giving rise to peptone, and later to leucin. It is a ferment, therefore, allied to the trypsin, rather than to the pepsin, of the animal organism.

Chelonespermum and Cassidispermum, proposed New Genera of Sapotaceae.

BY

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With Plates XI, XII, XIII, and XIV.

FOR some years past there have been some seeds of highly curious shape, and unknown origin, in the Kew Museum. It was not difficult to determine their affinity, and they were assigned without doubt to the Sapotaceae; but they could not be identified with any described members of the order, though possibly the flowering condition may have been described.

Our first knowledge of the native country of one of these singular productions was derived from a seed and a branch bearing two leaves, collected in the Fiji Islands by Mr. J. Horne, Director of the Department of Forests and Botanical Gardens, Mauritius, and sent to Kew in 1879.

In the small botanical collection brought home last year by the Rev. R. B. Comins, from the Solomon Islands, were seeds manifestly of the same genus as those already in the Museum; but as there was no flowering specimen of the tree that bore them, the only additional information they afforded was in relation to the area of the genus. As Mr. Comins was returning to the Solomon Islands, I particularly requested him to endeavour to procure specimens of this and some few other unusually interesting plants. He promised to do so, and has succeeded so far as to obtain specimens in a very late stage of flowering, as well as ripe fruits. He has also sent seeds of what is doubtless a second species of the same genus from the

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same group of islands. Mr. Horne's Fiji species is likewise quite different in foliage, and the Museum seeds of unknown origin, described below, increase the number of species to four, which I refer to *Chelonespermum*, and one which I have called *Cassidispermum*.

In the Solomon Islands these seeds bear a name signifying turtle-seed, which is a most appropriate designation, for they are so much like miniature turtles as to at once suggest the name. And I have chosen the Greek equivalent to designate the genus.

Considering the size and singularity of these seeds it seems surprising that nothing more is known of them. Almost every part of Polynesia has been so fully explored by various expeditions that one would have expected these objects to have attracted special attention. Possibly they are comparatively rare, though Mr. Comins states that he was told that *C. minor* was plentiful in the island of San Cristoval. Possibly, too, flowering specimens of some of them, at least, have been described and referred to existing genera. Nevertheless, I venture to found two new genera for them, partly because I believe they deserve generic rank, and partly because publication of descriptions and drawings may lead to further knowledge.

The natural order *Sapotaceae* has occupied the attention of several botanists since their publication in Bentham and Hooker's Genera Plantarum, in 1876, including Professor Hartog [1], Dr. W. Burck [2], Dr. L. Pierre [3], and Dr. H. Baillon [4]. The last named botanist monographs the order, so far as genera are concerned, and he defines sixty-four genera as against twenty-four by Bentham and Hooker. Baillon groups his genera in three numerically very unequal series, namely:—1. Bumelieae, fifty-four genera; 2. Illipeae, nine genera; and 3. Mimusopeae, restricted to the genus *Mimusops*.

[1] Journal of Botany, 1878 and 1879.
[2] Annales du Jardin Botanique de Buitenzorg, vol. v. 1886.
[3] Notes Botaniques: Sapotaceae, 1890.
[4] Histoire des Plantes, vol. v. 1891.

I sent Dr. Baillon rough sketches of two of the 'turtle seeds,' and in reply to my inquiries he wrote that he had never seen such Sapotaceae, but that he supposed they might belong to the section *Imbricaria* of the genus *Mimusops* as some of the species have crested seeds[1]. The Capucin, *Northea seychellana*, Hook. fil.[2], also approaches our plant in seminal characters in regard to size and the enormously large hilum. Baillon regards this as a species of *Mimusops*, rather than as an independent genus.

With regard to the position of *Chelonespermum*, the imperfect flowers collected by Mr. Comins are sufficient to indicate that it belongs to Baillon's group Illipeae, assuming the characters given are so far constant in the groups. As there were only four stamens, and some fragments of petals attached by the web of some insects to one of the old calyces, there remains only the calyx to determine which group it belongs to. Baillon describes the calyx of the Bumelieae as composed of five somewhat unequal sepals, quincuncial in aestivation; the calyx of the Mimusopeae as having six to eight valvate or imbricate sepals in two series; and the calyx of the Illipeae is described as of four sepals, imbricated in aestivation, two having both edges outside of the other two. The last is the condition in Mr. Comins's plant, but the seeds point to a close relationship to *Mimusops*, as suggested by Baillon; and should it eventually be deemed expedient to refer it and its immediate allies to *Mimusops*, the name *Chelonespermum* might be retained for sectional purposes.

So far as the materials go, the following is a description of the proposed new genus, *Chelonespermum*.

Flores hermaphroditi. *Calycis* segmenta 4, imbricata, 2 exteriora et 2 interiora. *Corolla* . . . . *Stamina* . . . antheræ basifixæ, ovato-oblongæ, apiculatæ. *Discus* obsoletus. *Ovarium* glabrum, 2-loculare, loculis uniovulatis. *Bacca* magna, obovoidea, carnosa; semen unicum, excentricum, sæpius compresso-ovoideum, compresso-ellipticum, vel compresso-orbiculare, facie ventrali hilo omnino tecta, hilo nunc plus minusve

[1] See Gaertner, De Fructibus et Seminibus Plantarum, iii. t. 206.

[2] Hooker, Icones Plantarum, xv. p. 57. t. 1473.

convexo infra medium nudo, medio umbonato costato vel unispinoso, supra medium grosse indurato-muricato vel spinoso, nunc longitudinaliter bilamellato, facie dorsali convexa testa castanea nitida marginata tecta, margine integra vel irregulariter dentata lobata aut spinosa; albumen nullum vel ad membranam redactum; embryo circumscriptione varius, cotyledonibus hilo parallelis latissimis crassis carnosis plano-convexis.

The floral characters, as far as I am able to give them, are drawn up entirely from *C. majus*, and may have to be modified when the flowers of the other species are known, or even the genus may fall. The two-celled ovary, with one ovule in each cell, associated with a four-lobed calyx, and the peculiar seed, are the points relied upon. As in *Sideroxylon globosum*, *Lucuma mammosum*, *L. Rivicoa*, and *Northea seychellana*, the hilum is very large, larger indeed than in any of the plants named. The seed is a large compressed dorsi-ventral body, the hilum covering the ventral portion, which is more or less furnished with vertical plates and spine-like protuberances, especially in the upper half, sometimes with a stout central spike-like production 9–12 lines long. The dorsal part is covered with a smooth, shining, brown or chestnut, exceedingly hard testa, having an extended margin a little overlapping the opaque corrugated hilum-portion, the edge being sharp and entire or more or less irregularly spine-toothed or lobed.

(1) **Chelonespermum majus**, Hemsl.

Arbor usque ad 80 ped. alta (fide Comins), ramulis ultimis floriferis crassissimis rugosis. *Folia* ad apices ramulorum conferta, longe petiolata, coriacea, obovato-oblonga, vel interdum ovato-oblonga, cum petiolo 4–8 poll. longa, obtusa vel subacuta, basi sæpius leviter oblique rotundata, primum præcipue subtus secus costam ferrugineo-tomentosa, demum glabrescentia, petiolo tereti ferrugineo-tomentoso 9–18 lineas longo. *Pedicelli* floriferi graciles, petiolos paullo excedentes, ut calyces ferrugineo-tomentosi. *Calycis* segmenta vix 3 lineas longa, late triangularia, subobtusa. *Fructus* pyriformis vel obovoideus, circiter 3½ poll. longus; semen circiter 3 poll. longum, hilo medio

longitudinaliter elevato et supra medium lamellato-spinoso, testæ margine supra medium in lobos paucos recurvos unguiformes producto; embryo ovatus, circiter 2 poll. longus et 1½ poll. latus, cotyledonibus crassis carnosis leviter umbonatis, apice breviter acuminatis, basi leviter auriculatis, margine supra medium obscure lobulatis.

Florida Island, Solomon group, Rev. R. B. Comins, 194. Native name *gae-vonu* or turtle-seed.

(2) **Chelonespermum minus**, Hemsl.

Semen compresso-ovoideum, vix 2 poll. longum et 1½ poll. latum, hilo elevato longitudinaliter distincte vel indistincte bilammelato cum lamellis transversis extus productis et versus apicem tuberculato, testæ margine saturato-castaneo irregulariter lobato nec spinoso; embryo ellipticus, 15–16 lineas longus, cotyledonibus crasso-carnosis (uno umbonato altero fovea magna excavato) apice rotundatis.

San Cristoval Island, Solomon group, Rev. R. B. Comins.

(3) **Chelonespermum fijiense**, Hemsl.

Arbor 50–60 pedalis (fide Horne). *Folia* coriacea, ut videtur, omnino glabra, longe petiolata, oblongo-lanceolata vel oblanceolata, cum petiolo 15–16 poll. longa et 4½–5 poll. lata, breviter obtuseque acuminata, sinuata, basi cuneata, venis transversis numerosis sat conspicuis, costa valida subtus elevata; petiolus bipollicaris, infra medium incrassatus. *Semen* compresso-ellipsoideum, circiter 2½ poll. longum et 1 poll. crassum, hilo versus apicem 1-spinoso, cætere lævi, testa castanea, prope marginem zona angusta pallidiore, ad marginem saturatiore, nitida, margine producto acuto supra medium obscure lobato; embryo ovalis, compressus, 2 poll. longus, medio circiter ½ poll. crassus, utrinque rotundatus, radicula minima.

Fiji Island, near the sea, Mr. J. Horne, 1878.

(4) **Chelonespermum unguiculatum**, Hemsl.

Semen orbiculatum, 2½–2¾ poll. diametro, hilo supra medium tuberculato-spinoso spino centrali valido circiter 9 lineas longo instructo, testa nitida saturato-castanea, prope marginem zona lata pallidiore, margine supra medium in lobos irregulares unguiformes producto.

Presented to the Kew Museum by the Rev. G. Henslow in 1882, without any indication of locality.

The fifth of these singular seeds I provisionally describe as a new genus.

Cassidispermum megahilum, Hemsl.

Semen fere sphæroideum, circiter 1½ poll. diametro maximo, hilo quam testa majore undique subæqualiter grosse indurato-muricato vel corrugato, processubus compressis, testa nitida pallide brunnea, margine vix pallidiore tenui basi emarginata supra medium irregulariter dentato-lobulata, lobulo terminali majore, cotyledonibus fere hemisphæricis cum testa et hilo angulum rectum formantibus.

Seeds presented to the Museum in 1874 by Mr. John Smith, the first Curator of the Royal Gardens, Kew, without any information of their origin; but in all probability they were from the Solomon Islands [1].

The name is given in reference to the resemblance to the insect genus *Cassida* when seen from above, though of course very much enlarged.

It is, perhaps, hardly justifiable to found a genus upon seeds alone; but this is a somewhat exceptional case. The seed is so different from *Chelonespermum* that it seemed undesirable to place it in that genus, and it is equally different from anything else that I am acquainted with. In *Chelonespermum* the broad flattened cotyledons are parallel to the dorsiventral seed-coat, if I may so call it, whereas in *Cassidispermum* the nearly hemispherical cotyledons are at right angles to the smooth and corrugated hemispheres of the seed-coat. In *Chelonespermum* the seed is attached laterally and the hilum vertical, and there is a much greater thickness of flesh or pulp on the hilum side or that side next the axis. The position of the seed in the fruit of *Cassidispermum* is unknown. In general appearance it is near *Calvaria* (*Sideroxylon*) *major* (Gaertn. Fruct. iii. t. 200), but that has copious albumen and thin cotyledons parallel to the differentiated faces of the

[1] Since the foregoing was put into type Mr. J. R. Jackson, the Curator of the Kew Museums, has brought to my notice two other seeds, exactly like those described, labelled 'Abyssinia, W. Plowden, Esq.'; but this brings us no nearer certainty as to their origin or native country.

seed, the testa is exceedingly thick, and the large hilum is represented as basal or inferior, instead of lateral as in *Chelonespermum*; and this position is confirmed by Dr. Baillon in his recent monograph. In *Lucuma—L. mammosum*, for instance—the large hilum is lateral as in *Chelonespermum*.

EXPLANATION OF THE FIGURES IN PLATES XI–XIV.

Illustrating Mr. Hemsley's paper on *Chelonespermum* and *Cassidispermum*.

PLATE XI.

Fig. 1. A branch of *Chelonespermum majus*, bearing the remains of a few flowers. Natural size.

Fig. 2. One of the floral remains. Enlarged.

Fig. 3. Back and front view of anthers found attached to the calyx by means of the web of some insect. Enlarged.

Fig. 4. Ovary from which the calyx has been removed. Enlarged.

Fig. 5. Vertical section of an ovary revealing one ovule much larger than the other, which ultimately disappears. Enlarged.

Fig. 6. Cross section of ovary and ovules showing attachment of the latter. Enlarged.

Fig. 7. Embryo enveloped in a broken film of tissue which forms a kind of marginal wing and is probably the remains of endosperm. Natural size.

Fig. 8. The same after removal of the film of tissue; the cotyledons slightly separated.

PLATE XII.

(All the figures natural size.)

Fig. 1. A ripe fruit of *Chelonespermum majus* in a dry condition.

Figs. 2, 3, and 4. Dorsal, ventral, and lateral views of seeds.

Fig. 5. A seed with figures engraved upon it by a native of the Solomon Islands.

Fig. 6. A seed from which the embryo has been excavated. Used as a match-box by the natives, according to Mr. Comins.

PLATE XIII.

(All the figures natural size.)

Figs. 1, 2, and 3. Different views of the seed of *Chelonespermum minus*.

Figs. 4 and 5. Different views of the embryo. The circular depression in figure 5 corresponds to the point of attachment of the seed.

Figs. 6, 7, and 8. Different views of the seed of *Chelonespermum fijiense*.

Fig. 9. Embryo with the cotyledons slightly separated.

PLATE XIV.

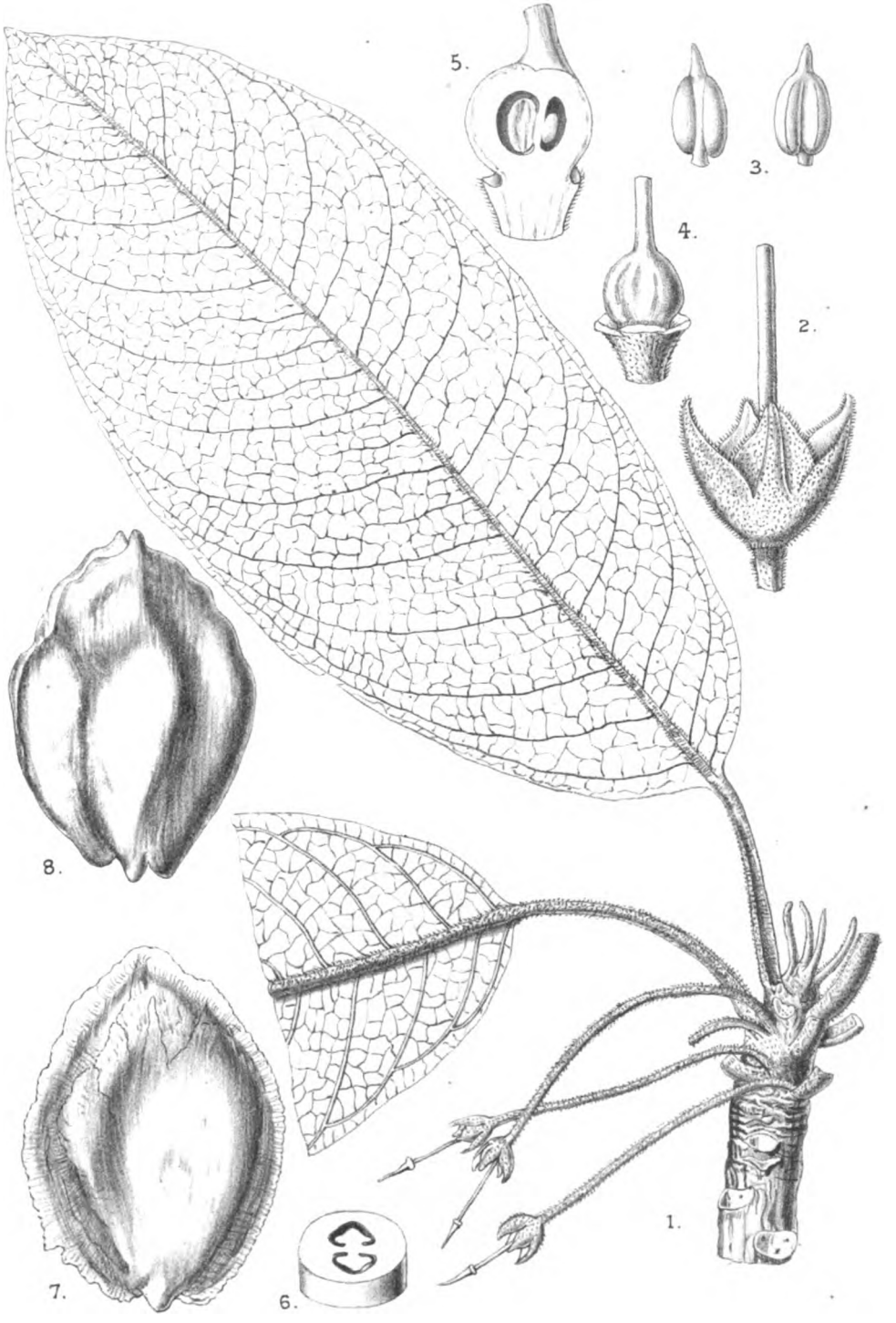
(All the figures natural size.)

Figs. 1, 2, and 3. Different views of the seed of *Cassidispermum megahilum*.

Fig. 4. Embryo which has lost its original shape through shrinking.

Figs. 5, 6, and 7. Different views of the seeds of *Chelonespermum unguiculatum*.

Fig. 8. Embryo, with the cotyledons slightly separated.



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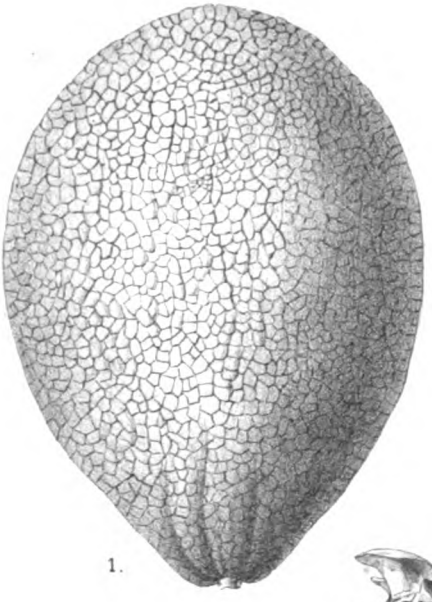
CHELONESPERMUM MAJUS, Hemsl.



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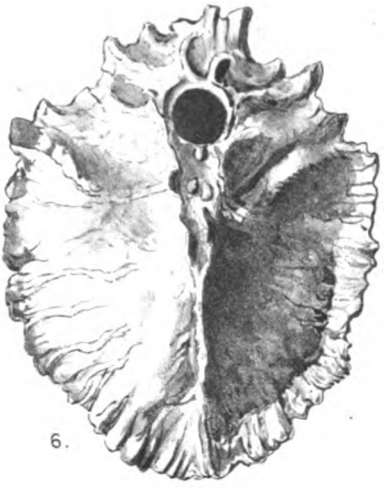

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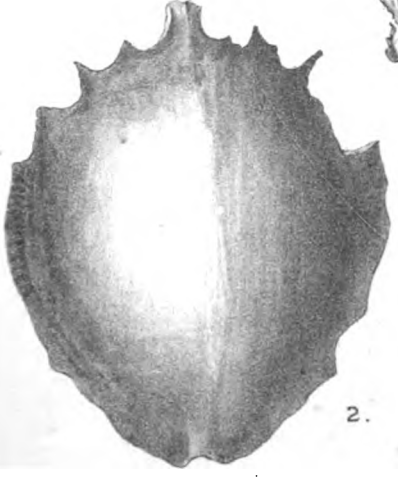

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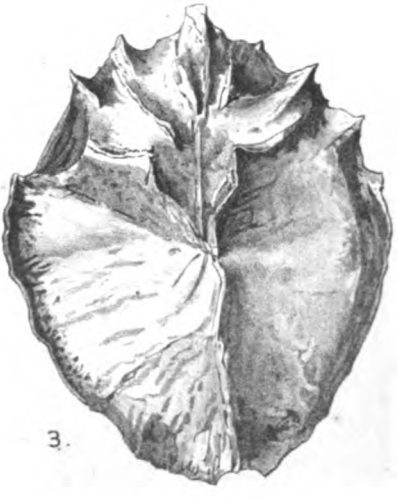

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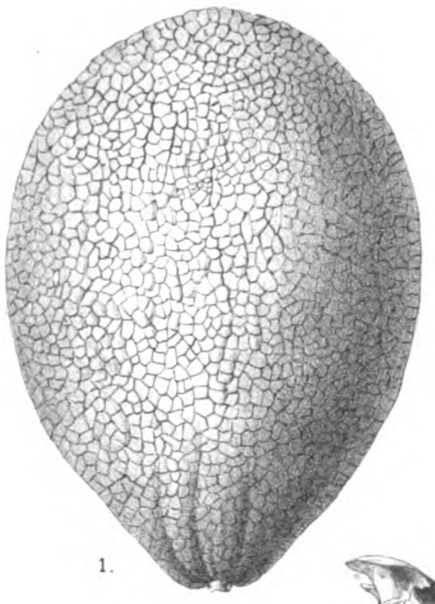
CHELONESPERMUM MAJUS, Hemsl.



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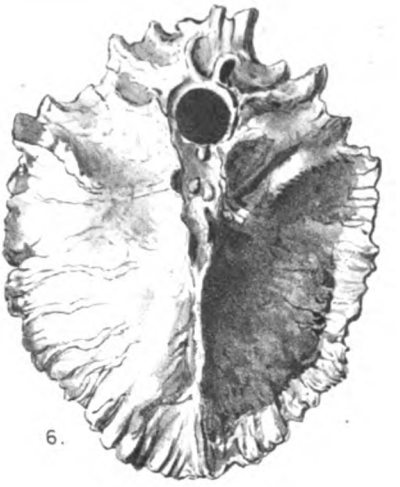

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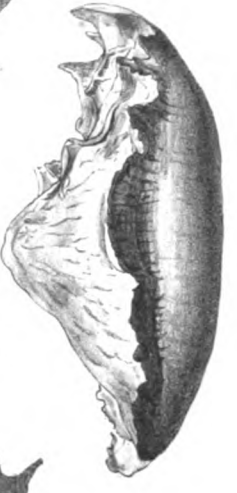

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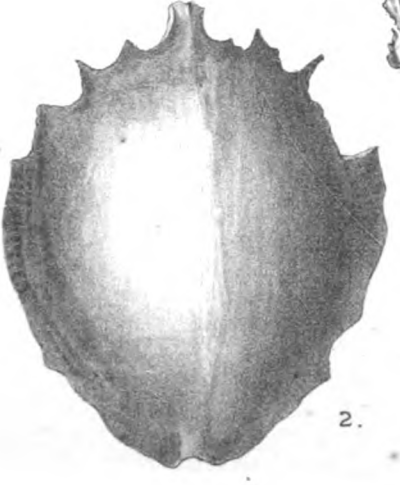

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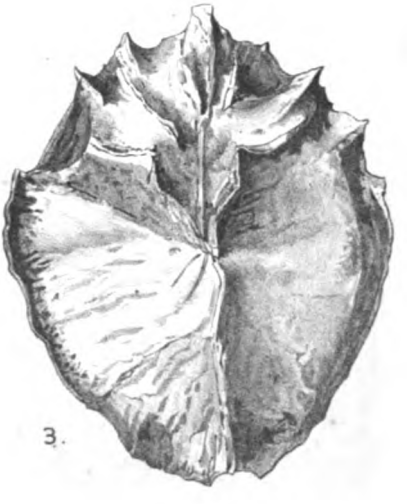

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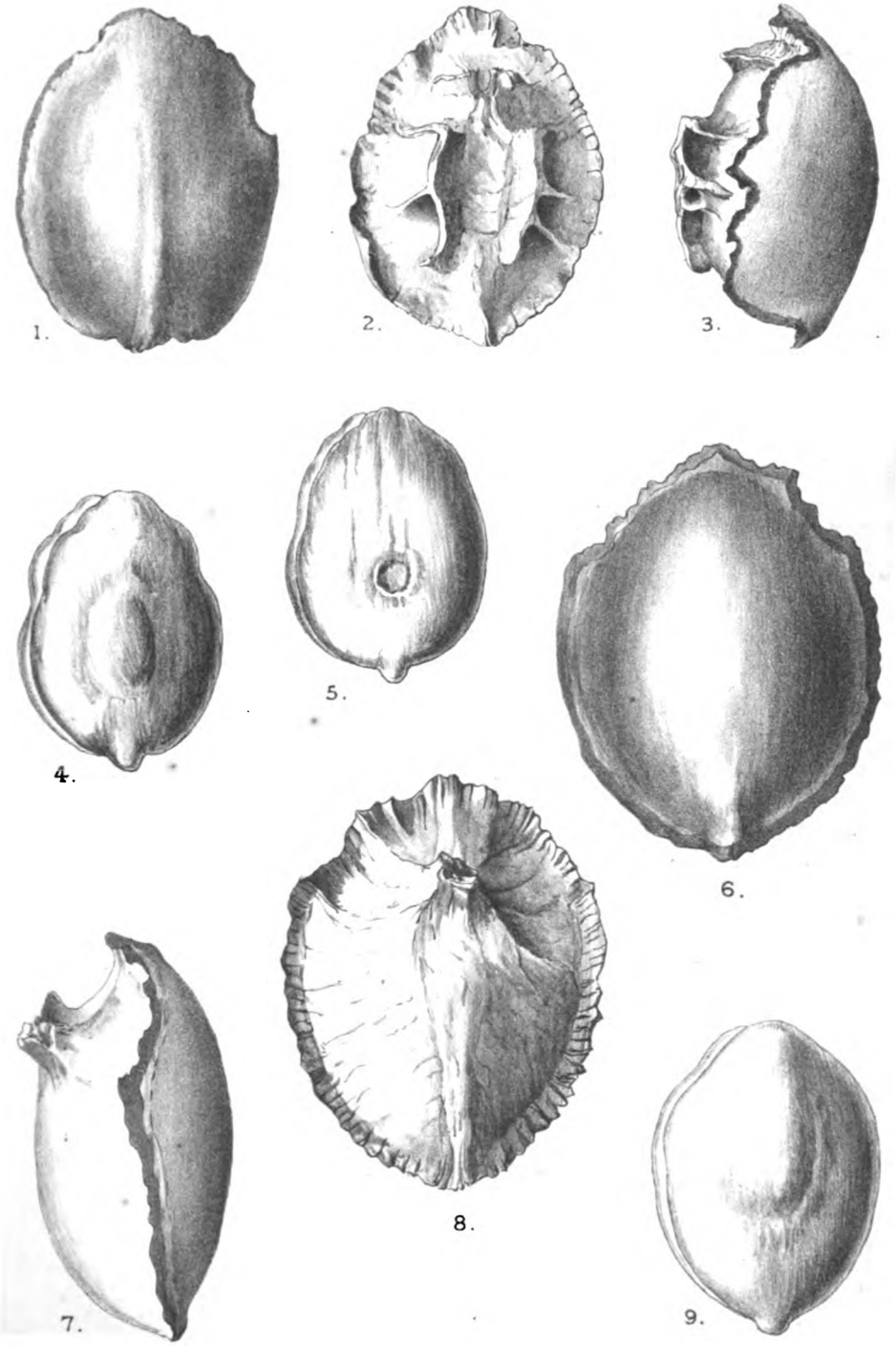

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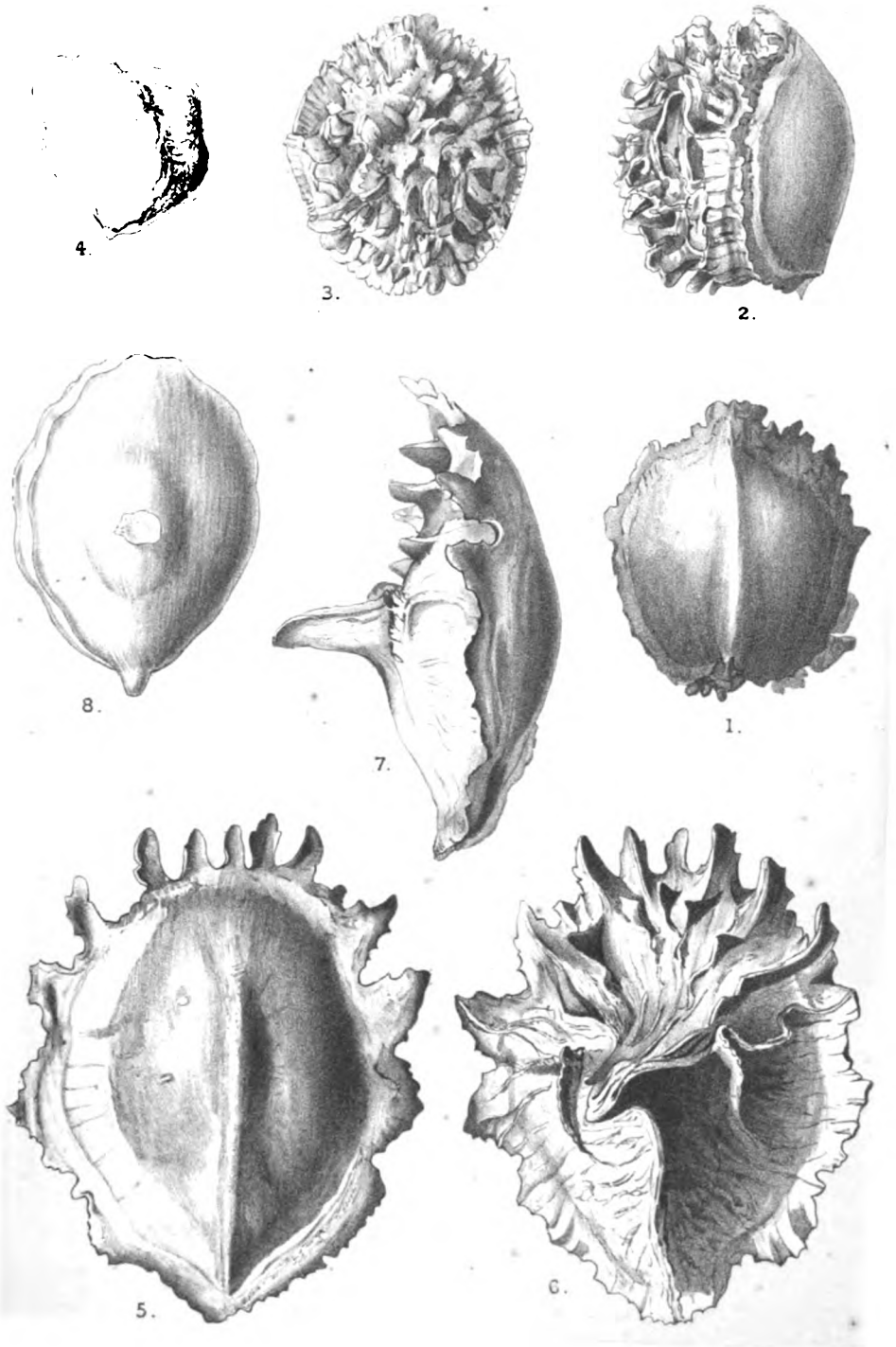
FIGS 1-5, CHELONESPERMUM MINUS, Hemsl
FIGS 6-9, CHELONESPERMUM FIJIENSE, Hemsl.



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FIGS 1-4, CASSIDISPERMUM MEGAHILUM, Hemsl
FIGS 5-8, CHELONESPERMUM UNGUICULATUM, Hemsl

NOTES.

ON ABNORMAL FLOWERS IN ONCIDIUM SPLENDIDUM.—The examples of abnormal flowers in Orchids are very numerous, and if I venture in this note to add yet another to the long list of recorded monstrosities, I do so because I think the case may serve to illustrate some points of interest.

During the present year I observed a spike of *Oncidium splendidum* on which two of the flowers were abnormal, all the others being normal. The lower of the two flowers (which were on the same orthostichy) exhibited an increased number of perianth-leaves. The

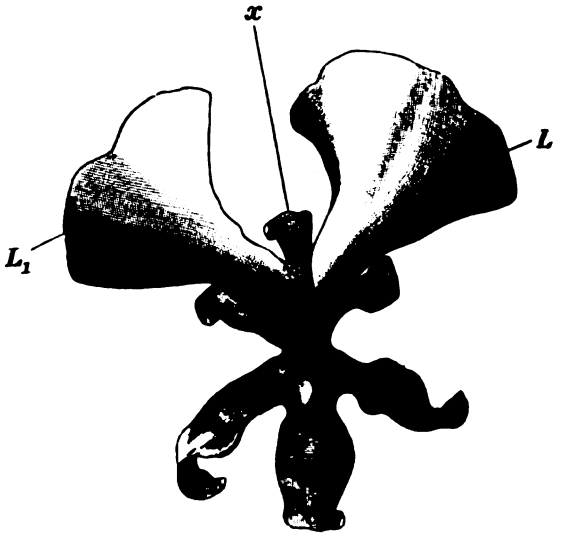

Fig. 3.

L L_1. Lobes of the split labellum. *x*. The new perianth-leaf.

ordinary flower possesses five sepaloid perianth-leaves, and a bright yellow labellum, but the flower in question showed six sepaloid leaves and a double labellum (see Fig. 3). It will be noticed that the labellum is split completely down to the base, and each half exactly resembles, on a slightly smaller scale, the labellum of an ordinary

flower. The new perianth-leaf arises in the space left by the diverging halves of the labellum, but slightly behind them, whilst it is also decidedly internal to the two posterior sepals on each side of it. Probably, therefore, it represents a later development, which arose in the space left vacant by the splitting of the primordium of the labellum and the divergence of its two lobes. The flower was normal in all other respects.

The second flower (Fig. 4) on the other hand exhibited a reduction in the number of its perianth-leaves. The two posterior sepals had coalesced, and had assumed a median position. The labellum was only very slightly developed, especially on one side, on which it bore

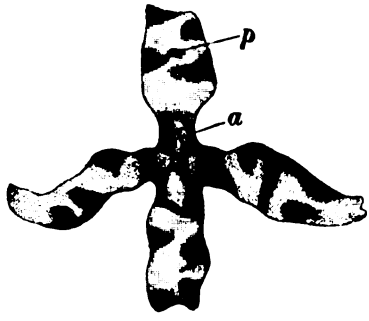

Fig. 4.

a, Abortive labellum with pollen-sac. *p*. The two coalesced posterior sepals.

a pollen-sac. It is probable that this pollen-sac may represent one of the normally suppressed postero-lateral stamens of the outer whorl. One of its special features of interest consists in its relatively late development, for though the flower was fully expanded, and the pollen was quite ripe in the normal anther, in this one the pollen-mother-cells were only beginning to become clearly differentiated.

Possibly the arrested development of the labellum may be due to the appearance upon it of the sporangium; just as the normally vegetative leaf of *Botrychium* assumes the contracted appearance of the sporophyll of this plant when sporangia occur upon it; or as the expanded petals of *Nymphaea* become contracted and filamentous as the pollen-sacs become more and more prominent. It may, however, be urged that the reduction in size is entirely due to the lack of space wherein to grow, owing to the coalescence of the posterior

sepals; but against this it may be said that the somewhat fleshy consistence, which characterises the reduced labellum, renders it difficult to see how one can account for its small size on this supposition, since its actual shape demands more room than if it had developed normally. On the whole, then, the former hypothesis seems the more likely one, namely, that the diminution of size of the labellum is the result of the occurrence of the pollen-sac or sporangium upon it; and this view is borne out by many other teratological facts connected with the abortion or alteration of ovules and pollen sacs in other flowers, as well as by the characters of fern-sporophylls, whenever they differ from the vegetative leaves.

J. BRETLAND FARMER, Oxford.

ON THE OCCURRENCE OF TWO PROTHALLIA IN AN OVULE OF PINUS SYLVESTRIS.—The diagram which accompanies this note represents a somewhat remarkable abnormality in the development of the ovule of *Pinus sylvestris*; and as I have not met with any notice of a similar case, either in this plant or in any of its immediate allies, it seemed worth recording. The abnormality consists in the occurrence of two distinct endosperms or prothallia in the same ovule. They are separated by a well-marked wall, which runs obliquely between them and is continuous with the lateral walls of the cavity containing them. The upper prothallium (that nearest the micropyle) is somewhat smaller than the other one, but both possess perfectly developed archegonia (*a*, in the diagram), and the protoplasm of the central cell in each archegonium exhibits the frothy vacuolation characteristic of that of a normally formed corpusculum.

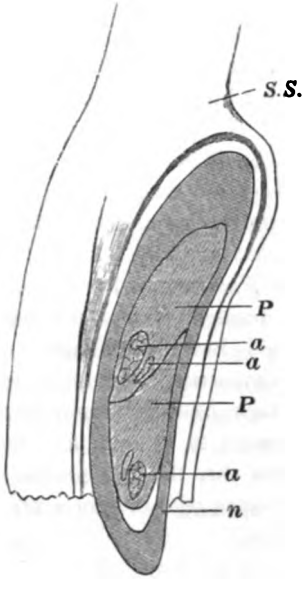

Fig. 5.

The question arises as to how the two chambers, in which the prothallia respectively lie, have been formed. Judging from the

obvious continuity of the dividing wall with the lateral walls of the prothallial chambers,—a continuity which is clearly shown through a series of mounted sections of the ovule under consideration, there can hardly exist a doubt that the two cavities result from a primary transverse division of the cell which would normally become at once the macrospore, but which in this instance has given rise to *two* macrospores. It will be remembered that in the normal development of this structure as described by Strasburger,[1] the embryo-sac mother-cell divides into an upper cell which undergoes a further division, and into a lower one which normally becomes at once the macrospore. Perhaps the ordinary behaviour of the latter cell is due to the supression of a further division, such as would have caused the original mother-cell to become the parent of four cells, as in the case of spore-formation in the Vascular Cryptogams. If this be so, the present exception to the ordinary course of development in *Pinus*, acquires a special interest as affording an example of a mode of spore-formation at least analogous with that which obtains in the higher Cryptogams. The fact, however, that *both* of the lower cells develop, instead of one of them becoming abortive like the two uppermost cells, shows that such a hypothesis should be received with caution, even if the development of the embryo-sac in Gymnosperms were far less uniform than is actually the case.

In some other members of the Coniferae, in *Thuja* for example, several mother-cells are differentiated, but only one macrospore normally reaches maturity. It might be suggested that the two chambers in this *Pinus*-ovule have possibly arisen by the development of two independent mother-cells, such as are formed in the plant just mentioned, but the intimate connection of the two superposed cavities renders a suggestion, which implies anything like an independent origin in their case, extremely improbable. But whatever explanation be admitted to account for their abnormal character, there can be no doubt that the two prothallia have been formed only after the two enclosing cells or spores were differentiated.

J. BRETLAND FARMER, Oxford.

ON THE SYNONYMY OF ANTHOCOMA FLAVESCENS, ZOLL.—A Labiate genus, *Anthocoma*, was proposed by Zollinger in

[1] Strasburger, Die Angiospermen und die Gymnospermen, p. 114.

1846 to accommodate a plant collected by him in Java. This plant (*Anthocoma flavescens*, Zoll.) was redescribed in the following year by Hasskarl who, however, at the same time suggested that it might be the same as Zollinger's own *Gomphostemma dichotomum*; this suggestion was adopted, without seeing a specimen, by A. de Candolle in 1848. Miquel in 1856, though pointing out that the accounts given by Zollinger and Hasskarl are opposed to this specific identification, nevertheless, and again without seeing specimens, has treated the plant as a *Gomphostemma*. On the other hand Bentham, who also has had to work without a specimen, has never admitted this reduction and, as recently as 1876, has suggested that, from the descriptions, the plant might perhaps be a *Phlomis*. The writer has had occasion to point out in another place[1] that *Anthocoma flavescens*, as described by Hasskarl, could not be a *Phlomis*, but owing to the absence of specimens and from the inadequacy of the published descriptions, was then compelled to leave its true position an open question.

Since writing the note referred to the writer has been enabled to examine specimens of this obscure plant kindly sent by Dr. Treub from the Buitenzorg Herbarium to that of Calcutta. These prove that *Anthocoma* is not entitled to generic rank, but at the same time show that it is neither a *Gomphostemma* nor a *Phlomis*.

The plant is in fact Miquel's own *Cymaria mollis*; as however this Java plant is conspecific with *Cymaria acuminata* from Timor, the species to which the name *Anthocoma flavescens*, Zoll. must henceforth be definitely referred as a synonym is *Cymaria acuminata*, Decaisne.

The full synonymy of this species is subjoined and is followed by a few critical remarks supporting the formal reductions. In presenting it the writer wishes to express his own gratitude to Dr. Treub for having afforded an opportunity of finally disposing of a synonym that has, for the past forty-five years, been a puzzle to students of Labiatae.

Cymaria acuminata, Decaisne, in Herb. Timor. Descript, 71 (1835); De Lessert, Icones Selectae, iii. 51, t. 86 (1837); Benth. *in* DC. Prod. xii. 602 (1848); Miq., Flor. Ind. Bat. ii. 992 (1856).

Cymaria mollis, Miq. Flor. Ind. Bat. ii. 992 (1856).

[1] Ann. Roy. Bot. Garden, Calcutta, iii. 231 (1891), footnote.

Anthocoma flavescens, Zoll. *in* Nat. en Geneesk. Arch. ii. 569 (1846); Hasskarl, Flora, xxx. 596 (1847).

Gomphostemma dichotomum, Zoll. et Mor.? Hasskarl, Flora, xxx. 596 (1847).

Gomphostemma dichotomum, A. DC. *in* Prod. xii. 552 (*ad calc.*) *et* xii. 700 (1848), *nec* Zoll. et Mor., Syst. Verzeichn., *nec* Walp., Rep. vi., *nec* Benth. *in* Prod. xii.

Gomphostemma flavescens, Miq. Flor. Ind. Bat. ii. 987 (1856).

Phlomis ? sp., Benth. *in* Gen. Pl. ii. 1216 (1876).

The description by Decaisne and the figure in De Lessert, which Decaisne drew, indicate a plant with glabrous leaves, whereas the Java plant (*Cymaria mollis*) has leaves hirsute above and below. But the *description* by De Lessert, which he states is based on fuller material, points out that even the Timor plant may have leaves puberulous on both surfaces. In Java too the character is variable, the examples of *Anthocoma flavescens* being more hirsute than some of those on which Miquel founded his *Cymaria mollis*. The character, which is the only tangible one in Miquel's diagnosis of the two plants, is at best a trivial one; the flowers and fruits of the Timor and the Java plants so precisely agree that it is impossible to doubt that they are conspecific.

In the Icones Selectae the nutlet figured is glabrous; Decaisne's own description, however, as well as those of De Lessert and of Hasskarl, indicate correctly that the nutlets are hirsute at the apex.

In Decaisne's original description, and also in the figure in De Lessert, the true condition of the anthers is expressed; these are not merely (as stated in Gen. Pl. ii. 1222) two-celled with cells becoming divaricate, but become ultimately by confluence one-celled. The condition of the anthers in *Cymaria elongata* is precisely the same[1]; probably therefore the character should be added to the generic description.

Hasskarl has described the corolla-tube of *Anthocoma flavescens* as hirsute within; the corolla-tube is however quite devoid of any annulus or hirsute patch within; the *filaments* are hirsute at the base in this species, as they also are in *Cymaria elongata*, and this condition may have led to the statement which, though inexact, has not produced any serious consequences. The anther-cells, probably from only those of

[1] Unfortunately the solitary specimen of *Cymaria dichotoma* at Calcutta is in fruit only, so that no opinion regarding its anthers can be expressed.

flowers that were too young having been examined, have been unfortunately described by Hasskarl as two-celled with parallel cells; it is doubtless this unhappy misapprehension that has led both De Candolle and Miquel to look upon *Anthocoma* as a *Gomphostemma*, and that has obscured so completely and so long the true affinities of Zollinger's plant.

DAVID PRAIN, Calcutta.

NOTICE OF BOOK.

E. STRASBURGER: UEBER DEN BAU UND DIE VERRICHTUNGEN DER LEITUNGSBAHNEN IN DEN PFLANZEN. Histologische Beiträge, III; Jena, Fischer, 1891.

Professor Strasburger, who for many years devoted himself almost exclusively to the investigation of the problems of minute histology, has in the present work made the most important recent contribution to the anatomy and physiology of vascular plants. If some of his former researches may claim a wider interest, as dealing with questions which are common to both the organic kingdoms, his latest book is, from a purely botanical point of view, of at least equal importance. This is owing, not only to the value of the special results attained, but also to the fact that this book presents the most striking example in botanical literature of combined anatomical and physiological investigation.

As a rule the anatomist and the physiologist work independently. The former either tends to ignore function altogether, or perhaps is too ready to infer function from structural evidence alone, without the test of experiment. The physiologist on the other hand has often trusted too exclusively to experimental methods, and has perhaps even treated anatomical details with a certain contempt. There are few men who are capable of equal success in both directions; among these few Prof. Strasburger is the most distinguished.

The motive of the book, and the point of view of the author, are made clear in the preface. Some years ago Prof. Strasburger began

experiments on the vexed question of the ascent of water in the wood. He became absorbed in the problem, and in order to obtain a firm basis for the investigation, found it necessary to undertake a minute anatomical examination of the conducting tissues. This part of the enquiry occupied fully two years (a short enough time considering the magnitude of the results attained), and involved the consideration of purely morphological questions. Subsequently, in the light of these anatomical data, the physiological experiments were taken up again, and led to conclusions of the greatest importance.

The author has made a point of strictly severing the morphological from the physiological side of his problem. He points out that the morphology of the tissues, like that of external organs, must be entirely uninfluenced by considerations of function. The task of morphology is to trace the derivation of one form from another, to refer different forms to a common origin. This is essentially a question of phylogeny; it can only be dealt with by means of the comparative method, and by the study of individual development. To these the reviewer would be disposed to add the direct evidence of palaeontology, which on the anatomical side is by no means unimportant.

The one object then of morphological anatomy is to establish the homologies of the tissues. There is also a physiological anatomy which has the equally important task of classifying the tissues by their functions. It is necessary to keep the two objects distinct, and to realise that a physiological classification has nothing to do with morphology.

Prof. Strasburger's clear exposition of the twofold purpose of anatomy is likely to be very serviceable. The mere description of internal structure, is in itself just as barren as that of external form, and anatomy in this narrow sense, has no claim to the rank of a science, as Nägeli long ago pointed out. The value of anatomy depends on the facts that it is at once an integral part of morphology, and the necessary basis of physiology.

The book contains about 1000 pages, which are almost equally divided between the anatomical and physiological portions. The first part begins with an extremely full investigation of the structure of the vegetative organs in Coniferae. It is only possible to enumerate a few of the most striking results here, though the completeness of the description is perhaps its greatest merit.

It is already known that many of the Abietineae have medullary rays of complex structure, the wood-rays containing water-conducting tracheides, as well as living parenchymatous cells. On the other hand we also know that most Coniferae have bordered pits on the tangential surfaces of the latest formed autumn-wood. The author shows that the development of these two structures varies inversely. All Conifers which are destitute of tracheides in their rays, form tangential pits on their autumn-wood, while those in which the ray-tracheides are best developed, have few or no tangential pits. Both structures in fact serve the same purpose of providing a radial connection between the water-conducting tissues of successive annual rings.

The author confirms Russow's observation of the constant presence of intercellular spaces, containing air, between the elements of the rays, both in Gymnosperms and Dicotyledons. The living cells of the rays communicate by pits with these spaces, which are continuous through the cortical tissues with the lenticels or equivalent openings in the periderm. Thus the respiration of the deep-seated living elements of the wood is provided for.

Among the most important of the author's discoveries is that of the physiological representatives of the companion-cells, in Gymnosperms, in which these elements, as such, are never present. The chief conclusions (some of which the author published in a previous paper) are here summed up as follows: (p. 55)

In the Abietineae the functions of the companion-cells are fulfilled by certain rows of cells belonging to the medullary rays of the phloëm. In a portion of the Cupressineae and Taxodineae these functions are divided between the specialized ray-cells and other series of elements forming part of the bast-parenchyma. In the remainder of these two tribes, and in the whole of the Taxineae and Araucarineae it is the bast-parenchyma alone which is concerned. In all these cases the function of the cells in question is inferred from their especially abundant protoplasmic contents, from the entire absence of starch when they are fully developed, from the fact that they reach maturity simultaneously with the sieve-tubes, and also become emptied and obliterated at the same time with them; lastly, from the fact that they are connected with the sieve-tubes by pits of peculiar structure, resembling one-sided sieve-plates. As regards the sieve-plates themselves, the author decides that in the Coniferae they are never really open in the functional sieve-tube. The plug of swollen middle-lamella

by which the continuity of the protoplasm is interrupted, does not, he believes, offer any hindrance to the passage of dissolved substances, though it renders the transference of living protoplasm impossible.

The callus is stated to be formed directly from the protoplasm; it is regarded as a by-product, rather than a reserve-substance; in some cases it becomes of functional importance as effecting the temporary closure of the sieve-pit.

The arrangement, both of the true companion-cells in Angiosperms, and of their representatives in Gymnosperms, shows that they cannot serve for the longitudinal conduction of food-substances. Their function is rather to receive the albuminous material conveyed by the sieve-tubes, and ultimately to pass it on to developing tissues.

The author finds that the arrangement of the sclerenchymatous elements in the bast of most Conifers is such as to exclude the possibility of mechanical function. In such cases these elements are regarded as serving simply for the deposition of excessive cellulose, formed as a necessary result of metabolic processes in the starch-containing cells (p. 77). It is difficult to believe in so great a waste of carbohydrate material, and some local mechanical use (e. g. the hardening of the bark) may perhaps be conjectured.

Prof. Strasburger points out that the primary structure of the phloëm in Abietineae resembles the permanent structure in Araucarineae and Taxineae. This anatomical fact, in connection with other morphological points, and with the results of Palaeontology, leads the author to regard the two latter families as relatively primitive forms, and the Abietineae as the most modified group of Conifers.

The careful re-investigation of the leaves of Coniferae has led to some interesting results, of which the most important is perhaps the discovery of 'albuminous cells' forming an extension of the phloëm of the leaf-bundles, just as the transfusion-tracheides form an extension of their xylem. These albuminous cells no doubt have the same function as the terminal phloëm-elements of the fine branches of the bundle-system in Angiospermous leaves. In both cases this function is to absorb from the mesophyll the nitrogenous products of assimilation, which are destined to be conducted elsewhere by the sieve-tubes.

In *Pinus* the central-cylinder of the leaf is alone continuous with the stem. Hence all the assimilated food must pass through the tissues of the cylinder (vascular bundles and conjunctive parenchyma). This observation leads to a general discussion of the internal morphology

of stem and leaf, which is of fundamental importance. All the parts of the central cylinder of the leaf in *Pinus* are continuous with the corresponding tissues of the stem, and ultimately with those of the root. It follows (p. 111) that in *Pinus* two tissue-systems, that of the central-cylinder and that of the primary cortex, run separately throughout the entire plant. This morphological separation coincides on the whole with distinctness of function. The cortical tissue is the great assimilating system; the central-cylinder assumes the function of conduction. 'As it is in *Pinus*, so is it in essentials in all vascular plants, while the distribution of the two tissue-systems in the plants, and their mutual delimitation, are also facts of general application.'

Thus the point of view of the French anatomist Van Tieghem is definitely adopted by the chief German authority; the central-cylinder is recognised as an anatomical region of the first order, of which the vascular bundles are subordinate parts. The importance of this conception, which profoundly modifies the anatomical teaching of Sachs and De Bary, is evident all through the book.

A section on the vegetative structure of Gnetaceae brings out the general agreement with Dicotyledons rather than with Gymnosperms. The chief anatomical characters which indicate the true gymnospermous affinities, are to be found in the structure of the phloëm, and in the indications of transfusion tissue in the leaves.

The investigation of the Cycadeae has not yielded much which is new. The structure of the phloëm is that of the simpler Coniferae.

The anatomy of a large number of dicotyledonous types is described in the fullest detail. The value of these minute researches can scarcely be fully appreciated except by those who are themselves engaged in such investigations, a remark which applies in a great degree to the whole book. The wood of Dicotyledons turns out to be even more complicated than was supposed before. In many of these plants, for example (e.g. *Salix*), both the medullary rays and the xylem-parenchyma consist of two kinds of cells. In the case of the rays they may be distinguished as horizontal and vertical cells. The former contain abundant starch, and communicate by pits with the intercellular spaces, but not with the vessels; the latter are destitute of starch in summer, and are in communication with the vessels, but not with the intercellular spaces. The former serve chiefly for the conduction of assimilated substances, and for gaseous interchange; the latter communicate with the tracheal system. This communication serves

a double purpose; first, to supply the living elements themselves with water, and with certain salts, especially phosphates, which they can directly assimilate (p. 868); and further, to allow of the passage of organic substances, especially carbohydrates, into the tracheae, by which they are rapidly conducted to the growing regions, at least during the spring (p. 894).

The Vine is studied with special minuteness, and among many points of interest the author calls attention here to the emptying of the sieve-tubes and companion-cells in winter, a fact which is quite inconsistent with the function of food-reservoirs, attributed to these elements by Frank and Blass.

The description of the wood of the Oak brings out a point of the highest morphological importance. The author regards the xylem generally as consisting primarily of two forms of tissue only—the tracheae and the parenchyma. In the great majority of woods the mechanical elements (fibres) belong to the parenchymatous system, as is indicated by their simple pits, and proved by the presence of transitional forms. In the Oak, however, and probably in all Cupuliferae, as well as in Rosaceae and a few other cases, the so-called fibres have bordered pits, and pass over through intermediate forms into the tracheides. In these cases, in fact, the mechanical elements are homologous with the tracheal system, while in most woods they have arisen by modification of the parenchyma. This shows well how misleading a physiological classification of tissues may be from a morphological point of view.

The author's conclusions as to the wood are also extended to the bast. Here we have on the one hand the cribral system, to which the sieve-tubes and their companion-cells (if present) belong, and on the other the parenchymatous system, from the modification of which the bast-fibres have been derived. In the Gymnosperms, however, we have seen that the functional equivalents of the companion-cells belong to the parenchymatous system. Similar views of the morphology of the phloëm had already been expressed by Lecomte.

The phloëm of *Cucurbita* has once more been fully investigated. Here also the author finds that the callus is derived directly from the protoplasm, its formation beginning in the pores of the sieve-plates. The function of the living protoplasmic layer lining the walls of the sieve-tubes consists, according to the author, in preventing diffusion

from the tube, and in providing material for the formation of callus in order to close the plates when necessary. From the fact that the upper phloëm of the bicollateral bundles in the leaf of *Cucurbita* is already empty at a time when the normal lower phloëm is in full activity, it is inferred that the former fulfils its function during the development of the leaf, serving to conduct to it the necessary food-supplies, while the normal phloëm is alone concerned in conveying the products of the leaf's own assimilation. It will be interesting to observe how far this distinction holds good in other cases of double phloëm.

In describing the anatomy of various Ranunculaceae the author calls attention to the close agreement in structure between the vascular bundles of this order, and those of the Monocotyledons.

The first monocotyledonous type described is the familiar *Zea Mais*. The course of the vascular bundles is traced in detail, and it is shown that each bundle thins out greatly before fusing with one below it. Hence at every point of fusion there is a marked constriction of the water-conducting channels. From this fact, as well as from many other observations and experiments pointing in the same direction, the author infers that very narrow tracheal strands are sufficient to conduct the ascending current of water. The large vessels are important for storage rather than for conduction.

The phloëm of the bundle, as well as its xylem, tapers in the downward direction. The effect of this is that each row of sieve-tubes and companion-cells is in its turn brought into contact with the surrounding parenchyma, to which it can thus pass on the nitrogenous food-supplies.

The anatomy of several Palms is investigated, and it is shown, in agreement with Eichler, that when growth in thickness takes place it is due entirely to the extension of the inter-fascicular parenchyma.

The Monocotyledons with true secondary thickening receive much attention. The author points out that the thickening-ring here differs from the true cambium of Dicotyledons or Gymnosperms in the fact that there is no single initial row of cells to the divisions of which all the secondary tissues can be traced. On the disputed question of the nature of the secondary 'tracheides' in these plants, Prof. Strasburger entirely confirms the opinion of Krabbe and Röseler, that these elements are really tracheides, formed by the elongation of single cells, and not 'short vessels,' arising by cell-fusion, as was maintained by Kny and others. Some valuable observations on the remarkable

secondary growth of the roots of these plants are recorded, some of which, however, were anticipated in the first edition of the author's 'Practicum.'

The anatomy of vascular Cryptogams is disposed of rather rapidly. As regards *Equisetum* a new interpretation of the structure of the bundle is given. It is also proved that here, as in Grasses, the intercellular space accompanying the xylem is always filled with water, not with air, even in those species in which each bundle is enclosed within an endodermis of its own.

Van Tieghem's interpretation of the prevailing structure of the stem in Ferns as 'polystelic' (each 'concentric bundle' of De Bary representing an entire cylinder like that of a root), is adopted by the author, who, however, differs from the French anatomist as to the morphology of the phloëm-sheath. This layer is regarded by Prof. Strasburger as constantly belonging to the cortex, while Van Tieghem finds that in many Ferns it is the homologue of the pericycle. That the phloëm-sheath and endodermis are often sister-layers is certain, but it is possible that in these cases both layers may belong to the cylinder, as was suggested by J. E. Weiss. These difficulties of delimitation occur in all morphological questions, and no doubt the problem is often insoluble.

In many Ferns the tracheides form a thick and uniform strand, in which no living cells are interposed, so that here at any rate there is no room for the vitalistic theory of water-conduction.

The central cylinder of *Lycopodium* is regarded as 'gamostelic,' in Van Tieghem's sense, i. e. as representing a fusion of a number of 'steles' like those of *Selaginella*. This view does not appear to rest on any sufficient developmental or comparative evidence, though as a mere description of the mature structure it is appropriate enough.

The author devotes a chapter to a summary of his anatomical results, but this has been to a great extent anticipated in our survey. We have already called attention to the adoption of the general anatomical conceptions of Van Tieghem. It is unnecessary here to follow the discussions which have arisen between the two investigators on points of detail. The idea of the cylinder or stele as a primary anatomical region, superior to the vascular bundles, appears to the reviewer to be a fruitful one, chiefly for two reasons. First, it enables us to understand the homologies between root and stem, which were to a great extent obscured by De Bary's treatment of the central-

cylinder of the root as a single 'radial' bundle. Secondly, it throws great light on the structure of the lower vascular plants, in which we may either have a single cylinder, not differentiated into individual vascu ar bundles at all, as in the simpler Ferns and in the fossil Lepidodendra, or a number of cylinders, each complete in itself. The confusion of these latter (under the name of concentric bundles) with the vascular bundles of the higher plants was another weak point in the anatomy of De Bary's school. On these main questions it seems pretty certain that the anatomy of the future will follow the lines of Van Tieghem and Strasburger; the differences between them are comparatively of trifling importance.

A chapter on the connection of the vascular bundles as affected by the growth in length and thickness in the stem and root, does much, with the help of the diagrams, to clear up this rather difficult subject. It is pointed out that the protoxylem of each new shoot is continuous, not with the protoxylem of the next older shoot, but with its later-formed wood. In this way only can a continuous water-channel be maintained, for the protoxylem of the older parts will have already become disorganized and useless, at a time when a new shoot is formed. Similar considerations apply to the phloëm. In the stem the new layer of thickening from the cambium, starts each year from the top, in immediate connection with the vascular bundles of the young shoots, and thence advances down the tree. The reason why the outer zones of secondary wood are always the most active in conduction, is because these alone are in direct continuity with the youngest shoots and their leaves. So far as the wood is concerned this is modified by the presence of tangential pits, and similar contrivances; in the phloëm there is usually no such provision for communication between successive zones; hence, as a rule, only the youngest layer of phloëm, which is in direct connection with the leaves, is functional. This does not of course apply to Monocotyledons without secondary thickening, in which the same phloëm may remain active for years.

A section on the width and length of vessels introduces the strictly physiological portion of the book. On the former point there was not much to be done. The widest vessels, as De Bary had already shown, are always pitted vessels with short joints. The greatest dimensions were found in a leguminous liane (*Mucuna*) where the vessels attain 0·6 mm. in diameter.

The determination of the length of the vessels was a more difficult

matter. The method adopted was to inject pieces of the stem with mercury, under pressure, and to observe the length of stem through which the mercury could be made to pass, and the number of vessels through which it escaped, on the lower cut surface. The general result is that while the vessels vary much in length they are on the whole much shorter than De Bary and most anatomists supposed. Among the longest vessels are those of the Oak, which are often two metres in length, and some of which may even extend the whole length of the stem. This, however, is an extremely rare case; in almost all plants the length of each continuous vessel is a mere fraction of that of the entire path of conduction.

Of the physiological half of the work the greater part is devoted to the question of the ascent of water in the wood. A repetition of the familiar 'ringing' experiments was first undertaken, with the result of proving once more that only the wood conducts the water-current, and that in the wood only the living alburnum is functional. In spite of this latter fact the author, on the ground of experiments to be mentioned below, does not admit that living cells take any part in the conducting process. The non-conductivity of dead wood depends on its comparative dryness, not on the absence of living elements.

Some general remarks on the whole question serve to define the author's point of view, and to introduce the experimental work. Prof. Strasburger, having convinced himself that the water-current passes through the *cavities* of the tracheae, was at first disposed to accept the 'vitalistic' theory, according to which the protoplasm of the living wood-cells, either by active contraction, or by its influence on osmosis, plays an essential part in pumping up the water. His experiments, however, have led him to the opposite conclusion, that the ascent of water in plants is a purely physical process, though one of which Physics is not at present able to give a full explanation.

The author has certainly established a very strong case, and in the face of his experiment the theories of Westermaier and Godlewski no longer appear tenable.

He summarises his results as follows (p. 539):—That the ascent of water in the plant is a physical and not a vital process was first proved by experiments with plants more than 10½ metres in height, which were caused to take up poisonous solutions. Corresponding results were attained with plants previously killed by other methods. The conditions necessary for the ascent of water are: (1) that the

cell-walls should be in a state of imbibition, (2) that the cavities of the tracheae should be to a certain extent filled with water, and (3) that they should be isolated, so as to exclude the entrance of air. Atmospheric pressure helps to keep the water suspended, but does not cause its ascent. Transpiration is only important in so far as it makes room for the ascending water. Should the supply of water be deficient, certain of the tracheal channels are emptied and closed. In such closed tracheae a very low pressure prevails, which is maintained until they can be refilled with water. The difference between the atmospheric pressure and that in the tracheae, great as it is, is not usually sufficient to force air into the emptied elements. Root-pressure is not immediately concerned in the ascent of water.

As the insufficiency of capillarity has long been established, we are led, as the net result of the most elaborate investigation of the question which has yet been made, to a purely negative result; the cause of the ascent of water in trees is still unknown. A great point is however gained, if we may take it as proved that the process is a purely physical one. The protoplasm, which is responsible for so much, has at least been relieved of one strictly mechanical function, and the problem of the sap is thus brought within a measurable distance of solution. The author's work does indeed afford some indications (e. g. the movement of the film of water between the air-bubbles and the walls of the tracheae) which, if taken up by physicists, may, he hopes, lead to the long-sought explanation.

The details of the experiments must be read in the original. A summary of them would far exceed the limits of this review. In every case the minute acquaintance which the author has gained with the anatomy of the plants investigated has proved to be of the greatest value, and gives a special character to his investigations.

The following passage, bearing on some of the most crucial experiments, may be quoted (p. 623):—'The fact that trees up to 20 metres in height, can, without the assistance of root-pressure, take up for weeks together a substance so poisonous as a 5–10 % solution of copper sulphate, and conduct it through their stems, which were of necessity killed within the first days of the experiment, shows clearly that the living elements of the wood can have nothing to do with the raising of water, and that this process is a purely physical one. On the other hand it may be assumed, on physical grounds, that atmospheric pressure and capillarity, even if taken together, are insufficient

to raise a fluid to a height of 20 metres. Thus the living elements do not take part in the raising of water within the plant; they may, however, excite "bleeding" by pumping additional fluid into vessels which are already full.'

Other striking experiments proved that water can be conducted through stems which have been killed for a great length by immersion in water at a temperature of 90° C. An ascent of liquid took place in spite of the fact that the transpiring organs were at a height of much more than 10 metres above the place of absorption, and separated from it by more than 10 metres of dead stem (p. 647).

Similar results were attained with plants which were completely dead, the only conditions necessary being that the dead tissues should be sufficiently injected with water, and their cell-walls sufficiently saturated (p. 663).

The author further proved that water can be raised by the plant without the help of atmospheric pressure. In these experiments the absorbing plants raised a column of mercury 67 cm. in height (in the case of Dicotyledons), and 70 cm. high, in the case of a Conifer. These mercury columns, with the addition of the column of water in the branch itself, were more than sufficient to counter-balance the atmospheric pressures (p. 791).

This review has already reached an extreme length, and it is impossible to notice the innumerable other points of interest presented by the physiological part of the book. The attention of the reader may however be specially called to the passages on the function of bordered pits (which are particularly adapted to keeping out the air from empty tracheae), on absorption from the soil (in which a *qualitative* power of choice is again claimed for the roots), and on the function of the tracheae in conducting assimilated food. Here the striking fact is brought forward, that in certain cases of 'ringing' the xylem is able completely to replace the phloëm in the conduction of nitrogenous and other organic food-substances to developing organs. This does not, however, affect the fact that under normal conditions this conducting function belongs to the phloëm.

In a section on annual rings the author shows that their formation is an inherited character, but that the degree of differentiation between spring- and autumn-wood depends on the intensity of the ascending water-current, for which channels have to be provided, and which acts as a stimulus on the developing cambial cells.

It is much to be desired that the present position of the whole question as to the ascent of sap in plants should be brought fully before English readers by some physiologist specially conversant with the subject. In the present review nothing more has been attempted than to point out how complete a revolution in prevailing views must be effected by Professor Strasburger's investigations.

In conclusion it only remains to congratulate the distinguished author on the brilliant success of his latest lines of research, and to recommend his book to the careful study of all botanists, as being the most important contribution of the last fifteen years at once to the anatomy and the physiology of the higher plants.

D. H. S.

The Chemistry of Chlorophyll,

BY

EDWARD SCHUNCK, Ph.D., F.R.S., F.C.S.

II.

IT is not without some reluctance that I accede to the request of the Editors of this Journal to write a short account of what has been done regarding the chemistry of chlorophyll since the publication of my former paper on the subject[1], not feeling quite sure that what is to be said on the subject will prove of interest to readers in general, that is to such as are not specialists.

In giving an account of the various recent memoirs on chlorophyll, I propose to refer to them under various heads, without regard to the respective dates of publication, beginning with those relating to the preparation and general properties of chlorophyll, and proceeding to those that treat of the various derivatives of the substance.

In a recent publication Armand Gautier[2] describes his method of obtaining what he calls crystallised chlorophyll. He extracts green leaves, that have previously been washed with water, with cold alcohol at 83 per cent. The green liquid is filtered and shaken up with animal charcoal. After standing for some days, the charcoal, which has taken up the greater part of the chlorophyll along with other matters, is filtered off and washed with strong alcohol, which removes a crystallisable yellow colouring-matter, and then treated with petroleum-ether or sulphide of carbon, in which

[1] Annals, vol. iii. p. 65–120. [2] Chimie Biologique. Paris, 1892.

the chlorophyll dissolves. The solution on spontaneous evaporation leaves the chlorophyll in the form of small crystals of an intense blackish-green colour, which are slowly changed in the light, becoming brown, then yellow, and lastly colourless; its consistence is a little firmer than that of fat; its composition corresponds to the formula $C_{40}\,H_{64}\,N_2\,O_4$. Chlorophyll, according to M. Gautier, is not the same in all plants, that of the dicotyledons is not identical with that of the monocotyledons, while that of the acotyledons is different again. The points in which these chlorophylls differ *inter se* are not mentioned; all that is stated is that the chlorophyll from the ordinary 'fern of the woods' is very easily decomposed when exposed to air and light. The properties of chlorophyll resemble those of bilirubin. Chlorophyll contains no trace of iron, but on being burnt it leaves about 1·75 per cent. of ash, consisting almost entirely of magnesium phosphate. The accuracy of some of these statements of M. Gautier may be doubted. That the chlorophyll from one order of plants may possibly differ from that of another order, that there are in fact several chlorophylls, has frequently been suspected, but most of those who have closely studied the subject have come to the conclusion that chlorophyll is always the same whatever be its origin. M. Gautier stands almost alone in asserting that there are three distinct substances to which the name chlorophyll has been assigned. As regards the so-called crystallised chlorophyll of M. Gautier, I feel pretty sure that it is a product of decomposition formed from chlorophyll during the process employed for its preparation. Its properties, so far as one is able to judge from the few details given, resemble those of Hoppe-Seyler's chlorophyllan. From the fact of its having a fatty consistence and its leaving a certain amount of ash, one may conclude that it is an impure product.

I may here refer to the interesting experiments of Hansen [1], who obtained by a peculiar process a substance which he

[1] Die Farbstoffe des Chlorophylls. Darmstadt, 1889.

calls 'chlorophyll-green,' and which according to him is the colouring-matter in a state of purity. Having myself paid some attention to the action of alkalis on chlorophyll, I have come to the conclusion that Hansen's process, in which caustic alkali plays a part, leads to a product which cannot be considered as unchanged chlorophyll, and I shall therefore defer what I have to say about it until I come to the derivatives of chlorophyll.

The elaborate paper of Professor W. N. Hartley, entitled 'The Spectra of Blue and Yellow Chlorophyll, with some observations on Leaf-green [1],' is chiefly devoted to a correction of the chlorophyll-spectrum as described and figured by previous observers. The author distinguishes blue chorophyll and yellow chlorophyll. The former corresponds to the ordinary chlorophyll of most authors, the latter to what has been called at various times xanthophyll, chrysophyll, or erythrophyll. The solutions of blue chlorophyll show two narrow bands close together in the red, usually represented as one. This splitting up of the band in the red may have been due to the use of barium hydrate in the preparation of the substance. According to Chautard, solutions of chorophyll on the addition of caustic alkali show two bands in the red in place of one. On the other hand, Professor Hartley states that by using neutral solvents, such as benzene, he obtained a blue chorophyll identical so far as the spectrum was con cerned with the other. The two colouring-matters of Professor Hartley, it should be observed, are not identical with the blue and yellow chlorophyll of Stokes and Sorby. The latter constitute together what may be called green chlorophyll, the terms blue and yellow being of course merely relative; they are separated by the use of various neutral solvents and closely resemble one another; blue chlorophyll, however, by decomposition with acids yields phyllocyanin, whereas yellow chlorophyll gives phylloxanthin, as I shall have again occasion to mention.

Timiriazeff[2] obtains from chlorophyll, by reducing agents

[1] Journ. of the Chem. Soc. lix. 106. [2] Comptes Rendus, cix. 414.

such as zinc and hydrochloric acid, a product which he names 'protophyllin,' and from which, according to him, chlorophyll may be reproduced by oxidation. It is contained, he says, in etiolated plants, from which it may be obtained by extraction with alcohol, the extracts showing no trace of the chlorophyll-band I, whereas band II is relatively dark and well defined. When kept in the dark and in an atmosphere of carbonic acid, the solution retains its yellow colour, but on exposure to light it turns green. Hence it has been inferred that the author considered the necessary oxygen to have been obtained by decomposition of the carbonic acid: but he does not, he says, go so far as this, it being possible there were traces of oxygen present in his solutions, which only acted on exposure to light. The author's researches have led him to conclude that the turning green (*verdissement*) of etiolated plants is due to the action of light, and that it is the rays absorbed by the chlorophyll that effect the decomposition of carbonic acid in plants. I confess that I am at a loss to understand the reactions described by M. Timiriazeff. By the action of hydrochloric acid, chlorophyll is decomposed, yielding phyllocyanin and phylloxanthin, and these by the further action of zinc and hydrochloric acid give red products which cannot be reconverted into chorophyll by oxidation, and though I have never made a special study of etiolin, the yellow colouring-matter of etiolated plants, I have never found any reason to suppose that it is converted into chlorophyll with or without the concurrence of light, the products of its oxidation being always colourless. The same author[1] shows by an ingenious experiment that it is the rays of light absorbed by the chlorophyll which chiefly promote the formation of starch in leaves. A plant having been kept in the dark for two or three days so as to allow all the starch contained in the chlorophyll-corpuscles to be absorbed, the image of a well-defined spectrum is thrown on one of the leaves contained in a dark chamber by means of a heliostat, an

[1] Comptes Rendus, cx. 1346.

achromatic lens, and a direct-vision prism. After some time the leaf is treated with boiling alcohol to extract the colouring-matters, then with tincture of iodine; the image of the spectrum of chlorophyll will then appear traced in starch iodide on the pale yellow ground of the leaf, the chlorophyll-band I being indicated by a well-defined line, those in the orange and yellow by an indistinct shading.

Henri Jumelle[1] finds that chlorophyllic assimilation is always much feebler with trees having red or copper-coloured leaves than with trees of the same kind bearing green leaves. Thus the leaves of the copper beech and the purple sycamore assimilate six times less than those of the common beech or sycamore. This explains the fact familiar to horticulturists that the growth of trees with red foliage is much less rapid than that of trees of the same kind having green leaves.

I shall now proceed to give a short account of such recently published memoirs as relate to the various derivatives of chlorophyll.

Tschirch[2] prepares a substance which he calls 'phyllocyanic acid' by making an alcoholic extract of grass, evaporating to dryness, and treating the residue with hydrochloric acid. It is evident from the mode of preparation that phyllocyanic acid is merely impure phyllocyanin. That it is an impure product may be inferred from the fact that it gives bright green compounds with copper and zinc, whereas it has been shown that phyllocyanin yields such compounds with metallic oxides only in the presence of organic acids. Tschirch prepares a normal solution of his phyllocyanic acid in alcohol (1 : 100,000), and having obtained an alcoholic extract of a measured square area of a leaf, as exactly uniform as possible, to which a drop of hydrochloric acid has been added, and having diluted the extract until its absorption-spectrum coincides with that of the normal solution, he is able to estimate the quantity of chlorophyll contained in the leaf.

In the paper on the chemistry of chlorophyll, published in this journal several years ago, I gave a general account of the

[1] Comptes Rendus, cxi. 382. [2] Chem. Centralbl. 1889, p. 996.

action of acids on chlorophyll. I there said that by decomposition with acids chlorophyll yields two coloured products—phyllocyanin and phylloxanthin. Of the former I gave a detailed description. The latter has since then been more minutely examined, the results being contained in a paper read before the Royal Society in June 1891[1]. Phylloxanthin is there defined as the 'product formed along with phyllocyanin by the action of strong acids on chlorophyll and left dissolved in ether when concentrated hydrochloric acid is added to an ethereal solution of the two substances, the phyllocyanin passing into the acid.' It is necessary to adhere strictly to this definition, in order to avoid confusion, the term phylloxanthin having been applied to more than one substance. The phylloxanthin of Fremy is a mixture of several colouring-matters, true phylloxanthin as just defined forming only one constituent of the mixture. Although the quantity of this substance formed by the decomposition of chlorophyll with acids is much larger than that of the phyllocyanin accompanying it, its preparation in a state of purity is much more difficult, and the product even at the best is never free from impurities of a fatty nature. An account of the mode of purification will be found in the paper referred to.

The properties of phylloxanthin resemble those of phyllocyanin so closely as to lead to the conclusion that the two substances must be nearly related, that they are perhaps isomeric bodies; the attempts made to convert one into the other were however unsuccessful.

When dry, phylloxanthin appears dark green, almost black, thus differing from phyllocyanin, which always shows a dark indigo-blue colour. It is amorphous, even under the microscope, though it may occasionally be obtained by very slow evaporation of its ethereal solution in small rosettes which are rust-coloured by transmitted light. When a very minute portion is placed on a glass slide, then moistened with ether under a cover-glass, it is seen to resolve itself under the microscope into a number of long whip-like filaments and

[1] Proc. Roy. Soc. vol. l. p. 302.

pseudo-crystalline needles, much curved and twisted, which are brown by transmitted light. Chlorophyllan, according to Hoppe-Seyler, shows the same behaviour under the microscope. Towards solvents phylloxanthin behaves like phyllocyanin. The solutions are fluorescent and when dilute exhibit a marked reddish tinge, of which nothing is seen in the case of phyllocyanin.

The ethereal solution shows five bands closely resembling those of phyllocyanin, as regards both position and relative intensity, with this difference, however, that the first and second bands lie further away from the red end than those of phyllocyanin, while the space between the fourth and fifth bands is so much darkened that when the solution is concentrated the two bands appear as one. Phylloxanthin remains unchanged when heated to 130°, but at 160° decomposition commences, and at 180° it is completely decomposed.

When phylloxanthin is burned it always leaves some ferric oxide behind, a fact which might seem to favour the notion that iron is a constituent in some form or other of chlorophyll, and that on decomposition of the latter with acid the iron passes into the phylloxanthin, forming a compound from which it cannot be separated by ordinary treatment. A chloroformic solution of phylloxanthin when exposed to sunlight in a loosely stoppered bottle is slowly bleached. When treated with concentrated hydrochloric acid, phylloxanthin is gradually dissolved, yielding a dark greenish-blue solution; the substance undergoes a change by the action of the acid, but is not thereby converted into phyllocyanin. Phylloxanthin yields a green compound when cupric acetate is added to its solution in glacial acetic acid, but no similar compound is formed when zinc acetate is employed. In this respect phyllocyanin behaves quite differently, yielding green or bluish-green compounds with zinc as well as with copper in the presence of acetic acid and other organic acids. Phylloxanthin dissolves easily in alcoholic potash or soda, yielding red solutions which on boiling turn green. The substance undergoes a change by the action of the alkali, but the

product formed has far less characteristic and well-defined properties than has phyllotaonin, the analogous product from phyllocyanin. It may indeed be said that in every respect phylloxanthin is from a chemical point of view a far less interesting substance than phyllocyanin. Though the two substances so closely resemble one another, it is certain that they are not formed simultaneously, though they may appear to do so, when a strong acid, such as hydrochloric acid, is employed in the decomposition of chlorophyll. When a little acetic acid is added to an ethereal solution of chlorophyll there is an immediate change of colour in the solution, accompanied by the formation of phylloxanthin; it is only after some time that phyllocyanin makes its appearance; at least such is the conclusion derived from spectroscopic examination of the solution.

The close resemblance subsisting between phyllocyanin and phylloxanthin may be explained by supposing that they are derived from two distinct though nearly allied bodies. The researches of Stokes, Sorby, and others have led to the conclusion that ordinary chlorophyll is a mixture of several colouring-matters, two of which Mr. Sorby has named 'blue chlorophyll' and 'yellow chlorophyll' respectively. In a written communication received from Sir G. Stokes, he informs me that he is convinced that by decomposition with acids blue chlorophyll yields phyllocyanin, whereas yellow chlorophyll gives phylloxanthin. It must be understood that the yellow chlorophyll of Sorby and the yellow chlorophyll, xanthophyll, &c., of other observers are quite distinct.

The literature of chlorophyll contains descriptions of several substances, the properties of which closely resemble those of phylloxanthin. One of these is Pringsheim's 'hypochlorin.' After immersing tissues containing chlorophyll in dilute hydrochloric acid, Pringsheim observed the formation, after some time, of peculiar brown, crystalloid, sometimes even crystallised, bodies, attached to, and proceeding from, the chlorophyll-corpuscles of the cells, and which he supposed to pre-exist in the latter, the acid merely serving to bring them

out. They constitute his hypochlorin. I have repeated Pringsheim's experiments with the leaves of various plants and found the phenomena under the microscope exactly such as he describes. On examining the properties of the hypochlorin obtained, more especially the absorption-spectrum of its solution, I arrived at the conclusion that they do not differ from those of phylloxanthin, that the two substances are in fact identical.

The memoirs recently published relating to the action of alkalis on chlorophyll are of more interest than those treating of the action of acids. In a memoir entitled 'Extraction de la Matière Verte des Feuilles[1],' Guignet describes a method of obtaining the sodium-compound of chlorophyll in dark green crystalline needles, of which he says that its solutions show exactly the same absorption-bands as those of ordinary chlorophyll. Following the directions given by him, I obtained a product which was quite amorphous, and I doubt whether it is possible to get any crystalline compound by the direct action of alkalis on chlorophyll.

Hansen, in his work on the colouring-matters of chlorophyll already referred to, proceeds on the assumption that chlorophyll is not altered by treatment with caustic alkalis, and he accordingly submits his crude product to a process of saponification. The colouring-matter of chlorophyll—that which by French and English chemists is called simply chlorophyll—consists according to him of a green colouring-matter and a yellow colouring-matter. To separate these from each other and from the fatty matters with which they are associated was his main object. Taking green leaves, preferably grass, he first extracts them with boiling water, which removes yellow colouring-matters and other impurities. The leaves, after pouring off the extract, are dried in the air, when they appear blackish-green. The dry material is then extracted with strong boiling alcohol, by which a solution of a splendid green colour is obtained. This, after some deposited fatty matter has been filtered off, is mixed with caustic soda lye

[1] Comptes Rendus, c. p. 434.

and boiled for some time in order to saponify the fats contained in it. After distilling off a great part of the alcohol, carbonic acid is passed through the liquid so as to get all the soda carbonated; the whole is then evaporated to dryness on the water-bath. A dark green mass, consisting of colouring-matters and soaps, is thus obtained, which is treated with ether; this extracts the yellow colouring-matter which on evaporation of the solution is left as a coral-red mass. The residue left undissolved by the ether is now treated for several days with a mixture of equal parts of alcohol and ether, which removes a great part of the sodium-soaps along with some green colouring-matter. The residue is then treated with a mixture of one part of absolute alcohol with ten parts of ether, to which phosphoric acid is added until no more colour is taken up by the liquid. The solution is then filtered, the ether is distilled off, and the alcoholic liquid is evaporated, when it leaves a shining dark green brittle residue[1]. This the author considers to be the green colouring-matter of chlorophyll in a state of purity. Its properties are as follows:—It is insoluble in water, benzol, and carbon disulphide, sparingly soluble in ether, but easily soluble in alcohol; the solutions have a splendid green colour and show six absorption-bands; the colour of the alcoholic solution gradually changes on the addition of acids, but much more slowly than that of an acidified alcoholic leaf-extract; it has the character of an acid forming compounds with bases, those with alkalis being soluble in water; its solutions show much greater stability when exposed to light than do alcoholic leaf-extracts; it contains nitrogen and iron.

I have obtained by a much simpler process a product which I consider to be identical with Hansen's. A description of my process will be found in the paper previously referred to[2]. The product obtained by the action of caustic alkalis on chloro-

[1] I have described Hansen's process at greater length than might have been thought necessary, thinking that the work containing his results might not be easily accessible to the readers of this journal.

[2] Roy. Soc. Proc. l. p. 312.

phyll, according to the process described, has very distinct properties, though resembling the parent substance in some respects. A substance presumably identical with it has been named 'alkaline chlorophyll,' but I prefer to call it simply 'alkachlorophyll.' It is obtained by my process in the form of an amorphous resin-like body, purple by reflected, bright green by transmitted light; it may be easily pulverised, yielding a dark green powder. It is insoluble in water, easily soluble in alcohol, ether, chloroform, benzol, aniline, and carbon disulphide, insoluble in petroleum-ether. Its solutions have a brilliant bluish-green colour and a marked red fluorescence; the ethereal solution shows six absorption-bands. It combines with alkalis, yielding compounds which are soluble in water. The sodium-compound is amorphous and has very much the appearance of the substance itself; its aqueous solution gives green precipitates with barium chloride, lead acetate, cupric acetate, and silver nitrate. Alkachlorophyll in solution shows a remarkable degree of permanence when exposed to the combined action of air and light, as compared with chlorophyll. An alcoholic solution of chlorophyll exposed to light in a loosely stoppered bottle lost its green colour in a few days, whereas a solution of alkachlorophyll of as nearly as possible the same strength exposed along with the other retained its colour for some time, and after some weeks still showed a faint tinge and traces of the absorption-bands peculiar to the substance.

The action of acids on alkachlorophyll is especially interesting, because the products to which it gives rise differ entirely from those derived from chlorophyll by decomposition with acids. When a small quantity of sulphuric acid is added to an alcoholic solution of alkachlorophyll the solution almost immediately loses its bright green colour, which changes to a dirty purple, and on standing an abundant granular deposit is formed, which, being filtered off and slightly washed with alcohol, may be dissolved in boiling alcohol. The solution on cooling deposits brilliant purple crystals which have the properties of ethyl-phyllotaonin; the

ethereal solution shows the absorption-bands of the latter substance and by decomposition with alcoholic potash they yield phyllotaonin. It appears therefore that whether we decompose chlorophyll with acid and subject the product of decomposition to the action of alkali, or whether we act on chlorophyll, first with alkali and then with acid, the final product is in both cases the same. It is almost certain, however, that it is the blue chlorophyll to which the formation of alkachlorophyll and consequently of phyllotaonin is due. The yellow chlorophyll must by the action of alkalis give rise to products which are not distinctly recognised during the processes hitherto employed. In my process, these products, if formed, probably remain in solution when carbonic acid is passed through the alkaline solution of chlorophyll.

It can hardly be doubted, I think, after what I have said, that chlorophyll does indeed undergo a metamorphosis, when subjected to the action of alkalis. It is not a case of mere combination, nor on the other hand of decomposition, strictly speaking, but rather of internal molecular re-arrangement, whereby the whole complex attains to a state of greater stability.

In consequence of this relatively greater stability, accompanied by general resemblance in properties, of alkachlorophyll, certain questions naturally suggest themselves, the solution of which is calculated to throw light on the composition of chlorophyll itself. Among these is the question whether chlorophyll by decomposition with acids yields any product soluble in water. This question could not be determined in the case of chlorophyll on account of the impossibility, so frequently dwelt on, of separating it from the impurities with which it is associated in alcoholic leaf-extracts, but with alkachlorophyll, which can be obtained relatively pure and in a form in which it can be easily manipulated, the case is different. I have made a few experiments with alkachlorophyll, decomposing it with sulphuric acid, and treating the liquid after the products insoluble in water had been filtered off in the usual manner, and I obtained a syrupy substance which

seemed to have the properties of an organic base. The experiments were, however, too few, and the amount of substance obtained too small to form a basis for positive conclusions. The observation, if correct, would tend to confirm the view taken by Hoppe-Seyler, who obtained cholin as a product of decomposition of his chlorophyllan, and hence concluded that chlorophyll itself might have a constitution similar to that of lecithin.

The statements as regards the action of aniline on chlorophyll, contained in the previous communication on the chemistry of chlorophyll [1], have been found on further investigation to be partly erroneous. For the true or more probable explanation of the phenomena which take place when green leaves are exposed to the action of aniline, the reader is referred to the paper [2] on 'The action of aniline on green leaves and other parts of plants.' I still think that in order to explain all the phenomena observed, it is necessary to suppose that chlorophyll itself undergoes some change when brought into contact with aniline while still within the vegetable cell. It is possible that the aniline acts in this case as a base, producing to some extent the same effect as an alkali would.

It remains to say a few words as to the new facts discovered of late with reference to the yellow colouring-matter accompanying chlorophyll in green leaves. These facts are but few. Hansen arrived at the conclusion that this colouring-matter is identical with that of etiolated plants, with that of faded leaves, and with that of yellow flowers: that xanthophyll, chrysophyll, erythrophyll, and carotin are different names for the same substance, that this substance may be obtained in regular rhombic crystals, and that its solutions are non-fluorescent and show two bands at the blue end, but none at the red end of the spectrum. Professor Hartley, on the other hand, states that his yellow chlorophyll gives brownish, fluorescent solutions, which show a weak absorption-band at the red end, as well as strong bands at the blue end, from

[1] Annals, iii. 1889. [2] Annals, vi. 1892, p. 167.

which it may be inferred that his substance was not altogether free from an admixture of blue chlorophyll. I find no allusion in recent memoirs to the observations of Stokes and Sorby, as to there being two distinct yellow colouring-matters accompanying chlorophyll. It is remarkable, though perhaps not altogether unfortunate, that so far we have not been favoured with any speculations as to the functions of these yellow colouring-matters in plants. Considering how little we really know regarding the function of chlorophyll itself in connection with its physical and chemical properties, such speculations would be somewhat premature.

On the Artificial Production of Rhythm in Plants[1].

BY

FRANCIS DARWIN AND DOROTHEA F. M. PERTZ.

THE instrument used for the observations here recorded is a modification of the klinostat, which we have named the *intermittent klinostat.*

In the ordinary instrument the plant is kept slowly rotating with the object of freeing it from geotropic and heliotropic curvatures. It is possible to imagine the action of the klinostat as being one of two distinct kinds. We may suppose that either the plant is not influenced by the stimulus because it is never long enough in one position, or that it is influenced by the stimulus the whole time, and does not curve because it is equally stimulated on all sides. In a research 'On the circumnutation of unicellular organs[2],' one of us observed *Phycomyces* on a vertical klinostat, and found that it exhibited a movement simulating circumnutation, but which was reversible by reversing the driving gear. It was clear that this movement was the result of heliotropic stimulation causing a regular series of slight curvatures by

[1] A short paper on this subject was read by us at the Cardiff meeting of the British Association, 1891.

[2] F. Darwin, Bot. Zeitung, 1881, p. 473.

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which the sporangium was carried round in a circle, like the free end of a circumnutating organ. Elfving's interesting paper[1] on the growth of the pulvini of grass-halms on the klinostat proves that the geotropic stimulus may in like manner be continuously felt[2]. It is clear that in some cases the plant is not strictly speaking freed from external stimuli, although no permanent curvatures are produced.

It was to obviate this difficulty that the intermittent klinostat was devised and made for us, at the Cambridge Scientific Instrument Company's works, by Mr. H. Darwin.

Round the horizontal spindle of a klinostat[3] a cord is wound, which passes over a pulley and is attached to a weight. If the weight is allowed to descend the spindle will rotate, and it is this arrangement which gives the motive power to the instrument. The weight, however, is not allowed to act continuously: by means of a clock-work escapement, the spindle is only permitted to make a half-revolution twice in every hour. A simple but efficient fan-governor is fitted to the spindle, and the rotation is thus rendered so gentle, that no harmful jar is communicated to the plant.

If we imagine a plant attached to the klinostat, it is clear that it will, during the first half-hour, receive a geotropic stimulus tending to make it curve in a certain direction, and that during the next half hour an equal and opposite stimulus will tend to undo the effect produced in the first period. Under this series of stimuli, an ordinary apogeotropic shoot remains approximately straight, exhibiting however a rhythm of slight curvatures in the vertical plane which will be here described. The first use that we made of the instrument was to investigate rectipetality. Vöchting has shown that an organ which has been allowed to curve geo- or heliotropically and is then cultivated on the ordinary klinostat, loses the bend and becomes straight. This power of accommodation

[1] Ueber das Verhalten d. Grasknoten am Klinostat.

[2] Schwartz's experiments on growth during slow rotation point to an opposite conclusion. See Untersuch. Bot. Inst. Tübingen, I, 1881.

[3] We use the pattern of klinostat described by one of us in the Linnean Society's Journal, 1881, vol. xviii.

he considered to be an inherent power and named rectipetality. Since, as above pointed out, it is by no means clear that plants rotating on the klinostat are truly freed from stimulation, it seemed desirable to test rectipetality with our new instrument.

A vigorously growing internode was fixed to the intermittent klinostat with its axis parallel to the spindle. The clock-work was ungeared, so that no rotation could occur, and the plant was left until a distinct geotropic curvature was visible. The plant was now arranged so that the plane of its curvature was horizontal, and the clock-work escapement was set in action. The plant is now subject to alternate opposite geotropic stimuli in the vertical plane, i. e. at right angles to the plane of the geotropic curvature which it has already undergone. If it were not for these opposing and equal stimuli the plant would obviously assume a distorted form owing to the superposition of a fresh geotropic curvature in a plane at right angles to the first. But since no such distortion can occur, the original curvature can be studied. Under these circumstances it was found, as in Vöchting's experiments, that the curvature flattens out, and the plant becomes straight. Since this unbending or straightening occurs in a horizontal plane it can be in no way influenced by gravitation. This result, confirmed by a series of experiments, convinced us of the existence of an inherent regulating power which leads to growth in a straight line [1].

Rhythm. We have succeeded in inducing a rhythmic condition by subjecting plants to alternate and opposite stimuli of a geotropic and heliotropic character. The geotropic curvatures, for which the above described form of the intermittent klinostat is employed, will first be considered.

Vigorously growing shoots of a Valerian, or scapes of the dandelion (*Taraxacum dens-leonis*) are fixed in bored corks in test-tubes of water which are attached to the klinostat so that the axis of the plant is parallel to the

[1] These results were briefly published in the Proceedings of the Cambridge Philos. Soc. 1891.

spindle. It is not found necessary to carry on the experiment in the dark; the axis of the plant being directed towards the window, heliotropic disturbance is sufficiently eliminated. An index is fixed to the free end of the shoot and its displacement in a vertical plane observed by means of a horizontal microscope[1], or simply with a millimeter scale.

The movements are at first irregular, and it is only after a varying period has elapsed that the half-hourly rhythm is produced. The following may serve as examples of the behaviour at first:—

EXP. I. MAY 17, 1889. *Valerian.*

P.	Minutes.	Movement.
I	30	Down
II	30	D
III	1	D
	14	Up
	14	D
IV	4	D
	23	U
	2	D

It will be well to give a few explanations with regard to Exp. I, so that the following records may be comprehensible. The vertical columns headed P, *minutes*, and *movement*, are divided into spaces by horizontal lines, each space representing half an hour: the first column 'P' simply gives the number of these half-hourly periods. It will be noticed that in the two last spaces observation was only carried on during twenty-nine minutes, made up by 14+14+1 and 4+23+2 minutes; this is because it is not always possible to record accurately either just before or just after the moment at which the klinostat makes its semi-turn.

[1] Each division of the micrometer eye-piece being equivalent to 0,024 mm.

During the first half-hour the Valerian curved downwards, when, at the expiration of this time, the half-turn of the spindle occurred, the curvature was immediately reversed. It may be well to point out, once and for all, that if the curvature of the shoot continues unchanged from the end of one half-hour to the beginning of the next, that is if it continues to curve in its old direction after a half-turn has been made, the symbol recording the direction will change. If the shoot is curving geotropically *upwards* and is suddenly rotated on its axis through 180°, it will, if it continues the curvature, be bending downwards. Similarly in Exp. I, since the two first half-hours bear the record *Down* (or D), it is clear that the direction of curvature changed at the turn of the spindle. This movement, as well as the temporary downward curve at the beginning of the two following periods, is due to the physical bending of the shoot, a point in our experiments which caused us some difficulty.

During the last 14′ of the third period, a downward curvature occurred, and the same movement fills up the last 2′ of the fourth period. These are clearly growth-curvatures due to after effect, but not as yet showing any relation to the half-hourly period.

The following experiment shows a similar state of things:—

EXP. II. MAY 20, 1889. *Valerian.*

P.	Minutes.	Movement.	P	Minutes.	Movement.
I	16	?	V	1	D
	14	D		10	U
				18	D
II	28	D	VI	3	D
III	3	D		8	U
	15	U		17	D
	10	D			
IV	25	U	VII	4	D
	3	D		10	U
				15	D

In this experiment it is clear that downward movement at the beginning of the half-hourly periods (absent in Period 4), is a physical effect. We may assume that but for the physical droop, the movement would have been continuous from one period to the next. We can therefore get an approximate idea of the growth of the shoot if we add each of these short periods of 'sagging' to the figures immediately below them. In this way we obtain the following figures, representing the times during which the shoot curved, first in one direction (A) then in (B) the opposite. Beginning at period 3, we see that it curved up for fifteen minutes, adding in the previous three minutes of sag, and calling this curvature A, we get—

A = 18, B = 35, A = 14, B = 29, A = 31.

Here we see the beginning of a half-hourly rhythm at the end of the fifth half-hourly period, that is after about three hours. This is shown by the fact that the shoot curved for twenty-nine minutes in direction B, then reversed the movement, and after thirty-one minutes again curved in direction B.

EXP. III. MAY 21, 1889. *Dandelion.*

P.	Minutes.	Movement.	
I	28	D	
II	30	D	46′ = A
III	16	U	
	13	D	23′ = B
IV	5	D	
	5	U	
	20	D	37′ = A
V	17	U	
	11	D	23′ = B
VI	3	D	
	9	U	
	13	D	
	5	not observed	

Here in Exp. III we get movements in direction A and B in the following proportions: A, 46′; B, 23′; A, 37′; B, 23′, showing a very irregular rhythmic movement.

EXP. IV. MAY 31, 1888.

P.	Minutes.	Movement.	
I	28	D	
II	13	D	
	17	—?	
III	28	U	A = 38′
IV	10	D	A = 38′
	15	U	B = 22′
V	7	D	B = 22′
	16	U	A = 16′
	5	D	A = 16′
VI	1	D	B = 32′
	26	U	B = 32′
VII	3	D	
	18	U	
	9	?	

In Exp. IV we see an irregular curving backwards and forwards of the shoot. It will be noticed that the record is not an easy one to interpret; thus the three minutes of downward curvature with which Period 7 begins might be supposed to be part of the B curvature with which Period 6 ends. It may, however, represent a physical sinking or sag, in which case it would have had to be added to the 18′ in the middle of Period 7.

EXP. V. MAY 11, 1891. *Dandelion.*

P.	Minutes.	Movement.	
I	17	—	
	3	D	
	10	U	20′ A
II	10	D	
	18	U	
III	30	D	69′ B
IV	21	U	
	7	D	32′ A
V	25	U	
	4	D	26′ B
VI	7	D	
	15	U	
	5	D	20′ A
VI	5	D	
	10	U	
	12	D	

Exp. V (in which there are some difficulties of interpretation) again shows an irregular rhythm approaching in some places to the half-hourly period.

The movement is more readily followed when expressed graphically. Fig. 1 gives the result of Exp. VI.

EXP. VI. MAY 9, 1891. *Dandelion.*

P.	Minutes.	Movement.	
I	10	D	
II	24	D	42′ A
III	18	U	
	12	D	25′ B
IV	13	U	
	15	D	28′ A
V	13	U	
	15	D	26′ B
VI	11	U	
	18	D	

VII and VIII no record

IX	13	?	
	9	U	
	9	D	
Klinostat not turned			30′ B
	21	D	
	37	U	
continued to move up			

The diagram is to be read from below upwards. It is divided by strong lines into spaces one above the other, representing the periods i, ii, iii, &c., of half-an-hour each: the finer horizontal lines subdivide the half-hours into spaces representing 10′ each. Taking the bottom space which represents the second period in Exp. VI, it will be seen that D and U, symbolising as before *down* and *up*, are on the right and left respectively. The line marked with an arrow-

head travels obliquely towards the D side showing that the plant was curving downwards for the final 24′ of the second half-hour. Now the klinostat makes half-a-turn, *which is shown by the D and U changing* sides. The line representing the movement travels on in approximately the same direction although it now represents an upward curvature. In the middle of the third period the direction changes and the plant curves downwards.

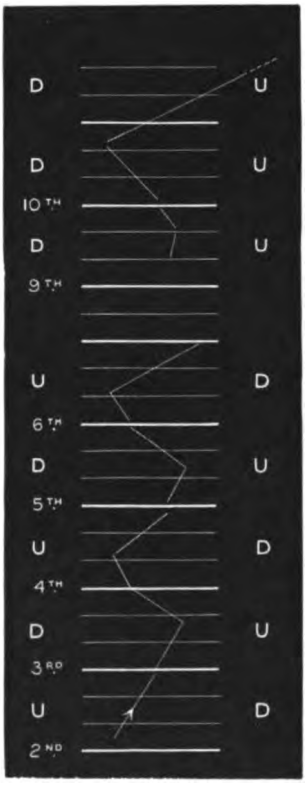

Fig. 1.

Observation was continued at the end of the ninth period; and the plant is seen to curve downwards during the last nine minutes. *The clock is now stopped so that the klinostat is not allowed to turn.* This is shown in the diagram by the symbols D, D, D for the ninth, tenth, and eleventh periods being all on the left, while the corresponding U, U, U are on the right. It will be seen that, in spite of the absence of the usual change of stimulus, the plant curves downwards for half-an-hour before it curves upwards permanently.

The diagram shows clearly enough that a rhythmic condition was set up during the fourth, fifth, and sixth periods. Owing to the difficulty of manipulation mentioned above (p. 248), the graphic method probably gives a truer record of the movement than the tabular summary. The change in direction of curvature occurs in the middle of the half-hour, and not at the end or beginning. This has already been shown in Exp. IV and V, and is a general feature of the rhythm.

But the most interesting point shown in Fig. 1 is that after

the clock had been stopped the plant gave evidence of an undoubted half-hourly rhythm. It is a most striking phenomenon to observe. The plant seems to be impervious to the geotropic stimulus, and only when it has fulfilled the tendency to bend in one direction for half-an-hour does it reverse its movement and curve upwards. In Fig. 1 only a single reversal takes place; no doubt when the alternation of the gravitation-stimulus ceases, the rhythm is soon destroyed; but in other

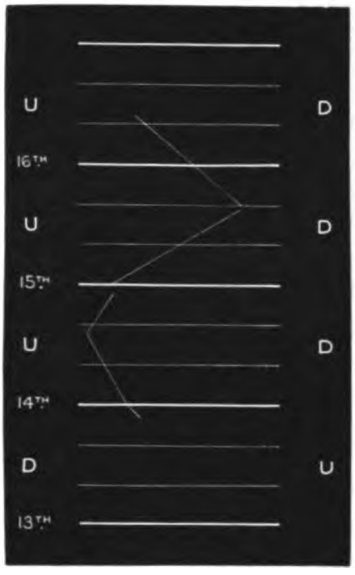

Fig. 2.

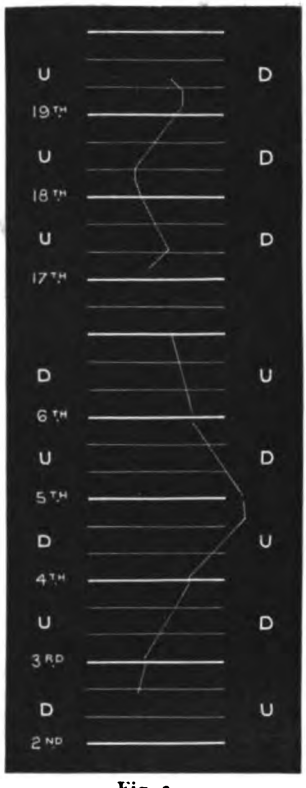

Fig. 3.

cases, as we shall show, the impression on the plant is of longer duration.

Fig. 2 is of interest as giving the termination of Experiment V, in which the movement was at first irregular.

The klinostat is stopped between the fourteenth and fifteenth half-hours, and the plant curved downwards for thirty-one minutes before it reversed and curved upwards.

Fig. 3 represents the movement of a dandelion-stalk observed on May 15, 1891 (Exp. VII). It is interesting because although the movement is at first very irregular, yet after eight to nine hours the half-hourly rhythm is so strongly impressed that two reversals of movement occur after the klinostat is stopped, at the end of the seventeenth half-hour.

It will be seen that at the end of the seventeenth half-hour the plant was curving upwards, and that it continued for exactly 30′ to do so. It then curved downwards for 29′ before it was again reversed and converted into a permanent apogeotropic curvature.

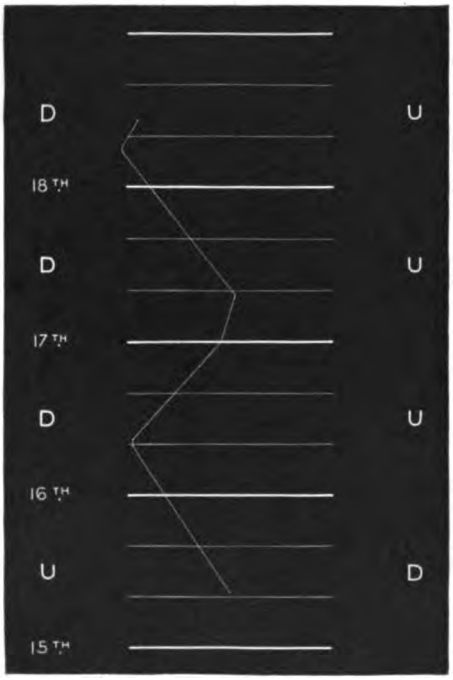

Fig. 4.

Fig. 4 gives the movement of a dandelion observed on May 22, 1891. (Exp. VIII.)

At the end of the sixteenth half-hour (when the klinostat was stopped) the stalk was curving upwards. It continued to do so until it had been moving in this direction for 28′, when it reversed, curved downwards for 29′, and then permanently bent upwards.

Fig. 5 gives the movement of a Valerian observed June 2, 1891. (Exp. IX.)

At the end of the thirteenth half-hour the plant was curving

downwards, the klinostat was stopped, and the stem continued to bend until it had completed thirty minutes when it reversed its direction, and bent permanently upwards.

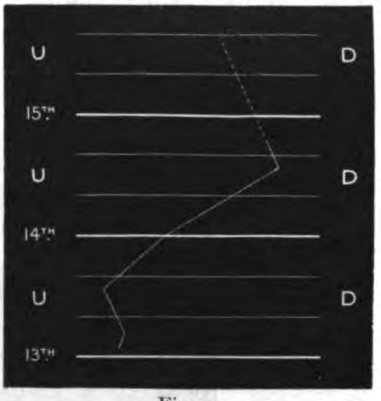

Fig. 5.

We now give a few examples of a rhythmic condition induced by heliotropic stimulus. The plants used were seedlings of the Canary Grass (*Phalaris canariensis*). The klinostat was arranged vertically, and as before made a half-turn every half-hour. In the following tables and diagrams + means a curvature towards, − away from the light.

EXP. X. MAR. 20, 1890. *Phalaris.*

P.	Minutes.	Movement.	
III	20	+	
	10	−	25′ A
IV	15	+	
	15	−	30′ B
V	15	+	
	15	−	30′ A
Klinostat stopped			
VI	15	−	
	15	+	x B
and continued		+	

These results are graphically given in Figure 6. It should be noted that when, in the heliotropic experiments,

the klinostat was stopped, the plant was rotated through 90°, so that the plane of movement was at right angles to the direction of incident light. *Phalaris* is heliotropically so sensitive that the impressed rhythm would be hardly perceptible without this precaution. For the sake of simplicity

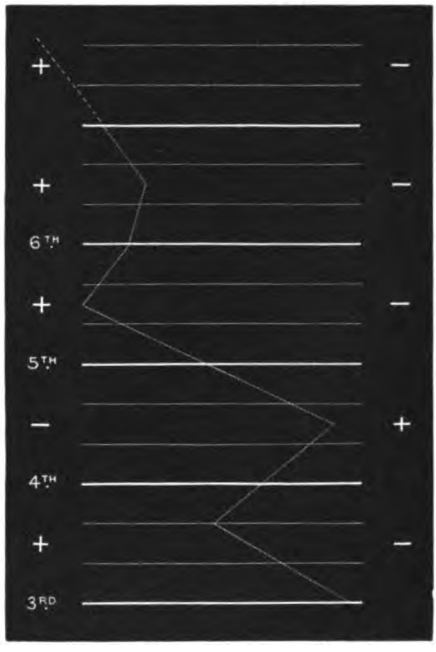

Fig. 6.

the symbols + and − are retained after the klinostat was stopped; thus during the last 15′ of the fifth half-hour the grass was actually curving away from the light; during the first half of the sixth period it is *shown* moving away from the light because its movement was a continuation of that curvature, although it was in fact moving across the line of light.

Another example may be given:

EXP. XI. MAY 20, 1890. *Phalaris.*

P.	Minutes.	Movement.	
I	30	−	
II	30	−	
III to IX	no observation		
X	22	?	
	8	−	31′ A
XI	23	+	
	7	−	
Klinostat stopped			30′ B
	13	−	
	10	no curvature	
	32	+	more than 32′ A
and continued		+	

Here we see a half-hourly rhythm was well established by the tenth half-hour, and that after the klinostat stopped, precisely 30′ elapsed before the movement was reversed.

The above examples may serve to show the nature of the movements induced by the intermittent klinostat.

Those who repeat our experiments must not expect uniform success, as there is undoubtedly a certain capriciousness in the results, which probably depends on varying degrees of vigour in the plants used. The experimenter must beware of employing, in geotropic experiments, shoots which from length or flexibility show a droop or sag when the klinostat is reversed. A considerable series of our experiments were vitiated apparently from this cause. We were also unfortunate in the loss or destruction of a valuable set of notes in which our most successful series of experiments were recorded. We

cannot therefore with any fairness give an estimate of the whole number of experiments which have failed and succeeded in showing a rhythm.

The following figures give some idea of the frequency of success.

Out of eighteen experiments on Valerian in 1890-91, nine, or one half, showed a rhythm.

Out of seven experiments on Dandelion in 1890, six showed rhythm.

Out of ten experiments (heliotropic) on *Phalaris* in 1890, seven showed rhythm.

We have worked at this subject at intervals for three years, and we cannot for a moment doubt that what we have seen is not an accidental result. To watch the movement of the free end of the shoot and to see it reversed exactly at the expiration of half-an-hour, is an experience so impressive as to compel belief. When a shoot is in a thoroughly rhythmic state, it is possible to prophecy to a minute at what time the reversal will take place. This can only be done with certainty when the klinostat is kept going and the alternation of stimuli keeps up the rhythm already acquired. We have frequently been strongly impressed by a result of this kind.

Given the fact that a to-and-fro movement of the plant, in a rhythm closely approximating to a half-hourly period, takes place in a fair proportion of cases, there seem but two possible explanations. (1) The theory we hold, namely, that the plant learns as it were the rhythm of the alternate stimuli impressed on it. (2) That the whole affair is a matter of chance; the plants used happen to circumnutate with a half-hourly period, which, therefore, *seems* to have been 'learnt' from the tempo of the klinostat. This view, which was suggested to us by a friendly critic, has thus much in its favour. It would certainly account for the cases in which no rhythm appeared, because from what we know of circumnutation we should not expect to find a half-hourly period except very rarely and by the merest chance. If this explanation were the true one we ought to have come across a regular rhythm

of say twenty or forty minutes' period. But this was not so, whenever a *regular* rhythm was set up, its period was half-hourly. Nor would such a theory account for the oscillations occurring in a vertical plane, whereas if they are geotropic in origin they must obviously occur in that plane.

Moreover, the movement induced by the klinostat is precisely the kind of result that might be expected from what we know of intermittent stimulation in plant-physiology. The best example of what we mean is the series of movements produced by the alternation of day and night. Take for instance the nyctitropic movements of leaves. The most striking feature about these movements is that the turning-points, the places where a reversal of movement occurs, are not at the beginnings of the periods, but in the middle of them[1]. Thus a leaf begins, in the day, to assume the night-position and in the early morning before it is light it begins to return towards the day-position. A graphic representation of the movement is a wavy line in which the crests and valleys of the waves occur in the afternoon and early morning. Precisely the same is true of the geotropic rhythm, as may be seen in Fig. 1[2]. Thus, whether we have a cycle of twenty-four, or of one hour, the result is the same, and it is not a little remarkable that it should be possible to make, with a klinostat and a dandelion stalk, a working model of the natural periodic movements of plants.

It is one of the most familiar facts about the periodic phenomena in plants, that the rhythm continues after the conditions which have built up the rhythm have ceased to act. Thus in the 'sleep' of flowers and of leaves, it is well-known that in constant darkness the periodic movements continue for some time. The same is true of the periodic variations in growth-rate.

[1] See the graphic representation of sleep-movements in Pfeffer's Die periodischen Bewegungen der Blattorgane, 1875.

[2] It should be noted that in the majority of our heliotropic experiments the moment of reversal of movement coincides much more closely, often exactly, with times at which the klinostat turns.

The fact, therefore, that the geotropic or heliotropic rhythms, induced by the intermittent klinostat, continue after the periodic stimulation has ceased is precisely what we should have expected from our knowledge of natural rhythms.

The comparison need not stop here, we may, without being fanciful, compare our results with certain periodic phenomena in our own lives. If a man is waked by a knock at his door every morning at six o'clock, he will continue for some time to awake at approximately the same hour, when he is no longer called. In such a case a man says that he wakes at six o'clock because he has 'got into the habit of it.' If this is a fair use of the word habit, which we cannot doubt, then we may equally apply the word habit to the periodic phenomena of plants, including our klinostat results.

We think, indeed, that our results may throw a certain light on such a periodic act as that of waking at a habitual hour in the morning.

It is always possible to suppose that the man wakes without being called, because the act of waking has become associated or adherent to some other features of the morning hours ; that his body (unconsciously) discovers it to be time to wake without the particular stimulus implied by a knock at the door. It would be impossible to place a man in such uniform conditions that such association or adherence is excluded. But in the case of a half-hourly rhythm imposed on a plant the case is different, there are no external changes occurring at half-hourly intervals which can help the plant to 'know' when the half-hour is passed. There must be a sort of internal chronometry in the case of the plant, that is to say, the reversal of the geotropic curvature must take place at the right time because it has been associated and become adherent to some processes (of nutrition possibly) in which the element of time comes in. What has here been said is the merest guess-work ; its speculative character, however, does not interfere with the fact that our rhythmic experiments point to a time-measuring quality in plants.

No one has thrown more light on the periodic phenomena

of plants than Pfeffer, whose admirable 'Periodische Bewegungen' is known to all physiologists. He uses the pendulum as an illustration: a pendulum is kept in action by the periodic application of force, and continues to swing in its proper rhythm for some time after the force has ceased to be applied. This, of course, is no explanation of the similar state of things in plants, but such an analogy is more useful than the statement that the rhythms in plants are explicable as the result of 'latent period' and 'after-effect.' The pendulum illustration fixes the attention on the right point, namely, the condition of equilibrium in the organism. A pendulum is a machine specially constructed to swing; and the organism must have a faculty of repetition, a power of swinging as it were, or it could not be periodic. This repeating power may be that fundamental property of living matter, which stretches from inheritance on one side to memory[1] on the other—a region too wide for the limits of our present paper.

Pfeffer has shown in a beautiful manner[2] the resemblance between a rhythmic plant and a pendulum. *Acacia lophantha*, like other sleeping-plants, becomes motionless in continued daylight; if it is now darkened the periodic movement of the leaves begins, but this movement does not continue nearly so long as if the plant had, before the artificial darkening took place, been exposed to normal alternations of day and night. The first case corresponds to a stationary pendulum set in action by a single touch; the second to an oscillating pendulum receiving a similar touch synchronously with its swing.

The rhythmic condition induced in our experimental plants has a special interest for us because of its possible bearing both on rectipetality and on circumnutation. One of us has sought to show elsewhere[3] that it is possible to look at these two forms of movement as different aspects of the same

[1] See Mr. S. Butler's Life and habit.

[2] Pflanzenphysiologie, ii. p. 263.

[3] F. Darwin, Presidential Address, British Assoc., Section D (Cardiff, 1891).

phenomena. Whether we believe (with Charles Darwin) that circumnutation is the basis of growth-curvature, or whether with Wiesner we reject such a belief, we must believe in the facts of rectipetality, that is, we must believe in a self-regulating power which keeps growth to a straight line. When a normally straight-growing shoot has become curved by excess of growth on one side, the regulating power leads to increased growth on the concave side, and thus tends to undo the curvature. Whether or no this has any connection with circumnutation, it shows a pendulum-like quality in longitudinal growth which may well serve as the basis for the induced rhythm described by us.

We are inclined to see in our results a confirmation of the main thesis of the *Power of Movement in Plants*, namely, that growth-curvatures are developments or exaggerations of circumnutation. We believe that it is because of the connection between growth-curvature and circumnutation, that an artificial rhythm can be built up by geotropic stimulation. If, as the authors of the *Power of Movement* believe, circumnutation can be converted under the action of a single stimulus into a one-sided movement, it is not unnatural that under intermittent opposite stimuli, a circumnutation should be moulded into the half-hourly to-and-fro movement which we have described.

On the Embryogeny of Angiopteris evecta, Hoffm.

BY

J. BRETLAND FARMER, M.A., F.L.S.

Fellow of Magdalen College, Oxford.

With Plate XV.

NOTWITHSTANDING the attention which has for many years been bestowed upon the Filicineae and their allies, the embryogeny of no member of the eusporangiate Ferns is as yet known; a circumstance, the cause of which is to be attributed to the difficulty of obtaining the plants in a condition suitable for investigation.

When in Ceylon last year, I took the opportunity of securing as much material as possible of prothallia of *Angiopteris*, with the view of studying the development of the sporophyte, and although my results are incomplete on some points, it has been possible to make out clearly the more important features presented by the embryo of this plant.

The prothallium is remarkably deep green in colour and somewhat orbicular in shape. It is not unlike the thallus of *Anthoceros*, with which my specimens were often associated; it commonly however reaches a large size, occasionally attaining to as much as three quarters of an inch in diameter. The

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development of the prothallium has been followed by Jonkman [1] from the germinating spore, and he observed the formation of the sexual organs. The *antheridia* occur on the upper and lower surfaces of the oophyte, though they are more freely distributed on the lower side. They arise from single superficial cells. Each of these divides into an inner and an outer cell. The former by repeated division gives rise to the mother-cells of the antherozoids, whilst the latter or outer cell divides in planes at right angles to the free surface, thus forming the cover-cells, by whose separation the antherozoids are eventually liberated from the antheridium. The *archegonia* are confined exclusively to the lower surface of the prothallium, and arise from the 'cushion' region, which is exceptionally large in this plant. A superficial cell divides periclinally into an outer cell from which the neck of the archegonium originates, and an inner cell from which the neck-canal and ventral-canal cells are successively cut off, leaving the oosphere at the base. The neck-canal-cell grows between the cells of the short neck forcing them apart. It ultimately divides into two transversely, and the resulting cells are often separated by a true wall, as was observed by Jonkman, and by Campbell [2] also in the case of *Osmunda* as an occasional occurrence. It is by no means invariable in *Angiopteris*, and in Fig. 2 there is shown a case in which the cell-wall, though it had begun to be formed, was not completed, and was drawn away with the shrinking of the protoplasm, from the lining walls of the neck-canal. The ventral-canal-cell, which is very large in this fern, is converted like the two neck-canal-cells, into mucilage, which on the addition of water, bursts open the archegonium. In a few cases I observed an apparent deviation from the course just described, inasmuch as the inner of the two cells resulting from the first division of the archegonial primordium seemed to have divided again, before forming the axile row of cells;

[1] Jonkman, De Geschlachtsgeneratie d. Marattiaceeën.

[2] Campbell, On the Prothallium and Embryo of *Osmunda claytoniana* and *Osmunda cinnamomea*, Ann. Bot. VI, p. 67.

the lowest of the three first cells would thus be the equivalent of the basal cell of Janczewski. My material was however unfortunately not sufficient in quantity to enable this question to be conclusively settled.

I had not the opportunity of observing the process of fertilisation, and unfortunately none of my material showed the earliest divisions of the oospore, though in preparations of the youngest embryos, their succession could be determined without much difficulty. The basal wall is formed as in *Isoëtes* and *Equisetum*, at right angles to the long axis of the archegonium, that is, in the plane of the prothallium. The next wall in order of succession I believe to be the median one; it is at right angles to the basal wall, and parallel to the axis of growth of the prothallium. This wall can easily be distinguished, even in advanced embryos, as a well-defined vertical line. The transversal wall is much more indefinite, and soon becomes quite unrecognisable as the embryo grows in size. New cell-walls succeed each other very rapidly, and without much regularity (cf. Figs. 3 and 4 which represent almost identical stages), and they are far less easily followed than in the common types of leptosporangiate ferns. I was unable to determine the presence of segment-walls in most preparations of young embryos, though they are indicated in some cases. No doubt this comparative irregularity is to be connected with the absence of well-marked apical cells from the members into which the young embryo becomes differentiated. These members originate from the octants in a way recalling strongly the typical fern-embryo, as will be at once seen from what follows.

The two anterior epibasal octants (i.e. the two anterior upper ones) give rise to the cotyledon. Of the two posterior epibasal octants one probably contributes the larger share in the formation of the stem, but, during the earlier stages at any rate, *both* are devoted to this purpose (Fig. 5 *b*). There is no single apical cell, and on each side of the median wall cells are seen clearly marked out by their contents and large nuclei, as merismatic cells. The foot originates from the

posterior pair of hypobasal octants, beneath the stem; its cells which border upon the prothallium afford a good example of digesting and absorbing cells; their contents and general appearance contrasting strongly with those of the surrounding prothallial cells.

The root is formed from one of the octants beneath the cotyledon, that is from an anterior hypobasal one. The sister octant merely undergoes a few irregular divisions and rounds off the embryo on that side. The root is first indicated by a triangular apical cell, and is best seen in sections cut at right angles to the basal and median walls. It offers considerable difficulty in tracing out the course of its further development, as the apical cell which is at no time very clear, is subsequently replaced in *most cases* by a group of initials (see Fig. 8), as I convinced myself by an examination of a number of sections specially cut obliquely, in order to determine this point. In some instances however I was unable to satisfy myself that a cell-group was formed at the young root-apex, and there seems no doubt that some variability exists in respect of the structure of the latter. This is well shown in the Figs. 13–17, which were drawn from transverse sections of the roots of young plantlets in which not more than two leaves had been found. It may easily be seen that not only does the number of apical cells vary, but also the direction of the early division-walls is very inconstant, even giving rise in one case (Fig. 15) to an appearance almost suggesting the presence of an apical cell. It is obvious however that this construction would break down if the attempt were made to derive the daughter-cells from such a supposed cell, even in the section figured, which was one most favourable to such a hypothesis. Possibly a connection may exist between the relative robustness of the root and the structure of its apex, and this may perhaps account for the discrepancies existing in the statements given by different authors. I regret that my own material was not sufficient to determine this point conclusively in the case of the young embryos, but we know that some latitude of variation exists in certain ferns, e.g.

Osmunda as described by Bower[1]; however this may be, it is a fact of some importance that in a number of cases at any rate, the root-apex in the embryo contains a group of merismatic cells, instead of the single apical cell so characteristic of the leptosporangiate ferns.

The vascular bundle of the embryo is formed at an early age, and is first differentiated in the cotyledon; it joins directly on to the bundle of the root, the first tracheids appearing at the point where the leaf-trace curves into the stem, and from thence fresh ones are differentiated in an upward and downward direction. The vascular bundle is accompanied in the cortex surrounding it, by rows of cells containing tannin (Figs. 11, 12). These are differentiated in the embryo at a very early age, long before it issues from the prothallium. Their development is best observed in the cotyledon, where they are seen to arise as cells, which elongate with the growth of the leaf, and finally coalesce by the disappearance of their transverse septa much as do the cells composing the laticiferous tissue of *Chelidonium*. I saw no instance of any lateral extension of these tannin-cells, though sometimes short blind protuberances are pushed between other cells of the cortex.

When the embryo has reached a certain size, it bursts the prothallium, the root boring through the lower surface, whilst the cotyledon and stem break through the cells of the upper surface. This manner of issuing from the oophyte serves at once to distinguish *Angiopteris* from those other ferns whose embryogeny is known; for it will be remembered that in them the cotyledon and stem appear through the archegonial region on the *lower* surface, and, so to speak, grow up round the edge of their prothallium. The peculiarity of *Angiopteris* in this respect may be connected with two facts in its earlier history; namely, first, with the occurrence of the archegonia at some distance behind the apex of the somewhat large oophyte, and secondly with the position of the basal wall which separates the shoot and root portions of the embryo, it

[1] Bower. Comparative examination of the meristems of Ferns: Ann. Bot. III, p. 310.

being formed in this plant in a plane parallel to, instead of at right angles to, that of the prothallium, as in most ferns.

Fresh leaves and roots speedily arise on the young plantlet, the second leaf appears nearly opposite to the first one, and immediately above the first root. Its own root emerges just beneath the cotyledon. The third leaf arises (continuing the spiral) close to the side of the cotyledon and its proper root also emerges on the opposite side of the stem. The first two leaves are destitute of stipules, but these structures appear at once on the third leaf, where they are relatively large, and quite functional. The leaf-stalks, especially in the stipular region are covered with hairs containing a large quantity of tannin.

EXPLANATION OF FIGURES IN PLATE XV.

Illustrating Mr. Farmer's paper On the Embryogeny of *Angiopteris evecta*, Hoffm.

[B = Basal wall. M = Median wall. T = Transversal wall. × = Apical cell.]

Figs. 1, 2. Archegonia.

Fig. 3. Consecutive sections through a very young embryo.

Fig. 4. Median section through a similar embryo.

Fig. 5. Section of embryo cut parallel to transversal wall; (*a*) through the cotyledonary end, (*b*) through the stem-apex (shaded).

Fig. 6. Longitudinal section of older embryo with root-cell.

Fig. 7. Section, almost transverse, through embryo showing apical cells of the root.

Fig. 8. Embryo cut obliquely with apical cells of root.

Fig. 9. Median longitudinal section of embryo in the prothallium, with tannin-cells (shaded) in cotyledon.

Fig. 10. Section (not quite median) of embryo in the prothallium.

Figs. 11, 12. Longitudinal sections of part of the cotyledon showing tannin-cells (shaded).

Figs. 13–17. Transverse sections of root-apices of young plantlets.—The curved wall in Fig. 15 is unusual. The arrangement of the cell-walls in Fig. 17 is irregular.

Figs. 18–23. Young plantlets in various stages of development. In Fig. 21 two embryos are shown to have been formed on one prothallium.

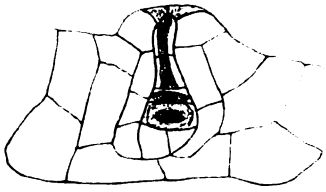

Fig. 1.

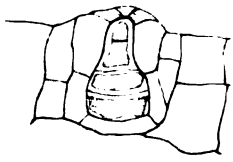

Fig. 2.

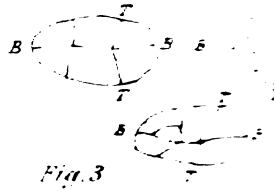

Fig. 3

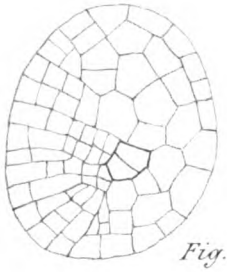

Fig. 7.

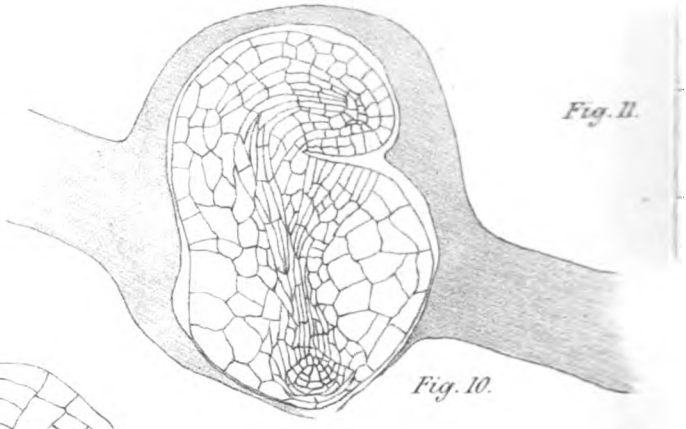

Fig. 11.

Fig. 10.

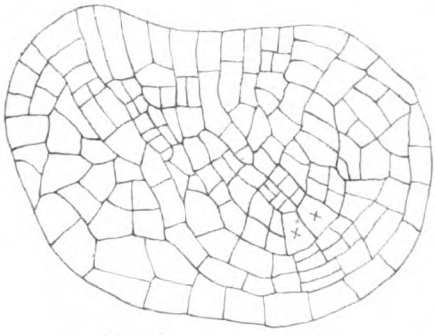

Fig. 8.

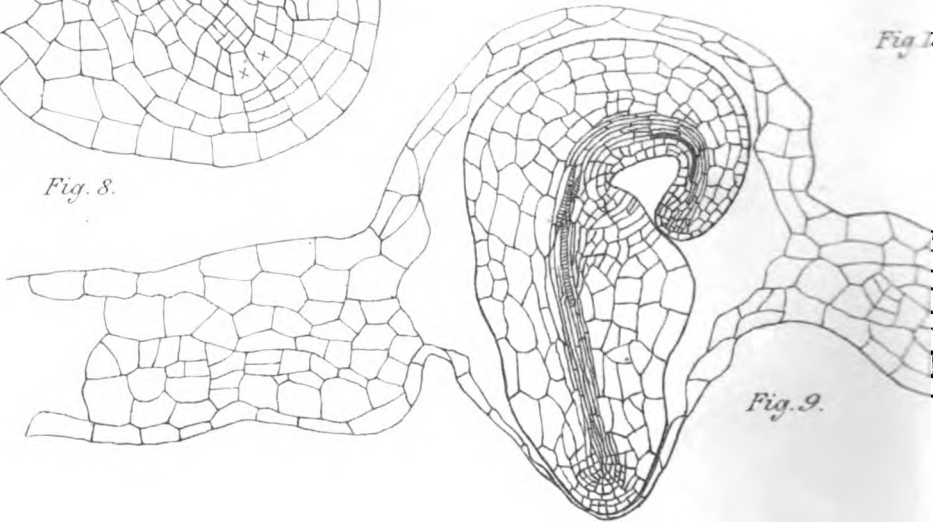

Fig. 9.

Fig. 18

Fig. 19.

Fig. 20.

Fig. 21.

J. B. Farmer, del.

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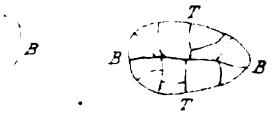

Fig. 4.

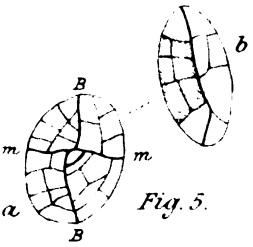

Fig. 5.

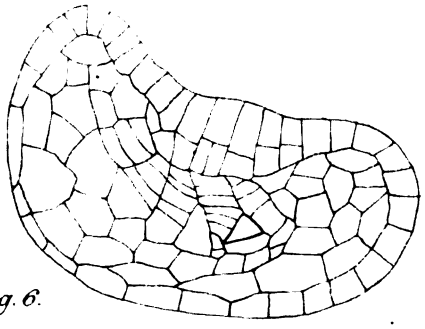

Fig. 6.

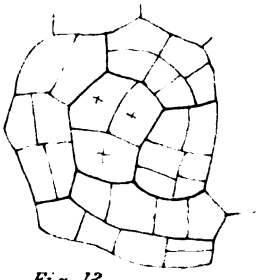

Fig. 13.

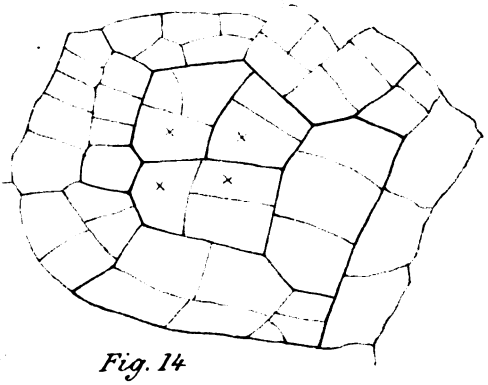

Fig. 14

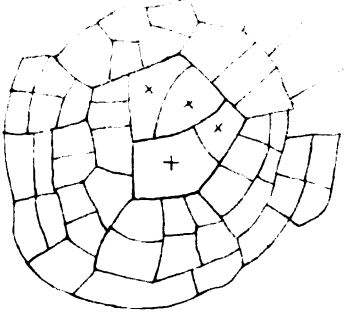

Fig. 15.

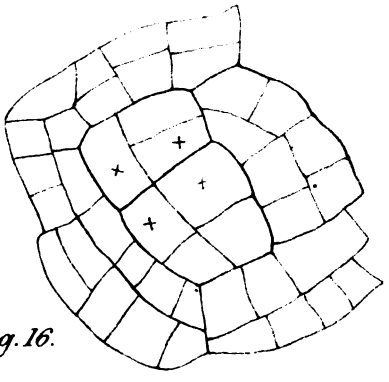

Fig. 16.

Fig. 22.

Fig. 23.

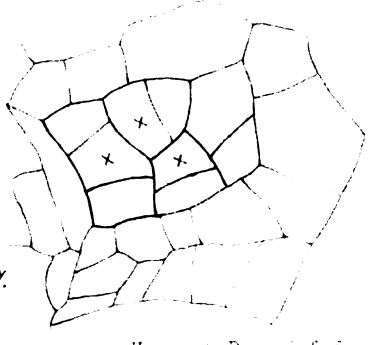

Fig. 17.

On the Staminal Hairs of Thesium.

BY

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With Plate XVI.

IN the Genus *Thesium* and in several allied Genera of the Natural Order Santalaceae there occur, associated with the stamens, curious groups of hairs. These have from time to time received the attention of morphologists, who have discussed their homology, but no account has yet been given of their precise structure and significance. It is proposed to fill this lacuna by a narration of the facts gathered from an examination of a large number of species of *Thesium*, and of such other genera of Santalaceae as possess hairs of the same character. Their service in the mechanism of the flower will be considered, and their morphological value briefly discussed.

Fig. 9 (a longitudinal section of *Thesium alpinum*) shows the position of these hairs and their relation to the anthers in a well known species. The stamens are inserted on the simple epigynous perianth-tube, they are equal in number to the lobes of the perianth and are opposed to them. The hairs arise immediately behind the stamens.

Character and Position of the Staminal Hairs.—Two varieties of these small yellow hairs, or 'staminal hairs' as I shall call them for distinction, are to be found in the genus *Thesium*:—

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(1) those which are comparatively short and thick and directed downwards towards the base of the style (as in Fig. 14), and (2) those which are long and slender, and directed upwards towards the top of the anther (as in Fig. 9).

Both the classes indicated are illustrated by material preserved in alcohol, kindly forwarded from the Cape by Mr. Harry Bolus, F.L.S. The species sent were—*Thesium capituliflorum*, Sond. and *spicatum* L. belonging to the first class; and *Thesium paniculatum*, L. and *debile*, R. Br. to the second. *Th. alpinum*, also belonging to the second class, I have had at my disposal both in a fresh condition and preserved in alcohol. For all other species mentioned in this paper I have depended on herbarium specimens from Kew.

In *Thesium capituliflorum* the perianth consists of five lobes united into a tube at the lower part, but free above, the apex of each lobe being much thickened so as to form a 'hood' over the anther, as shown in Fig. 14, *c*. From the margins and upper part of the hood depend long cellular filaments, thicker above, but tapering towards the free end, which reach to the stigma and form a veil in front of the anthers (Fig. 14, *fil.*). These consist of elongated cells set end to end, three or four thick at the upper part, but in single file below. The anthers are large and almost fill up the cavity of the perianth behind the pendent filaments. The stamens are inserted near the base of the perianth, one opposite each perianth-segment, and at about the level of their insertion is a fringe of yellow hairs which completely encircles the inner surface of the base of the perianth. These are the staminal hairs. The longest and most numerous hairs appear to proceed from ten groups, one on either side of each stamen, but the groups are connected together and form a continuous fringe, as shown in the transverse section of a flower at this level (Fig. 15). The hairs are two or three deep, and directed downwards towards the base of the style which they almost touch (Fig. 14, *a*). They consist of two portions: an enlarged base, the 'basal cushion,' which is sunk below the surface level of the perianth, and a projecting, slender, finger-like portion, which is con-

tinuous with the basal cushion and tapers towards the free extremity. Here it exhibits three remarkable constricted rings (Fig. 6, c_1, c_2, c_3), and a small rounded terminal cap (*c*), an arrangement which appears to serve a particular purpose, as will be demonstrated later. Apparently the hairs are unicellular, for I was unable to find a septum, or even indications of one, between the broad basal cushion and the projecting portion of the hair, at any stage in the development of the flower. Each hair is filled with droplets of a yellowish-green semi-fluid secretion, lying in a protoplasmic meshwork; these droplets produce the yellow colour of the hairs. In older flowers the hairs are colourless and empty, and their terminal caps appear to have been broken off. The cuticle of the hair is thin, and exhibits no special markings. The basal cushion, as well as the projecting portion of the hair-cell, contained globules of the yellow secretion; but in this species I was unable to find a nucleus, although in the surrounding enlarged cells they became conspicuous after staining with methyl-green.

The staminal hairs in *T. spicatum* closely resemble those just described. They are shorter and thicker, and consequently the three terminal constrictions are more evident (Fig. 2, c_1, c_2, c_3). In many, the terminal cap is seen to be broken off, the separation always occurring along one of the constricted zones. In Fig. 3 the separation has taken place at the middle one, in Fig. 4 at the lowest. In all cases where it has happened only the empty membrane of the hair remains, all the yellow secretion having escaped. The basal cushions of these staminal hairs are large, and the secretion developed in them forms large globules which separate out from the protoplasm of the cell. I was able to distinguish a nucleus in the basal portions of two of the hairs (Fig. 1, *n*). In a transverse section of the flower, at the level of the hairs, the long axis of the enlarged base is seen to be at right angles to the surface (Fig. 1, *bas*), but in a longitudinal section the base has a more circular figure (Fig. 2, *bas*). The grouping has been carried further in this species, the ten chief regions

being no longer connected with one another, but forming isolated patches, one on either side of the insertion of the stamen.

Many pollen-grains may usually be seen adhering to the free ends of the staminal hairs in both instances, especially in older flowers after the terminal caps have become detached and the hairs emptied and collapsed. Two such hairs with the accompanying pollen-grains are shown in Fig. 4.

In *Thesium debile*, where the free portions of the staminal hairs are much thinner and longer than in either of the above cases, I noticed one hair in which the terminal cap had not been completely separated, but remained attached at one side (see Fig. 5, iii). From the general appearance of the hairs in all the species I examined it would seem that the secretion is always liberated in this remarkable way, though owing to the preservation of the material in spirit, or by drying, the partially detached minute caps may have been mechanically removed.

In the first class of staminal hairs, of which *T. capituliflorum* and *spicatum* may be regarded as typical, the capped ends remain free and unconnected with the anthers, since they are inclined downwards towards the base of the style; but in the second class this is not so. They are much more abundant, very long and slender, and form only a single group behind each of the stamens (as in Fig. 10), above its point of insertion. The hair-like portions pass up behind the stamens and usually become adherent to the tops of the anthers, sometimes so firmly that they cannot be detached without rupture (cf. Fig. 9 and 11). As types of this class the staminal hairs of *T. debile* and *paniculatum*, and of *T. alpinum* are here briefly described.

T. debile.—The staminal hairs of this flower are long and slender and directed upwards towards the top of the anthers. They are arranged in five groups, one behind each of the stamens, the basal cushions appearing much elongated in a direction parallel to the surface in a longitudinal section of the perianth, and about two deep; but with the longer axes in the opposite direction in a transverse section, forming

a slight prominence above the perianth-surface, and consisting of from twelve to sixteen cells side by side. The secretion is very much the same as before, but the globules seem more finely divided. The filamentous portions spring from the upper end of the basal cushions and are much thinner than in the preceding cases; they are quite filled with small globules of the secretion and extend almost to the top of the anther; the free ends are constricted three or four times (Fig. 5). The terminal caps break off, and in one case I noticed that the cap had not entirely separated from the hair, but remained attached at one side (Fig. 5, iii).

T. paniculatum.—The staminal hairs resemble those of *T. debile* but are more numerous, and still longer and thinner, passing up behind the anther and over the top. They are arranged in five groups, one behind and just above each stamen. The secretion is abundant, the globules being very finely divided. In a longitudinal section of the perianth the basal cushions appear somewhat square and two or three deep; in a transverse section they are flattened from side to side, and there are from twelve to fourteen in a row. The free ends show three constricted zones and a rounded terminal cap, as before, but they are not so conspicuous, owing to the narrowness of the hairs. The hairs are situated directly above the xylem of the vascular bundle which runs up the centre of each perianth-segment.

T. alpinum.—The perianth is much elongated and only 4-fid; consequently there are only four groups of staminal hairs, which generally resemble those of *T. debile* and *paniculatum*, but are more elongated and slender; the filamentous portions of the hairs pass over the tops of the anthers and bend over to touch the stigma (Fig. 9). Their free ends are constricted in the usual manner. Owing to their upward direction the filamentous portions are necessarily cut off in a transverse section of the flower, such as that shown in Fig. 10. I have therefore represented them by dotted lines, in order to indicate their length and breadth, as if they had been bent forwards into a horizontal position.

The staminal hairs of other genera closely allied to *Thesium*, e. g. of *Osyridocarpos* and *Thesidium* seem to differ very slightly from those already observed. They are in both these cases very slender and directed upwards, and although the basal cushions are not very distinctly defined in *Osyridocarpos*, the constrictions and rounded terminal cap at the free end are particularly clear. In the male flowers of *Thesidium* and in *Comandra* the perianth hairs are attached to the top of the anther—in *Comandra* so strongly that they cannot be removed without breaking.

In *Arjona* the character and arrangement of the hairs is slightly different. They are short and thick, and spring from various levels behind the stamen, forming a column covered by short upwardly directed hairs. The cuticle of the hair has pronounced spiral markings, and constrictions occur at intervals throughout the whole length of the hair, giving it a decidedly septate appearance. The free ends are not attached to the top of the anthers, for only the highest ones attain to that level.

In *Quinchamalium*, the flower of which is otherwise very similar to that of *Arjona*, no staminal hairs are present.

I have not been able to find any very satisfactory figures of the staminal hairs in *Thesium*. The best are those of *T. pratense*, by Nees [1], who correctly shows their appearance and position behind the stamens, and of *T. stelleroides* and *T. aureum*, by Jaub and Spach [2], where the hairs are well shown, attached to the tops of the anthers. There is also a small diagram of a longitudinal section through the flower of *T. alpinum* in Engler and Prantl's 'Pflanzenfamilien' (Santalaceae) which correctly indicates the position of the hairs in that species. In other cases they are represented as springing from a common vertical axis which originates behind the stamen (see figures of *T. linophyllum* and *T. ebracteatum* by Reichenbach [3]; of *T. multicaule* by Ledebour [4], &c.); but I have not been able to find any examples of this arrangement.

[1] Gen. Flor. Germ. 1835. n. 48.
[2] Ill. pl. Bot. t. 104. 300.
[3] Iconogr. Bot. 1827.
[4] Ic. Fl. Ross. t. 237.

The nearest is in *Arjona*, but here each hair is attached separately to the perianth itself. In many diagrams of *Thesium* flowers, the hairs are omitted entirely, e.g. *T. rostratum*, by Reichenbach[1]; *T. intermedium* and *T. humile*[2].

Development of staminal hairs.—In the youngest flower-buds which I examined, the staminal hairs were already formed and projected from the surface of the perianth, but the basal cushions were only slightly, if at all enlarged. In a very young bud of *T. spicatum* the thick downwardly directed hair as yet only contained very dense granular protoplasm, and looked as if it were merely a much modified and elongated epidermal cell. The protoplasm had become contracted by the alcohol and had receded from the membrane. The constrictions at the free end had already made their appearance. A later stage of the same species showed that the basal cushions were larger and that the constrictions at the free end of the hair had become more pronounced; the secretion was being formed by the protoplasm in the filamentous portion of the hair as well as in the thicker basal part. The secretion first becomes apparent as a number of very minute, yellowish globules, embedded in the granular protoplasm; these coalesce to form larger masses until very little protoplasm remains, and the hair is mature as shown in Fig. 2. The formation of the secretion within the hairs bears a strong resemblance to that of the fatty globules in adipose tissue of animals.

In Fig. 6. an intermediate stage is given in the development of the hairs of *T. capituliflorum*, where the globules are as yet only scantily formed. In all these young stages the contents of the cell were more or less contracted away from the membrane by the action of the spirit in which they had been preserved.

In older flowers the hairs are usually empty, and shrivelled in appearance, but the layer of cells immediately below the epidermis, and bordering on the upper side of the basal

[1] Iconogr. Bot. 1827.

[2] Gussone Plant. rarior. t. 20.

cushion becomes enlarged in the same direction as do the basal cushions themselves, so that it is difficult to draw the line between them when the secretion has escaped. The enlarged cells can ultimately be seen extending from the basal cushions of the hairs under the epidermis as far as the apex of the perianth. In *T. paniculatum* the side-walls of the empty basal cushions become plaited, but this does not seem to occur always in other species.

Nature of the secretion.—I have only investigated the nature of the secretion of the staminal hairs in two species of *Thesium*, viz. *T. paniculatum* and *spicatum*, which had been preserved in spirit, since I was unable to obtain sufficient dried material for the purpose. However, these constituted typical examples of the two classes of staminal hairs, and the secretion appeared to be very similar in both.

In mature flowers it is yellowish green in colour, and more or less clear, especially in the first mentioned, where the globules are more finely divided. In young hairs, and in *T. spicatum* it has a more granular appearance.

With a one per cent. solution of osmic acid, the secretion stains black, demonstrating the presence of an oil; but I was unable to completely dissolve it in either chloroform or benzole, although in the former case only a small dark granular residue was seen after mounting in spirit.

With picric-Hoffmann's-blue only negative results were obtained, although the surrounding protoplasm became deeply stained. After treating with an alcoholic extract of alkannin (which stains best when freshly made), the globules became bright crimson, and this colour could be obtained even after the sections had been immersed for some time in potash solution. It would therefore appear that some form of resin is present. Treatment with iodine solution only gives rise to a deep brown colour.

I therefore conclude that the secretion is of the nature of a balsam,—a mixture of a resin and an ethereal oil; similar globules are frequently to be seen in the subepidermal cells, where large quantities of tannin are also present.

Morphological value of the staminal hairs.—Very little has been published about the nature of these peculiar staminal hairs in *Thesium*; it has been observed that they are frequently attached to the back of the anthers, and in some closely allied genera, e. g. *Leptomeria*, the hairs are said to actually penetrate the loculi of the anther and to emerge on the other side. Some authors considered them to be part of the androecium, but Bentham and Fenzl maintained that they originated as an outgrowth from the perianth.

A. De Candolle mentions some cases in which the hairs were not joined to the anthers (e. g. in *Osyris*), but he could find no case in which they were not attached to the perianth, and therefore confirms Bentham and Fenzl's view. He considers that they originate in the first place at the point of insertion of the stamen on the perianth, and that they become separated from the stamen later; in proof of this he instances the fact that in the female flowers of *Osyris*, which have no stamens, the hairs are absent, and appears to consider the development of stamens necessary for their production. Reissek describes and figures a monstrous species of *Thesium* in which the stamens had become transformed into buds; the hairs were present at the base, as usual, but were not connected in any way with them, and this he argues demonstrates their independent origin.

By tracing the development of these staminal hairs and comparing sections taken through them in various directions, it seems to me that they are merely modified cells of the perianth which have become enormously elongated, and in the first series of species where the hairs are short and bend downwards towards the base of the style, they obviously have no direct connection with the stamen. The position of the hairs frequently near the throat of the perianth-tube and opposite the perianth-segments renders it highly probable that they do not represent a rudimentary corolla, as has been suggested; and they are rather to be regarded merely as emergences from the perianth.

Before considering the function of these peculiar structures,

there are other structures in flowers of *Thesium* to be noticed, which seem to be closely correlated with the staminal hairs.

The *pendent filaments* of the perianth-lobes in *T. capituliflorum* have already been mentioned in the general description of that flower. They are very long and composed of elongated cells forming a thick column at the upper part, three or four cells in breadth, and gradually taper off towards the free end (Fig. 14, *fil*). In young flowers the cells are oblong and regular; but in older stages they become corrugated. The apices of the perianth-lobes are much thickened in this species to form a 'hood' over the anther (Fig. 14, *c*); the style is short, and the pendent filaments reach to the stigma.

In *T. spicatum* the characteristics of the former species are more pronounced; the pendent filaments are very abundant and form a thick veil in front of the anthers.

In *T. alpinum*, which possesses staminal hairs of the second class, the inner surface of the perianth-segments is covered by a thick layer of elongated cells, somewhat conical in shape, as shown at *e* in Fig. 7 and 9. These cells also line the lateral flaps of the segments which fold over the anther in front, and enclose it in a cylindrical chamber open above and below. Fig. 7 shows a transverse section of the perianth-lobe taken at the level *D* in Fig. 9. There is no thickening or 'hooding' of the perianth-apex in this species, and the stigma is almost on a level with the top of the anther, the perianth-tube and the style being both much elongated.

In *T. paniculatum* the apex of the perianth-segments is thickened, and from its lower surface and lateral margins hang small projections, composed of groups of four or five rounded cells. Fig. 8 shows two of these groups situated on the lateral margins of two adjacent segments. (In the same figure is seen the way in which the segments dovetail together before the flower is fully opened; this arrangement seems to be general throughout the genus, and may also be seen indicated in Figs. 9 and 14.)

In *T. debile* the perianth-filaments are longer, being composed of six or eight cells set end to end, but only one cell thick. As before they hang from the thickened perianth-apex and from the lateral margins of the segments. In this species the stigma is farther from the top of the perianth than before, but the filaments almost reach it, in consequence of their greater length.

This flower seems to lead naturally up to that of *T. capituliflorum*, and the five species described seem to fall into two converging series, thus:—

I. *Thesium spicatum* and *capituliflorum*, with
- (*a*) downwardly directed thick staminal hairs, arranged in groups on either side of the stamens;
- (*b*) long pendent perianth-filaments, several cells thick;
- (*c*) short style, scarcely reaching above the base of the anthers, or below them;
- (*d*) apex of perianth-segments much thickened.

II. *Thesium debile, paniculatum* and *alpinum*, with
- (*a*) upwardly directed staminal hairs, long and slender, arranged in groups behind the stamens;
- (*b*) short projecting perianth-filaments, or none at all;
- (*c*) style reaching above the base of the anthers;
- (*d*) apex of perianth-segments only slightly or not at all thickened.

In order to see if the other members of the genus bore out this classification with regard to the structure of the flower, I examined about forty-five other species of *Thesium* and neighbouring genera. These all fell into the series, in the order given below, and the following gradations were exhibited in passing from one to another. Starting with *T. himalense*, and passing downwards to *T. scabrum* (a longitudinal section of whose flower is represented in Fig. 11).

(1) The staminal hairs become shorter and a little thicker, at first passing completely over the anther, and then barely reaching to its top.

(2) The style, at first very long and projecting beyond

the anthers, becomes shorter, only reaching to their lower part.

(3) The apex of the perianth-segments, at first curved inwards and the lateral margins either prolonged into flaps or inflexed, becomes gradually thickened to form 'hoods' over the anthers.

(4) The stamens are gradually inserted lower down in the perianth.

(5) The epidermis lining the upper part of the perianth, at first either thickened or consisting of elongated cells becomes prolonged into small projections of rounded cells, as in *T. paniculatum* (Fig. 8). The cells are elongated in *T. selaginеum* and arranged end to end in single file. In *T. debile* the arrangement is the same, but the filaments are longer. In *T. glaucum* the filaments are irregularly two cells thick.

T. funale (Fig. 12) forms the starting point of the second part of the series. Passing from this up to *T. spicatum* the following gradations are seen:

(1) The staminal hairs become much shorter and thicker, pass downwards towards the base of the style, and become arranged in two groups one on either side of the stamen, instead of behind it, as in *T. funale* (which must be regarded as an intermediate form, since some of its perianth-hairs are directed upwards).

(2) The style is very short, and does not reach to the base of the anthers (except in *T. capituliflorum*).

(3) The apex of the perianth is thickened to form a 'hood.'

(4) Stamens become inserted at the base of the perianth.

(5) The pendent filaments are very long and thick, almost reaching to the stigma, and being of the same kind as described in *T. capituliflorum*.

The inflorescence in the first part of the series is loose and spreading, but it becomes more compressed, and in the second part consists of densely packed heads of flowers.

Function of the staminal hairs.—The correlations of the

other structures of the flower with the modifications of the staminal hairs, as shown in the above series, seems to throw some light on their probable use to the plant. A. De Candolle[1] in 1857 called attention to the hairs, and suggested that they probably served some part in the pollination of the flower; they have been regarded as a nectary[2]; and in Engler and Prantl's 'Pflanzenfamilien' (Santalaceae), p. 206, it is stated that they probably serve as a collecting apparatus for the pollen, and 'are specially fitted to prevent the pollen-grains from falling into the cavity of the flower.'

Insect visits have been observed in *Thesium* by Müller[3], and that this is probably the usual method of pollination may be inferred from the presence of a nectar-secreting 'disc' in some of the species, e.g. *T. funale* (*nec*, Figs. 12 and 13), and *T. triflorum*. In the genus *Thesium*, the disc is usually not well developed, but in neighbouring genera, e.g. *Osyridocarpos* and *Comandra*, it is quite conspicuous, and is frequently drawn out into lobes between the perianth-segments.

In *Arjona*, *Comandra*, and other genera in which the disc is developed, the staminal hairs are present in great abundance. In *Comandra*, Nutt. (Bastard Toadflax), the adherent disc is said to have 'five free lobes, stamens inserted between these, opposite the lobes of the calyx, to the middle of which the anthers are connected by a bundle of threads[4].' Hence the function of the hairs can scarcely be that of a nectary merely, this office being performed by the cells of the disc.

Again, it might be argued that they act as supports to the stamen, being firmly bound in so many cases by their secretion to the anthers, and so preventing any displacement of the stamens. I incline to the view that the close adhesion of the staminal filaments to the anthers is incidental, and due rather to the nature of the secretion, than to any special function of support.

[1] Note sur la fam. Santal. in Bibl. Univ. Genève, 1857.
[2] Mert. and Koch. Deutsch. Flor. p. 281.
[3] Engl. and Prantl's Pflanz. (Sant.) p. 208.
[4] Asa Gray, Bot. U. S. p. 397.

That the staminal hairs do serve as collectors of pollen may be inferred from the fact that usually many pollen-grains are entangled in them, especially in older flowers in which the terminal caps have broken off, and in which the secretion has consequently escaped, as seen in Fig. 4. In the plants constituting the second part of the above series, the pollen would be held at about the level of the low stigma, so that an insect visitor would either take pollen from the hairs, or leave pollen on the stigma according to circumstances, the same position serving for either; and the grouping of the hairs on either side of the stamen would also facilitate the catching of the pollen as it falls from the anther. The long pendent perianth-filaments may serve as guides to the insect, leading it to the lower part of the flower, and the 'hooding' of the perianth would, in this case, probably prove sufficient obstacle to prevent the insect passing behind the stamen, and so missing the stigma.

In the plants constituting the first part of the series, the staminal hairs have been modified apparently, for a different function.

They are more numerous, and are grouped behind the stamen, instead of on either side. The 'hooding' is not well developed, and in many cases is altogether absent, while the stamens are inserted high up in the perianth. Perhaps in this series the staminal hairs act as an obstruction, preventing the insect passing behind the stamen. They may also help to catch and hold the pollen, and this function would account for the large amount of secretion they contain, for in many cases the hairs and pollen grains were seen glued into an irregular mass above and behind the anther by the extruded secretion. The perianth-filaments are very short and thick, or absent altogether, but if their purpose be as before, to guide the fertilizing insect, the greater length and prominence of the style and stigma, together with the position of the anthers, inserted high up in the perianth-tube, would render them less necessary. The correlation in the two cases seems obvious; but it is exceedingly difficult to judge what part the various structures play in different cases.

PROPOSED CLASSIFICATION OF THE GENUS THESIUM, ACCORDING TO THE STRUCTURE OF THE FLOWER.

THE material examined in the elaboration of the series described above, on which is based the foregoing explanation of the part played by the staminal hairs, consisted, except in the case of five species which have been detailed more fully, of dried specimens, which had to be boiled for some minutes and then placed in spirit before sections could be cut; it was probably on this account that I was unable to find any staminal hairs in them, in which separation between the terminal cap and the remaining portion had not been completed; most likely they had been broken off during the drying. However, in most cases the constrictions at the free ends were clearly marked, especially in those flowers in which the hairs were directed downwards.

The pendent filaments of the perianth, after boiling, swelled up until they resembled those of the spirit specimens, and probably had undergone very little change during the process of drying. When very abundant they appeared to be usually white in colour, and to press back the segments of the perianth, as shown in Figs. 11 and 13, and, as it were, to keep the flower open.

The order Santalaceae is divided into three sub-orders—the Anthoboleae, Osyrideae, and the Thesieae. In the Anthoboleae the ovary is superior; in the Osyrideae it is more or less inferior, the perianth is not greatly elongated, and an epigynous disc is usually present. *Comandra* was the only genus in this sub-order which I examined. The Thesieae, however, show an advance on these older types by their lengthened perianth, inferior ovary, and the insertion of the stamens high up in the perianth-tube.

The sub-order Thesieae, is defined as possessing a 'disc' whose limits are usually not distinctly defined, and with 2–3 ovules upon the placenta[1]. It consists of the following genera, which differ but slightly from each other :—

[1] Engl. and Prantl, Pflanzenfam. (Santal.).

Osyridocarpos.
Thesidium.
Thesium.
Arjona.
Quinchamalium.

The genus *Thesium* is divided by Bentham and Hooker into three sections:—*Frisea*, *Euthesium*, and *Psilothesium*. The classification given in the 'Pflanzenfamilien' (Santalaceae) is based on that of Bentham and Hooker, but the section *Psilothesium*, which consists of the only two American species of the genus, is included in *Euthesium*, thus:—

Sect. 1. *Frisea*, with thick perianth-filaments, consisting of about thirty-one species, all from S. Africa.
  Sub-sect. 1. With upright staminal hairs behind the stamens.
  Sub-sect. 2. With no staminal hairs behind the stamens.
Sect. 2. *Euthesium*, with no perianth-filaments.
  Sub-sect. 1. (*Euthesium*, Benth.), flowers in leaf axils or terminal, widely dispersed through the Old World, including a few S. African, and the Australian and S. American species.
  Sub-sect. 2. (*Aetheothesium*, Benth.), with umbellate inflorescences or crowded heads of flowers. All S. African species.

I have arranged the species I have examined in the following converging series, based merely on the structure of the flower, as already described, *T. scabrum*, *T. gnidiaceum*, and *T. funale* forming the connecting links between the two series.

Those in the upper part of the larger series correspond generally to the sect. *Euthesium*, since they possess no perianth-filaments; those in the smaller series fall under the sect. *Frisea*.

Quinchamalium.
Arjona.
Osyridocarpos natalensis.
Thesium himalense.

T. macranthum.
T. brasiliense. (*Comandra.*)
T. pratense.
T. rostratum.
T. divaricatum.
T. ebracteatum.
T. alpinum.
T. australe.
T. madagascarense.
T. tenuis simum.
T. humile.
T. tauricolum.
T. kotschyanum.
T. crassifolium.
T. euphorbioides.
T. racemosum.
T. ericaefolium (*Thesidium exocarpoides*).
T. triflorum
T. compressum.
T. cystoseiroides.
T. paniculatum.
T. leptocaule.
T. strictum.
T. selagineum.
T. debile. *T. spicatum.*
T. lineatum. *T. junceum.*
T. capitatum. *T. carinatum.*
T. glaucum. *T. capituliflorum.*
T. squarrosum. *T. funale.*
T. gnidiaceum. *T. scabrum.*

In addition to the five distinguishing points previously detailed, as we descend the series from *T. himalense* to *T. gnidiaceum*, the inflorescence, at first loose and scattered, becomes compacted, the flowers being arranged in spikes, and closely crowded together.

The plants at the lower end of the series are only found in

S. Africa, but ascending, they are found successively in Asia Minor, Persia, S. Europe, and Australia; then in Central Europe and Afghanistan; the mountains of temperate Europe and in E. Siberia, and lastly in S. America and high up on the Himalayas.

In the second series, passing from *T. funale* to *T. spicatum*, the inflorescence is very crowded, the flowers being collected together into capitula, or dense spikes.

These plants are only to be found in S. Africa.

I have placed in the series the closely-allied genera of *Quinchamalium*, *Arjona*, *Comandra*, *Osyridocarpos*, and *Thesidium*.

Thesidium only differs from *Thesium* in the dicœcious character of its flower; it is a native of S. Africa, and falls naturally into the series.

Osyridocarpos forms a connecting-link between Thesieae and Osyrideae, the flower is very similar to that of *Thesium*, except perhaps for the presence of a slightly developed disc; but the leaves differ considerably, and the distribution does not accord with that of the rest of the series, *Osyridocarpos* being only found in S. Africa and Abyssinia.

Comandra, which resembles *Thesium* in its flower, but has a very distinct disc, is found in Hungary and N. America, and agrees generally with the Thesiums of Central Europe. *Arjona* and *Quinchamalium* resemble the S. American species of *Thesium* in the general shape of the perianth, although the staminal hairs are modified in the one case, and altogether absent in the other. They are only to be found in South America.

From the considerations given above it would seem probable that the ancestral home of the genus *Thesium* is S. Africa, where the species are at the present time more abundant than anywhere else, and that as they spread further north, their perianth became elongated and they lost their perianth-filaments, these no longer being required. The suggestion that the family Grubbiaceae represents an ancestral type of Santalaceae[1] would also strengthen this view, if correct, since

[1] Pflanzenfamilien, p. 229.

the lower converging species of the series of *Thesium* given above would most closely resemble the members of its solitary genus *Grubbia*, with their small perianth and short truncate or slightly-lobed style, with their closely-packed inflorescences, and with their stamens inserted at the base of the perianth. The species of *Grubbia* are natives of S. Africa.

EXPLANATION OF FIGURES IN PLATE XVI.

Illustrating Miss Ewart's Paper on the Staminal Hairs of *Thesium*.

Fig. 1. *Thesium spicatum*, L. Part of transverse section of flower, taken through the level of the staminal hairs. The basal cushions of the hairs are large, and contain protoplasm and globules of secretion, *b*. The filamentous portions are inclined downwards, and are consequently cut across in a transverse section. Faint markings are shown on the cuticle at *a*. *n*, nucleus; *bas*, basal cushion of staminal hair; *f*, stamen filament. × 195.

Fig. 2. Do. Longitudinal section of flower, through a single staminal hair. The constricted zones c_1, c_2, c_3, and the terminal cap, *c*, are well shown. *bas*, basal cushions of hairs; *a*, cuticle of hair; *g*, inner surface of base of perianth. × 125.

Fig. 3. Do. Free end of staminal hair, from which the terminal cap and upper part have broken away, the separation taking place along the constricted zone c_3. *a*, cuticle of hair. × 450.

Fig. 4. Do. Two staminal hairs from an old flower. The terminal caps have broken away and the secretion has escaped, leaving the empty cuticle which shows slight markings. A mass of pollen-grains, *pol*, are entangled with the hairs. *e*, epidermis covering the inner surface of the perianth. × 300.

Fig. 5. *Thesium debile*, R. Br. Free ends of staminal hairs at various stages: i, without terminal cap, separation having taken place at c_2; v, the same, but separation having occurred at c_1; iii, terminal cap incompletely separated; ii, iv, typical ends of hairs. × 490.

Fig. 6. *Thesium capituliflorum*, Sond. Longitudinal section of very young bud, passing through a staminal hair. *bas*, basal cushions of hairs; *p*, protoplasm of hair; *d*, *b*, globules of secretion; *a*, cuticle of hair; *g*, base of perianth, inner surface. × 280.

Fig. 7. *Thesium alpinum*. Diagrammatic transverse section of perianth-segment, at level *D* in Fig. 9. The anther, *n*, is enclosed by lateral flaps, *fl*, of the perianth, lined on both sides by elongated cells, *e*. *v*, vascular bundles. × 44.

Fig. 8. *Thesium paniculatum*, L. Part of transverse section of flower, passing through two adjacent perianth-segments. From the inner margins of each

project groups of rounded cells, *fil*, and the characteristic interlocking of the segments is shown at *j*. *e*, outer epidermis; *i*, inner epidermis. × 200.

Fig. 9. *Thesium alpinum*. Longitudinal section of flower (diagrammatic). In this Fig. and in Figs. 11 and 14, the section is taken approximately through the median plane, so that in the pentamerous flowers the section passes through the centre of the perianth-segment on one side, i.e. that on which the stamen is represented, and through the lateral portion of the segment on the other. In the flowers with only four perianth-segments (e.g. *T. alpinum*), the section passes through the centre of both. *a*, filamentous portion of staminal hair; *bas*, basal cushions of staminal hair; *c*, apex of perianth-segment; *e*, inner epidermis of perianth, above stamen insertion; *f*, stamen filament; *n*, anther; *st*, style. × 23.

Fig. 10. Do. Transverse section, through level *A–B* in Fig. 9. The filamentous portions of the staminal hairs were necessarily cut off, in consequence of their upward direction, but are represented by dotted lines, showing their relative length and thickness, as if they had been bent downwards into a horizontal position. The vascular bundles are represented by darker shading. *bas*, basal cushions of staminal hairs. × 56.

Fig. 11. *Thesium scabrum*. Longitudinal section of flower (diagrammatic; see description of Fig. 9). The stamen filament is inclined towards the style. Lettering as in Fig. 9. *fil*, perianth-filaments. × 20.

Fig. 12. *Thesium funale*. Longitudinal section of flower (diagrammatic; see description of Fig. 9). The perianth-filaments, *fil*, are very long and thick. At the sides of the short style and base of the perianth are darkly-coloured cells, which possibly function as a nectary, *nec*. Lettering as in Fig. 9. × 35.

Fig. 13. Do. View of interior of flower, two perianth-segments being removed. The position and extent of the nectary (?), *nec*, are shown. × 18.

Fig. 14. *Thesium capituliflorum*. Longitudinal section of flower (diagrammatic; see description of Fig. 9). The 'hooding' of the perianth, *c*, is pronounced; the perianth-filaments are very long; the staminal hairs, *a*, are directed downwards. Lettering as in Fig. 9. × 24.

Fig. 15. Do. Transverse section of flower, through level *A–B* in Fig. 14. The enlarged basal cushions of the staminal hairs form a more or less complete ring round the perianth. The filamentous parts are directed downwards or horizontally, and are not so liable to be cut away in the section as in *T. alpinum*. *f*, stamen filament; *b*, globule of secretion; *a*, cuticle of hair. × 64.

Fig. 1.

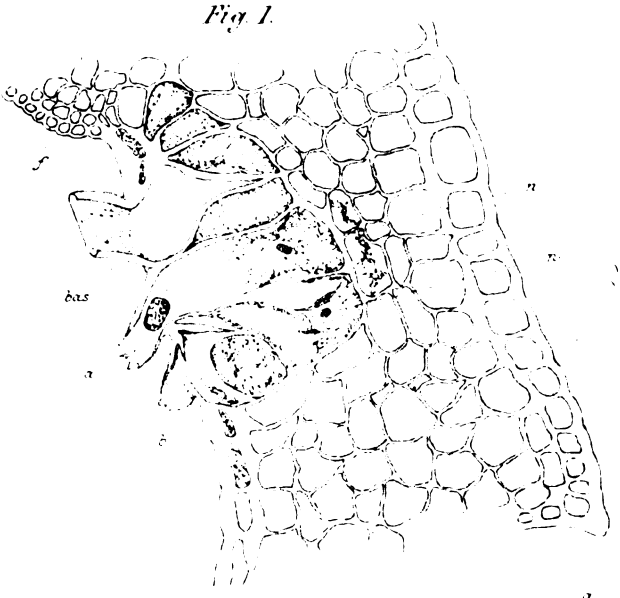


Fig. 3.

Fig. 2.

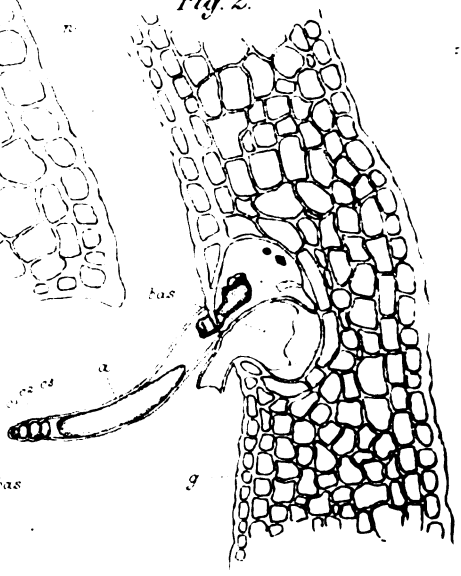


Fig. 10.

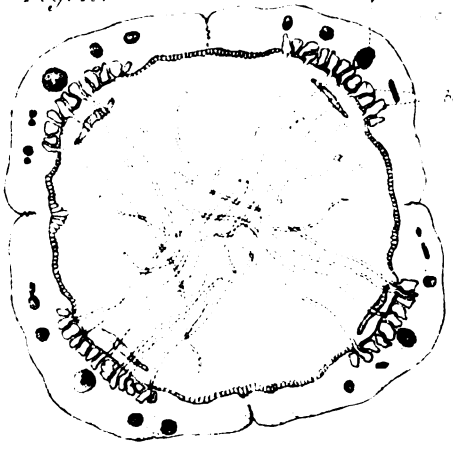


Fig. 13.

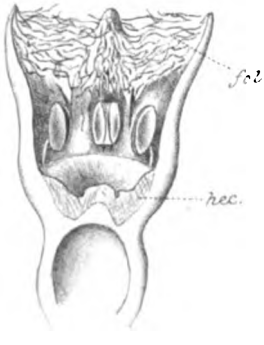


Fig. 11.

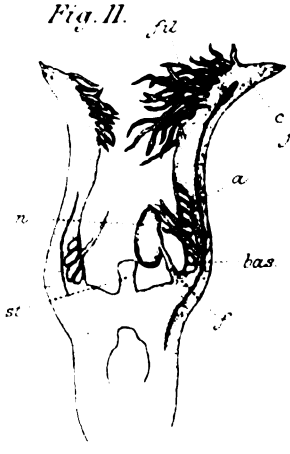


Fig. 1

Fig. 4.

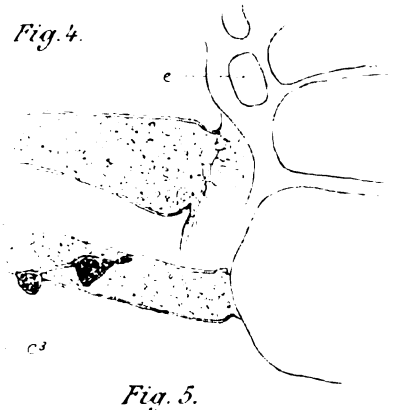


Fig. 6.

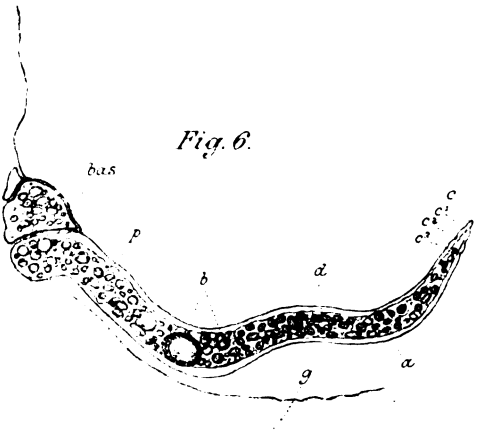


Fig. 5.

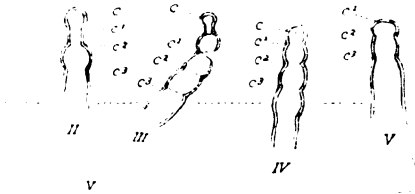


Fig. 8.

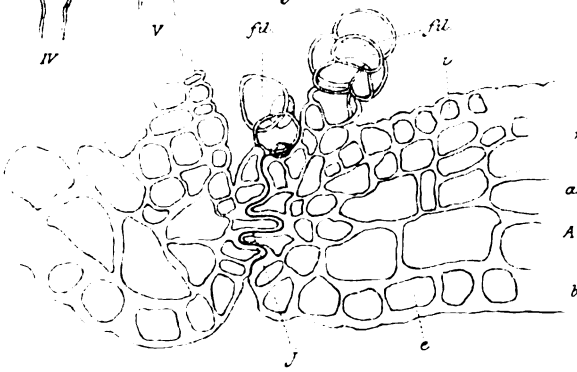


Fig. 9.

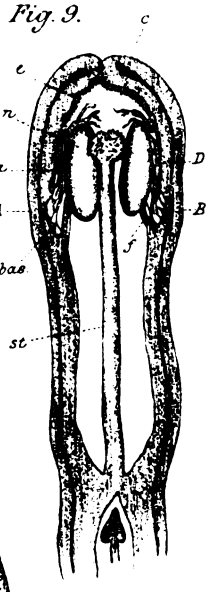


Fig. 14.

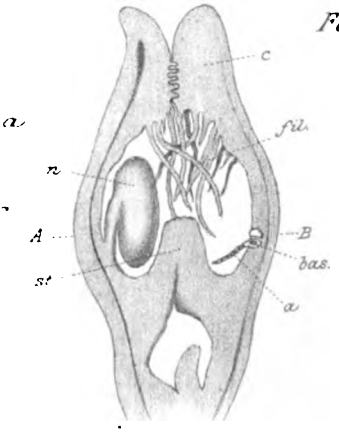
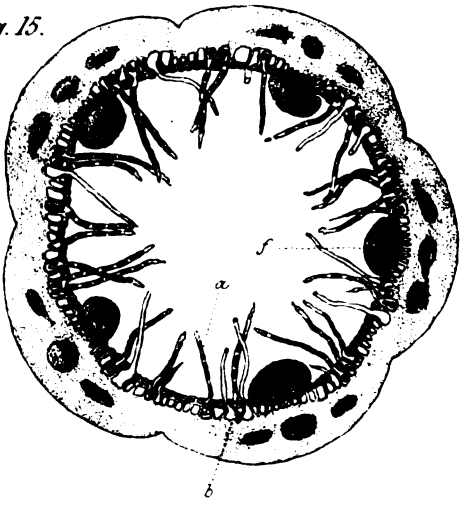


Fig. 15.



University Press, Oxford

On the Sonerileae of Asia.

BY

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With Map, Plate XVII.

WHEN working out the Melastomaceae of Borneo with the intention of giving an enumeration of them to include a considerable number of new species which the Kew Herbarium received recently from Dr. G. D. Haviland of Sarawak, and to show their geographical and phylogenetic relations to those of the remainder of Malaya and of Asia in a general way, I met with various difficulties, which arose partly from the artificial arrangement of the species of *Sonerila* in Cogniaux's monograph of Melastomaceae, and partly from what appeared to me a sometimes very narrow and not always uniform view of the conception of the species. But with this reservation, nobody can more appreciate M. Cogniaux's elaborate work than I do. Any comparative study, however, be it for phytogeographical purposes or for the object of eliciting the phylogenetic relations of a large number of more or less closely-allied species, must be very difficult, if not valueless in its results, if merely based on an arrangement which has chiefly the determination of species for its object. Such a one does not spare us the trouble of reworking the larger genera for all questions which concern the natural relationship of their species. Much could be done, I think, in this direction by keeping separate what is required for mere naming, and for comparative studies of the kind

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indicated. The former might find its best place in a clavis which serves exclusively practical purposes, and may be as artificial as these require; but the latter ought to prevail exclusively in the arrangement and the sequence of the described species. Thus not only the scientific value of such monographs would be highly increased and much trouble spared to those who subsequently take up the same order or a part of it for any special study, but it would likewise be to the benefit of those who use it for determination, as all the more closely allied forms would be kept together, which is quite impossible in an artificial arrangement. It was in compliance with this want of a more natural arrangement of the Sonerileae for my special object that the present paper originated.

There are ninety-eight species of Sonerileae described in Cogniaux's monograph, to which I add the twenty species of *Veprecella*, which I consider to belong to this tribe, not to Oxysporeae. I further add seven new species, which were not taken up by Cogniaux or appear as varieties with him. Thus the number of Sonerileae known at present would be 125. As forty-two, or about one quarter, are African, only eighty-three come into consideration in this paper. They are all limited to tropical Asia, with the exception of *S. papuana*, Cogn., which is a native of Western New Guinea.

The Asiatic Sonerileae, as described in Cogniaux's monograph, belong to the genera *Sonerila* with seventy-two, *Sarcopyramis* with one, *Phyllagathis* with two, and *Brittenia* with one species. *Brittenia* was described first in this monograph; the other genera are admitted generally and even by H. Baillon, who inclines otherwise to rather a wholesale reduction of genera in Melastomaceae. I may premise here that I shall establish in this paper a fifth and a sixth genus on *S. Fordii*, Oliv., and on *S. peperomiifolia*, Oliv., species enumerated by Cogniaux in a special section, *Anomalae*. With this exception, the limitation of the genera of the Asiatic Sonerileae does not need any discussion, and I can proceed forthwith to the classification of the species of *Sonerila* itself. These

appear in Cogniaux's monograph under four sections:— *Genuinae*, *Sonerilopsis*, *Oxycentria*, *Anomalae*. *Sonerilopsis* comprises three, *Oxycentria* one, *Anomalae* three species. Of these, each of the species of *Anomalae* belongs, in my opinion, to a different genus; *Oxycentria* is known only from a description by Miquel, and is very doubtful; *Sonerilopsis* forms a very natural division, whilst *Genuinae* consist of rather heterogenous elements which point at least to two different lines of descent. The greatest complication is exhibited within the section *Genuinae*, and especially in their first sub-division, which is characterised as 'caulescentes; folia consimilia, in eodem jugo subaequalia,' and comprises forty-five species. In discussing them I shall not follow the sequence of Cogniaux's monograph, but dispose them in the arrangement which appears to me the most natural.

SONERILA, *Roxb.*

Distrib.: Western Ghats, from Bombay southwards; Central and South-west Ceylon; Chota Nagpore; tropical and sub-tropical Himalaya from Kumaon eastwards; from the Khasia Mountains and Assam eastwards to South China, and throughout the Eastern peninsula; Malayan Archipelago to New Guinea and the Philippines.

I. **Group of S. zeylanica.** Central and South-west Ceylon; the most southern part of the Western Peninsula; Borneo.

1. *S. zeylanica*, W. et Arn. (Syn. *S. pumila*, Thw., *rostrata*, Thw., *cordifolia*, Cogn., *rhombifolia*, Thw., *glaberrima*, Arn., *affinis*, Arn.), Central and South-west Ceylon; Borneo.

S. zeylanica was originally established by Wight and Arnott on specimens from Ceylon. Two years later Arnott described two more species in Hooker, Comp. Bot. Mag. 307: *S. affinis* and *S. glaberrima*. To these were added *S. rhombifolia*, *rostrata*, and *pumila* by Thwaites, in Enum. Plant. Zeyl. 109 (1859), and *S. cordifolia* by Cogniaux in his monograph, all found in Ceylon. Of these seven species, only *S. zeylanica*, *affinis*, and *rhombifolia* were admitted by C. B. Clarke in Hooker, Flora of British India, II. 530. Cogniaux, how-

ever, in his monograph enumerates all seven as distinct species, and puts them even into different sub-divisions. After a careful examination of the material in the Kew Herbarium, which includes all the type-specimens but one, I have come to the conclusion that all these species should be reduced to *one* species.

S. affinis was established on specimens collected by Col. Walker. They are perhaps a little more robust than those which were named by Wight and Arnott *S. zeylanica*, and their anthers are more attenuate. There are no other differences whatever. It was the shape of the anthers which evidently induced Thwaites, Clarke, and Cogniaux to follow Arnott's view concerning these two species. I, however, find differences in the shape and length of the anthers of the same amplitude within what is considered to be typical *S. zeylanica*. In fact, both species represent one unbroken series of forms varying with anthers 4–4·5 mm. long and produced into a slightly curved beak, to anthers not more than 1·5 mm. long, cordate-ovate and more or less obtuse. The specimens with smaller anthers are generally weaker, but some plants of this form, raised at Kew from seeds sent by Thwaites, maintained their short obtuse anthers, notwithstanding the luxuriant development of the vegetative parts. Both forms grow evidently together, as appears from the localities indicated on the labels. The species next described was *S. glaberrima*, Arn. I have not seen the type-specimen of it which is preserved in Delessert's Herbarium in Geneva. But Thwaites identifies it with his *S. rostrata*, whilst Cogniaux brings it close to *S. rhombifolia*. These two species were brought into different divisions by Thwaites on account of the shape of the leaves, which he says are asymmetric in the former and symmetric in the latter. This, however, is no reliable character. Symmetric leaves occur in *S. rostrata*, as well as in *S. affinis* and *S. zeylanica*, and on the other hand *S. rhombifolia* has also occasionally some asymmetric leaves, just like those which prevail in *S. rostrata*. In fact, the difference of both of them is limited to the more robust habit and the larger leaves in

S. rhombifolia. The venation of the leaves, their texture and serrature, the shape, size and colour of the flowers are exactly the same, so that I am disposed to consider them as mere individual variations, the more, as the type specimens are derived mostly from the same place, Hinidoon. Thus also *S. glaberrima* evidently belongs to the same species. Next *S. pumila* follows as a new species in Thwaites' enumeration. It is merely a dwarf form of the typical *S. zeylanica*, and was found in the more elevated parts of Ceylon. Clarke made it a variety of *S. zeylanica*, but I should not go even so far, but consider it as a form which might be derived anywhere from the typical *S. zeylanica*, when this is exposed to unfavourable conditions of growth. Cogniaux re-established it as a species apparently on account of the supposed smoothness of the seeds. But smooth and glandular-dotted seeds may be found in the same capsule. The last member of this difficult set is *S. cordifolia*, Cogn. It was named originally *S. zeylanica*, W. et A., *forma cordifolia* by Thwaites *in sched.*, and indeed it is nothing but a rather flaccid form from the Singhe Rajah Forest, probably from a very shady place. The leaves are more rounded at the base, and sometimes even cordate, but the same may be observed occasionally in typical *S. zeylanica*. The anthers are of the short acute type.

For these reasons I bring the whole set of these hardly distinguishable forms into one species, *S. zeylanica*, and maintain only the most marked ones as varieties, viz.:

(*α*) v. *vulgaris*, Stapf: anthers short, acute or obtuse, not over 3 mm. long, leaves with distinct more or less spreading serrature (Syn. *S. pumila*, Thw., *S. cordifolia*, Cogn., *S. zeylanica*, W. et A. *sensu strictiore*).

(*β*) v. *affinis*, Stapf: anthers more attenuate or rostrate, 3·5–7·5 mm. long, leaves with usually distinct, very spreading serrature (Syn. *S. affinis*, Arn., *S. rhombifolia*, Thw.).

All the localities hitherto known for *S. zeylanica* in this broad sense are limited to the centre and the South-west of Ceylon, in elevations from 600–1800 m. It is the more remarkable that this plant was recently found by G. D. Havi-

land near Quop in Sarawak, and in a form which is absolutely identical with that which was described by Thwaites as *S. pumila.*

2. *S. Brunonis*, W. et Arn. Central Ceylon; Tinnevelly Distr.

3. *S. wightiana*, Arn. (Syn. *S. wightiana, sensu strictiore*; *S. arnottiana*, Thw.; *S. tomentella*, Thw.; *S. hookeriana*, Arn.) Central Ceylon; Western Peninsula, northwards as far as the Anamally Hills.

Whilst the formation of hairs is either entirely suppressed or limited to a few small scattered bristles on the leaves in *S. zeylanica*, it assumes a far more intensive development in a very closely allied set which otherwise differs but very little. The difference is indeed so trifling that further investigation on the spot may prove the existence of an uninterrupted connexion between this hairy set and that of *S. zeylanica.* The former, which I comprise under the name of *S. wightiana*, Arn., is closely linked to *S. zeylanica* by a form which was called *S. tomentella* by Thwaites, and only differs by the almost tomentose covering on the stem, the petioles along the nerves on the back of the leaves, on the pedicels and on the calyx. But whilst there is—at least as far as our knowledge goes at present—a distinct though slight gap between *S. tomentella* and *S. zeylanica*, the former runs completely away into a still more tomentose form, with more robust habit and a stem more or less woody at the base, *S. wightiana*, Arn., and passing this stage arrives finally at the extreme development of trichomes in *S. hookeriana*, Arn. *S. tomentella* was collected in the Saffragam District (Thwaites, C.P. 2616), *S. wightiana* and *S. hookeriana* both on Adam's Peak (Thwaites, C.P. 3907; resp. 426, 173), all within the area of *S. zeylanica.* From the same locality, Adam's Peak, another species was described as *S. arnottiana* by Thwaites. The type-specimens of it are represented on three sheets in the Kew Herbarium (Thwaites, C.P. 2615). They are identical with those of *S. tomentella.* These specimens vary remarkably in the thickness of the tomentum, and one or two plants are almost glabrescent. These forms, however

do not link this set closer to *S. zeylanica.* They show rather a tendency to branch off in a different direction which is marked by the enlargement of the capsules, which at the same time become comparatively narrower. This glabrescent form from Adam's Peak, which was not separated by Thwaites, approaches very near to *S. tenella* Bedd. or *S. arnottiana*, v. *tenella*, C. B. Clarke in Hook. Fl. Br. Ind. III. 2. 532, if it is not identical, and this again seems to be the same as *S. Brunonis*, W. et Arn., as C. B. Clarke has already pointed out. If I do not sink this latter species into *S. wightiana*, it is because the material of it preserved in the Kew Herbarium is rather poor, and because the capsules differ more in the direction indicated above than those of any other form of this set. Beddome's *S. tenella* was collected in the Anamally Hills, *S. Brunonis* near Courtallam, in the Tinnevelly District, and on Adam's Peak. Thus *S. wightiana, sensu latiore*, with its numerous but quite inseparable forms, appears within the area of *S. zeylanica*, and linked rather closely to it; but it extends in a slightly modified form to the Southern part of the Western Ghats. This is the case with a still more modified and perhaps specifically distinct form, *S. Brunonis*, which may for the present still stand as a species.

4. *S. hirsutula*, Arn. Central Ceylon.

This is a rather well-marked species, closely allied to *S. wightiana*, but stouter, very hairy and with larger very hirsute leaves, much larger flowers and longer, more acuminate or rostrate anthers.

5. *S. Clarkei*, Bedd. Tinnevelly Distr.

It approaches, like the former, very near to *S. wightiana*, but it differs by its leaves which are more narrowed into the base, by its larger flowers, and by its longer, rostrate anthers, which resemble those of *S. hirsutula.*

These four species (2–5) *exhibit a very remarkable parallelism with regard to the shape of the anthers when compared with the varieties of* S. zeylanica. They correspond in *S. Brunonis* and *S. wightiana*, where they are 2–3·5 mm. long, with those of *S. zeylanica* v. *vulgaris* : and in *S. hirsutula*

and *S. Clarkei*, where they attain 6–7 mm., with those of the variety *affinis*.

II. **Group of S. Gardneri.** Central and South-west Ceylon.

The species of this group are small, more or less woody undershrubs with sessile or subsessile leaves with 3–7 basal nerves and short comparatively broad capsules. The tendency to forms with short and long anthers is also here very obvious, another parallel to the two series in the *zeylanica*-group.

1. *S. Gardneri*, Thw. Central Ceylon, 1500 m.

It is distinguished by very short petioles, broadly ovate leaves, a tomentum exactly like that in *S. wightiana*, and short anthers, and particularly by its *broad, ellipsoid capsules*. There is a variety *firma*, Triana, with sessile leaves which approaches closely the following species.

2. *S. robusta*, Arn. Central Province, 1800–2100 m.

3. *S. Harveyi*, Thw. Central Province, 1800 m.

S. robusta has capsules more like those of *S. wightiana*, and the anthers are long and rostrate (5–6 mm.). The hairiness of the stem, the foliage, and the inflorescence, is subject to great variation, and it is sometimes almost suppressed (v. *glabricaulis*, Thw.). *S. Harveyi* is very similar, but it has short anthers (3·5–4 mm.), and it is still more glabrous. Further investigation will probably prove it to be a slight variety of *S. robusta*, to which it stands in an analogous relation as *S. zeylanica* v. *vulgaris* to v. *affinis*.

4. *S. lanceolata*, Thw. South-west Ceylon, 300 m.

It is the most aberrant form of the group, quite glabrous with sessile lanceolate indistinctly crenate leaves and almost obconical capsules more like those of *S. Brunonis*.

The group is separated from that of *S. zeylanica* by a very distinct gap; its members, however, are (with the exception of *S. lanceolata*) in the closest relationship to each other. *They show tendencies in their variation quite parallel to those which become evident in the first group*, and they inhabit also the same area.

III. **Group of S. versicolor.** South-west Ceylon; Western Ghats northwards to the Nilgherries.

Whilst in all species mentioned hitherto the lateral nerves

part from the middle nerve at the very base of the leaf, or nearly so, the venation assumes a different character in this group, which otherwise approaches that of *S. zeylanica*, particularly the peninsular species *S. Brunonis* and *S. Clarkei*. The lateral nerves branch off higher up, the leaves becoming thus 3–7-plo-nerved, and even penninerved (in *S. versicolor*).

1. *S. versicolor*, Wight (*S. axillaris*, Wight). Nilgherries. A very well-marked species much resembling *S. Brunonis*, but with penninerved leaves. *S. axillaris*, Wight, was derived from the same locality as *S. versicolor*, the Sispara Ghat in the Nilgherries, and is identical with it.

2. *S. travancorica*, Bedd. Travancore, 1300 m.

3. *S. elegans*, Wight. Nilgherries.

S. travancorica differs from *S. Clarkei*, to which it is linked nearest, in the nervation and size of the leaves; habit, tomentum, size and shape of the flowers, anthers and capsules being exactly the same. If we imagine a leaf of *S. Clarkei* much increased in the lower part, and that part provided with the vascular bundles necessary for its nutrition and its mechanical strength, we should get a leaf like that of *S. travancorica*. This species was separated from *S. elegans* chiefly on account of its supposed eglandular tomentum, but there are glandular hairs of exactly the same form also in *S. elegans*, and both species are extremely closely allied, if altogether separable.

4. *S. pilosula*, Thw. South-west Ceylon, 300–600 m.

The *versicolor*-group is represented in Ceylon by *S. pilosula*, a species very near to *S. elegans* and *S. travancorica* as far as foliage and flowers are concerned, but evidently of a much more flaccid habit and a somewhat different tomentum. From *S. versicolor* it differs in the 5–7-plo-nerved (not penninerved) leaves.

The species forming this group are closely allied to each other. The nearest affinity outside the group is to *S. Clarkei* and *S. Brunonis* on the side of the *zeylanica*-group and to *S. speciosa* in the following group. Their anthers are long acuminate or rostrate, like those of *S. Clarkei*. They are

smallest in *S. versicolor* (5 mm.), larger in *S. pilosula* (6 m.), and still larger in *S. elegans* and *S. travancorica* (6–8 mm.).

IV. **Group of S. speciosa.** Western Ghats, northwards to Mysore.

This comprises three species distinguished by their robust habit, large flowers, and by the venation of the leaves which are 3–7-nerved, not 3–7-plo-nerved as in the former group.

1. *S. speciosa*, Zenk. Western Ghats from Courtallam to Mysore.

It is a very well-marked species, nearest allied to *S. elegans.*

2. *S. grandiflora*, R. Br. Nilgherries.

The most distinct form of the whole series as far as the habit is concerned. It is an almost shrubby plant with perfectly glabrous, subcoriaceous leaves.

3. *S. Bensonii*, Hook. f. Malabar Ghats (precise locality unknown).

It is nearer to *S. speciosa* than to *S. grandiflora*, but differs from all species hitherto mentioned by the number of the stamens, both whorls being developed. The great importance of this species for the phylogenesis of *Sonerila* will be discussed later on. I wish here only to accent its affinity to *S. speciosa* and *S. grandiflora*, which is so great that it would appear to me quite unnatural to separate it and to make it a group by itself.

The mutual affinities of these first four groups may be expressed thus: the group of *S. zeylanica* is linked on one side to that of *S. Gardneri*, both exhibiting a parallel divergence in their staminal structure, and to that of *S. versicolor* in another direction, and by means of that group to a still more different set, the *speciosa*-group, which has its most aberrant type in *S. Bensonii.* In these two groups, however, no remarkable differentiation in the staminal structure exists, this being uniform and corresponding with the set of the long anthers in group I and group II. All the species of these four groups are limited to Ceylon and the Southern half of the

Western Ghats with the exception of *S. zeylanica* which occurs also in Borneo. Thus it intrudes into the area of another group of close affinity although of, at least for the present, sufficiently marked character. This is the:—

V. **Group of S. tenuifolia.** From Malacca and Sumatra to Borneo.

1. *S. tenuifolia*, Bl. From Malacca and Sumatra to Borneo. *S. tenuifolia* is exceedingly like *S. zeylanica* v. *affinis* in habit, and differs chiefly in the more campanulate (instead of obconic) shape of the calyx and the short capsule. The anthers are acute, but neither acuminate nor rostrate, and about 4 mm. long, being thus intermediate between those of the two varieties of *S. zeylanica*. The species was found in Borneo at Sarawak by Hullett and Haviland and on Kinibalu, at 1800 m. by Low. It is, in contradiction to *S. zeylanica*, remarkably uniform.

This group is represented by several species, but we know yet very little of them, and it is only with reluctance that I link to it several of the Malayan species which I know only from description.

2. *S. laeviuscula*, Zoll. & Mor. Java, Celebes.

3. *S. biflora*, Zoll. & Mor. Java, Billiton.

4. *S. Impatiens*, Becc. Sarawak.

5. *S. purpurascens*, Becc. Sarawak.

6. *S. triflora*, Cogn. Sarawak.

S. Impatiens, *purpurascens*, *laeviuscula*, and *biflora* were placed near *S. rhombifolia*, and seem, as far as I can judge from the descriptions, to form a link closely attaching the *zeylanica*-Group, whilst *S. triflora* appears almost identical with *S. tenuifolia*.

7. *S. insignis*, Bl. Sumatra. This species, which was put nearest to *S. speciosa* by Cogn., belongs, as far as I can deduce from the description, probably to the *tenuifolia*-group.

The affinity of this group and the first is in any case so great that I expect both will appear one when more complete material is to hand.

VI. **Group of S. maculata.** From Nepal to South China and southwards to Sumatra.

In *S. pilosula* a link is given which connects the western group of *S. versicolor* with an eastern one of very great range of distribution, of which *S. maculata* may be considered the type.

1. *S. maculata*, Roxb. From Nepal and the Khasia Mountains to Upper Assam.

The difference between it and *S. pilosula*, Thw., is limited to the presence of glandular hairs, the coarser serrature of the leaves, and the generally stouter habit in the former.

2. *S. brandisiana*, Kurz. Thounggyen River, Amherst District.

This species was referred by Clarke and Cogniaux to *S. maculata*, but it differs really more from it than very many of the species which were admitted by these authors do from each other. The stem is short, fleshy, and rather densely covered with the large scars of the fallen leaves. These are broadly lanceolate, with a much more attenuate or almost decurrent base. It is nearer allied to *S. picta*, or *S. margaritacea*, than to *S. maculata*.

3. *S. picta*, Korth. Sumatra to Mergui.

A well marked species.

4. *S. rivularis*, Cogn. Tonkin.

Nearest allied to *S. picta*, but differing by a taller habit, longer petioles, comparatively shorter leaves and a little larger flowers.

5. *S. cantonensis*, Stapf, Prov. of Canton.

Herba monocarpica, 5–15 cm. alta, simplex vel fere a basi parce ramosa. Caulis nigrescens setulis patulis inferne laxe, superne dense vestitus. Folia symmetrica aequalia, petiolo setuloso-hirsuto, 5–10 mm. longo suffulta, ovata acuta, basi cuneata vel subrotundata, argute serrata, supra subglabra, infra setulis in nervis aspersa, nervis secondariis utrinque 2 in parte tertia infima ortis, 3–4·5 cm. longa, 1·5–2 cm. lata. Cymae distincte et bifarie circinnatae pedunculo 1–2 cm. longo

suffultae, demum quidem glaberrimae. Flores ignoti. Capsula obconica 5–7 cm. longa, leviter obtuseque costata, laevissima, pedicello aequilongo suffulta.

In monte Jing ti Shan, West River, Prov. Canton, C. Ford.

Closely allied to *S. rivularis*, but much smaller, and with a different tomentum.

6. *S. margaritacea*, Lindl. Moulmein?

This species is known only from cultivated specimens. The seeds from which they were raised were probably sent from Moulmein by Lobb.

7. *S. Parishii*, Stapf. Moulmein and Amherst District. (Syn. *S. picta* v. *Lobbii*, Clarke.)

Herba monocarpica, 2–2·5 dm. alta, simplex vel parce fere a basi ramosa. Caulis plus minusve dense tomento rufo vestitus. Folia symmetrica, subaequalia petiolo adpresse tomentello, 1–3 cm. longo suffulta ovata, acuta vel subacuminata, basi cuneata, argute serrata, supra setulis paucis aspersa, infra secundum nervos tomentella, nervis secundariis utrinque e dimidio inferiori ortis, 2·5–8 cm. longa 1·5–7 cm. lata. Cymae terminales pedunculo 2–3 cm. longo suffultae, sub anthesi umbellatim contractae, deinde in circinnos singulos protractae, tenuiter glanduloso-tomentellae. Pedicelli 4–6 mm. longi. Calyx primo subtubulosus, mox dilatatus obovatus vel obconicus, glabrescens vel tenuiter tomentellus, 5–7 mm. longus, dentibus triangularibus parvis. Petala ovata, acuta, 6–7 mm. longa. Stamina 3, antheris rostrato-acuminatis, 6–7 mm. longis. Capsula (immatura) angulato-obovato, 5–7 mm. longa, costis tenuibus 3.

Moulmein District: Mount Moolyet, 2100 m., Parish; high forests on the Thounggyen River, Lobb.

S. Parishii differs from *S. picta* by the more slender habit, longer petioles, the much coarser serrature of the leaves, the more ovoid and shorter calyx. A state of it with smaller leaves, collected on the Thounggyen River by Lobb, was called *S. picta* v. *Lobbii* by C. B. Clarke.

8. *S. secunda*, R. Br. Tavoy, Moulmein.

Closely allied to *S. maculata*, from which it differs only by

the shorter petioles and very slender peduncles and pedicels. Cogniaux describes it as having 'folia valde asymmetrica,' but that may be a slip, as they are hardly asymmetric at all, particularly in the type-specimens of Wallich (4094).

VII. **Group of S. linearis.** South-west Ceylon; Chota Nagpore; Kumaon to South China and the Philippines, southwards to Penang.

1. *S. linearis*, Hook. f. Moulmein, 900 m.

2. *S. Guneratnei*, Trim. South-west Ceylon.

These two are characterised by very narrow one-nerved leaves, an almost ovoid calyx, and slender, sub-cylindric capsules. They are annuals with a thin, somewhat wiry stem. The former was found on Mount Gerai in Moulmein, the latter was discovered by Trimen in the Pasdun Corle in Ceylon. Both species resemble each other in so high a degree that they are, in my opinion, undistinguishable. It is true, Cogniaux attributes to *S. linearis* opposite, and to *S. Guneratnei* alternate leaves. But I find the leaves in both as a rule in whorls of four, but sometimes of three, and sometimes they are opposite. If I keep them separate for the present, it is solely because the fruit of *S. Guneratnei* is not known, and it might be that it constitutes a differential character.

3. *S. angustata*, Triana. South-west Ceylon.

The leaves are broader than in *S. Guneratnei*, but still lanceolate, with coarse crenations and a distinct middle nerve besides two very faint side ones. The flowers are not known, but the capsules diverge very clearly from the *linearis*-type towards that of the *zeylanica*-group. It was indeed named first *S. rhombifolia* v. *angustata* by Thwaites *in sched.*

3. *S. erecta*, Jack. Penang to Moulmein.

Whilst *S. Guneratnei*, and still more *S. angustata*, point to a close affinity with the *zeylanica*-group from Ceylon, we find in this and the following species types, the evolution of which lies in a different direction, and ends, if I may say so, blind, without links towards any other group. *S. erecta* is distinctly different from *S. linearis*, but its close affinity is still clear

enough. It has the same almost wiry stem, and the same shortly pedicelled slender capsules. The leaves are mostly opposite, but there occur also whorls of four. They are broader, more finely serrate, and have distinct side nerves on each side. Besides, the whole plant is more or less hairy, the stem particularly along two opposite commissural lines, the leaves on both sides. The species was removed very far from *S. linearis* by Cogniaux in his arrangement, on account of the anthers being shorter. They are indeed 4–4·5 mm. in *S. linearis*, and 2·5 mm. in *S. erecta*, and besides, they are more acuminate in the former. But we have seen of what little importance this character is in other groups. It really cannot have much weight when compared with the connecting characters.

4. *S. stricta*, Hook. f. Moulmein.

This plant is in a similar relation to *S. linearis* as *S. erecta*. It has generally broader leaves, but sometimes they become almost as narrow as those of *S. linearis*, and assume then a very similar nervation. They are not whorled, but opposite. The plant is much smaller and more slender. The inflorescence and the capsules are quite the same; the flowers, however, are smaller, and the anthers shorter (2 mm.), acute, acuminate or rather obtuse. The plant has fine bristly hairs and is puberulous along the commissural lines of the stem.

5. *S. tenera*, Royle (*S. brachyandra*, Naud.). Garhwal eastwards to South China and the Philippines; Chota Nagpore.

With *S. stricta* a plant was combined as a variety by C. B. Clarke which certainly is most closely allied to it, but differs by broader, very indistinctly serrate leaves, a less strict habit, and shorter, more obtuse anthers. It was called *S. stricta*, v. *burmanica*, and founded on specimens from the Khasia mountains. But this plant is absolutely identical with Royle's *S. tenera*, which was found first in Kumaon and near Dehra Dun. It was also collected by C. B. Clarke in Chota Nagpore, and it extends over Manipur and Burma to Hong-Kong, and re-appears in the Philippines, where Gaudichaud collected it near Manila. These specimens from the

Philippines were described by Naudin as *S. brachyandra*. I have not seen the Philippine plant itself, but the figure given by Naudin (Ann. sc. nat. 3 sér. XV. t. 18. f. 2), leaves no doubt whatever about its identity with *S. tenera*.

VIII. **Group of S. squarrosa.** Khasia Mountains.

It comprises two very aberrant and very well-defined species.

1. *S. squarrosa*, Wall. Khasia Mountains. It has a short, branched stem, which evidently hides in moss. The stem is covered with the scars of the fallen leaves in the lower two-thirds, and with a dense foliage in the upper third. At the base of the lanceolate leaves, brown pointed bristles rise, one on each side, like stipules. Otherwise the plant is quite glabrous. The flowers are arranged in axillary (sometimes apparently terminal) cymes, or these are reduced to a single flower. Calyx, petals, and anthers are similar to those of *S. linearis*, but the capsule is more like that of *S. zeylanica*. The pedicels, however, are in the mature state thicker and very distinctly articulate at the base.

2. *S. arguta*, R. Br. Khasia Mountains. The leaves are very similar to those of *S. squarrosa*, but more membranaceous. They are arranged and accompanied by bristles at their base, as in the former species. The stem is thinner and more fragile, and hides also in moss. The inflorescence is always reduced to a single flower. The peduncle, however, bears still 1–2 of those minute bracts which support the flowers in the cymes of *S. squarrosa*. The calyx is narrower, as in *S. squarrosa*, but besides, there is hardly any difference in the flowers of the two species. But the capsule differs more. It is but faintly ribbed, much elongated, and has a thinner pericarp.

IX. **Group of S. scapigera.** South-west Ceylon. Western Ghats, from the South to Bombay. From Malacca to the Khasia Mountains and to Sikkim.

This group consists chiefly of scapigerous forms, which diverge remarkably from those mentioned hitherto. But there are some species, west and east of the Bay of Bengal, in which

the formation of the '*scapus*' is but indicated, not perfect, and these are the nearest links towards the remainder of *Sonerila*. It is a very remarkable fact that the species forming this group constitute two series which exhibit a striking parallelism, one beginning in Ceylon with a caulescent type, and extending in scapigerous forms to Bombay, and the other starting from Malacca, also with a caulescent species, and reaching in scapigerous types to Sikkim. I shall follow both series separately.

a. Western series.

1. *S. pedunculosa*, Thw. South-west Ceylon.

The reduction of the stem is imperfect. The leaves, however, show a distinct tendency towards crowding above the ground. But these clusters of leaves are connected by generally long hypogaeous or epigaeous weak and flaccid internodes which root sometimes. The leaves are more or less penninerved. The inflorescences are supported by slender and simple, generally long, peduncles. The flowers resemble those of *S. zeylanica*, and are not particularly characteristic. The capsules, however, are short, indistinctly ribbed, and they have a thin pericarp, and exhibit after the seed-scattering a very peculiar appearance, evidently in consequence of their anatomical structure.

2. *S. Rheedii*, W. et Arn. Travancore to North Canara. C. B. Clarke and Cogniaux brought this species to *S. Wallichii*, Benn. The specimen of Wallich's herbarium (4076), named *S. Rheedii*, and quoted by Wight and Arnott under *S. Rheedii*, undoubtedly belongs to *S. Wallichii*; but the authors meant the plant figured by Rheede in the Hortus Malabaricus. This, however, is not a stemless plant. It agrees exactly with the specimens collected by Johnston at Cochin, by Wight at Quillon and by Talbot in Curwar in North Canara. It has a short erect or succumbent fleshy stem, with leaves very much like those of *S. pedunculosa*, but with a more distinctly pinnate nervation and longer, more rostrate anthers. The capsules agree with those in *S. pedunculosa*. The shortening of the stem, and in consequence the crowding of the

leaves above the ground, is sometimes as evident as in *S. pedunculosa.*

3. *S. Wallichii,* Benn. Anamally and Baba Badun Hills, above 900 m.

4. *S. scapigera,* Dalz. Baba Badun Hills to the Bombay Ghats. In *S. Wallichii* the stem is reduced to a very short rhizome which is rather thick and covered with fibrils. The leaves, few in number and often very unequal in size, rise from the rhizome as true 'radical' leaves, and with them the scapelike peduncle. They are long petioled, always more or less cordate, and exhibit sometimes even a tendency towards becoming peltate. The nervation is similar to that in *S. Rheedii*; three nerves spring from the base on each side of the middle nerve, whilst another pair and 3–5 alternate nerves spring higher up. It is the type of the nervation which was meant by Bentham (Bennet et Brown, Pl. rar. Jav. p. 215) under the term 'heteroneura.' But sometimes the first two are alternate or, on the other hand, some of the upper ones opposite. *S. scapigera* differs by smaller leaves, generally longer anthers and the narrower white margin of the capsules. The anthers vary from 3·5 to 6·5 mm. in length, against 3 mm. in *S. Wallichii.* There is, however, but scant material of *S. Wallichii* before me, and future investigation may possibly prove the latter to be only a large-leaved variety of *S. scapigera.*

5. *S. rotundifolia,* Bedd. Anamally Hills.

This species is also closely allied to *S. scapigera.* It has a very small and short rhizome from which the leaves and the peduncle spring, the former being orbicular-ovate and purplish beneath and very similar to those of *S. scapigera.* Also the inflorescence is the same, but more reduced, often to a single flower. The anthers are smaller (2·5–3·5 mm.) and not rostrate.

β. Eastern series.

6. *S. Griffithii,* C. B. Clarke. Malacca.

It is a caulescent form, like *S. pedunculosa* and *S. Rheedii,* found growing with mosses in dripping places on rocks on

Mt. Ophir. It seems to form a short and thick rhizome from which thin often very long branches spring which creep in or upon the moss or the soft ground. Their internodes are sometimes 2–3 cm. long and bear small leaves which soon disappear leaving scars, from the callous margins of which tender rootlets spring occasionally. Towards the end of these branches the internodes become suddenly shortened and consequently the leaves crowd just as in *S. pedunculosa,* whilst the axis ends with a long-peduncled inflorescence. This consists of a cyme which is reduced sometimes to a single flower. The flowers are as in *S. rotundifolia*, but the anthers are rostrate, 3–4 mm. long. The capsules differ more. They are distinctly ribbed and destitute of the characteristic white margin of the allied western species.

7. *S. nudiscapa*, Kurz. Mergui Archipelago; Tenasserim.

The stem is reduced to a very small and short rhizome from which the leaves and 1–3 peduncles spring. The leaves are few, very thin, and show the same venation as those of *S. Griffithii.* The flowers are smaller, the anthers not rostrate, 2·5–3 mm. long, the capsules as in the former, but almost sessile and with faint ribs and a thinner pericarp.

8. *S. amabilis*, Kurz. Tropical Himalaya of Sikkim, to 1200 m.

Very closely allied to *S. nudiscapa.* C. B. Clarke says in a note to a specimen collected in the Rungbee Valley, near Darjeeling, it has a bulbous root. This material is not sufficient to come to a decisive opinion, but from Treutler's specimens it appears quite clear, that these tubers are part of the stem, in fact tubershaped rhizomes. There is, for instance, one specimen with three such tubers, each about 2·5—3 mm. in diameter, in connexion. They are joined by a very short internode. The first and second bear root fibrils, the third besides them a single leaf, whilst the next internode ends with a cluster of 'radical' leaves and two peduncles.

9. *S. khasiana*, C. B. Clarke. Khasia mountains, 900–1500 m.

The rhizome emits short creeping or succumbent branches with 2–3 pairs of leaves above the ground and a terminal inflorescence which overtops, more or less, the leaves. The rhizome is much shortened and tuberlike, or it consists of a few tubers which are connected by slender internodes. The stem near the nodes is covered with reddish spreading bristles like those of *S. squarrosa* and *S. arguta*. The leaves are of the type of *S. Griffithii*. The flowers are rather larger than in this species, the anthers 4·5–5 mm. long and acuminate, the capsules ovoid-ellipsoid with a thin pericarp and rather faint ribs.

10. *S. violaefolia*, Hook. f. Moulmein.

A much stouter plant than any of the former. The innovations are, as far as the material allows us to conclude, of the same character as in *S. khasiana*. The leaves are crowded at the ends of the branches of the rhizome. They are larger, and of a firmer texture than in the remainder of the group, but of the same type. The inflorescence is cymose, first umbel-shaped, but afterwards circinoid in consequence of the lengthening of the sympodium. Flowers and capsules are as in *S. khasiana*.

In all the species mentioned hitherto the leaves are destitute of transversal venation. The tertiary nerves are faint, sometimes hardly visible, and rise at acute angles from the middle and the secondary nerves. They are curved towards the apex and branch into an exceedingly tender network of venules. Only in *S. secunda*, *maculata*, and *picta*, the outer two or three secondary nerves are more or less distinctly joined by nervules which diverge at more obtuse angles, thus approaching slightly the typically transversal nervation of the following species.

X. **Group of S. obliqua.** (Subgen. *Sonerilopsis*, Miq.) Malayan Peninsula from Perak to Singapore; Sumatra, Borneo.

1. *S. obliqua*, Korth. Area of the group.

2. *S. teysmanniana*, Miq. Sumatra.

The species constituting this group are distinguished by the presence of six stamens. In *S. obliqua* three are of a different shape and colour. Both kinds seem to be fertile, but the pollen in the yellow and smaller anthers is not isodiametric in a wet state, but decidedly shorter in two directions, the axes being 15 : 10 : 10, instead of 15 : 15 : 15. The larger and purple stamens are episepalous and thus correspond with the one series present in the three-staminal Sonerilas. The plant is an erect, but rather flaccid and somewhat succulent annual, and inhabits wet rocks and dense shady forests. The leaves are often very asymmetric and those of one pair very unequal in size. They are thinly membranaceous and often become patched like those of the *maculata*-group. They have 2–3 side nerves entering the blade at the very base and connected with each other and with the middle nerve by distinct transversal nerves which diverge at an angle of 70–90° and run straight or with a slight flexure to the next outer nerve. The axillary inflorescence consists of moderately long-peduncled circinoid cymes. The capsules are sessile with six obtuse ribs in the upper two-thirds and of the shape of short inverted pyramids. They are not unlike those of *S. maculata*, but shorter. *S. teysmanniana* appears from the description to be exceedingly near *S. obliqua*.

3. *S. junghuhniana*, Miq. Sumatra.

It is said to have less unequal leaves and anthers, and these are all yellow and sagittate at the base.

XI. **Group of S. moluccana.** From Penang throughout the Malayan Archipelago to Western New Guinea.

It is distinguished by very unequal leaves, one of each pair being much reduced, by a usually dense strigillose tomentum and short subsessile capsules which are generally bullate-rugose. The larger leaves are mostly more or less asymmetric and sometimes contracted above the base, and the larger half is often produced into a rounded auricle which overlaps the petiole.

1. *S. moluccana*, Roxb. Malayan Peninsula, south of Penang, Sumatra, Java, Billiton, Borneo.

2. *S. beccariana*, Cogn. Sarawak.
3. *S. velutina*, Cogn. Sarawak.
4. *S. borneensis*, Cogn. Sarawak.
5. *S. hirtella*, Cogn. Sarawak.

S. moluccana is monocarpic with a short rooting stem and rather crowded leaves. The small ones are often only a few millimeters in diameter and early deciduous. There occurs in Sumatra, Java, and South Borneo, a variety which is distinguished by a much scantier and shorter tomentum on the stem and the petioles, and, at least in the Borneo specimens, almost glabrous leaves. It was called *S. begoniaefolia* v. *pilosula* by Triana (Trans. Linn. Soc. xxviii. 77). I call it *S. moluccana* v. *pilosula*. Very near to *S. moluccana* come: *S. beccariana*, Cogn., chiefly differing by narrower stronger leaves and distinctly pedicelled flowers and capsules; *S. velutina*, Cogn. with a softer and shorter tomentum; then *S. borneensis*, Cogn. and *S. hirtella*, Cogn. The latter two, of which I saw only *S. borneensis*, are evidently very closely allied, and I doubt very much whether they can be separated specifically. They approach at the same time also very closely to *S. beccariana*, and I should not be surprised if these four Sarawak species should prove in future to belong to one multifarious set of inextricable forms.

6. *S. parviflora*, Cogn. Sarawak.

Unknown to me. It has unequal but symmetrical leaves.

7. *S. heterophylla*, Jack. Sumatra, Java.
8. *S. tuberculifera*, Cogn. Sumatra.

The narrow-leaved Sonerilas of this group are represented in Sumatra and Java by a similar form, but with conspicuously sinuate-dentate leaves and very short axillary cymes which often are reduced to fascicles or clusters of two to five flowers. This is *S. heterophylla*, from which *S. tuberculifera* differs only by shorter and broader leaves. It is probably nothing but a state or variety of *S. heterophylla*.

9. *S. integrifolia*, Stapf. Perak.

Herba erecta. Caulis nigrescens, breviter et adpresse strigillosus. Folia valde inaequalia; majora subsessilia oblique

obovato-oblonga, breviter acuminata, basi brevissime cordata *margine integerrimo*, 10–12 cm. longa, 3–5 cm. lata, supra glaberrima, infra in nervis adpresse setulosa, nervis secundariis duobus basilaribus in latere exteriore, uno subbasilari et uno altius orto in latere interiore, minora minima, decidua. Circini pedunculati axillares, strigillosi. Flores ignoti. Capsulae sessiles vel sub-sessiles, breviter turbinatae, bullato-tuberculatae, 5 mm. longae. Perak, C. Curtis (1302). Well characterised by its entire leaves.

10. *S. papuana*, Cogn. Western New Guinea. Only known to me from description.

All these specimens are closely allied and form a very natural group. But I find it very difficult to link them to any of the previous groups.

XII. **Group of S. magnifica** (Subgen. *Oxycentria*, Miq.). West Sumatra.

1. *S. magnifica*, Miq.

The only species, known from a description of Miquel. It is a very aberrant form, or perhaps no *Sonerila* at all. It has six anthers with an acute spur at the back, and a narrow panicled inflorescence with short contracted branches.

S. Helferi, C. B. Clarke. Tenasserim.

The specimen representing this species is in too imperfect a state to ascertain its natural position in the genus.

Two species which differ very remarkably were brought to the genus *Sonerila* by Oliver, although with some reluctance. Cogniaux referred them to his section, *Anomalae*, but they are no more *Sonerila* than, for instance, *Sarcopyramis* or *Phyllagathis*. They have *tetramerous* flowers and two whorls of inappendiculate stamens, the outer of which are longer and purplish. Both were found in Southern China in the province of Canton, *S. Fordii* on the Lo Fau Shan (at 930 m.) and *S. peperomiifolia*, above Ookaisa, near the summit of the Mausan Mountains, at 690 m. They are by no means closely allied, but belong, in my opinion, to two different new genera which occupy a position at *Sonerileae* similar to that of *Sarcopyramis* and *Phyllagathis*. I call them *Fordiophyton* and

Gymnagathis, and treat them in connexion with *Sarcopyramis* and *Phyllagathis*.

FORDIOPHYTON, *Stapf.*

Flores tetrameri. Calycis parce pilosuli tubus *obpyramidatus*, lobi 4 *decidui membranacei majusculi*. Petala ovata. *Stamina* 8, *inaequalia*; antherae dimorphae, exteriorum staminum *e basi biloba* longe lineares apice uniporosae, interiorum 3–7-plo breviores, ovato-oblongae, omnes inappendiculatae. Ovarium semiadnatum, 4-loculare, vertice exsculptum, marginibus membranaceis coronatum. Stylus filiformis, stigmate incrassato. Capsula ignota.

Herbae erectae, habitu *Sarcopyramidis*, simplices, inflorescentiis exceptis glabrae carnosulae, caule tetragono. Folia petiolata, ovata, serrulata, 5–7 nervia. Flores majusculi, albidi vel rosei, bracteati, in cymis valde contractis primo capituliformibus, demum circinatim expansis, solitariis vel cymose vel subracemose aggregatis dispositi.

Distr.—South China.

1. *F. cantonense*, Stapf. (Syn. *Sonerila Fordii*, Oliv.; Cogn. Mel. 516.) Lo Fau Shan, prov. of Canton.

2. *F. Faberi*, Stapf. South West China.

Circa 3 dm. alta. Folia *oblonga*, unius paris subinaequalia, 8–12 cm. longa, 2–3 cm. lata, acuminata, *basi rotundata*, tenuiter serrulata, *venulis transversalibus vix conspicuis*, petiolo 1·5–4 cm. longo. Inflorescentiae terminales et in ramulis axillaribus dichasicae, ramis deinde in circinos excrescentibus. Calyx tenuiter membranaceus, tubo 10–12 mm. longo, lobis ovatis *acutis* 4 *mm. longis* roseis. Petala saturate rosea, 8–10 mm. longa. Stamina majora antheris 12 mm. longis *lobis basilaribus acutis*, minora antheris vix 4 mm. longis. Capsula ignota.

Mt. Omei, prov. of Sechuan, 1050 m., E. Faber.

The more important differences from *F. cantonense* are given in italics.

SARCOPYRAMIS, *Wall.*

This is a very well-marked genus, which approaches nearest to *Fordiophyton*. It is monotypic and so well defined that it never has been questioned.

1. *S. nepalensis*, Wall. Nepal, Sikkim (800—2700 m.); Khasia Mountains, Silhet, Manipur, Mishmi Hills.

GYMNAGATHIS, *Stapf.*

Flores tetrameri. Calycis glaberrimi *tubus turbinato-campanulatus, dentes breves, late triangulares.* Petala ovata, obtusa, plerumque mucronulata. *Stamina* 8, *inaequalia;* antherae dimorphae, exteriorum staminum *e basi breviter decurrente* longe lineares, apice uniporosae, interiorum 3-plo breviores, lineari-oblongae, omnes inappendiculatae. Ovarium semiadnatum, 4-loculare, vertice exsculptum, margine membranaceo quadri-lobulato coronatum. Stylus filiformis, stigmate incrassato. Capsula brevis, obpyramidata, tenuiter 8-costata, laevis, valvulis late rotundatis dehiscens. Semina ignota.

Herba *acaulis*, rhizomate brevi crasso. Folia longe petiolata, *crassiuscula*, late ovata vel subcordata, integra, 7–9-nervia. Flores albi roseo-suffusi, majusculi, in cymis saepe ad florem unicum reductis longe pedunculatis solitariis vel subracemose aggregatis dispositi.

Distr. South China.

1. *G. peperomiifolia*, Stapf (Syn. *S. peperomiifolia*, Oliv.; Cogn. Melast. 516). Mausan Mountains, Prov. of Canton.

PHYLLAGATHIS, *Blume.*

Distr. From Tenasserim to Sumatra; China, Tonkin, Borneo.

A well-defined genus with tetramerous (rarely trimerous?) flowers, having two equal whorls of stamens and winged calyx-teeth; plurinerved leaves with very distinct transversal venation; and contracted capituliform or umbel-shaped cymes. Cogniaux says in his diagnosis of the genus, 'Folia opposita vel *terminali solitario.*' Their leaves are typically always opposite, but in *P. rotundifolia* one leaf of the uppermost pair is suppressed as a rule, leaving at its place only a small naked bud, or no trace at all. Then the leaf appears terminal, bearing the peduncle laterally at the base of its petiole, which seems to continue the axis.

1. *Ph. rotundifolia*, Bl. From Tenasserim to Sumatra.

2. *Ph. gymnantha*, Korth. South-east Borneo.

3. *Ph. tonkinensis*, Stapf (Syn. *S. tonkinensis*, Cogn. Mel. 1184). Tonkin.

This plant has all the characters of a true *Phyllagathis*, but for the calyx-teeth not being bristly. They have the characteristic dorsal wing though it is a little smaller than in the other species, and exactly the same anthers and similar capsules as *Ph. rotundifolia*.

There is very probably another *Phyllagathis* in South China. Unfortunately the specimen in the Kew Herbarium has only ripe capsules, no flowers. It has a short creeping stem with a rufous tomentum, long petioled almost orbicular-cordate leaves, and long peduncled umbel-shaped cymes. It seems to come near *Ph. tonkinensis*. It was collected by R. Swinhoe in the interior of the province of Fokien.

There is no doubt that *Ph. rotundifolia* is much more remote from *Ph. tonkinensis* and *Ph. gymnantha* than these are from each other, so that two groups can be distinguished within the genus, one in Sumatra and the Malayan Peninsula and the other in Borneo, Tonkin, and South China.

BRITTENIA, *Cogn.*

Distr. Sarawak.

I know this genus only from the description and a tracing of a leaf, which I owe to the kindness of M. Cogniaux. It exhibits evidently the habit of *Phyllagathis* which it resembles in the venation of the leaves, the inflorescence and the winged calyx-teeth. But the flowers are *pentamerous*; the ten stamens are equal, and the anthers have an appendix in front and a long spur at the back. The fruit is not known. But I suspect that a specimen with ripe capsules, sent by Dr. G. D. Haviland from Sarawak, should be referred to *Brittenia*. The leaves exhibit exactly the same venation as Cogniaux's tracing shows, and the capsules are 5-merous, arranged in an umbel-shaped cyme which is supported by a long peduncle. It is true G. D. Haviland states on the label 'stamens 8, blue.' Unfortunately he did

not send any flowers, and I suppose he had a flowering specimen of a *Phyllagathis* before him, when he wrote down these remarks. This error is the more probable as the specimen he sent very much resembles *Phyllagathis* in habit, and the capsules of that specimen are without exception pentamerous. Only in a few cases one valve is distinctly smaller, but not quite suppressed.

1. *B. subacaulis*, Cogn. Sarawak.

Before I try to draw any conclusions from the facts here stated it will be useful to summarise the main points. I think I cannot do it better than by means of a scheme, given at the end of this paper, which expresses in a concise way the arrangement which I think best represents the natural differentiation of the Asiatic Sonerileae. I do not pretend to give a table showing the descent of these genera and species. This would be far more than I can possibly prove. It is nothing more than a short transcription of the rather long exposition I have been compelled to give. If there result from it in the end any suggestions as to the phylogenesis of the Sonerileae they will be the more valuable as they are derived solely from facts.

In order to simplify the scheme I have divided it into two parts. One part shows the arrangement of the species of *Sonerila*, the other of the remainder of the Asiatic Sonerileae. The species are arranged within the groups so as to indicate roughly their greater or lesser affinity. Double lines mean that the species thus connected form probably one uninterrupted series of forms: single lines, that the affinity is very close although there is a distinct gap between them: whilst the names of species which occupy a more isolated position are not linked at all. Thus the attention of botanists who intend to study the subject further, and particularly of those who are in a position to pursue it on the spot, will be drawn immediately to the more critical and questionable species. The lines connecting the groups show in a similar way the degree of affinity and the direction in which they approach

each other most. Where the mutual approximation is doubtful the line is stippled.

The more important facts which we may grasp from this scheme may be stated thus:

1. The species of *Sonerila* group round eleven types. The number at present known is 61, of which 10, however, are in so close a relationship to other species that it is very probable that they will be reduced, with further material.

2. The species belonging to one group are closely or very closely allied, or they assume a comparatively isolated position: but their affinity to at least one of the other species of the group is greater than towards any species of any other group.

3. The species of one group cannot be arranged as a rule in a linear series, but they are linked variously to each other.

4. One or several species of one group show a closer affinity to another group, whilst the remainder diverge more or less from this line of connection, thus forming the blind terminations of the ramification of the group.

5. The groups are connected to each other in an analogous way.

6. The groups of *S. linearis*, *squarrosa*, *scapigera*, and *maculata* converge towards a line which is occupied by the groups of *S. zeylanica*, *tenuifolia*, *Gardneri*, *versicolor*, and *speciosa*.

7. The groups of *S. obliqua* and *S. moluccana* approach each other more than any other group, but they hold an isolated position within the genus.

8. Within the groups of the *zeylanica-speciosa* line we find a species in which both whorls of stamens are developed, *S. Bensonii*; and the same is the case in the two or three species of the *obliqua*-group to which *S. moluccana* and its allied are linked.

9. In all other Sonerileae besides *Sonerila*, both staminal whorls are developed.

From these facts we may, without losing ourselves in too uncertain speculations, draw a few conclusions with regard to the phylogenesis of these Sonerileae.

1. Thus the 3-staminal Sonerileae appear as the reduced offspring of forms with two penta-, tetra- and trimerous staminal whorls. *Brittenia*, *Phyllagathis*, *Gymnagathis*, *Fordiophyton*, and *Sarcopyramis* are still at this earlier stage of evolution. In *Sonerila* it is preserved only in *S. Bensonii* and in the species of the *obliqua*-group.

2. The Sonerilas belong to two different lines of descent which have diverged from a common stock probably in very remote times. One line may be traced back to an origin which is indicated at present by *S. Bensonii*, or the *speciosa*-group; the other may be supposed to have started from a type near to or identical with the *obliqua*-group.

3. Therefore the subgeneric name *Genuinae*, should be applied to the first set; *Sonerilopsis* to the rest, viz. the groups of *S. obliqua* and *S. moluccana*. Of these two, *Sonerilopis* is decidedly nearer to the old type, which is represented by the genera *Brittenia*, *Phyllagathis*, *Gymnagathis*, *Sarcopyramis* and *Fordiophyton*, with which it has the characteristic nervation of the leaves in common.

These deductions are supported in a very remarkable way by the geographical distribution of the Asiatic Sonerileae. The list at the end of this paper shows this more clearly. In this list Nos. 1–49 comprise the *Sonerila* § *Genuinae*, 50–71 the *Sonerila* § *Sonerilopsis* and the remaining genera. Out of the 49 *Genuinae*, twenty-three are found in Ceylon and the Western Ghats, and only ten in the Malayan Archipelago and the Malayan Peninsula, and these belong [all but three] to the *tenuifolia*-group, which is so closely allied to the western *zeylanica*-group, and one is *S. zeylanica* itself.

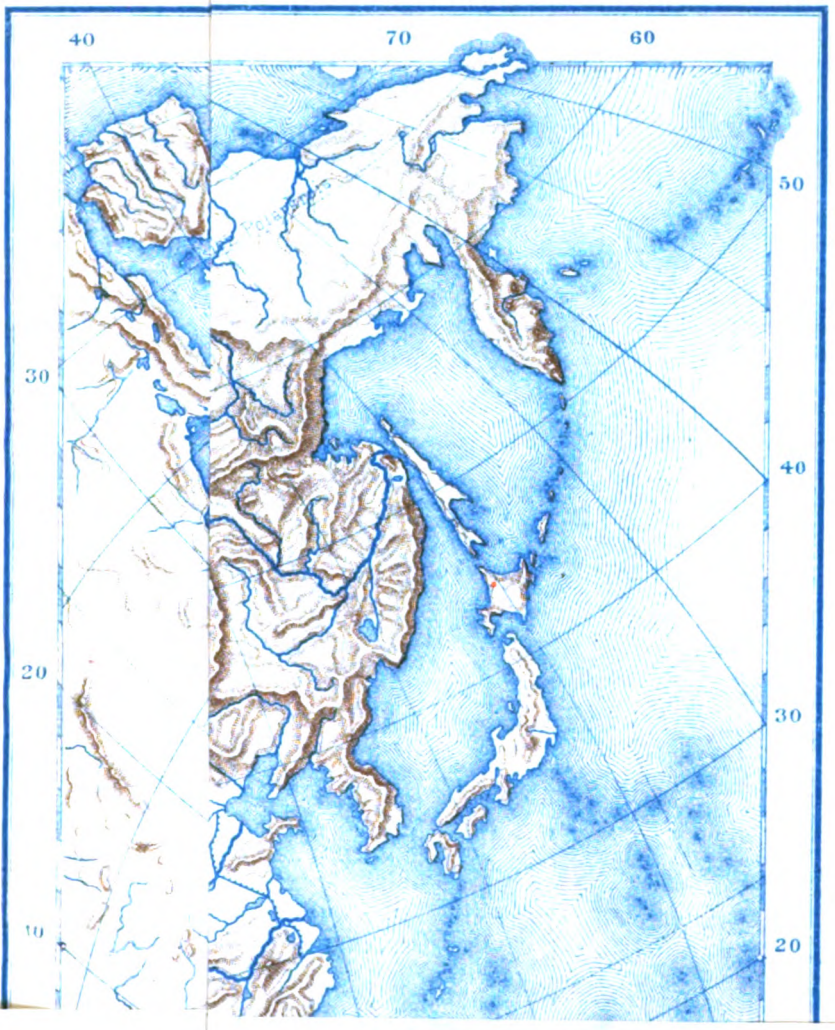
The groups of *S. linearis* and of *S. scapigera* are also represented by a few species in Ceylon and the Western Ghats, whilst that of *S. maculata* approaches, partly at least, *S. pilosula*, again a Ceylon species. On the other hand

there are no close relations between these groups and § *Sonerilopsis*.

Not a single species of the subgenus *Sonerilopsis*, or of the genera *Brittenia*, *Phyllagathis*, *Gymnagathis*, *Sarcopyramis*, and *Fordiophyton*, is found in the Western Peninsula or in Ceylon. *Sarcopyramis* and *Fordiophyton* almost meet in South-West China, and we may look there for their centre of evolution, whilst *Gymnagathis* and *Phyllagathis* meet in South-East China, and *Phyllagathis*, *Brittenia* and § *Sonerilopsis* in the Malayan Archipelago. Probably they sprang from the old continent in which China and Malaya joined each other, a connection which is indicated likewise by numerous zoo- and phyto-geographical and even geological facts. In an analogous way we may assume a centre of evolution for the § *Genuinae* either in Ceylon and the Southern Ghats, or in a hypothetical connection of land which probably has existed between this part of India and Malaya. But this centre is evidently much younger and must probably also be traced back in the last instance to this Sino-malayan continent.

It is not my intention to take into consideration the relations which exist between the Asian and the African Sonerileae. But I must point to the very significant fact that the only connection which exists between them lies through Madagascar, and that the Asian Sonerilas are linked to the African ones by way of their oldest and least reduced type—the pentamerous *Brittenia*,—which is linked closely to the likewise pentamerous *Gravesia* of Madagascar.

Thus the Asiatic Sonerilas may be finally regarded as true Sino-malayan types, and as a typical instance with which the distribution of numerous larger or smaller groups of plants of similar origin may be paralleled.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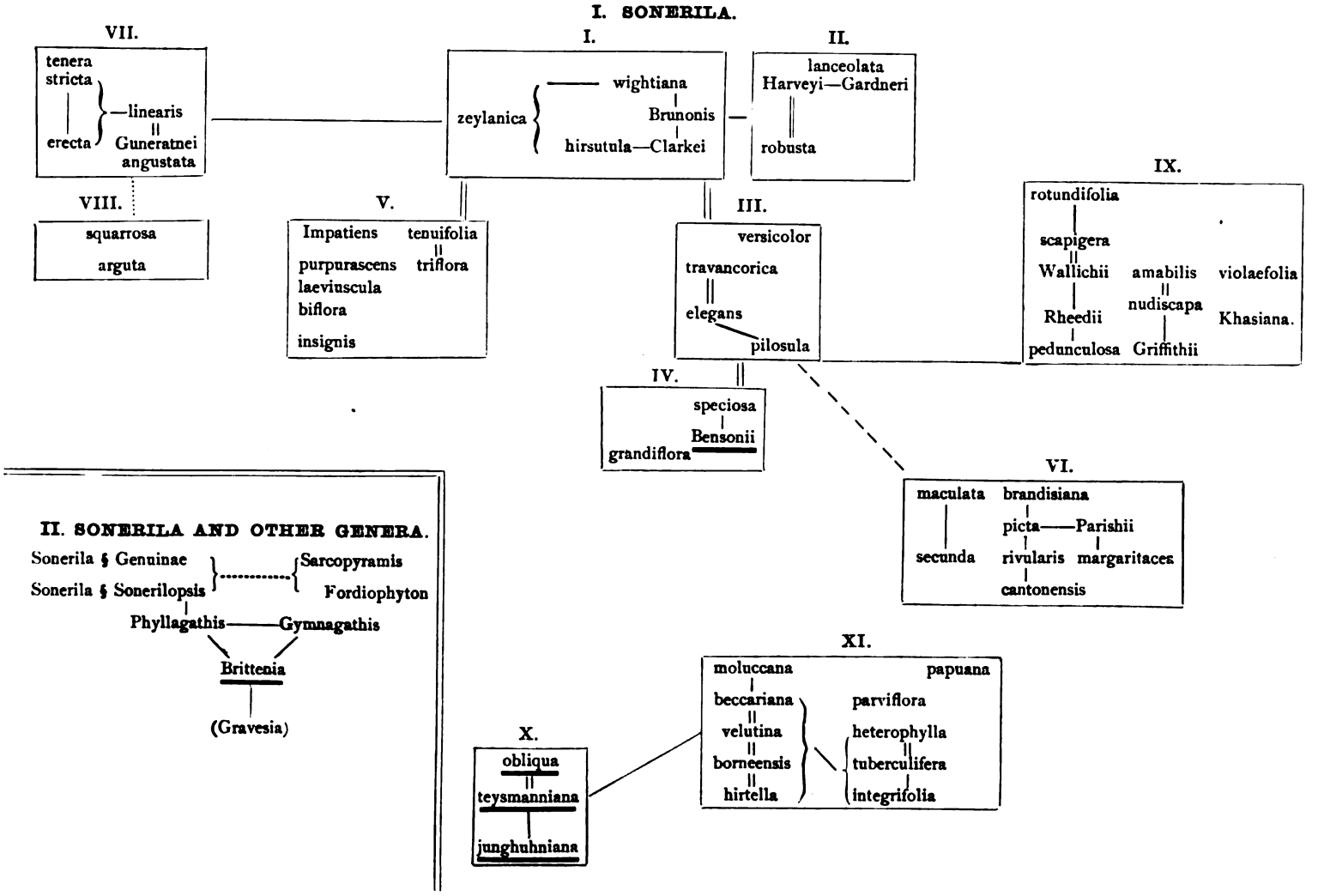
Scheme of the Natural Differentiation of the Asiatic Sonerileae.



		Ceylon.	Western Penins.	Chota Nagpore.	Himalaya.	Khasia to Assam and Burmah.	Moulmein to Tenasserim.	Malaya.	New Guinea.	China, Tonkin.	Philippines.
Sonerila § Genuinae.											
I.	1. zeylanica	1						1			
	2. Brunonis	1	1								
	3. wightiana	1	1								
	4. hirsutula	1									
	5. Clarkei	1									
II.	6. Gardneri	1									
	7. robusta	1									
	8. Harveyi	1									
	9. lanceolata	1									
III.	10. versicolor		1								
	11. travancorica		1								
	12. elegans		1								
	13. pilosula	1									
IV.	14. speciosa		1								
	15. grandiflora		1								
	16. Bensonii		1								
V.	17. tenuifolia							1			
	18. laeviuscula							1			
	19. biflora							1			
	20. Impatiens							1			
	21. purpurascens							1			
	22. triflora							1			
	23. insignis							1			
VI.	24. maculata				1	1					
	25. brandisiana						1				
	26. picta						1	1			
	27. rivularis									1	
	28. cantonensis									1	
	29. margaritacea						1				
	30. Parishii						1				
	31. secunda						1				
VII.	32. linearis						1				
	33. Guneratnei	1									
	34. angustata	1									
	35. erecta						1				
	36. stricta						1				
	37. tenera			1	1	1				1	1
VIII.	38. squarrosa					1					
	39. arguta					1					
IX.	40. pedunculosa	1									
	41. Rheedii		1								
	42. Wallichii		1								
	43. scapigera		1								
	44. rotundifolia		1								
	45. Griffithii							1			
	46. nudiscapa						1				
	47. amabilis				1						
	48. khasiana					1					
	49. violaefolia						1				
		13	12	1	3	5	10	10	-	3	1

	Ceylon.	Western Penins.	Chota Nagpore.	Himalaya.	Khasia to Assam and Burmah.	Moulmein to Tenasserim.	Malaya.	New Guinea.	China Tonkin.	Philippines.
Sonerila § Genuinae. Total ...	13	12	1	3	5	10	10	–	3	1
	23									
Sonerila § Sonerilopsis.										
X. 50. obliqua							1			
X. 51. teysmanniana							1			
X. 52. junghuhniana							1			
XI. 53. moluccana							1			
XI. 54. beccariana							1			
XI. 55. velutina							1			
XI. 56. borneensis							1			
XI. 57. hirtella							1			
XI. 58. parviflora							1			
XI. 59. heterophylla							1			
XI. 60. tuberculifera							1			
XI. 61. integrifolia							1			
XI. 62. papuana							1	1		
63. **Fordiophyton** cantonense									1	
64. ,, Faberi ...									1	
65. **Sarcopyramis** nepalensis .				1	1					
66. **Gymnagathis** peperomiifolia									1	
67. **Phyllagathis** rotundifolia .						1	1			
68. ,, gymnantha .										
69. ,, tonkinensis .							1		1	
70. ,, sp.									1	
71. **Brittenia subacaulis** ...							1			
	–	–	–	1	1	1	15	1	5	–
	13	12	1	4	6	11	25	1	8	1
	23									

On Habenari-orchis viridi-maculata, Rolfe, hyb. nat.

BY

R. A. ROLFE, A.L.S.

Assistant, Herbarium, Royal Gardens, Kew.

With Plate XVIII.

THE subject of the present note is an extremely interesting plant which was sent to Kew for determination by Cecil H. Spencer Perceval, Esq., Longwitton Hall, Morpeth, in July 1891. It was found in a field at Longwitton, Northumberland, on the west side of Trench New Plantation (or Spencer's Plantation) in July 1891, together with *Orchis incarnata*, *O. maculata*, *Habenaria viridis*, *H. chlorantha*, *H. bifolia*, and *Listera ovata*. That it was none of these species, nor indeed any other British one, was at once apparent, and on careful examination it was seen to be so precisely intermediate between *Habenaria viridis* and *Orchis maculata*, or, more correctly speaking, perhaps, to present such an unmistakable combination of the characters of these two species, as to leave no doubt that it was a natural hybrid between them. How far this is the case may be seen in the annexed careful drawing by Miss Smith (Plate XVIII). Fig. 1 shows the hybrid, and Figs. 2 and 3 its supposed parents. In general shape, the flower of the hybrid bears a considerable resemblance to that of *Orchis maculata*, especially in the spreading sepals, and the shape of the lip, yet the latter organ has the narrower more acute side lobes, much exceeding the small median lobe, which strongly indicates the influence of the other parent. And as regards colour, the same influence was unmistakable. Instead of the pale lilac or nearly white shade of the *Orchis*, there was a strong suffusion of pale green

which masked, but did not altogether obliterate, the former colour. The spur is remarkably modified, both in shape and size, having neither the long slender and tapering form of the *Orchis*, nor the very short saccate form of the *Habenaria*, but a linear-oblong, very slightly clavate body barely over a line in length. With regard to the anther, the only really essential difference between the two genera, the balance of characters is rather in favour of the *Habenaria* parent. The two cells are quite parallel, and the glands are exposed, i.e. not enclosed within a pair of pouches, as in *Orchis*, nor do the two cells slightly diverge upwards, as in *Orchis maculata*. There is, however, either a slight abnormality in the development of the tissue at this point, which causes the glands to be more than usually exposed, as shown in the drawing, or else a shrinking of tissue has taken place before the drawing was made. This point was not carefully observed until afterwards, when the specimen was not absolutely fresh. The pollinia, however, are normally developed, as shown in the drawing.

The occurrence of this hybrid is very interesting, as natural hybrids appear to be very rare in Britain, though *Orchis latifolio-maculata* has been recorded from Hampshire (Townsend, Fl. of Hampsh., p. 341) and from Plymouth (Rolfe in Gard. Chron., 1889, pt. II, p. 10). Nor have I succeeded in finding any record of the occurrence of this particular hybrid on the continent of Europe. The one to which it is most closely analogous has been called *Platanthera Erdingeri*, Kerner (Verh. zool.-bot. Ges. Wien, XV, p. 229, t. 4, figs. 4–9), a natural hybrid between *Habenaria viridis* and *Orchis sambucina*, found on the Plateau des Klauswaldes, in Austria.

As the present plant is a hybrid between species of two distinct genera, it may be of interest to call attention to other instances of generic hybrids among Orchids. At least four such cases are known in a wild state; namely, hybrids between *Aceras* and *Orchis*, *Serapias* and *Orchis*, *Lælia* and *Cattleya*, and between *Cattleya* and *Epidendrum*. The first is a natural hybrid between *Aceras anthropophora* and *Orchis militaris*, found in the forest of Fontainebleau. Of the second,

several instances have been recorded, namely, between *Orchis laxiflora* and three different species of *Serapias*, *S. Lingua*, *S. cordigera*, and *S. longipetala*; also the last named with *Orchis Morio* and *O. militaris*; and *O. Morio* with *Serapias Lingua*. Between *Lælia* and *Cattleya* three well-marked cases are known; namely, *Lælia purpurata*, with both *Cattleya guttata* and *C. intermedia*, from the province of Santa Catharina, S. Brazil; and the last named with *Lælia boothiana*, from a region somewhat further north. The last of the series is a natural hybrid between *Cattleya Skinneri* and *Epidendrum aurantiacum*, found together with its parents in Guatemala. Between *Habenaria* and *Orchis* three other examples have been recorded, besides the two already mentioned, namely, *Habenaria Conopsea*, with both *Orchis latifolia* and *O. pyramidalis*, and *H. odoratissima* with *O. maculata*.

Under cultivation several other generic hybrids have been raised; namely *Sophronitis* with *Cattleya*, *Phaius* with *Calanthe*, *Zygopetalum* with *Colax*, and *Hæmaria* with *Dossinia*, *Macodes*, and *Anæctochilus*, while between *Lælia* and *Cattleya*, mentioned above, several other combinations have been effected.

Some of the natural hybrids recorded in books are, to say the least, doubtful, but there are many instances of which no reasonable doubt can exist, and as four disputed cases have been actually confirmed by direct experiment under cultivation, we must at least allow that some of the recorded instances are genuine, and that by careful examination it is possible to trace their origin.

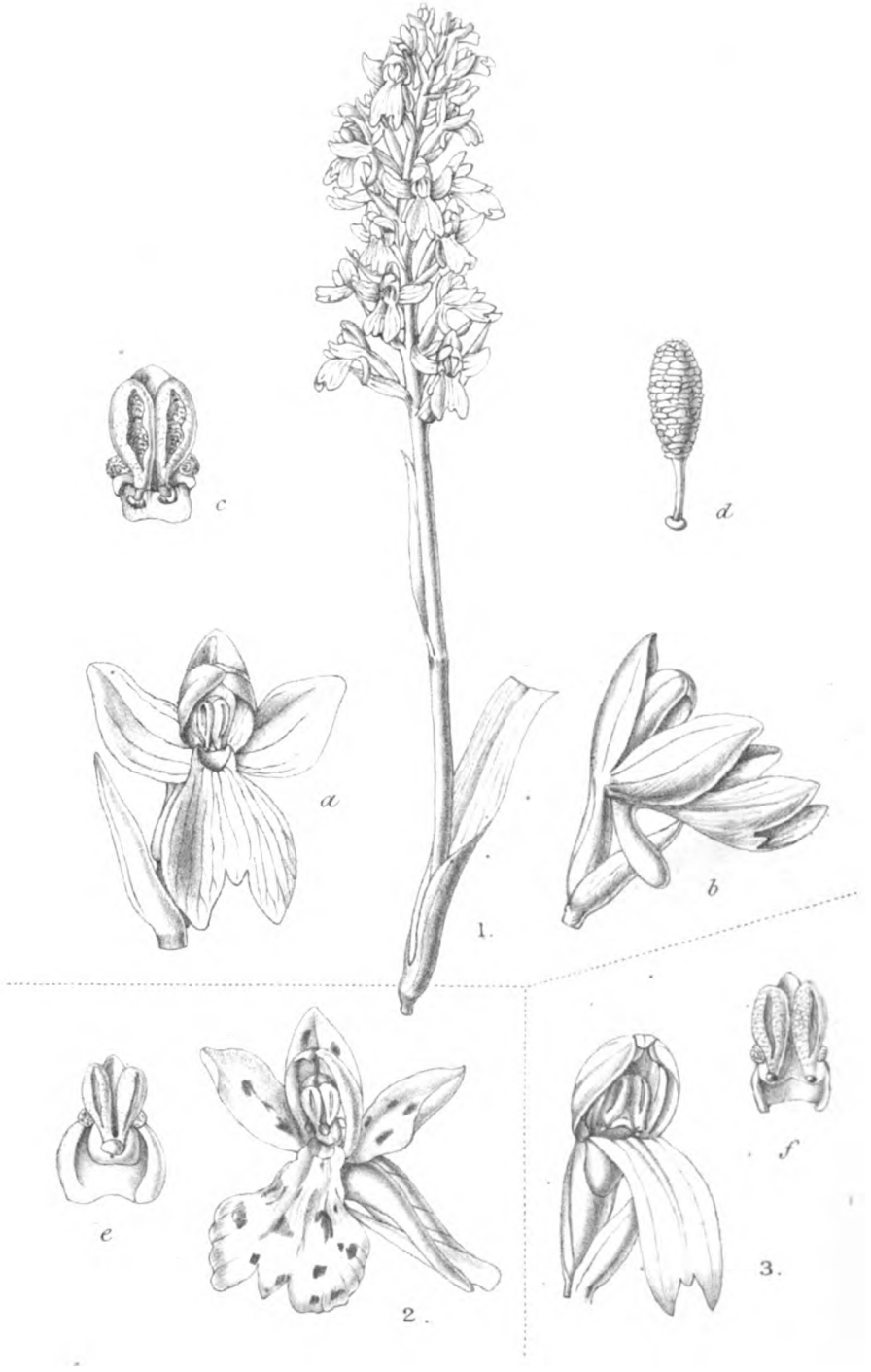
EXPLANATION OF FIGURES IN PLATE XVIII.

Illustrating Mr. Rolfe's paper on *Habenari-orchis viridi-maculata*.

Fig. 1. *Habenari-orchis viridi-maculata*, nat. size, *a*, flower seen from front; *b*, ditto seen from side; *c*, column showing the anther-cells with protruded glands; *d*, pollinium.

Fig. 2. Flower of *Orchis maculata*. *e*, column of same.

Fig. 3. Flower of *Habenaria viridis*. *f*, column of same.



M. Smith del

University Press, Oxford

ROLFE.—ON HABENARI-ORCHIS VIRIDI-MACULATA.

Nematophycus Storriei, nov. sp.

BY

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With Plates XIX and XX.

THE anatomical structure and mode of occurrence of primaeval plants cannot but be of great interest to the student of nature. For many years past the existence, in the Carboniferous age, of gigantic club-mosses and other fern-allies has excited our wonder; and we are now led to believe that, in the older rocks of the Devonian and Silurian periods, forms existed whose structure points to a close connection with the algae.

Pieces of these fossil plants, of great size, occurring in the lower Devonian of Canada, were originally described by Sir William Dawson under the name of *Prototaxites*[1]. This name was generally found unsuitable, and Mr. Carruthers, in a well-illustrated paper published in the Monthly Microscopic Journal of October, 1872, pointed out the algal affinities of the plant, and changed the generic name to the more applicable one of *Nematophycus*.

It may be noted, in passing, as a remarkable fact, that the present genus and *Pachytheca*, long suspected to be parts of the same plant, and occurring, for the most part, in the same beds, form, with *Chara* and a few unicellular organisms, the

[1] Geol. Surv. Can., Fossil Plants, 1871.

sole representatives of the algae which exhibit structure in the fossil state.

The protracted discussion between Sir William Dawson, on the one hand, and Mr. Carruthers on the other [1], together with the careful anatomical description by Professor Penhallow [2], has presented us with a clear impression concerning the nature and affinities of *Nematophycus*, as far as these can be judged in the total absence of reproductive organs.

Thus far, the only detailed descriptions have related to one plant—*Nematophycus Logani*; and, consequently, the full treatment of any new species will add considerably to our conceptions of this remarkable genus.

While examining the slides of *Pachytheca* in the possession of Mr. Storrie at Cardiff, specimens of *Nematophycus* were placed before me, which appeared to me to differ in several important points from the published descriptions of *N. Logani*, and a subsequent examination of a specimen of the latter fossil, kindly communicated to me by Sir William Dawson, confirmed me in this impression. The description of this new species forms the subject of the present paper; and, as a tribute to the energy displayed by Mr. Storrie in collecting the specimens, and the skill with which he has prepared the microscopic sections, both of this fossil and of *Pachytheca*, I propose to call the Cardiff fossil *Nematophycus Storriei*.

The locality and horizon from which the Cardiff specimens were collected have been detailed in a former paper on the structure of *Pachytheca* [3]. The small pieces of *Nematophycus* are found in the same layers as the specimens of *Pachytheca*, and occur in the Tymawr quarry near Cardiff, in rocks of about Wenlock age.

[1] Literature quoted by Penhallow:—Journ. Geol. Soc. xv. 484; Aug. 1881, 482; May 1882, 104; Geol. Surv. Can. 1863, 401; 1871, 16; 1882, II. 107; Can. Nat. (New Ser.) vii. 173; Ann. and Mag. Nat. Hist. 5, ix. 59; M. Micr. Journ. viii. 160; x. 66, 208; xi. 83; Quart. Journ. Micr. Sc. xiii. 313; Amer. Nat. v. 245, 185; see also Dawson Geol. Hist. of Plts.

[2] On *Nematophyton* and allied forms, &c., Trans. Roy. Soc. of Canada, VI. iv. 1888.

[3] Annals of Botany, v. 145, 1891.

It may be well, before proceeding, to give a summary of the characters on which the genus *Nematophycus* was founded, after which it will be easier to form an intelligible idea of the structural details peculiar to *N. Storriei*. The species already instituted are *N. Logani*, Dn.; *Hicksii*, Eth.[1]; *laxum*, Pen.[2]; but from the two latter we can learn little at present. *N. laxum* is described from a few small pieces of fossil wood found associated with *N. Logani*, while the state of preservation in *N. Hicksii* is anything but what could be desired. These two 'species' will, however, be referred to in the sequel.

For the present, then, I shall confine myself to a reference to *N. Logani*, because this is the only species of which any clear idea or good illustrations exist.

Nematophycus Logani.

Nematophycus Logani occurs in the form of fragments, frequently of large size, in the Lower Devonian rocks of Canada. The substance of the plant, as its name implies, consists of a number of thread-like cells. These appear as undivided, elongated, sinuous tubes, with the rarest exceptions entirely separate from one another. The tissue therefore forms a mass of interwoven filaments, recalling more or less vividly the structure of *Codium* and other non-septate Siphoneae (Fig. 1).

In this latter plant the separate cells arise independently, as in the tissue of a fungus, and may be considered as separate plants. But it is not altogether safe to conclude that *Nematophycus* belongs to this class of plants, because such 'hyphal tissue' forms a large proportion of the older thallus of *Fucus* and other Phaeophyceae.

The tubes of *N. Logani*, moreover, may be readily divided into two classes, according to their diameter and the thickness of their walls. Between the larger cells, visible under the lowest power, may be detected, on further examination, a dense network of much narrower tubes (Figs. 2, 3).

[1] Quart. Journ. Geol. Soc. of London, 1881.
[2] Penhallow, Trans. Roy. Soc. Can., VI. iv. 1888.

The presence of these two well-marked kinds of cells is characteristic of *Nematophycus Logani*; and, as will be seen in the sequel, this forms a separating character between this species and *N. Storriei.*

The transverse section of *Nematophycus Logani* is characterised by the presence of a number of more or less radially arranged spaces. These appear like clefts in the tissue, of fairly constant width, but varying considerably in their radial extension (Fig. 4).

At first sight they appear to be regions where the tissues have decayed, but a closer examination occasionally reveals the branching and subdivision of a larger tube—leading Professor Penhallow to suggest that these spaces are the points of junction between the otherwise distinct larger and smaller tubes.

There appears, under a low power, in the transverse section, to be a series of well-marked concentric rings (Fig. 4). This is caused by the considerably diminished diameter of the larger tubes in these zones. It is natural, from our knowledge of exogenous stems, that the term 'rings of growth' should be applied to these. There is always a gradual change in the diameter of the tubes in these regions, as opposed to the sudden widening of the spring-wood in dicotyledonous stems. Occasionally a double ring is met with, such as usually accompanies the formation of new shoots during the summer in plants with secondary thickening.

Beyond these peculiarities of structure, the whole trunk may be said to be fairly homogeneous. No distinction seems to have been noted between the structure of the central portions of the stem and that of the peripheral parts, such as would appear if there were a distinct bark [1].

The tissues described so far seem to belong to an alga of large size with general siphoneoid characters.

No traces of leaves or roots seem to have been discovered. Possibly the thallus was not flattened into 'leaves,' although it is hardly probable that the plant was devoid of roots or hapteres.

The anatomy thus indicated is derived from a careful

[1] Penhallow, loc. cit., p. 42.

examination of the specimens and a perusal of the descriptions of Professor Penhallow and Mr. Carruthers. The plates illustrating the former paper are unfortunately of little use, and those drawn by Mr. Carruthers in the Monthly Microscopic Journal, October 1, 1872, form at present the only reliable illustrations of this fossil. I have, accordingly, prepared a few figures exhibiting the main points of structure under discussion, so that the difference between the present plant and *N. Storriei* will be rendered more evident.

The summary of the structure of *N. Logani* given above represents the present state of our knowledge on the subject. Although, in the main, I am perfectly convinced of the correctness of Professor Penhallow's description, there are several points on which I am not so well satisfied, and which I shall discuss in the sequel. I am not quite content, for instance, to regard the spaces as functionally branching depôts for the junction of larger and smaller tubes. No single instance have I seen of a 'large tube' being connected with a 'small tube.' Both the larger tubes and the smaller undoubtedly branch, and the smaller tubes appear to be divided by transverse walls.

NEMATOPHYCUS STORRIEI, nov. sp.

The pieces of this fossil, on which the present description is based, were all obtained by Mr. Storrie from the Tymawr quarry, near Cardiff, and prepared for the microscope by the same gentleman.

The beds in that quarry, as already noted, are considered by Professor Sollas to belong to Wenlock[1] age[2]. The fragments of fossilized wood occur as small, broken, waterworn bits, imbedded in a crumbling argillaceous matrix, with accompanying specimens of *Pachytheca*. The stems of *N. Logani*, on the other hand, are described as occurring in great blocks much resembling the massive trunks of Carboniferous age.

[1] Annals of Bot., v. 146.

[2] Sollas, on the Silurian District of Rhymney and Pen-y-lan, Cardiff; Quart. Journ. Geol. Soc., xxxv. p. 475, 1879.

The microscopic examination of the present species has been rendered difficult on account of the smallness of the area of the sections; but the structure is preserved in a most remarkable manner, rendering the slides much more amenable to photographic methods than those of *N. Logani*. In general character the plant consisted of a number of separate interlacing tubes, undivided, usually unbranched, but of varying size. The tubes cannot be sharply divided into large and small, as is the case in *N. Logani*, but the spaces between the larger tubes contain those with thinner walls and of smaller diameter (Figs. 5, 6, 7, 8).

Scattered through the tissue are 'spaces,' as in *N. Logani*. But the spaces in *N. Storriei* are more or less isodiametrical in transverse section, as contrasted with the radiating spaces of *N. Logani*. From the smallness of the specimens I have not succeeded in satisfying myself regarding the existence of 'rings of growth.'

Having regard to the characters enumerated thus far, we notice in *N. Logani* all the appearances of a secondary tissue. The regularity of the larger tubes, the growth-rings and the radiating spaces at right angles to them, might, indeed, appear to indicate the secondary tissue of a form having the scattered spaces and varying loosely arranged tubes of *N. Storriei*. With regard to the periphery, it is exceedingly doubtful whether it is represented among the specimens examined. In one or two cases, however, an indentation of the surface is accompanied by a complete change in the direction and arrangement of the tubes—a fact which would seem to indicate that the indentation was caused by a surface-wound during the life of the plant. If such be the case, it may be confidently affirmed that there is no bark or definite external layer in *N. Storriei*.

Such are the main characters of *Nematophycus Storriei*. In the matter of *branching*, we find, as pointed out by Professor Penhallow in *N. Logani*, that the spaces are the regions where this is most evident. In fact, while it requires great care to detect such branching of the large tubes in the latter fossil, in

the present case it is a marked and striking character. As will be seen in the transverse, and more especially in the longitudinal sections figured (Figs. 9 and 10), there is a perfect network of tissue in some of the spaces.

But branching occurs elsewhere. It is not confined to the spaces, but may be met with in the ordinary tissues far from any space, as will be seen from Fig. 11. It is, however, rare in such positions. In passing, it is certainly worthy of note that, although the branching is so excessive in these spaces, yet the small-tube net-work of *N. Logani* is absent in the present species, although the structural details are well preserved. Therefore, Professor Penhallow's suggestion, that the spaces are functionally regions of union between the larger and smaller tubes, must be regarded with caution. One of the peculiarities of the slides of *Nematophycus Storriei*, not noticeable in Sir William Dawson's slides, is the presence of numerous filamentous bodies, of short length and great tenuity, appearing to have their origin in the walls of the larger tubes (Figs. 12 and 13). The presence of these bodies is always accompanied by an apparent disintegration of the tubes. Occasionally they form a network, and forcibly call to mind the hyphae of a minute fungus parasitic on the walls of the tissue. The resemblance is emphasized by the presence of spherical punctate bodies, much resembling masses of spores (Fig. 14), which occur in the cavities of the tubes. Occasionally a tube is seen to arise from such a mass (Figs. 7 and 14). The appearances alluded to seem to be sufficiently striking to be figured, although my present feeling is against their plant-nature. It seems possible that they may belong to some such mineral form as the 'trichites' seen in certain rock-sections. I have not, however, met with them in the sections of *Pachytheca*, although the lithological character of the two fossils may be considered identical.

As already remarked, no single case has come to my knowledge of the connection of the larger with the smaller tubes in *Nematophycus Logani*. The nearest approach to such a union

is seen in Fig. 15. The large tube, at this point, appears to give rise to branches of nearly the same diameter as the small tubes. At a short distance from this point the smaller tubes are branching considerably; and, from a careful examination of the sections, I find numerous cases of small tubes branching in the spaces (Fig. 16). The branches are, however, all uniform in size; and where a large tube divides, its branches have much the character of the similar branches of *N. Storriei*.

The smaller tubes appear to me to be segmented. All search after such dividing walls in the larger tubes has been fruitless, and the many delusive appearances, in examining a fossil section under high powers, has made it difficult to be certain regarding this point.

I have succeeded, however, in observing appearances in isolated tubes which have convinced me that the smaller tubes are really divided by transverse walls (Fig. 16). The relation of large tubes to small tubes remains a mystery, and the introduction of transverse walls into the smaller tubes renders the classification of the fossil a matter of difficulty.

Such are the results of my examination of slides prepared from a single specimen forwarded from Canada. An exhaustive study of the numerous slides in Sir William Dawson's collection would be necessary before the correctness of these observations could be determined.

With regard to the function of the radiating spaces of *N. Logani*, I have a certain amount of difficulty in accepting Professor Penhallow's suggestion of 'branching depôts.' I do not think, at any rate from the illustrations appealed to, that the connection between large and small tubes has been 'proved[1].' I have shown that branching is not confined to the spaces in *N. Storriei*. The following contradictions regarding allied forms are to be noted. In *N. laxum*, where the smaller tubes are excessively numerous, 'there are no spaces at all.' In *N. Storriei*, where there is no small-tube network, the spaces are particularly numerous and the branching well-marked. It appears to me, in consideration

[1] Penhallow, loc. cit., p. 43.

of these difficulties, that it is admissible to seek for another explanation of the existence of spaces in the tissue. It is not probable that they have anything to do with reproduction, from their internal position and the absence of every trace of spore-like bodies; but it is quite in accordance with the structure of existing algae that the spaces might have *some connection with the aeration of the plant.* The branching of the tubes at these points would certainly not militate against such a function, in fact would rather be looked for. I do not, however, think it altogether impossible that these openings in the tissue had somewhat the significance of the medullary rays of higher plants, as suggested by Sir William Dawson. The interchanging of material between different parts of the plant-tissues would probably, even in an alga, be assisted by such channels. But if these openings have this character, we should expect to find the majority of included tubes running in the radial direction, and such is not the case.

It has occurred to me that the specimens described under the name of *N. laxum* might possibly be hapteres or clinging organs of *N. Logani*—although such a suggestion is perhaps premature, before having seen the fossil. The absence of air-spaces in such a part of the plant would not be surprising.

The specimens of *N. Hicksii*, so far described, have been in a very poor state of preservation. From the general character of the tissues and the mode of occurrence of the specimens in the Pen-y-glog quarry near Corwen—where I have carefully collected the fragments—I consider it possible that *N. Hicksii* and *N. Storriei* belong to one and the same species. I do not feel justified at present, however, in classing them together, and have preferred to introduce a new specific name to avoid possible future confusion. An examination of the single slide exhibiting structure, preserved in the Jermyn Street Museum, exhibits peculiarities which are not present in the slides of *N. Storriei.* There is certainly the appearance of transverse walls in the tubes, which here, as in *N. Storriei*, are of one kind. There is frequently a curious transverse sculpturing on the

walls of the tubes, calling to mind scalariform thickening[1]; and curious triangular bodies are met with whose relations are obscure. Of these appearances I find a rough drawing in my note-book, which will suffice to attract attention to the details mentioned (see Fig. 17).

ST. JOHN'S, ANTIGUA, W. I.
March 14, 1892.

[1] Mentioned by Etheridge, Q. J. G. S., Aug. 1881.

EXPLANATION OF FIGURES IN PLATES XIX AND XX.

Illustrating Mr. Barber's paper on *Nematophycus Storriei*.

Fig. 1. *Nematophycus Logani*—longitudinal section from a photograph. × 45.
Fig. 2. do. do. do. × 110.
Fig. 3. do. transverse section do. × 90.
Fig. 4. do. do. do. × 45.
Fig. 5. *N. Storriei* do. do. × 45.
Fig. 6. do. longitudinal section do. × 45.
Fig. 7. do. transverse section from a drawing. × 160.
Fig. 8. do. do. showing the fractured edge of a specimen, from a photograph. × 160.
Fig. 9. *N. Storriei*, transverse section through a 'space,' from a drawing. × 160
Fig. 10. do. longitudinal section through a 'space,' do. × 250
Fig. 11. do. do. showing branching of the tubes away from a 'space,' from a photograph.
Fig. 12. *N. Storriei*, longitudinal section, from a drawing.
Fig. 13. do. transverse section of a single tube, from a drawing. × 800.
Fig. 14. do. spore-like bodies, from drawings.
Fig. 15. *N. Logani*, sketch of a 'space' with large tube branching and small tubes branching.
Fig. 16. *N. Logani*, small tubes branching and divided by transverse walls, from drawings.
Fig. 17. *N. Hicksii*, rough drawings of the Jermyn St. Museum specimen.

Fig. 1.

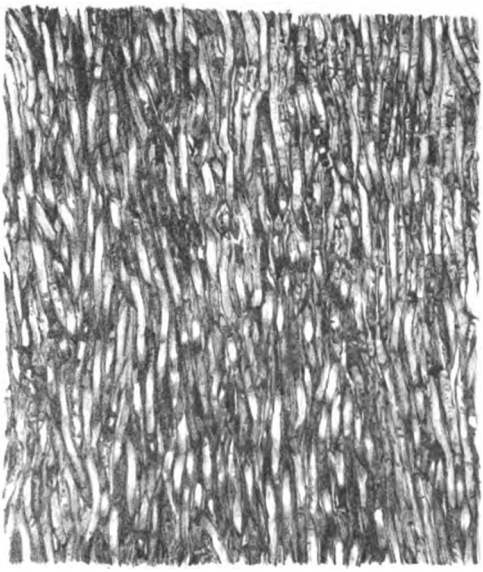


Fig. 2.

Fig. 3.

Fig. 5.

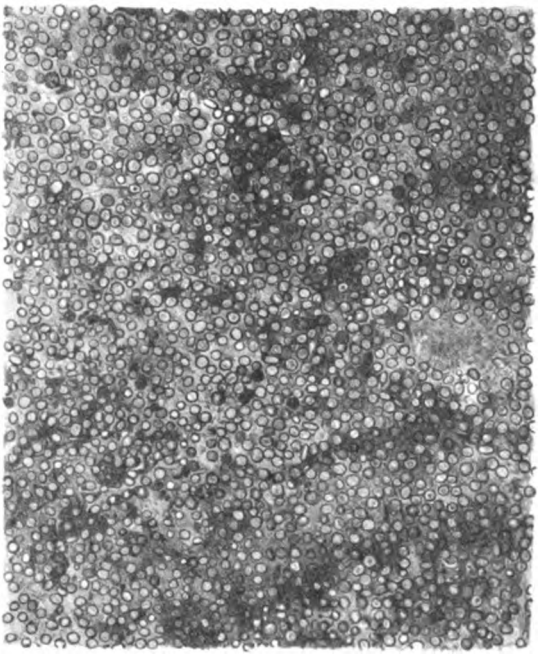
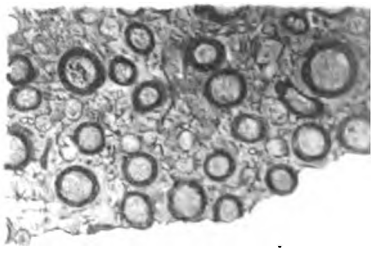


Fig. 8.



From photo. & drawn by T. A. Barber.

BARBER. — ON NEMATOPHYCUS.

Fig. 3.

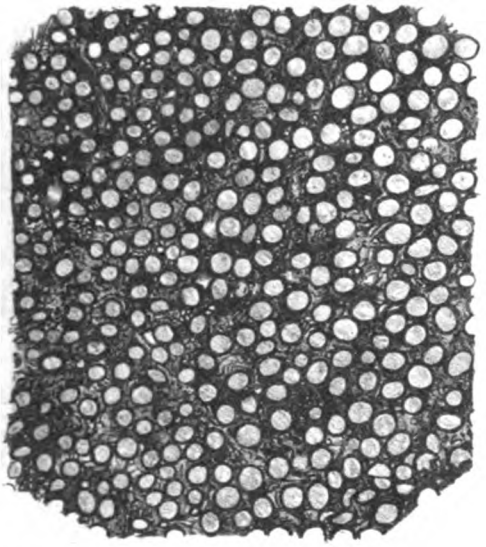
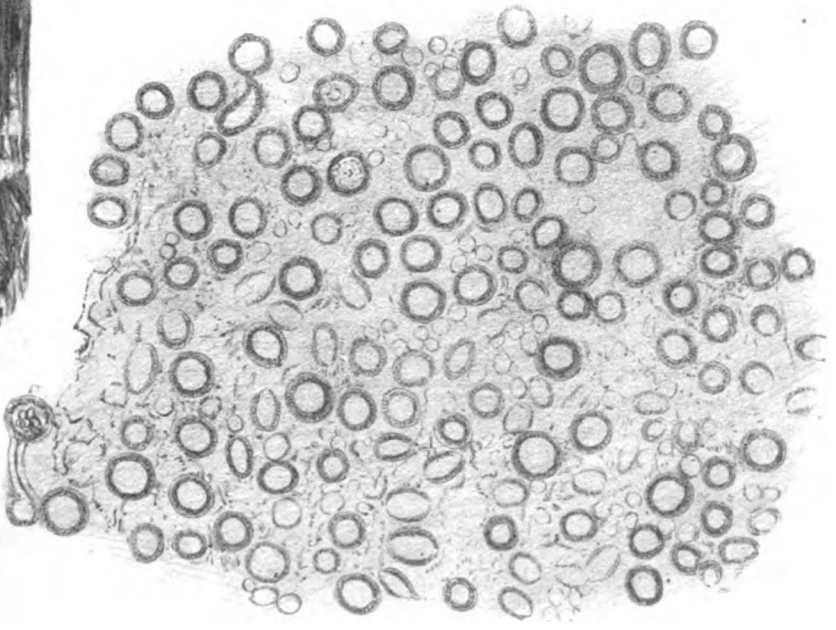


Fig. 4.

Fig. 7.



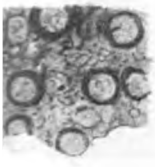


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Fig. 9.

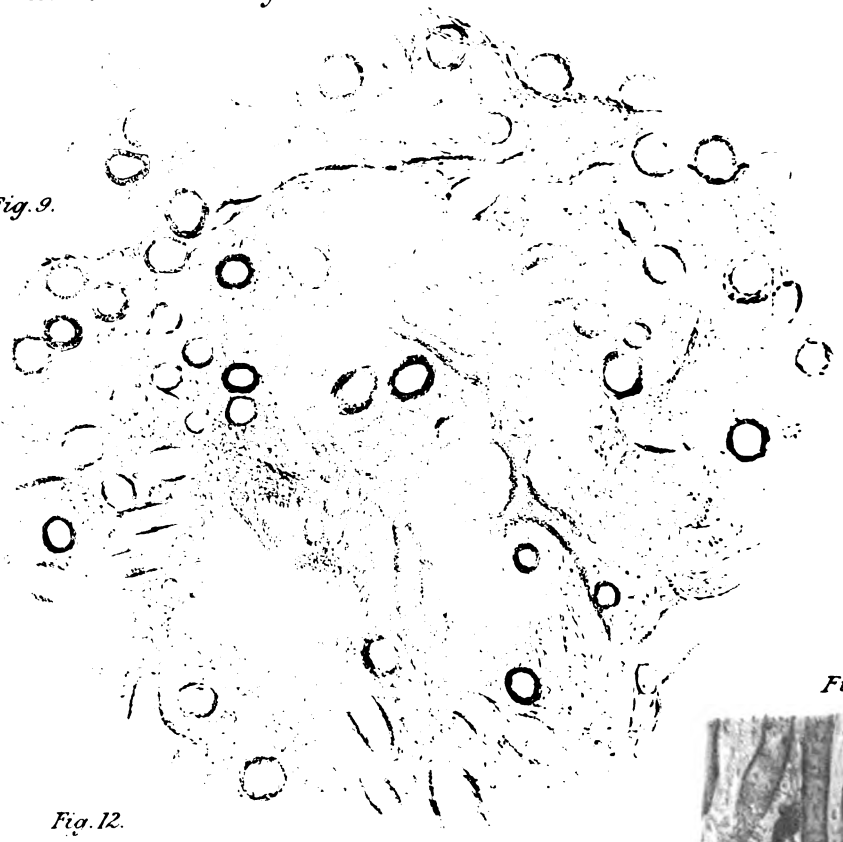

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 16.

Fig. 13.

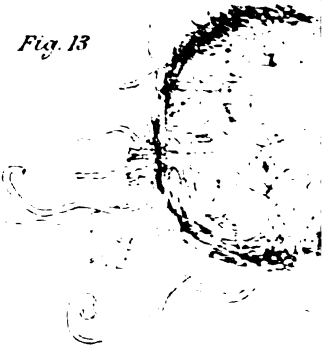

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Fig. 14.

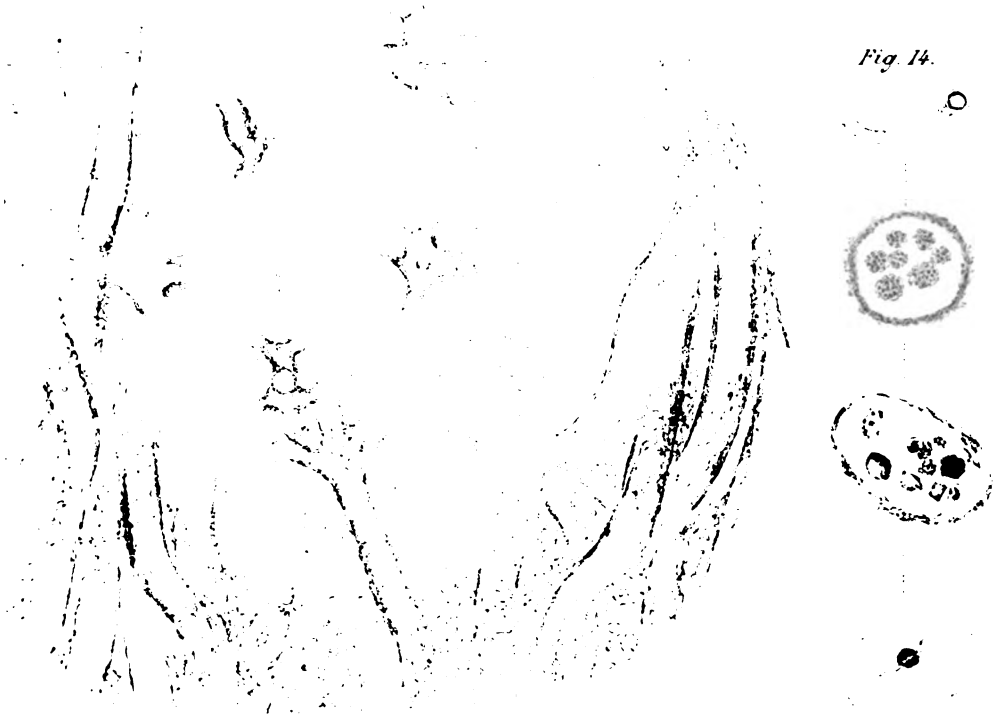

Fig. 15.

Fig. 17.

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Development of the Frond of Champia parvula, Harv. from the Carpospore.

BY

BRADLEY MOORE DAVIS.

With Plate XXI.

THE investigation, the results of which are set forth in this paper, was begun at the suggestion of Dr. William A. Setchell, at the Marine Biological Laboratory, Woods Holl, Massachusetts, in the summer of 1891. The greater part of the work, however, was done in the botanical laboratory of the Leland Stanford Junior University at Palo Alto, California. To Dr. Setchell, and Dr. Douglas H. Campbell I wish to express my thanks for many helpful suggestions which they have given me in my work.

But little was known about the minute structure of this interesting alga, representative of a very curious group, until 1886, when a memoir by Prof. F. Debray[1] appeared. In 1887 a paper by Robert P. Bigelow[2] was published upon the same subject, written without knowledge of Prof. Debray's memoir.

Previously to this time, papers bearing upon the subject of

[1] Debray, F., Recherches sur la Structure et le Développement du Thalle des *Chylocladia*, *Champia* et *Lomentaria*. Bull. scientif. du Départ. du Nord, ix. pp. 253–266, 1886.

[2] Bigelow, R. P., On the Structure of the Frond of *Champia parvula*, Harv. Proc. Amer. Acad., vol. xxiii. p. 111, 1887.

[Annals of Botany, Vol. VI. No. XXIV. December, 1892.]

the structure and manner of growth of this alga and allied species had been published by Nägeli, Berthold, and Wille. It will be of interest to compare later their views with those of Debray and Bigelow. Debray and Bigelow arrived independently at the same conclusions in regard to the apical growth and the development from the growing-point of the structures characteristic of the adult frond of this species. My own observations are in accordance with their conclusions, and a brief *résumé* of their investigations had best be given here.

They found that the tip of the adult frond was occupied by a group of actively growing cells. These cells were often disposed in three distinct circles. The inner circle was composed of four to six cells, which met at a point at the apex. Each of these cells gives rise to a file of segments, which is cut off behind it, and which can be traced for some distance from its proper initial cell. These are the primary files of segments. Just behind the initial cells of the first circle is a second circle of initial cells, which usually have their pointed ends wedged in between the initial cells of the first circle. Each initial cell of the second circle gives rise to a file of segments. These are the secondary files. Again, just behind the second circle and between the primary and secondary files of segments, is a third circle of initial cells, double the number of the first or second circle. Each of these cells also gives rise to a file of segments.

The segments cut off from the initial cells soon undergo a very important division. This usually occurs when the segment is separated from its generating cell by one sister-segment. A cell-wall is formed across the segment perpendicular to its greatest diameter and parallel to the surface of the frond. The segment is thus divided into an inner and an outer cell.

The cortex of the frond is developed from the outer cells of the segments. These divide irregularly by cell-walls perpendicular to the surface of the frond. This division first begins when the segment is separated from its initial cell by from

two to four segments. This division of the outer cells of the segments breaks up the files of cells which radiate from the group of initial cells at the apex of the frond. The files of cells are not distinguishable after the division of the segments. The division of these cortical cells tends to lengthen the frond.

As the frond thus grows in length, the cells which were cut off on the inside, when the segments divided in the plane parallel to the surface of the frond, lengthen into internal filaments or hyphae. The inner cells lengthen to keep pace with the growth of the frond. Every segment adds a cell to a hypha when it divides into the inner and outer cell. The frond increases in length by the division of the outer cells, and the inner cells lengthen as the frond grows. These inner cells form the long hyphae which traverse the length of the hollow frond on the inside. The hyphae begin near the apex of the frond, under the files of segments, which are cut off from the initial cells. Of course there are as many hyphae as there are initial cells.

The cells of the hyphae are often attached to the inside of the frond by one or more small cells, and usually have upon them, on the side nearest the axis of the frond, one large pear-shaped cell, the bulb-cell. The bulb-cells develop when very near the summit of the frond, and when several of them upon different hyphae meet and touch, they become fastened together, and by farther growth form the transverse diaphragms, so characteristic of the species.

Some of the botanists who have published papers on the structure of this alga and allied species have held views as to their manner of growth which differ widely from the view of Debray and Bigelow. Carl Nägeli [1] believed that *Lomentaria kaliformis* had an apical cell at the tip of each branch, and that this divided by oblique partitions to form the frond.

Dr. G. Berthold [2] described and gave a diagram of the tip of *Champia parvula.* He pointed out that there was a group

[1] Carl Nägeli, Die neuern Algensysteme, p. 246, 1847.

[2] Dr. G. Berthold, Beiträge zur Morphologie und Physiologie der Meeresalgen. Jahrbücher f. wiss. Botanik, Bd. xiii. p. 686, 1882.

of apical cells, which he thought were arranged in the following definite manner. At the apex of the frond were four apical cells which formed a cross. In the angles of the cross were four more apical cells, and behind these two sets and between the apical cells were eight more. Each apical cell gave rise to a file of segments. His view is similar to that held by Debray and Bigelow, although the latter do not find such extreme regularity in the arrangement of the apical cells. Berthold also showed that there was a hypha for every apical cell.

N. Wille[1] has described a conical apical cell in *Lomentaria kaliformis*, which divides by walls in several directions and thus gave rise to segments. By the division of these segments into inner and outer cells, and the continued division of the outer cells, the cortex and internal hyphae are developed. Debray has also studied *Lomentaria kaliformis*, and believes that there is a group of initial cells in this species as well as in *Champia parvula*. Debray has written a second memoir[2], in which he treats at length of the structure of the fronds of the allied genera of *Chylocladia*, *Champia*, and *Lomentaria*.

All the publications upon the subject treat only of the adult frond, and the development from the spore has not been carefully studied in any of the three allied genera above mentioned. It was in the hope that the careful study of the development of the spore of one of these curious algae might throw some light upon the relationship of these three genera, that this investigation was undertaken.

The Carpospore; its Germination and Segmentation.

Fruiting plants of *Champia parvula* are found throughout the middle of summer, and towards the latter part of the

[1] N. Wille, Beiträge zur Entwickelungsgeschichte des physiol. Gewebesystems bei einigen Algengattungen. Bot. Centralblatt, vii, xxvi. p. 86, 1886.

[2] Debray, F., Sur la Structure et le Développement des *Chylocladia*, *Champia*, et *Lomentaria*, 2ᵐᵉ mémoire. Bull. scientif. de la France et de la Belgique, Tom xxii. p. 399, 1890.

season reach a large size, the tufts being four to five inches in diameter. On these plants the older portions of the frond bear empty cystocarps, and the younger portions cystocarps which are just mature or in process of development. The ripe spores may be easily obtained in large quantities by leaving the fruiting plants over night in a dish of sea-water. In the morning the bottom of the dish will be sprinkled with the reddish spores, which have been thrown out during the night. This ejection of the spores occurs only at night, as was proved by experiment. Many of the spores, when they escape from the cystocarps, settle down on the mother-plant and develop there into young plants, not unfrequently reaching the length of an inch and a half. Most of the stages in the segmentation of the spore (Plate XXI, Figs. 4–10) were obtained from the parent plant, and all of the young plants come from the same source. There could be no doubt about the identity of these small plants with the mother-plant, for one could find them in all stages of development, from the newly-shed spore to the plants showing all the characteristics of the adult frond, with diaphragms and hyphae.

Several attempts were made to grow the spores, but they never developed beyond a 4-celled stage. The method of sowing was to place the spores on a glass slide in the bottom of a dish of sea-water, and then to keep a small stream of sea-water constantly running through the dish. The spores are heavy, and readily remain on the slide. Those which germinated attached themselves to the slide, so that they could be killed, hardened, stained, and mounted in balsam, by simply dipping the slide into the various fluids. There was silt suspended in the salt water, which settled over the spores, and probably prevented their farther development.

The spores, which measure ·05–·08 mm. in diameter, are spherical, and filled with dark red chromatophores (Fig. 1).

The nucleus lies in the centre, making that portion of the spore appear lighter and brighter in colour. It was noticed that the smaller spores germinated more readily and appeared

more mature, being provided with a firmer wall, and with denser cell contents.

The first sign of germination is the formation of a thick hyaline wall around the spore. In the adult plant the cell-walls on the outside of the frond are thickened into a hyaline layer. This layer is developed directly from the hyaline coat of the spore. The formation of the hyaline coat serves to attach the spore to the substratum, and the cell-division then begins almost immediately.

The first division of the spore takes place in a plane perpendicular to the substratum (Fig. 2), and this is followed soon after by another division, at right angles to the first, but also perpendicular to the substratum (Fig. 3). The spores enter the 2-celled stage about twenty-four hours after their ejection from the cystocarps, and then enter almost immediately into the 4-celled stage. Many examples of these two stages were found on the adult cystocarpic plants. The youngest stages were usually to be found towards the end of the branches of the frond, especially around those cystocarps whose contents had but a short time before been discharged. All the stages more advanced than the 4-celled condition were obtained from the parent cystocarpic plant.

The cystocarpic plants, from which the later stages were taken, were prepared for study by fixing the plants in ½ per cent. hot chromic acid, and were preserved finally in 70 per cent. alcohol. Very little difficulty was experienced when this material was imbedded in paraffin for sectioning. There was but little cell-shrinkage, but some care was necessary to keep the chamber inside the frond from collapsing.

The early stages in the segmentation of the spore were studied while on the adult frond. Small pieces of the frond, which contained the young plants, were cut off and mounted in 50 per cent. glycerine. By turning the piece of the frond over and over it was a simple matter to get both top and side views of the different stages. In drawing the figures, all sketched with the camera lucida, I have disregarded the substratum, which in every case, except Figs. 1, 2, and 3. was the

parent plant. The boundary of the outside hyaline layer, which is simply the thickened outside cell-walls, has been indicated in the figures. This layer is perfectly homogeneous, showing no striations, which is also true of the other cell-walls. The measurements were all made with a Zeiss micrometer eye-piece.

The third division in the segmentation of the spore seems to be in a plane parallel to the substratum, making an 8-celled stage out of the 4-celled stage. Fig. 4 gives a side view of the 8-celled stage. The fourth stage, 16 cells, Fig. 5, is not a common one, and probably does not last long, but changes quickly into the fifth stage, represented in Fig. 7, which consists of 28 cells.

As shown in Fig. 5, the 16-celled stage develops from the 8-celled stage, by each of the eight cells being cut by cell-walls parallel to the substratum. The cell-walls are probably slightly oblique, inclining towards the centre of the embryo. Optical sections of later stages seem to indicate this (see Fig. 14). In the 16-celled stage the first indication of the formation of an organ of attachment appears. This is indicated by a slight bulging of the four bottom cells of the embryo. Fig. 6 represents a top view of the 16-celled stage, and shows the four cap-cells with the four cells just below them. The fifth stage, Fig. 7, is but slightly different from the one preceding it, Fig. 5. Each of the eight cells, arranged in a zone between the two groups of four cells at the top and bottom, divides by a perpendicular cell-wall. The slight processes, which appeared at the bases of the four bottom cells, have now been cut off as four separate cells, from which are to arise the four organs of attachment.

With the next stage (Fig. 8) we may consider the segmentation of the spore complete. In the two figures, 7 and 8, numbers have been given to the cells, which seem to be the same or to have had the same origin. It will be seen that the only change that has occurred has been at the base of the embryo.

The four cells, numbered 5 in Fig. 7, have each given rise

to three cells, while the four cells directly above them (numbered 4) have been divided first into eight cells by walls parallel to the substratum, and then each of the four upper cells have been divided by a perpendicular wall.

Disregarding the thick gelatinous coat, it is probable that the segmented spore is about the same size as the freshly discharged spore. This is illustrated by the series, Figs. 1–9, all made under the same magnification, namely 450 diameters. The ripe spores themselves vary from ·05 to ·08 mm. in diameter, and one would expect a corresponding variation among the different stages. Up to this time there has been no forward growth of the young plant, and all the cell-division has been concerned with establishing the young plant firmly upon its support by means of the four small holdfasts. There has been nothing like that apical growth which is peculiar to the adult.

Professor Debray[1], in his second memoir, mentions having germinated the tetraspores of *Chylocladia kaliformis*, but he did not follow their development carefully. He remarks that they divide in an irregular manner, while preserving their spherical shape. In my own experience I have found the earlier stages (Figs. 2–7) very common and with the greatest symmetry of form, but, of course, with later stages there is much greater likelihood of irregular cell-division. I have found in the few examples of stages five and six (Figs. 7 and 8) that I have examined (for these stages are passed over quickly, and are consequently rarer and are also more difficult to manipulate), that there are sometimes slight irregularities in cell-division. Often some of the cells, by their more rapid growth, have crowded their neighbours a little out of the proper symmetrical arrangement. However, these irregularities were usually at the base of the plant, and in all cases there were four cap-cells at the top of the young plant. It is from these four cap-cells that the apical growth begins, and from them that the group of initial cells arises.

[1] Debray, Bull. scientif. de la France et de la Belgique, Tom xxii. p. 415.

Development of the Growing-Point.

Immediately after the segmentation of the spore, the growth of the young plant is more or less irregular. The cells at the base of the plant frequently divide very irregularly, and serve to strengthen the four organs of attachment. The space between the four small holdfasts becomes sooner or later filled in with cells, and a disc-shaped base is formed. One specimen was noticed in which the four holdfasts were present unaltered in a plant half a millimetre long.

A plant, ·15 mm. long, is figured in Fig. 9. The cells which probably agree in origin are numbered to correspond with Fig. 8. The apex of the young plant at this stage is very small, and is almost completely covered by the four cap-cells. At this time the growth of the young plants is very rapid.

Because of the size of the young plants it soon became evident that it would be impossible to get satisfactory top and side views of the apex that would show how the groups of initial cells arise. Further study was carried on by means of microtome-sections.

The plants had been well fixed in chromic acid, and very little difficulty was experienced in preparing them for sectioning. The young plants were very small, so for convenience they were always left attached to a short piece of the old frond on which they had grown. They could be much more easily handled when thus attached to a short piece of the mother-plant.

The specimens were all stained in alum-carmine; the colour given them enabled the specimens to be oriented in the paraffin blocks more easily. They were treated with absolute alcohol in the usual way, and prepared for the paraffin by treatment with turpentine and solutions of paraffin in turpentine. In some cases the stain produced by alum-carmine was sufficient to show all the points, but usually it was necessary to stain again on the slide, and no stain was found so satisfactory for this purpose as Bismarck-brown. The

sections were cut serially on a Minot microtome ·01 mm. in thickness. There was no cell-shrinkage, when care was taken not to let the paraffin get too hot, but with large specimens there was always danger of the hollow frond collapsing.

Transverse sections of the young plants will first be considered, for it is these alone that show how the group of initial cells arises. The smallest plant sectioned was ·10 mm. long, and there were ten sections in the series. Eight of these sections are shown in Fig. 10, in the order in which they were cut. This plant was more advanced than that shown in Fig. 6, although it was not so long. Its base was disc-shaped, and was not raised on four organs of attachment, as the case figured in Fig 9.

The first section, Fig. 10 *a*, shows the four initial cells at the apex of the frond, arranged in the form of a cross. They are numbered 1, 2, 3, 4, and appear also in the second section, Fig. 10 *b*. The four initial cells are the same as the four cap-cells of the segmented spore. They have divided several times, giving rise to daughter-cells behind them, and are now smaller than they were at first.

The other sections are very interesting, because there is an apparent division of each section into four parts. In the stained specimen this division was very noticeable, much more so than can be represented in outline-drawings, for the older cell-walls between the four divisions stained much more heavily. I have drawn in each figure two straight lines to mark the extent of the four divisions. It will be remembered that in the later stages of the segmentation of the spore there were always present four clearly marked divisions, each of which had a cap-cell at the top, and at the bottom was terminated by an organ of attachment. It is interesting to find this division into four parts present for so long a time in the young plants.

Fig. 11 shows the first two sections from another slide of a plant ·15 mm. long, and Fig. 12 is the first section of a plant ·17 mm. long. In both of these plants the four initial cells derived from the four cap-cells are distinct. They are num-

bered 1, 2, 3, and 4 in these figures, as in all the other figures of cross-sections.

Now it will be apparent that as the young plant grows, the top, which was at first small enough to be covered by the four cap-cells, would finally become so broad that it would include some of the daughter-cells derived from the four initial cells.

The four initial cells become smaller in size, and as the apex increases in area they become slightly separated behind. This allows some of the cells behind to crowd in between them, and then they assume the functions of initial cells, and daughter-cells are cut off behind them.

If one or two of the four primary initial cells are not quite so large as the others, they are very likely to be pushed aside by this crowding forward of the cells behind them, and thus the apex will be occupied by five or six initial cells. If the four primary initial cells are of equal size and are symmetrically arranged, the cells behind them will not be able to displace them, but will take up their position just behind them, but with their points wedged in between them.

It is interesting in this connection to notice Berthold's[1] views as to the arrangement of the apical cells. He thought that they were always arranged in a regular manner. There were four initial cells at the apex; behind this group was a second group consisting of four cells placed in the angles of the cross formed by the first group of four cells. Behind the second group were eight more initial cells, arranged between the files of segments, derived from the eight initial cells in front of them. Such a case might happen if the development were perfectly symmetrical, although usually the growth is irregular, and results in there being a circle of 5–6 initial cells at the apex.

Fig. 11 shows the first two sections of a plant ·15 mm. long. In this plant there are four initial cells, numbered 1, 2, 3, 4, at the apex. Another cell, numbered 5, by its shape and posi-

[1] G. Berthold, Beiträge zur Morphologie und Physiologie der Meeresalgen. Jahrbücher f. wiss. Botanik, Bd. xiii. p. 686, 1882.

tion seems about to push its way into this group of four initial cells. In Fig. 12, the first section of a plant ·17 mm. long, the four original initial cells are not symmetrically arranged, and it is evident that the cell numbered 5, and perhaps also 6, would soon have become members of the first circle of initial cells. The first four sections of a plant ·30 mm. long are shown in Fig. 13, and in that plant the tip may be said to have five initial cells. Probably the cells numbered 1, 2, 3, and 4 are the original four. Here the position of the cells numbered 6, 7, 8, and 9 indicate that they will be, if they are not already, initial cells in the second circle.

The young plant starts out with four initial cells. These were the cap-cells of the segmented spore. The initial cells cut off segments behind them, which divide rapidly and by their growth increase the area of the apex of the frond. The cells at the apex of the frond crowd one another, and some of them are forced to take position between the four apical cells. One or two of them usually wedge their way in between the four initial cells and finally become part of the first circle of initial cells. Other cells remain with their points placed between the cells of the first circle and take on the functions of initial cells and become the second circle of initial cells. A third circle of initial cells will be developed in a similar manner when the area of the apex of the frond becomes large enough to allow of its formation. After the groups of initial cells are well established the segments derived from each initial cell form files of cells radiating from the apex.

The development of the hyphae and diaphragms will now be considered. I was very much surprised to find that they were the last structures of the frond to develop. It certainly seems that in *Champia parvula* they are developed some time after the initial cells have been differentiated and have assumed their function. The cells that are derived from the initial cells form a continuous tissue with the cells at the base of the plant, a tissue that is continuous with the cortex of the adult frond. This fact would seem to indicate that the hyphae

are secondary structures developed to strengthen the cortex. The usual view has been to consider the initial cells morphologically as the ends of the hyphae, and the cortex as a tissue developed from the hyphae.

Longitudinal sections of young plants ·36 mm. long were cut, which were without a trace of hyphae and of course without diaphragms, as the latter are developed from hyphae. Sections of plants ·28 mm. long were also cut and these had both hyphae and diaphragms.

The figures 14–18 tell the story of the development of the hyphae. Fig. 14 is an optical section of the fully segmented spore, shown in Fig. 8. Fig. 15 is a longitudinal section of a plant ·20 mm. long, and it would probably appear in cross-section in about the stage of Fig. 12. The comparative length and breadth of the initial cells lettered x is shown. In Fig. 16, which is a section of a plant ·25 mm. long, we have the first beginnings of two hyphae: x and x^1 are the two initial cells, and just behind them may be seen the segments which have been cut off from them. Segments 1^1, 2^1, 3^1 have each been divided by a wall parallel to the surface of the cortex, cutting off the cells of the hyphae lettered h^1, h^2, h^3. Segment 1 on the left-hand side of the figure has not divided, but from segment 2 a hypha (*h*) has started. It is worth noticing that the segments 1^1, 2^1, and 3^1 and 1 and 2 are part of the same tissue, which extends to the base of the plant, and that the cells below them have no connection with the hyphae. From the appearance of the ends of the hyphae it is probable that they creep down a little on the inside of the frond, see Figs. 16 and 17.

Fig. 17 is a figure of a longitudinal section through a plant ·32 mm. long. The first diaphragm (lettered *d*) has been formed, but the section was cut a little obliquely and does not show the relation of the cells of the hyphae to the cortex as well as does Fig. 18. It is interesting, however, on account of the two hyphae lettered *h*, both of which seem to have grown down along the inside of the frond from the apex. The series of four transverse sections shown in Fig. 13 are

probably of a plant in about this same stage of development. The sections *b*, *c*, and *d* of this figure show on the inside cross-views of the hyphae.

Perhaps the most instructive figure is Fig. 18. The plant from which this was taken was ·28 mm. in length. In this the initial cells are lettered x and x^1, and the segments derived from each initial cell are numbered to correspond. The typical arrangement of the segments and of the cells which are cut off from them to form the hyphae is, I think, apparent. Segment 1^1 has not cut off its hyphal cell; all the rest have. In the older segments (4, 5, 3^1 and 4^1) the cortical portion of the segments has again divided, and this illustrates very beautifully the manner in which the cortex grows.

In this specimen, Fig. 18, there is one well-developed diaphragm, lettered d^1, and also the beginnings of the second diaphram, lettered d^2. At this last point some of the hyphal cells have met and become fastened together. By the farther division and growth of these hyphal cells, keeping pace with the widening of that portion of the frond, the second diaphragm would have been developed. One bulb-cell, lettered *b*, is shown in this section, attached to a hypha (*h*). The development of the bulb-cells has not been seen in these young plants, but there is no doubt, from the investigations of Debray and Bigelow, that they arise from hyphae.

I wish again to call attention to the lateness of the development of the hyphae. The cap-cells of the segmented spore often divide several times before the hyphae appear. That this is so is proven by the fact that the initial cells of a young plant are much smaller than the cap-cells of segmented spores. Compare Figs. 14 and 15, both drawn under the same magnification; the segmented spore (Fig. 14) is ·08 mm. long, the young plant (Fig. 15) is ·20 mm. long. The hyphae, when they first appear, are very small (see Fig. 16) and insignificant. If the hyphae are to be considered as structures which are directly derived from the initial cells and which give rise to the cortex of the adult frond, one would hardly expect to find them to be the last structures formed in the development of

the plant. They start simply and increase in size and importance as the frond has need of their support and the support of the diaphragms derived from them.

All the important structures of the adult frond are now present in the young plant, and in its farther growth the initial cells simply repeat the cell-divisions that I have already described. It will be noticed that all the stages which I have figured and described, although taken from a number of cystocarpic plants, form a complete series from the spore to a stage that is in all essentials identical in structure with the adult frond, which has been so carefully studied by Debray and Bigelow.

PALO ALTO, CALIFORNIA.
March, 1892.

EXPLANATION OF FIGURES IN PLATE XXI.

Illustrating Mr. Davis' paper on *Champia*.

N.B. All figures drawn with an Abbé camera. Figs. 1–9 magnified 450 diameters. The sections were cut with a Minot microtome, were ·01 mm. thick, and were mounted in benzole-balsam.

Fig. 1. A spore drawn soon after its discharge from a cystocarp and while in sea-water; the light spot in the centre marks the position of the nucleus; specimen ·05 mm. in diameter.

Fig. 2. First division of the spore; viewed from above; drawn from living specimens in sea-water; showing hyaline coat.

Fig. 3. Second division of the spore; viewed from above; specimen drawn when in sea-water.

Fig. 4. Third stage in segmentation of the spore; viewed from the side; glycerine preparation.

Fig. 5. Fourth stage in segmentation of the spore; 16 cells; viewed from the side; glycerine preparation.

Fig. 6. Top view of the same stage as Fig. 5; glycerine preparation.

Fig. 7. Fifth stage in segmentation of spore; 28 cells; viewed from the side; glycerine preparation.

Fig. 8. Last stage in segmentation of the spore; 44 cells; viewed from the side; glycerine preparation, specimen ·08 mm.

Fig. 9. Side view of young plant ·15 mm. long; glycerine preparation. The cells are numbered to indicate probable homologies with Figs. 7 and 8.

Fig. 10. A series of transverse sections of a young plant ·10 mm. long; ten sections in the series, eight shown; the four divisions of the young plant included between the straight lines drawn across the sections; initial cells numbered 1, 2, 3, 4; stained with alum-carmine. × 250.

Fig. 11. First two sections of a young plant ·15 mm. long; primary initial cells numbered 1, 2, 3, 4; stained with Bismarck-brown. × 250.

Fig. 12. Tip of young plant ·17 mm. long; cells numbered 1, 2, 3, 4, the primary initial cells; 5 and 6 probably would soon be initial cells; stained with Bismarck-brown. × 250.

Fig. 13. Series of four sections of a young plant ·30 mm.; initial cells numbered 1, 2, 3, 4, 5, perhaps also 6, 7, 8, 9. In sections *b*, *c*, and *d* are seen cross-sections of hyphae; stained with Bismarck-brown. × 250.

Fig. 14. Optical section of the last stage in segmentation of spore; from same specimen as Fig. 8; ·08 mm. long. × 450.

Fig. 15. Longitudinal section of young plant ·20 mm. long; × and $\times^1$ initial cells; stained with Bismarck-brown. × 450.

Fig. 16. Longitudinal section of young plant ·25 mm. long; × and $\times^1$ initial cells, the corresponding segments numbered; the hyphal cells lettered *h*; stained with alum-carmine. × 500.

Fig. 17. Longitudinal section of young plant ·32 mm. long; × and $\times^1$ initial cells; *h*, hyphae which have crept down on the inside of frond: *d*, diaphragm; stained with alum-carmine. × 500.

Fig. 18. Longitudinal section of young plant ·28 mm. long; segments and initial cells numbered and lettered as in Figs. 16 and 17; d^1, first diaphragm; d^2, second diaphragm just forming; *b*, bulb-cell on its hypha *h*; stained with alum-carmine. × 500.

Fig. 1.

Fig. 2.

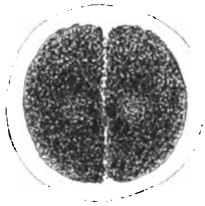
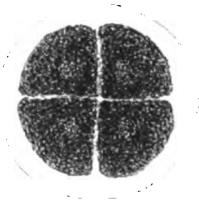


Fig. 3.



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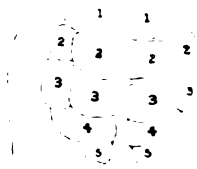


Fig. 4.

Fig. 5.

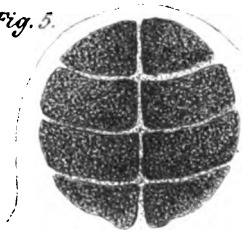


Fig. 6.

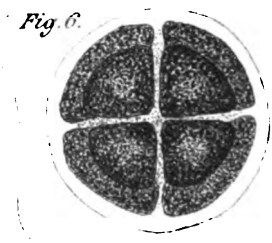


Fig. 8.

a

Fig. 12.

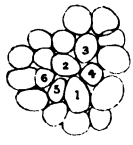
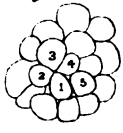
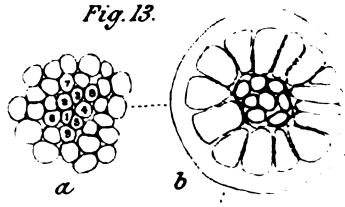


Fig. 11.



b

Fig. 13.



a *b*

Fig. 14.

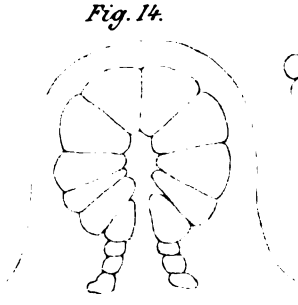
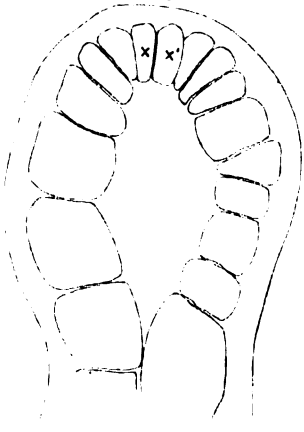
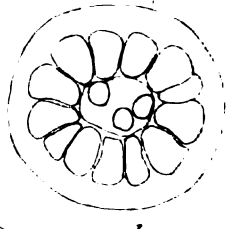


Fig. 15.





d

Fig. 16.

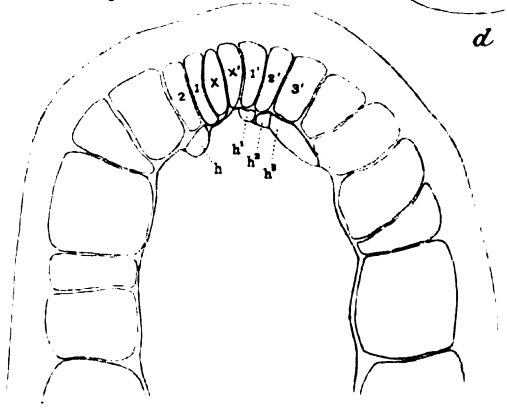
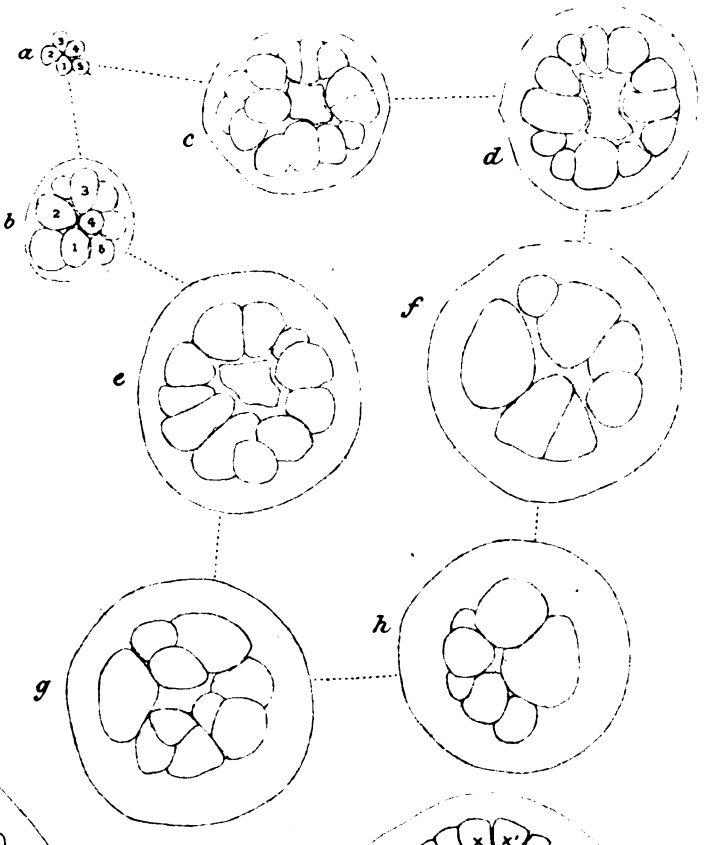


Fig. 9.

Fig. 10.



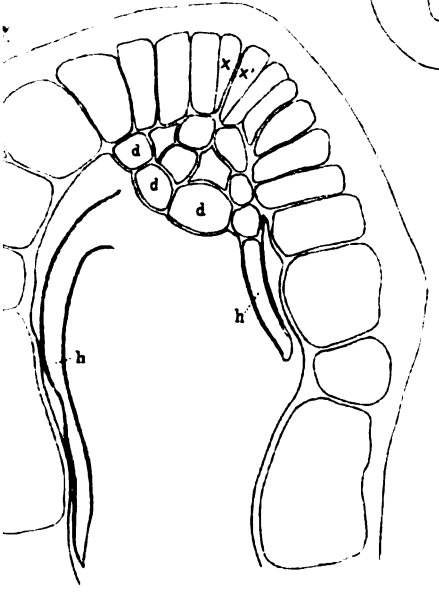
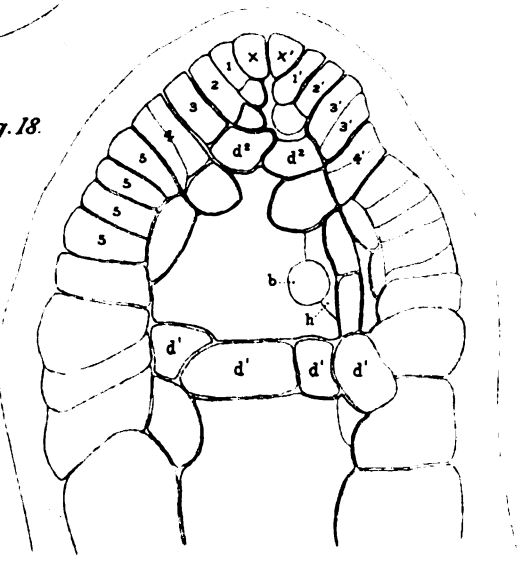


Fig. 18.



University Press, Oxford

On the Simplest Form of Moss,

BY

KARL GOEBEL,

Professor of Botany in the University of Munich.

With Plate XXII.

As the result of previous researches, I had come to the conclusion that the peculiarities which mark the earlier stages in the development of the Moss-plant are of great importance as indicating homologies between the Mosses and other groups of plants. The germinating spore, namely, does not directly give rise to a Moss-plant, but to a protonema which, on its first discovery, was thought to be a filamentous Alga; and even in those forms (*Sphagnum*, *Andreaea*) in which the protonema is not filamentous, it has been shown to be produced by the modification of a filament, a conclusion which is also applicable to the development of the protonema in the Liverworts [1], where it exhibits much more marked adaptation to various external conditions than is the case in the Mosses. In some remarkable forms of Liverworts the plant bearing the sexual organs is only an appendage of the protonema. Thus, in certain forms of *Metzgeriopsis* [2] (which are closely allied to *Lejeunea*), the leafy shoot does not, as is generally the case,

[1] Goebel, Ueb. die Jugendformen der Pflanzen, Flora, 1889.

[2] Goebel, Morphol. und Biol. Studien, Ann. du Jard. Bot. de Buitenzorg, VII. 1887.

[Annals of Botany, Vol. VI. No. XXIV, December, 1892.]

constitute the main portion of the Liverwort, but is merely the portion which bears the sexual organs to which the leaves serve as involucres. These forms seemed to me to represent a primitive type.

If, now, we suppose a case in which the formation of the sexual organs is deferred to a later stage of development, whilst the leaves function as assimilatory organs, the result in such a case would be to shorten the protonemal stage of the life-history, a state of things which is actually realised in the Mosses. The simplest form of Moss would then be one in which the sexual organs are directly borne, with or without an involucre, on a filamentous protonema.

It would seem to be scarcely probable that such a form is still to be found among existing Mosses; but, as a matter of fact, it does exist under the name of *Buxbaumia*. This Moss is usually included among the higher Bryineae, but erroneously, for the sexual generation is so wonderfully simple that it very nearly comes up to the hypothetical ideal of the simplest primitive Moss.

The protonema of *Buxbaumia* resembles that of the other Mosses; it is peculiar only in that the filamentous branches frequently anastomose.

The male plant has no stem: it consists of a branch of the protonema bearing a single terminal antheridium. The antheridium differs in its form from that of Bryineae, and resembles that of *Sphagnum* and of many Liverworts in that it is globular and is borne on a long stalk; it is invested by a leaf forming a conchiform involucre. The leaf, which is destitute of chlorophyll, differs in the arrangement of its cells from the leaves of the Bryineae in that it has at its apex, not a two-sided apical cell, but cells arranged in slightly diverging anticlinal series. The habit of the male plant is, in fact, such that, were it found occurring alone, it would be classed as an Alga without much hesitation.

The female plant is somewhat more highly developed. On a mass of tissue, which represents a rudimentary stem, is borne an archegonium surrounded by several involucres which

resemble that of the male plant, but are peculiar in that the marginal cells can grow out into protonemal filaments. This case is similar to that of certain species of *Trichomanes*, where some branches of the filamentous prothallium develop into flattened cellular expansions which reveal their filamentous origin in that their marginal cells grow out into filaments, and to that of the protonema of *Sphagnum*.

That the female plant should attain a higher degree of development than the male is naturally correlated with their respective functions. The male plant has but a short existence ; when it has produced and has set free the spermatozoids, it perishes. The female plant has to protect and nourish the sporogonium during its development, for which a period of 7–8 months is necessary; consequently the plant must attain a relatively high organization.

The organization of the sporogonium, likewise, is rudimentary as compared with that of the true Bryineae; it somewhat recalls that of the sporogonium of *Sphagnum* and of *Andreaea*, and especially that of *Diphyscium* the second genus of Buxbaumieae. It has no true seta, but merely an absorbent organ which penetrates into the rudimentary stem of the Moss-plant ; this organ gives off a number of rhizoids which absorb nourishment from the stem. Consequently in this form the calyptra is ruptured, not by the elongation of the seta as in the Bryineae, but by the expansion of the theca of the sporogonium.

From these facts it appears that *Buxbaumia* is an ancient type of Moss which still retains a number of primitive characters. It is also of interest in that it recalls the simplest form of the sexual generation of the Ferns. The Hymenophyllaceae, among the Leptosporangiate Ferns, are forms which have a filamentous prothallium on which cellular archegoniophores are borne ; the archegoniophore is homologous with the Moss-plant, since, as has been briefly indicated above, the Moss-plant was itself originally only an archegoniophore. In most other Ferns the filamentous stage of the prothallium is very brief, because the development of the

with those of the sporophyll in the lower. Directly the abortion of the sporangia is induced, the sporophyll at once exhibits the characters of a foliage-leaf.

At present I can see in the sporophylls of *Ophioglossum* and of *Helminthostachys* nothing more than very highly modified portions of leaves. In *Ophioglossum* the sporangia are imbedded in the sporophyll, whilst in *Helminthostachys* and *Botrychium* they are superficial, a difference which finds its parallel in that which exists between the highly modified stamens of the Angiosperms with their imbedded pollen-sacs and those of the Cycadeae with superficial pollen-sacs which present some external resemblance to the sporangia of the Vascular Cryptogams. Moreover, in *Ophioglossum palmatum* the sporophylls are still clearly recognisable as leaf-segments.

EXPLANATION OF FIGURES IN PLATE XXII.

Illustrating Professor Goebel's paper on the Simplest Form of Moss.

Figs. 1 and 2. *Botrychium Lunaria*, two pinnae of the sterile portion of the leaf on which sporangia (*sp*) have made their appearance.

Figs. 3–5. *Onoclea Struthiopteris*, a sporophyll artificially converted into a foliage-leaf.

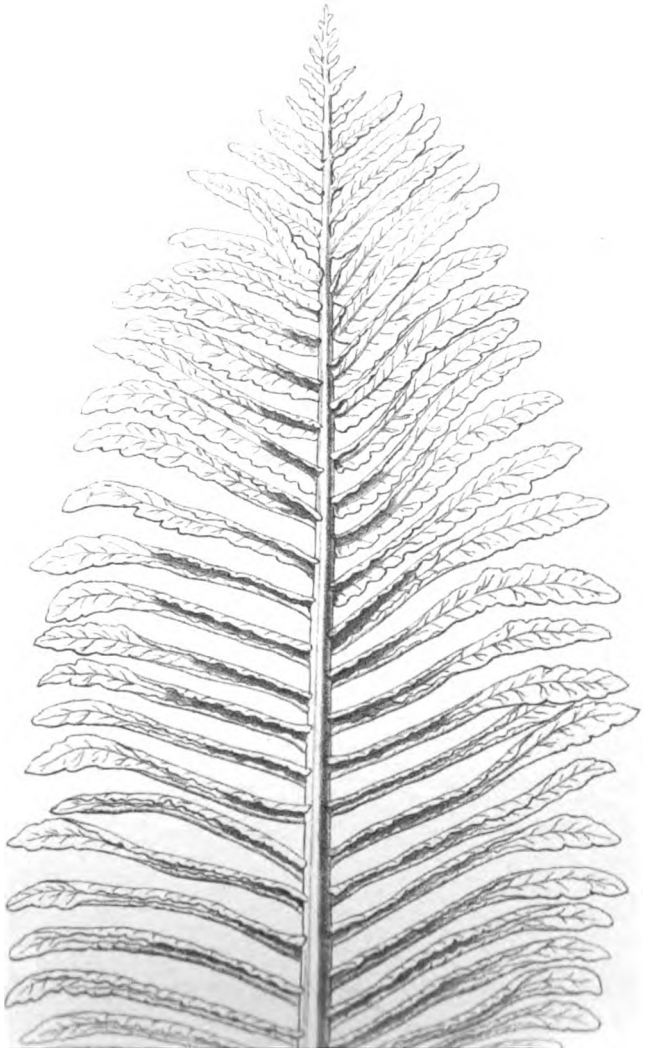


Fig. 5

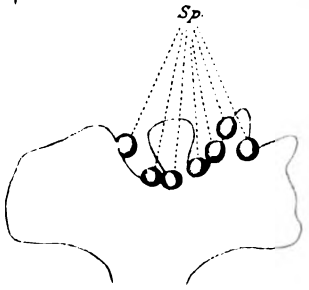


Fig. 1.

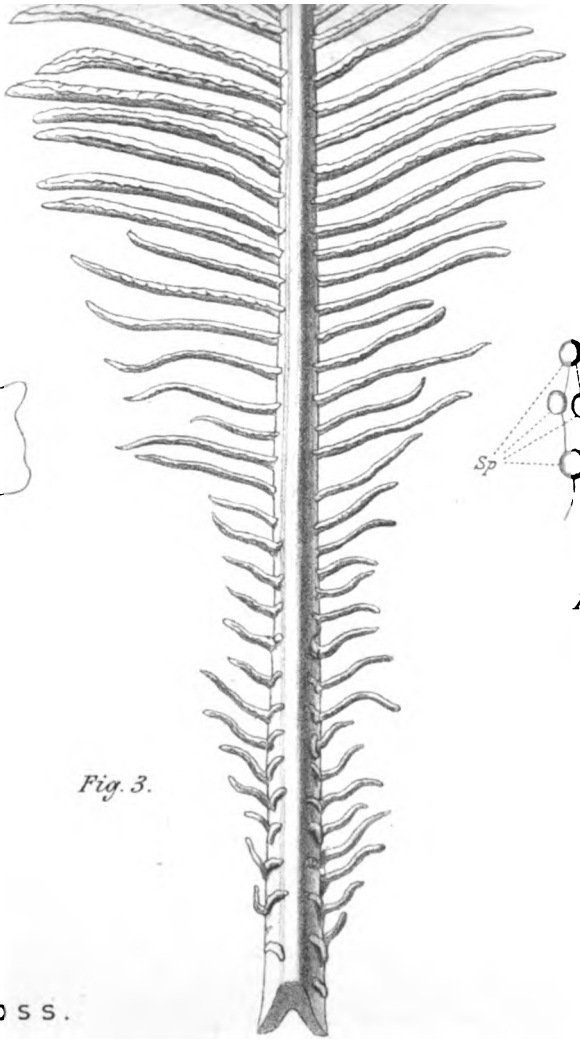


Fig. 3.

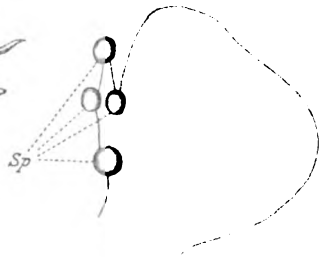


Fig. 2.

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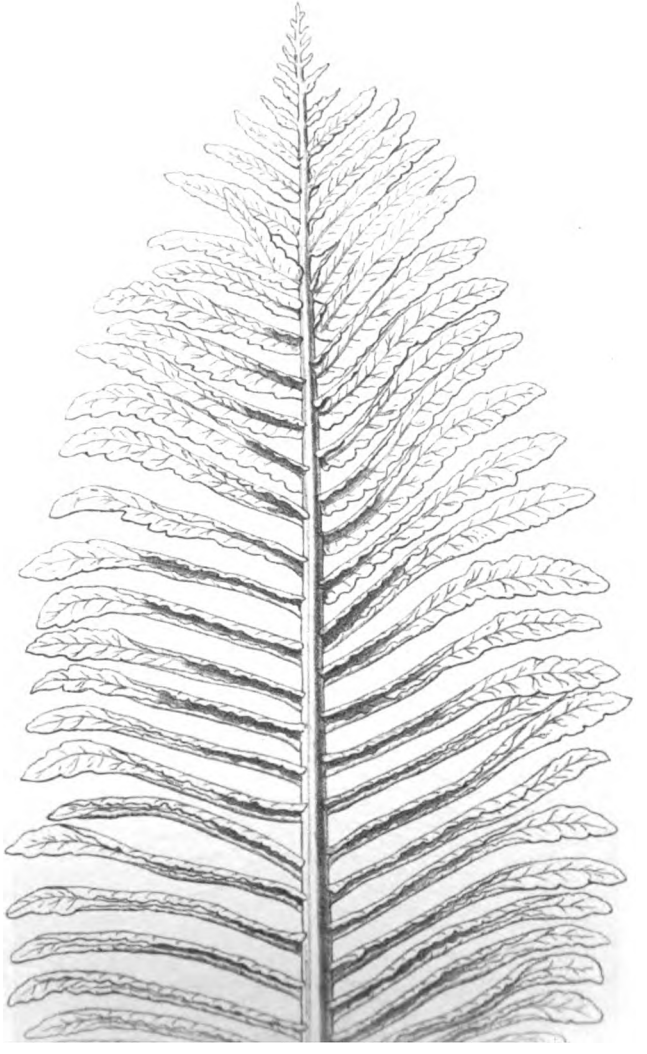


Fig. 5

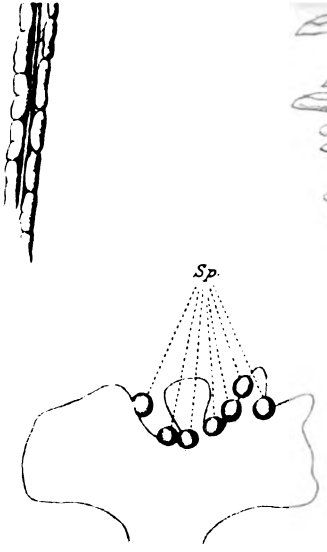


Fig. 1.

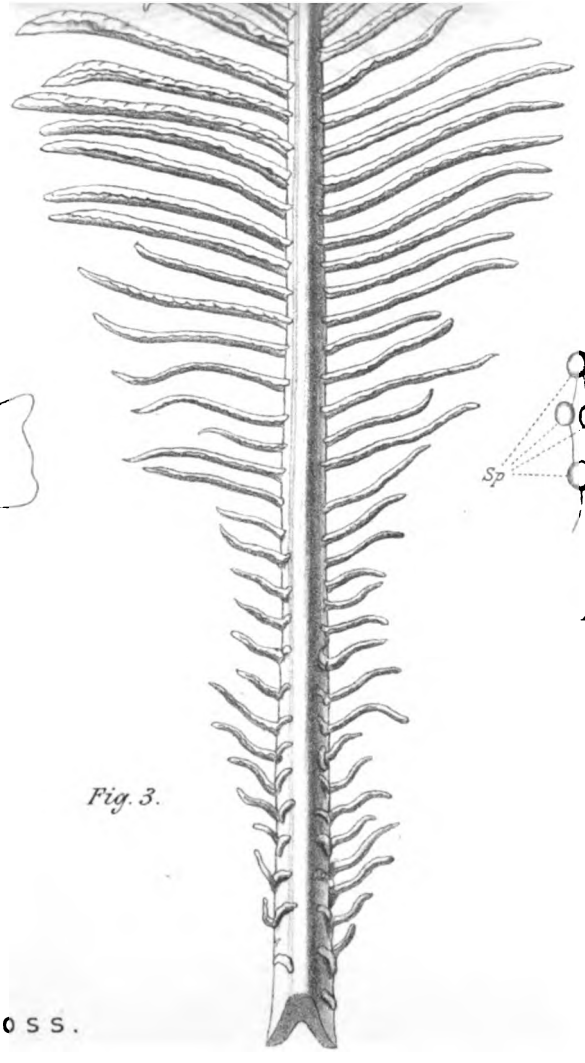


Fig. 3.

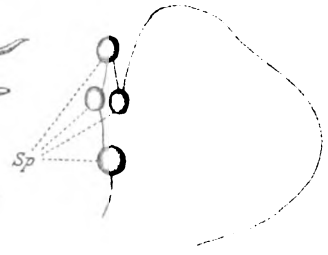


Fig. 2.

University Press, Oxford.

Stenogramme interrupta, (C. Ag.) Montg.

BY

T. JOHNSON, D.Sc., F.L.S.,

Professor of Botany in the Royal College of Science, Dublin.

With Plate XXIII.

THE genus *Stenogramme* (*stenos*, narrow, *gramme*, line) was founded by Harvey in 1841 to include a Californian sea-weed, in which the fruit had the anomalous form of a nerve-like, or midrib-like, interrupted line in the thallus-segments. Of the two species of *Stenogramme*, *S. interrupta*, (C. Ag.) Montg., and *S. leptophylla*, J. Ag., *S. interrupta*, though a rare British red alga [1], is to be found growing in Plymouth waters, and may be obtained, in all stages, in tolerable plenty by dredging in 5–7 fathoms in Plymouth Sound, west of the Duke Rock buoy. In spite of the unusual form and position of the cystocarps, the female plants have not been described since Harvey examined the ripe fruits; the male plants, beyond a passing reference in the Études Phycologiques [2], have not been described; and doubts still exist as to the characters of the tetrasporangiate plants.

Tetrasporangiate Plant.

The tetraspores are cruciate (Fig. 2): the tetrasporangia are so arranged as to form wart-like sori of varying size and outline, irregularly scattered over both surfaces of the thallus

[1] Harvey, Phyc. Brit., Pl. CLVII.

[2] Thuret et Bornet, Études Phycologiques, p. 82.

[Annals of Botany, Vol. VI. No. XXIV, December 1892.]

(Fig. 1 *s*). Each sorus consists of innumerable vertical rows of cells, the individual cells in each row being the mother-cells of the tetraspores, each ultimately dividing into four tetraspores, cruciately arranged. As the sori arise from the superficial cells of the thallus by repeated horizontal divisions, it is not difficult to see how, by examination of young sori in certain stages (Fig. 3 *s*), one may be led to consider such a sorus as a mature one showing zonate tetraspores, a mistake which has been more than once made in the case of *S. interrupta*[1].

Male Plant.

In a male specimen of *Stenogramme interrupta* the antheridia are recognisable, in a fresh plant, as pale patches occurring in the upper part of the thallus on opposite sides of it (Fig. 4). Each antheridium is nearly as broad as the thallus-segment on which it occurs, may be as long as or longer than it is broad, forms a slight elevation on the surface of the thallus and is homogeneous, i. e. consists of closely applied spermatium-mother-cells, without intervening sterile thallus-cells (Fig. 5). Towards the edges of the antheridium there is, as is to be expected, a tendency to the interruption of the homogeneity. I saw nothing to suggest that the spermatia are not quite normal (Fig. 6).

Female Plant.

S. interrupta is readily distinguished from all other Florideae by the midrib-like, or nerve-like, more or less continuous simple or forked line found running along the centre of the segments—more especially the upper ones—of the thallus of the female

[1] In Grevillea III (1874), E. M. Holmes described and figured, as he then thought for the first time, tetrasporic plants of *S. interrupta*. In Grevillea IV (1875), E. P. Wright showed that Harvey had described and figured such plants in his Phycolog. Austral. (IV. Pl. CCXX, Figs. 2–5), and that he had in his Nereis Bor. Amer. (Part II. p. 162) acknowledged the priority of the late Miss Gifford as the discoverer of the tetrasporic plants. I refer to this matter, to prevent others from falling into the same mistake as I nearly did through finding in De Toni's Sylloge Algarum a reference to Holmes' paper but not to Wright's correction, and also to state that I find (Fig. 3) the sori on *both* surfaces of the thallus as Montagne did, and as Holmes states he was, himself, unable to do.

plant (Fig. 8). This 'fertile line,' as it may be called, increases in distinctness as the fruits form, becomes more convex, and may finally become very irregularly swollen (Fig. 9). It was with considerable interest that I began, in the autumn of 1889, the microscopic examination of the nature of the fertile line. The ordinary sterile thallus of *S. interrupta* shows, in cross-section, 4–6 layers of cells, of which the inner 2–3 layers consist of larger cells. Where the line is, the thallus is as many as 12 layers thick, the internal cells being much smaller, more numerous, and relatively richer in contents than elsewhere (Fig. 10). Microscopic examination shows that, when the line is only just visible to the naked eye, the female organs or procarpia are already beginning to appear, and that where the line is absent the procarpia are also absent. The gaps in the fertile line, to which the plant owes its specific name, are due to the absence of procarpia or to the non-fertilisation of procarpia present, in different regions of the fertile line. By making sections through the line in different directions, at the right stage, the procarpia are seen to be very numerous, extending, in almost continuous series, along the whole length of the line, 3 or 4 deep, on both surfaces of the thallus (Fig. 10). Thus, in a fertile line an inch long, there must be several hundred fertilisable procarpia. Each procarpium has the more usual floridean characters, and consists of a curved 3–4-celled special carpogenous branch, of which the free terminal cell is the carpogonium, from which the trichogyne grows out in the usual way, projecting, for some distance, on the surface of the thallus (Fig. 11). The curvature of the carpogenous branch is such as to place the carpogonium near to the surface of the mother-cell of the branch. This mother-cell is a large medullary cell, well marked by its rich protoplasmic contents. After the spermatium has come into contact with the trichogyne and fertilisation of the carpogonium has taken place, the trichogyne is cut off by a septum from the fertilised carpogonium, which becomes connected by an ooblastema-filament with the mother-cell of the carpogenous branch – the auxiliary-cell (Fig. 11). The cell, thus resulting from the

fusion of the fertilised carpogonium and auxiliary cell, becomes the central cell of the cystocarp, increases in size, and sends off from its surface, more especially the under surface, numbers of radiating nucleated, septate meta-ooblastema filaments (Fig. 12, *a*, *b*, *c*). The two or three carpogenous cells between the auxiliary cell and the carpogonium, which represent the 'trichophore' of some writers, take no part in the formation of the cystocarps. To return to the consideration of the rest of the fertile line:—by the time the procarpia are quite ready for fertilisation the fertile line has become more conspicuous, partly owing to an increase in the number of superficial layers of cells, which layers of cortical cells ultimately form the general fruit-wall, partly owing to an increase in the size and richness of contents of the medullary cells of the line (Fig. 13). These accessory reproductive cells become more or less widely separated from one another, and though they, apparently, do not contribute directly to the formation of the carpospores, they must play a very important part in the supply of nutriment to the developing cystocarps. Before the procarpia are fertilised these medullary cells of the line are very full of food-materials: when the carpospores are just beginning to appear, their contents have become quite sparse. After the cystocarps have begun to form, delicate septate vegetative filaments make their appearance amongst the medullary cells; these branch and connect non-adjacent medullary cells with another. Similar filaments are to be observed in other Florideae, e.g. Cryptonemiaceae, and are not to be mistaken for fertilising filaments.

The nuclei of the meta-ooblastema filaments are derived from the central cell of the cystocarp. This central cell becomes multinucleate by the repeated divisions of the nucleus which results, judging by analogy, from the fusion[1] of the nucleus of the fertilised carpogonium with the nucleus of the auxiliary cell. As the septate meta-ooblastema filaments increase in importance, the rich medullary cells part with most of their contents to the developing cystocarps, and

[1] I saw many cases of fusion of these two cells, sometimes several cases in the same section, but was not fortunate enough to see their nuclei fusing.

thus help to make room for the growth and ramification of the meta-ooblastema filaments, in the medullary part of the fertile line, where they form, ultimately, dense aggregations of small rounded carpospores (Figs. 15, 16) which are solitary or in chains of 2 or 3.

Examination of a fertile line, at its fullest development, shows a thick cortex formed of vertical rows of cells, the fruit-wall, pericarp, or involucre, enclosing a dense, more or less free, granular mass (Fig. 16). This mass, which appears at first sight, and when only slightly magnified, to be more or less continuous, is, in reality, made up of a large number of distinct cystocarps. The different cystocarps are formed independently of one another, each being directly derived, as the result of a single act of fertilisation, from its own procarpium. Thus, what appears to be a single fruit, shows itself, on examination, to be a collection of fruits with a common fruit-wall.

Systematic position.

I do not propose to enter into a detailed consideration of the bearing of these investigations on the systematic position of the genus. *Stenogramme* was placed, with expressed doubts, by J. G. Agardh [1] in Ordo VI Rhodymenieae, with such genera as *Rhodymenia* and *Plocamium*. It is now placed by Schmitz [2] with *Phyllophora*, *Gymnogongrus*, *Ahnfeltia*, and *Actinococcus* in the Tylocarpeae of the group Gigartininae, of which *Rhodymenia* and *Plocamium* are not members. The plant *Stenogramme californica*, for which the generic name *Stenogramme* was originated in 1841 by Harvey, proved to be, as Harvey admitted, identical with a plant discovered at Cadiz by Cabrera, and described in 1823 by C. Agardh as *Delesseria interrupta*, a name which was altered by Montagne to *Steno-*

[1] J. G. Agardh: Sp. Alg. II, 2, p. 373.

[2] F. Schmitz, Syst. Übersicht d. Gattungen d. Florideen, 1889. My own investigations of *Stenogramme* agree in the main with those of Schmitz, judging from an interchange of views by correspondence. My examination of the genus was almost completed some two years ago, but the results were not published as I was waiting for the appearance of Schmitz's amplification of the work just cited.

gramme interrupta. In Webb's Otia Hispanica, 1853, Montagne gives a life-size illustration of a female plant of *S. interrupta*, which is much more like *S. leptophylla*, J. Ag., of which I have seen Australian specimens collected by J. B. Wilson, than *S. interrupta*, (C. Ag.) Mont.

Summary.

1. *S. interrupta* is distributed through the temperate zones of the Northern and Southern hemispheres, extending to New Zealand in the south, and Scotland and the north of Ireland in the north. It has a well-established habitat in Plymouth Sound, on stones and shells, in 5–7 fathoms.

2. Tetraspores, antheridia, and procarpia are found on distinct plants, and on both surfaces of the thallus.

3. The tetraspores are cruciately arranged, and occur in irregularly placed sori.

4. The antheridia form broad, flat, homogeneous patches of spermatia.

5. The procarpia are very numerous, of comparatively simple form, and have a unique position, as part of a fertile line extending more or less continuously along the centre of the thallus segments.

6. The mother-cells of the carpogenous branches, large medullary cells rich in contents, constitute the auxiliary cells. The fertilised carpogonium becomes fused by an ooblastema-filament with its auxiliary cell. The resulting cell becomes the central cell of the developing cystocarp, and, after repeated divisions of its nucleus, sends out radiating septate, nucleated, meta-ooblastema filaments, which ultimately form dense aggregations of small rounded carpospores.

7. The medullary part of the fertile line consists, before the procarpia are fertilised, of numerous rich cells which take no direct part in the formation of the cystocarps, but must contribute very materially to their development.

8. The cystocarps are formed independently of one another, as the result of the development, subsequent to fertilisation of their own procarpia.

EXPLANATION OF FIGURES IN PLATE XXIII.

Illustrating Professor Johnson's paper on *Stenogramme interrupta*.

Fig. 1. A portion of a tetrasporic plant, slightly magnified: *s* sorus, *s'*, empty one.

Fig. 2. Three tetrasporangia: *ts* shows the tetraspores cruciately arranged. × 350.

Fig. 3. Cross-section through two young sori, on opposite sides of the thallus. × 350.

Fig. 4. Male plant, natural size: *a*, antheridium.

Fig. 5. Portion of antheridium in surface view, showing mother-cells of spermatia. × 400.

Fig. 6. Vertical section through two antheridia. × 200.

Fig. 7. Portion of sterile surface of thallus: contents of the superficial cells not shown. × 400.

Fig. 8. Portion of female plant showing fertile lines, *f. l.* The dark spots in the lines indicate young cystocarps. × 10.

Fig. 9. Figure to show relative thickness of ripe fertile line: *t*, thallus, *l*, the wavy fruit-wall.

Fig. 10. Cross-section through the fertile line *f. l.* Five or six procarpia are indicated. × 80.

Fig. 11. Procarpium just after fertilisation: *t*, trichogyne with remains of spermatium, cut off from *c*, the fertilised carpogonium-cell now fused with *m. c.*, the medullary mother-cell of the carpogenous branch of which the two cells *c' c''* later disappear; *t. c.* ordinary thallus-cells; *o*, surface of thallus. × 500.

Fig. 12. *a*, *b*, *c* various young central cells of cystocarps: *m. f.* meta-ooblastema filaments. × 350.

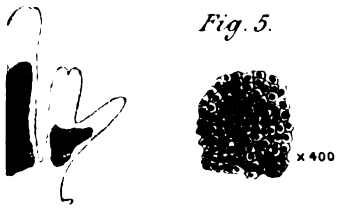
Fig. 13. Vertical section of fertile line to show the rich medullary cells *m'. c'.* at the time of fertilisation of the procarpia. × 80.

Fig. 14. A single medullary cell of Fig. 13. × 300. The peg-like projections represent points of connection with adjacent cells.

Fig. 15. Carpospores. × 500.

Fig. 16. Vertical section of ripe fertile line. The central granular-looking mass represents many cystocarps from which carpospores have become free here and there: *f. w.* fruit-wall. × 50.

Fig. 5.



g. 11.

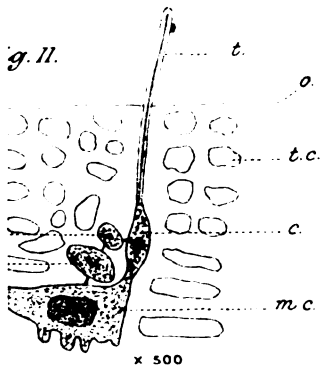


Fig. 12.c

Fig. 15.

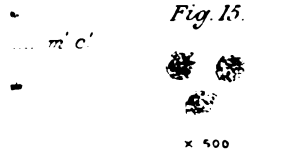


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Fig. 1.

Fig 2

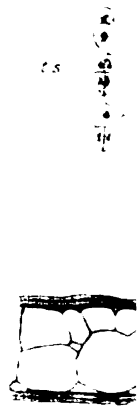

Fig. 8.

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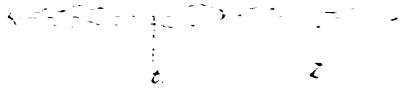

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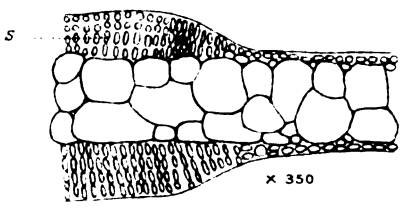
Fig. 3.



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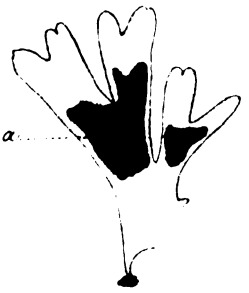

Fig. 5.

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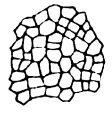


Fig. 6.

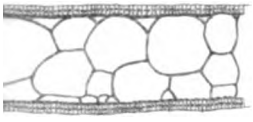


Fig. 10.

Fig. 11.

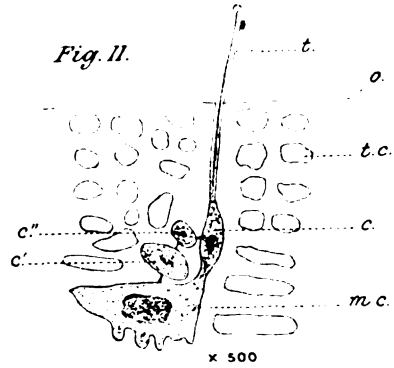


Fig. 12a

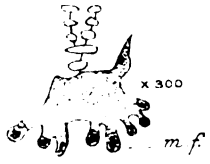


Fig. 12b

Fig. 12c

Fig. 13.

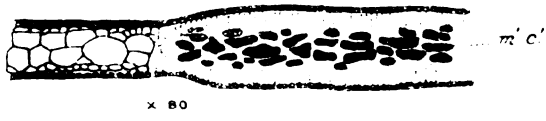


Fig. 15.

Fig. 14.

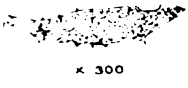
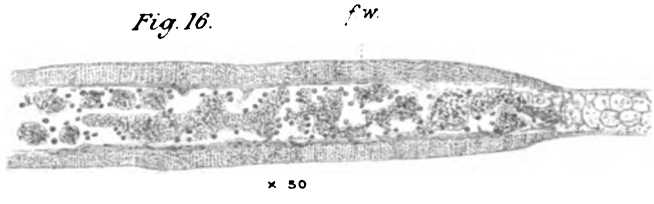


Fig. 16.



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A Drift-seed (Ipomoea tuberosa, L.).

BY

W. B. HEMSLEY, F.R.S.,

Principal Assistant, Herbarium, Royal Gardens, Kew.

With Plate XXIV.

IN most books treating of *Ipomoea tuberosa* it is recorded as a native of tropical Asia, Africa, and America; but Mr. C. B. Clarke[1] has separated the Old-World plant under the name of *I. kentrocaulos*, characterised by having a much smaller capsule without the greatly thickened pedicel; smaller elliptic-oblong sepals; and smaller, almost glabrous, seeds. Whether these characters are constant the specimens are insufficient to determine satisfactorily; but typical *I. tuberosa* with thickened pedicels, very broad lignified sepals, large capsule, and hairy seeds, as figured here in Plate XXIV, was early cultivated in English hothouses[2], and in India, Hongkong, Mauritius, South Africa, and other warm countries inhabited by English people. In foliage and flowers there are no obvious differences in the dried state.

Although this is termed here a drift-seed, it is not so in the sense of several of its congeners, which are common sea-shore

[1] Hooker's Flora of British India, iv. p. 213.

[2] See Transactions of the Horticultural Society of London, i. p. 184, plate 11, and the Botanical Register, plate 768.

[Annals of Botany, Vol. VI. No. XXIV, December 1892.]

plants in the tropics, and frequently spring up from seeds that have floated hither and thither on the sea, and finally been cast ashore to fulfil their destiny. Indeed, it is not essentially a shore-plant, but rather a climber of lofty trees; yet its seeds are not uncommonly met with in the drift of the Caribbean Sea, and they are sometimes carried far up into the north Atlantic by the Mexican Gulf stream. This is one of the points of interest attaching to it to be discussed here; another is the dimorphic or trimorphic development of the seeds. The latter phenomenon may be described first. Normally there are four seeds closely appressed and forming together a spheroid, each seed having two vertical facets and a convex back. Sometimes only two or three seeds are developed, and they are correspondingly different in shape; and not unfrequently only one is formed. When the latter is the case, the one seed assumes the size and nearly the shape of the four seeds combined, differing in being more depressed. It is also slightly furrowed at right angles into four quarters, resembling the four seeds; and the basal hilum is very large and oblong in outline. The furrows probably correspond to the septa of the ovary, which disappear at an early stage of the development of the seed or seeds. Instances of the abortion of some of the ovules and similar adaptations of the developed seed or seeds to the size and shape of the seed-vessel are probably not uncommon; and, as Mr. C. B. Clarke reminds me, some of the Commelinaceae exhibit this peculiarity to an equally remarkable degree with *Ipomoea tuberosa*.

As already stated, some of the species of *Ipomoea* are among the commonest of seaside plants in the tropics, and from actual observation it is known that their seeds will bear long immersion in salt water, or rather float on it, without losing their germinating power. Further, it has been ascertained that the seeds often germinate after being cast ashore. *Ipomoea pes-caprae* is a notable example, being found on sandy shores, including the most remote islets, throughout the warmer zone. Their seeds are well adapted for long journeys by water, having a dense, almost crustaceous testa, which protects the

highly developed, green embryo, and have a hollow centre, which gives them buoyancy.

I am not aware of the existence of any record of the self-colonisation of *Ipomoea tuberosa*, nor of its being carried by ocean currents to the shores of Europe; but Lieut.-Col. H. W. Feilden sent a seed of it to Kew last year with the following extract from his 'Journal of twenty years ago.'—'This seed is probably from the West Indies, and drifted by Gulf Stream to the Hebrides, and has, or used to have, a peculiar virtue attached to it by the inhabitants of the Long Island. The Gaelic name signifies Mary's Bean, and of course refers to the Blessed Mother. The belief was, and I daresay still lingers amongst the Celtic Roman Catholic people of the Long Island, that this seed clenched in the hand of a woman labouring with child would ensure easy delivery. I got this seed from a woman in the island of North Uist, and she said it had been in the possession of her mother and her grandmother.'

It would be interesting to know whether this is one of several or many instances of this seed being thrown up on the Hebrides. One would not expect it to possess a Gaelic name, and have the reputation for the virtue ascribed to it, from a solitary example.

EXPLANATION OF FIGURES IN PLATE XXIV.

Illustrating Mr. Hemsley's paper on *Ipomoea tuberosa.*

Fig. 1. A ripe capsule subtended by the enlarged somewhat lignified sepals.

Fig. 2. A cluster of four seeds seen from above.

Fig. 3. „ „ below.

Fig. 4. A single seed.

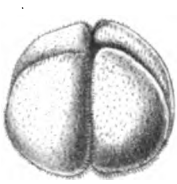
Fig. 5. A cross-section of the same showing the folded cotyledons embedded in the albumen, and the central cavity.

Fig. 6. A single seed, where only one is developed in a capsule, seen almost horizontally.

Fig. 7. The same seen from below.

Fig. 8. The same in cross-section.

Figures 5 and 8 enlarged; the rest natural size.



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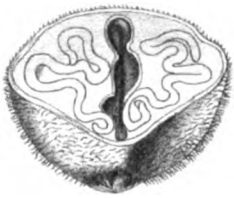

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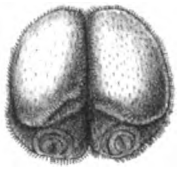

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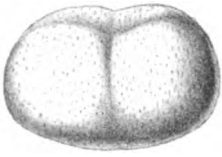

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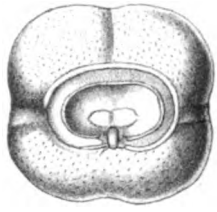

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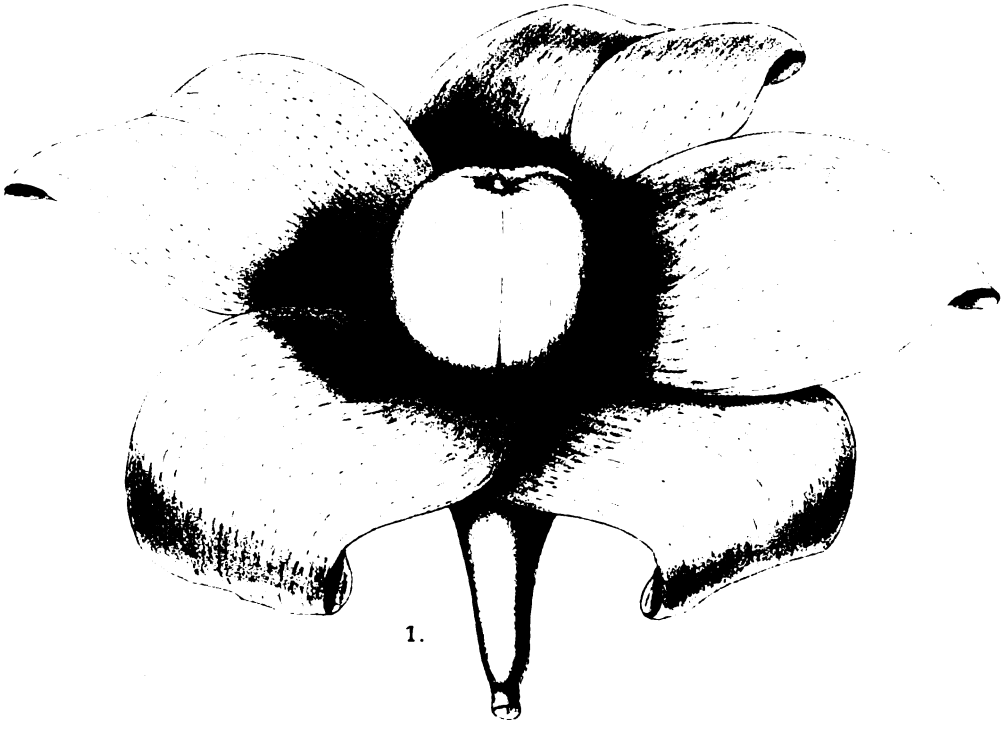

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M Smith del

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HEMSLEY. — ON IPOMOEA TUBEROSA.

NOTES.

ON THE CAUSE OF PHYSIOLOGICAL ACTION AT A DISTANCE[1].—Most vegetable organs are sensitive to the influences of the environment and respond to these stimuli, as long as they are capable of growth, by bending in a definite direction. In fact, they generally feel asymmetrical distribution of matter or energy around them. Thus, the geotropic, heliotropic, hydrotropic, haptotropic curvatures arise, which are familiar to vegetable physiologists.

But the very interesting phenomena described two years ago by Elfving did not appear to belong to any of the known categories, and led this distinguished botanist to the assumption of a new force revealing itself by 'physiological action at a distance'—as he terms it.

He found that pieces of iron and, to a less degree, of zinc or aluminium, as well as different organic substances, such as sealing-wax, rosin, roots of living plants, attract the growing sporangium-bearing filaments of *Phycomyces nitens*, a well-known fungus belonging to the Mucorini. All other metals tried by Elfving were inactive, whereas the filaments of *Phycomyces* itself showed a mutual repulsion.

The latter fact, which I had often observed, I always ascribed to negative hydrotropism. So the question arose whether the attractions discovered by Elfving are not due to a similar cause. For, just as we know that a surface which emits moisture repels the *Phycomyces* filaments, it seemed probable that moisture-absorbing substances should produce the reverse effect and attract them. Now iron certainly absorbs aqueous vapour whilst rusting, and its peculiar action on *Phycomyces* might thus be simply a case of hydrotropism.

I have tested this view by a great number of experiments, and I submit for the inspection of the members of this section photographs showing the behaviour of *Phycomyces* towards different

[1] Read before Section D of the Brit. Assoc. on the 5th of August, 1892.

substances. The theory thus established not only enables one to explain the known facts, but also to predict unknown ones.

It is easy to demonstrate that any modification of iron which lessens its capacity of rusting at the same time diminishes its attraction on *Phycomyces*; polished steel scarcely attracts, and nickeled steel does not do so at all.

China clay, which is very hygroscopic, attracts energetically, but china exhibits no attraction. One of the most striking instances is that of agate and rock-crystal. Although both are essentially formed of silica, the Japanese physicist Jhmori[1] has shown that the former is very hygroscopic, whereas the latter is not so. And, as was to be foreseen, agate strongly attracts the *Phycomyces*, though rock-crystal is perfectly inactive. I might quote many similar facts, if necessary. Thus, sulphuric acid, sulphate of copper, &c., are strongly attractive. Certain bodies which are only moderately hygroscopic, as white soap, lose or gain moisture according to the degree of dampness of the surrounding atmosphere; and in the first case they repel *Phycomyces*, in the second they attract.

The sensibility of *Phycomyces* is, in fact, so great that it may actually be used as a reagent to test the existence of hygroscopic power. Having noticed that camphor very distinctly attracts the filaments and thymol does not (although both of these substances have a deleterious action on them), I was led to anticipate that camphor is hygroscopic—a fact which, though unknown to chemists, was confirmed by careful weighing.

Lastly, the theory may be tested in another way. Unlike the filaments of *Phycomyces*, the roots of higher plants are positively hydrotropic. Then, as might be expected, they bend away from iron instead of being attracted by it.

All these experiments succeed also in a saturated atmosphere, which shows that hydrotropism is not due, as generally admitted, to differences in the hygrometric state of the air. But, the discussion of this point, as well as certain deductions relative to the physical phenomenon of hygroscopicity, must be reserved for a detailed paper on this subject.

To sum up the general results: the apparently mysterious action of iron on *Phycomyces* is nothing but a matter of hydrotropism; and hydrotropism itself (negative or positive) is the bending of a

[1] Wiedemann's *Annalen*, 1887.

plant-organ towards the point, not where it will find a minimum or maximum of moisture, but where it will, within certain limits, transpire most or least.

LÉO ERRERA, Brussels.

BOTANICAL NOTES.

No. 1. **ON THE THORNS OF RANDIA DUMETORUM, LAM.**—*Randia dumetorum* is a Rubiaceous plant widely distributed in tropical East Africa, India, the Malayan Archipelago, up to China, including Formosa and Hong Kong. The plant is very common on Dane's Island, Whampoa, where my observations were made.

Position and arrangement of the thorns.—The leaves of this plant are opposite as in most Rubiaceae. In the axil of each leaf is a branch, or bud, directly above which a thorn frequently occurs; so the thorns are supra-axillary. As a rule there is a distinct, often considerable, difference in the size of the two leaves at a node. The smaller leaf not uncommonly decays and drops off early in life. The thorn above this leaf is invariably inserted closer to the leaf-axil than is the thorn above the larger leaf. This is shown

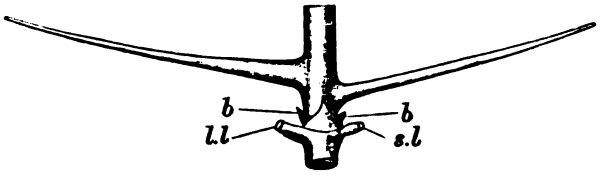


Fig. 1.

in the Fig. 1, in which *l.l.* and *s.l.* are the stalks of the large and small leaves respectively, and *b.b.* are the axillary buds. Thorns never occur in relation with the first pair of leaves of a branch, and they are occasionally not developed in connection with pairs of leaves higher up the branches; frequently a thorn occurs above only one of the leaves at a node, in which case it almost always is situated above the larger leaf.

Morphological Significance of the Thorns.—As far as the *position* of the thorn is concerned it might be a trichome, an emergence, or an accessory branch. It is not very exceptional to find in plants a protective outgrowth of the cortex above the axillary bud

and the thorn might readily have been derived from such an outgrowth. But the following facts prove that the thorns are accessory branches:

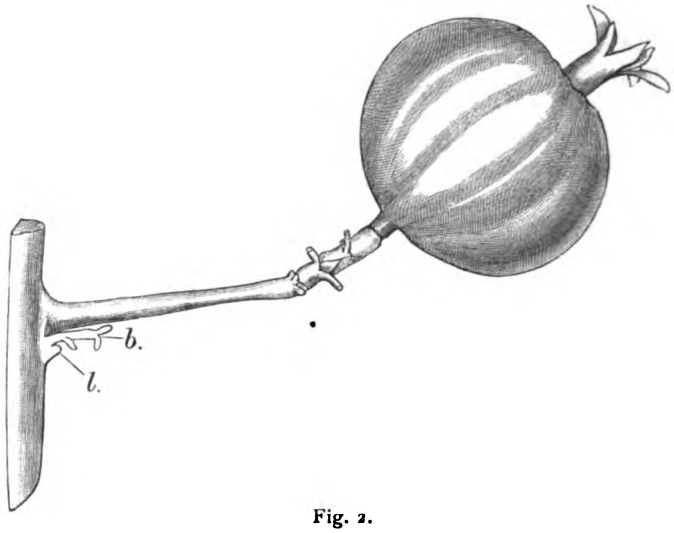


Fig. 2.

(i) *The thorn may develope into a shoot and bear leaves.* The thorn may become either a 'long shoot,' or a 'dwarf shoot' terminating in a flower. Fig. 2 shows a thorn converted into a

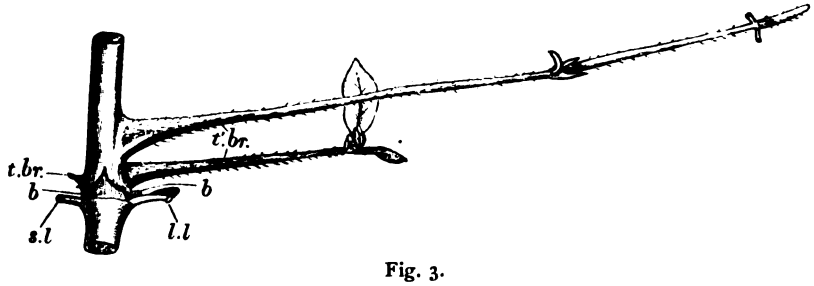


Fig. 3.

shoot and ending in a fruit. The ordinary axillary bud may develope as well; in one case I saw two superposed thorns changed into branches, as is shown in Fig. 3 in which *t. br.* re-

presents the two thorn-branches, above the large leaf, and *t. br.* the arrested thorn-branch above the smaller leaf.

(ii) *The thorn arises and grows like a shoot.* It appears as a multicellular outgrowth in the axil of a leaf, and the deeper layers of cells take part in its formation. It is only by the subsequent intercalary growth of the shoot bearing the thorn that the latter assumes its supra-axillary position. The thorn in connection with the smaller leaf arises later than that above the larger leaf at a node, hence it is not carried so far up the stem by the intercalary growth of the stem. The thorn differs from an ordinary branch in that it developes at once and does not wait till next season as an axillary bud does. It grows out in the form of a long slender structure which tapers to a fine growing-point. At first it is strongly hyponastic and is directed upwards parallel to the shoot which bears it: by subsequent epinastic growth it comes to stand more or less at right angles to the axis. A thorn-branch developes in precisely the same manner; but it at length bears a pair of leaves, placed in the transverse plane as on ordinary branches, which appear relatively

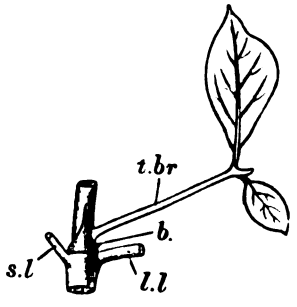


Fig. 4.

late. As is seen in Fig. 4, the first pair of leaves are inserted at the top of a long internode, which for brevity I will term the 'thorn-internode,' whereas an ordinary branch arises as a short broad outgrowth on which two leaves appear at once. Hence even old thorn-branches can be recognised as such by the long basal internode ('thorn-internode') with which they commence; this is clearly seen in the second figure. The typical leafless thorn does not differ from an ordinary shoot in that it only grows for a brief and

definite period, for this is also the case with the axillary shoots which terminate in flowers. But the thorn or thorn-branch has a peculiar feature in the pronounced dorsi-ventral nature of its base, as exhibited by its hyponasty and epinasty, and by its contour not being circular but elliptical with the long axis of the ellipse in an antero-posterior plane.

(iii) *The structure of a thorn agrees with that of a shoot.* It consists of epidermis (or periderm), cortex, vascular cylinder, and pith. But there are anatomical differences between the thorn and an ordinary shoot. The hairs on a thorn drop off much sooner than they do from a shoot, or even from a thorn-branch, and the cuticle thickens more rapidly. In the thorn the protoxylem has only a few narrow spiral vessels; and the later formed xylem consists of parenchyma and radial lines of thick-walled prosenchyma with small pits. In a shoot the primary annular and spiral vessels are more numerous and slightly larger; in the later-formed xylem wide vessels are interspersed amongst the thick-walled prosenchyma-cells which have larger pits. The sieve-tubes of the shoot have wider lumina. The 'thorn-internode' stands midway between a shoot and a thorn in structure, as there are a few wide vessels amongst the wood-prosenchyma. In the young stage there is no apparent difference in the structure of a thorn and a thorn-internode already possessing leaves. This shows that leaves are not formed on thorns because the vessels are wider and more numerous, for these distinctions do not appear till after the leaves have been formed. I know no case in which it can be so clearly shown that the structure of the wood depends on the extent of the assimilating and transpiring surface; here we can compare two identical members formed at the same time and under the same external circumstances, for we can examine a thorn and a thorn-internode arising at the same node.

Attention has already been directed to the dorsi-ventral nature of the thorns and 'thorn-internodes': these members also exhibit remarkable peculiarities in connection with the assumption of their ultimate position. Under all circumstances the thorn, or the 'thorn-internode,' assumes a position approximately at right angles to the shoot bearing it. Thus these structures assume their position independently of the two great directive influences of light and gravitation; and this is the more clearly seen when the thorn becomes a branch, in which case the subsequently formed internodes have their direction

governed by light and gravitation. I noted one especially striking series of thorn-branches which were on the lower surface of a horizontal lateral shoot. The 'thorn-internodes' pointed vertically downwards, i. e. at right angles to the shoot; but the remaining internodes of these thorn-branches were directed horizontally, that is at right angles to the thorn-internode and parallel with the lateral shoot and pointing towards its apex. Thus each thorn-branch was shaped like an L, doubtless because of the marked positive heliotropism of the later formed internodes.

Biological Significance of the Thorns.—The thorns are protective, and their particular *rôle* appears to be the defence of the young branches which arise in connection with the same leaves as themselves. They accomplish their end by (1) standing out at right angles to the stem or having a slightly ascending direction: (2) developing in the year of their origin and speedily becoming hard and lignified. In accordance with their particular function they are absent when there is no axillary branch to protect: for example, the bud in the axil of the smaller leaf frequently does not develope, and then there is no supra-axillary thorn: neither do the buds of the lowest pair of leaves of a shoot grow out, and here again thorns are absent.

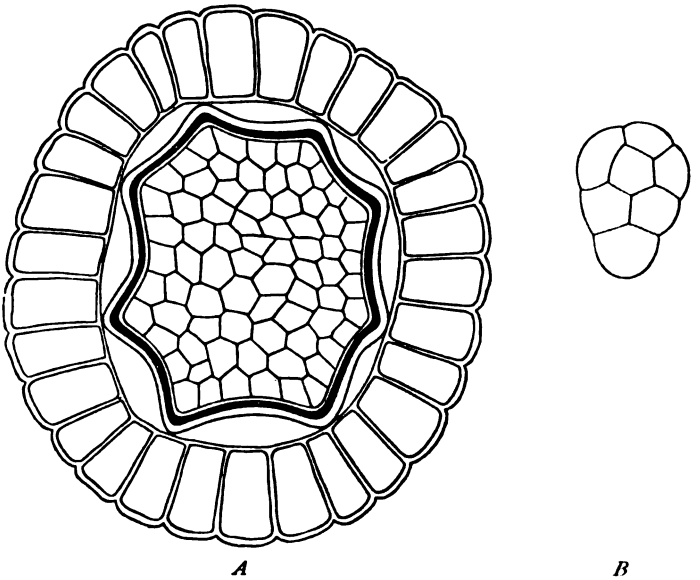
The Atrophy of the Leaves.—The unequal size of the two leaves at a node is by no means a peculiar phenomenon, but it is interesting to find a plant actually in the process of diminishing the size of certain of its leaves. In some shoots of *Randia dumetorum* and other species of *Randia*, no difference exists between the two leaves at a node. But on the other hand extremes in the other direction exist in which there is marked difference in size and the smaller leaf falls off early in life. Connected with the atrophy of the leaf is the postponement of the development of the thorn, or its total absence, and the frequently permanent dormancy of the axillary bud. It is very suggestive that coffee-planters have ascertained by experience that the best method of cultivating another Rubiaceous plant, the coffee-plant, is to do just what *Randia dumetorum* seems to be aiming at, namely to nip off one leaf from each of the successive internodes so as to make the leaf-arrangement alternate. The postponement in the development of dwindling organs may be also seen in the late appearance of the first pair of leaves on a thorn-branch.

No. 2. **ON A MONSTROUS FLOWER OF NELUMBIUM SPECIOSUM, WILD.**—Masters and Penzig record the occurrence of double flowers and petaloid stamens as the sole monstrosities known amongst flowers of *Nelumbium speciosum*. In a flower of this plant which I obtained at Whampoa (Southern China) there is a considerable metamorphosis in the carpels, so the specimen appears worthy of description. Unfortunately before I saw the flower all the parts had withered and dropped save a few of the inner stamens and the carpels. The stamens display several stages of petalody. The least modified stamen consists of a thin filament, the upper portion of which bears a very narrow elongated four-winged petaloid process: above this region the filament is continued as a thread and bears at its summit a club-shaped, slender, unlobed, 1-chambered anther. Within the papillose epidermis of the anther are several layers of cells with brown cuticularised walls; the cells in the centre are thin-walled, are densely filled with starch, and are connected with the brown-walled cells only at certain spots by strands of starch-containing cells. A more petaloid stamen exhibits a broad flattened portion, much longer than an ordinary stamen, possessing at the middle of its broad summit a tiny knob which represents the anther. The knob is 2, 3, or 4-lobed, but is only 1-chambered. Its structure resembles that of the club-like anther previously described except that there are smaller masses of starch-containing cells, and they are interspersed with spongy parenchyma the cell-walls of which are partially converted into mucilage. The petaloid portion has neither brown-walled subepidermal cells nor aggregations of starch-containing cells. In a still more modified condition of the stamen the knob is reduced to a minute dark-coloured tooth. In the stamens the pollen-producing tissue is probably represented by the masses of starch-containing cells, and not by the brown-walled cells; though the latter, when isolated, look something like incompletely developed pollen-grains.

The carpels are changed into tubes, about two inches in length, each with a slit-like aperture at its apex. Style, stigma and ovules are not differentiated.

No. 3. **ON THE EMBRYO OF PETROSAVIA, BECCARI.**—*Petrosavia* is a small Liliaceous plant described by Beccari as parasitic on roots. It was only known to occur in Borneo, but recently Mr. Ridley has discovered the plant in the Malay Peninsula, at Perak, and

has amplified Beccari's description. He suggested a search for the hitherto unknown embryo and kindly supplied me with seeds for the purpose. The excessively minute seed has an external layer of large cells with cuticularised walls which easily separate from the inner portion of the seed. This latter part of the seed has some seven or eight ridges and furrows, and is laterally in contact with the outer layer of cells only at the tops of the ridges, excepting at its two ends where a few thin-walled cells intervene between it



A *B*

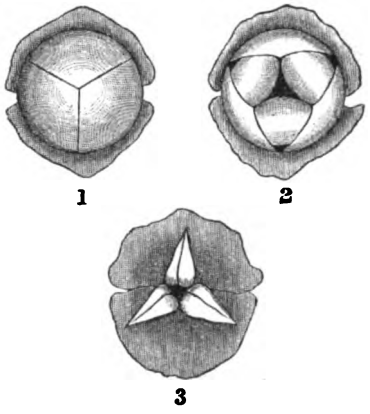
Fig. 5.

and the outer layer. A transverse section (diagrammatically represented by Fig. 5 *A*) shows that the extreme hardness of this core of the seed is caused by two thick, glistening, suberised membranes which are separated by a space containing a brown colouring-matter (partially tannin). In this space I could recognise neither protoplasm, nor cell-walls crossing it, so I suppose it is an intercellular space due to the disintegration of the middle lamella; but an examination of stages younger than those which I possess, may show that the space really represents a disorganised layer of flattened cells. Within the inner of the two membranes lies the endosperm

consisting of thin-walled cells rich in oil. At the micropylar end is the minute inconspicuous embryo, scarcely the size of an ordinary endosperm-cell (Fig. 5 *B*). It is shaped like a somewhat flattened pear and consists of but few cells, the one at the radical or suspensorial end being relatively large and hemispherical. Beyond this there is no differentiation into definite parts, and the embryo is nowhere more than two cells thick. In its minute size and excessive simplicity the embryo resembles that of many other parasites and saprophytes, as may be seen by the surface view which is here given.

PERCY GROOM, Oxford.

THE DISTRIBUTION OF THE SEED IN CLAYTONIA.—In studying the biology of the flower in *Claytonia* it was noticed that



CLAYTONIA ALSINOIDES

Fig. 1. Capsule before dehiscence, from above (×6).
„ 2. „ shortly after dehiscence.
„ 3. „ after ejection of seeds.

the ripe seeds were thrown with some violence out of the capsules. The mechanism was therefore investigated, and proved to be almost identical with that occurring in *Montia minor* (in the same natural order), as described by Urban[1]. The species examined were *C. alsinoides* and *C. sibirica*, both growing in the Cambridge Botanic

[1] 'Ueber d. Schleudereinrichtung bei *Montia minor*.' Jahrbuch des Königl. Bot. Gartens zu Berlin, vol. iv, 1886, p. 256.

Garden. As it is intended to discuss the biology of the flower in a forthcoming paper, the fruit only need be considered here. After fertilisation, the peduncle of the flower, previously erect, bends downwards through about 180°, and again becomes erect when the fruit is ripe. The persistent green calyx encloses the fruit, which (Fig. 1) is a small oval or spherical capsule, splitting loculicidally into three valves. It contains usually, in its single loculus, three seeds, which lie, forming a triangle, one across each of the slits between the valves (Fig. 2). They are ovoid in shape, rather more convex on the outer side; the surface is slightly tuberculate, but is very slippery, possessing a high polish.

The valves fall back after dehiscence until the seeds are fully exposed, and as they become dry their sides move inwards, towards one another, just as occurs in the fruit of the Violet. The effect is at first to press the seeds tightly against one another, until presently the resistance to slipping, offered by their tuberculate surfaces, is overcome. When this happens, one of the seeds, probably whichever happens to stand the highest, is shot out. In some cases two are ejected together, or even the whole three. Those which remain behind usually fall into the jaws of the still closing valves, and are finally ejected by these when the pressure becomes considerable. The distance to which the seeds are thrown is usually from a metre to a metre and a half. This was determined by allowing specimens to explode on a smooth table and noting the point where the seed first touched it after ejection.

After the explosion the valves are completely folded in upon themselves, one side usually overlapping the other (Fig. 3).

J. C. WILLIS, Cambridge.

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