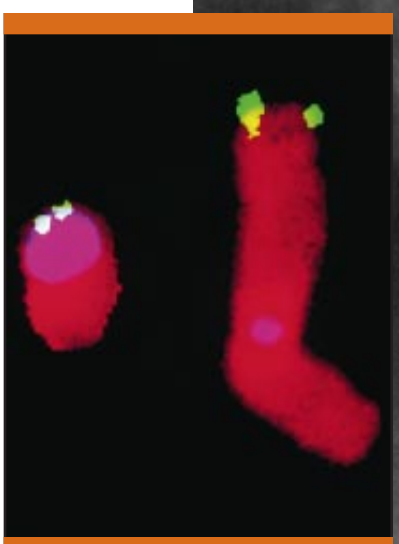
The Y chromosome causes an embryo

The Key to Masculinity



ORIGINS OF MALENESS lie on the Y chromosome, shown in the inset with the much larger X chromosome. Males normally have one Y and one X chromosome, whereas females normally have two Xs. The dyed spots at the tops of the chromosomes mark the pseudoautosomal regions.

to become male by directing the development of the testes. But new research indicates that the Y does much more

by Bruce T. Lahn and Karin Jegalian

What a difference a Y can make. In humans, the tiny Y chromosome—one of the smallest of the 46 chromosomes that carry all our genes—essentially dictates a person's sex. Genes on the Y chromosome trigger the development of male features—everything from the formation of the testes and the ability to produce sperm to the emergence of secondary sexual characteristics, such as facial hair and a deep voice. Without a Y chromosome and its resident male-forming genes, an embryo will develop as a female. Thus, at the most fundamental level, the problem of understanding the biological differences between the sexes can be reduced to a problem of understanding the Y chromosome and how it encodes maleness.

Under a microscope, the Y chromosome appears as a small rod about one third the size of the X—the other sex chromosome, present in both males and females. (Males, who normally have one X and one Y chromosome, are designated XY; females normally have two Xs, hence the XX designation.) Although it has been known for decades that it takes a Y to make a male, until about 10 years ago scientists believed that the human Y chromosome contained few genes aside from those that determined maleness. This view stemmed, at least in part, from the relative abundance of "junk" DNA—repetitive DNA sequences that do not encode any proteins—on the Y chromosome.

But on closer examination, using powerful molecular techniques, we and other investigators have since discovered that the Y chromosome is not quite as genetically barren as previously thought. At last count, researchers have identified 21 genes on the human Y chromosome, a figure that is probably more than half the true total. These genes fall into three distinct groups based on the roles they play in the body. The first group contains a single gene that, on its own, steers an embryo into developing as a male by directing the formation of the testes. The second group consists of 10 genes, active only in the testes of adult males, that may be involved in sperm production. The remaining 10 genes perform mostly "housekeeping" functions that keep cells throughout the body running smoothly. These genes are closely related to genes found on the X chromosome. The presence of these "X-homologous" genes reinforces the theory that the X and Y chromosomes evolved from a common X-like ancestor before the Y acquired its male-specific genes and adopted its role in sex determination.

For much of human history, the riddle of what makes a baby a boy or a girl has been debated in nursery rhymes and folklore. Various legends attributed sex determination to the phases of the moon and the time of day when conception occurred. Scientists turned their attention to this question only about 100 years ago, when genetics emerged as a discipline.

At about that time, biologists first saw the sex chromosomes inside cells. Microscopic examination of cells had established that the chromosomes in most multicellular organisms exist in pairs and that the two partners line up side by side during meiosis—the type of cell division that occurs during the formation of eggs or sperm. But while studying insect cells, researchers noticed that in males, one chromosome did not seem to pair with any partner. In 1891 German biologist Hermann Henking labeled this anomalous structure "X," and a decade later American scientists identified the structure as a sex chromosome. A few years later Nettie M. Stevens of Bryn Mawr College and others, again studying cells from male insects, spotted a very small chromosome that appeared to be the X chromosome's missing partner. The mismatched chromosome pair subsequently became known as X and Y.

The Master Switch

That the Y chromosome on its own has the power to trigger male development in humans became clear in the late 1950s. In one study researchers discovered that individuals who possess three sex chromosomes—two Xs and one Y (XXY)—always develop as males. Conversely, individuals with only one X chromosome and no Y chromosome—designated as XO individuals—develop as females. These cases of unusual sex chromosome compositions demonstrate that in humans the presence of the Y leads to male development and its absence leads to female development.

These observations implied that the Y chromosome bears one or more male-determining genes that somehow set in motion the cascade of developmental events that makes a male. Discovery of the male-determining gene, however, did not happen until the 1980s, when researchers started to study sex-reversed individuals.

Sex reversal occurs when a person's morphological sex—the sex he or she appears to be—is the opposite of what his or her chromosomes seem to predict: in other words, an XY female or an XX male. Researchers estimate that about one in every 20,000 individuals is sex-reversed. Many such individuals grow up unaware of their condition; very often the first time they learn of it is when they go to a clinic with fertility problems (the sex reversal makes them unable to conceive). Using molecular analyses, two groups of researchers—one led by David C. Page of the Whitehead Institute for Biomedical Research and the other by Peter Goodfellow of the Imperial Cancer Research Fund—found that the sex chromosomes in many sex-reversed individuals have in fact undergone genetic rearrangements that are not detectable under a microscope. In many XX males, one of the X chromosomes

HORNICK/RIVLIN STUDIO (*photograph*); DEPARTMENT OF CLINICAL CYTOGENICS, ADDENBROOKS HOSPITAL/SPL/PHOTO RESEARCHERS, INC. (*inset*)

carries a small piece of the Y abnormally stitched onto it. Similarly, in many XY females, the Y chromosome is missing a small fragment. The male-determining gene or genes, the researchers reasoned, must therefore lie within that small region.

The search ended in 1990, when Goodfellow and his colleagues identified the *sex-determining region Y,* or *SRY.* This gene appears to be the master switch controlling male development in all mammals, including humans. Investigators led by Robin Lovell-Badge of the Medical Research Council's National Institute for Medical Research in London subsequently showed that injection of this single gene into fertilized mouse eggs is enough to turn a genetic female—an XX individual—into a male.

SRY encodes a protein that binds to DNA, suggesting that it exerts its effect on male development by regulating the activity of a number of genes. Those genes then somehow spur the embryonic bipotential gonad—the primitive tissue from which both male and female sex organs derive—to develop into testes rather than ovaries. The testes, once formed, in turn induce all other male features. In humans the two hormones secreted by the testes, testosterone and anti-Müllerian duct hormone, prompt the development of virtually all other male sex-specific characteristics, including the external genitalia, facial hair, deep voice and male skeletal structure.

Although researchers have had *SRY* in hand for nearly a decade, no one has yet identified any genes that are directly controlled by the master switch. In part, the fact that *SRY* acts only transiently in a highly complex system during early embryonic development has made it difficult to determine exactly how this gene functions to make a male.

Making Sperm

Given *SRY*'s pivotal role in dictating male development, it is tempting to think of the gene as being functionally interchangeable with the entire Y chromosome. But the situation is not that simple. Another critical aspect of maleness, the production of sperm, requires the participation of other genes on the Y chromosome.

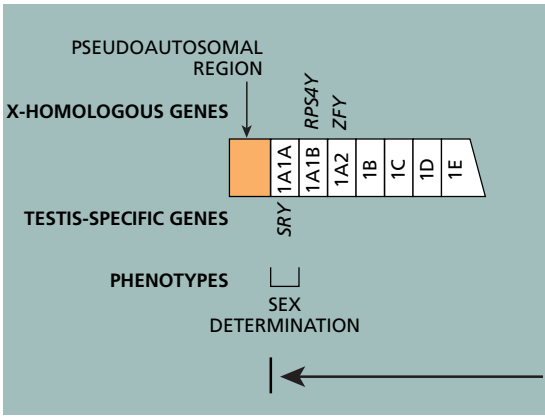
In 1976 clinicians Luciano Tiepolo and Orsetta Zuffardi of the University of Pavia in Italy first noticed that some infertile men who had no detectable sperm in their ejaculate—a condition called azoospermia—also had abnormally short Y chromosomes. The researchers reasoned that the part of the Y chromosome deleted in these patients harbored a gene or genes essential for making sperm. But these patients developed as males because their shortened Y chromosomes carried the *SRY* gene.

More recently, using molecular techniques for revealing much smaller deletions, our laboratory group and others have shown that at least three regions of the Y chromosome harbor genes essential for sperm production. Deletion of any one of these three regions—called azoospermia factors a, b and c—can lead to infertility caused by defective sperm production. One of the regions, azoospermia factor c, is deleted in one of every 5,000 to 10,000 newborn males; the other two regions are deleted much less frequently. To understand how the deletions in the Y chromosome block sperm development, the genes in the deleted regions have to be identified. Several laboratories, including our own, are trying to understand the precise functions of these genes in sperm development and how infertility may result from their deletion.

Because infertile men cannot father children, the deletions in the Y chromosome represent new mutations rather than inherited traits. But this may not always be the case, thanks to rapid advances in assisted-reproduction techniques. A small amount of normal sperm can be found in the testes of some infertile men with Y chromosome deletions. Using a procedure called intracytoplasmic sperm injection (ICSI), doctors can retrieve these isolated sperm cells and inject them directly into eggs to achieve fertilization. Although ICSI may offer these otherwise infertile men the only hope of having biologically related children, their sons will unfortunately carry the same abnormal Y chromosome and will in all likelihood be naturally infertile. In time, society may need to grapple with the long-term effects of such reproductive intervention [see "Of Babies and the Barren Man," on page 74].

The Y chromosome's contribution to biology is not limited to processes that are specific to males. This small chromosome also harbors genes that contribute to fundamental processes in each and every cell, processes that are common to the two sexes.

The existence of such general-function genes on the human Y chromosome was first postulated in 1965 by Scottish biologist Malcolm A. Ferguson-Smith, then at the University of Glasgow. Ferguson-Smith was studying Turner syndrome, a congenital disorder in which an individual possesses only one sex chromosome, the X. Such XO females are characterized by many anatomical abnormalities, including short stature, a webbed neck and a failure to develop secondary sexual characteristics, such as breasts and pubic hair. Although Turner syndrome occurs in approximately one in every 3,000 live births, an estimated 99 percent of XO embryos abort spontaneously. Ferguson-Smith proposed that the abnormalities seen in Turner syndrome were caused by insufficient levels of proteins that are produced by some specific "Turner genes" present on the missing sex chromosome.



Where X = Y

What does Turner syndrome tell us about the Y chromosome? First, let us review a bit of basic biology. In female mammals, one of the two X chromosomes in each cell is shut down so that its thousands of genes no longer direct the production of proteins. The rationale for this scheme—known as X inactivation—is simple. Females have two X chromosomes, whereas males have only one. And because the amount of protein produced from a gene is typically proportional to the number of copies of that gene in the cell, without X inactivation females would have twice as many X-encoded proteins as males. This could spell disaster for a complex organism that depends on tightly balanced levels of gene activity.

X inactivation thus allows the two sexes to achieve comparable amounts of activity from X genes.

Getting back to Turner syndrome: If the second X chromosome in females is shut down, why should an XO woman differ from an XX woman? As Ferguson-Smith hypothesized, and others later confirmed, X inactivation in females is not complete. A small subset of genes on the X chromosome always escapes inactivation and directs protein production from both chromosomal copies. So XO individuals wind up with half the normal amounts of these important proteins.

But males also have only one X, and yet they manage to evade the characteristics of Turner syndrome. Again, Ferguson-Smith hypothesized, and others later confirmed, that the genes that escape X inactivation have close counterparts, or homologues, on the Y chromosome. These genes are involved in processes fundamental to all cells, such as the production of essential proteins. In most cases, the homologues on the X and Y chromosomes encode proteins that are about 90 percent identical in their amino acid sequences. Although these homologous genes are considered possible "Turner genes," none of them has yet been definitively linked to a specific aspect of Turner syndrome.

It was not always the case that the presence of a Y chromosome dictated the sex of an organism. In many reptiles—crocodiles, alligators, and some turtles and lizards, for example—the sex of the embryo is determined by the ambient temperature. So how did the sex chromosomes arise?

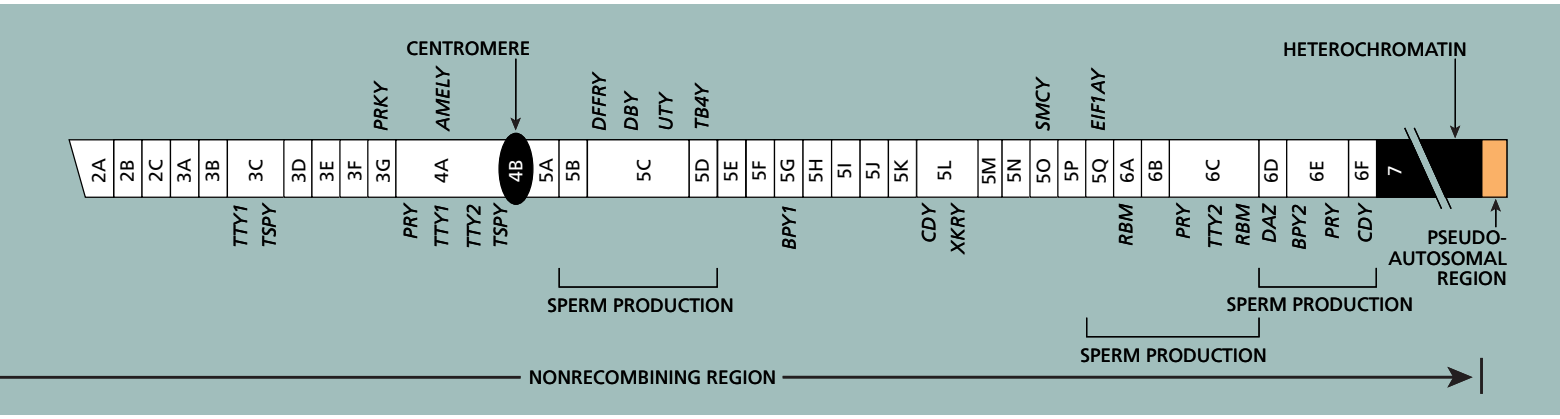
Where Did Y Come From?

Because the X and Y chromosomes share homologous genes, they are thought to have evolved from a pair of identical chromosomes. When mutations or genetic rearrangements gave rise to the male-determining *SRY* gene—or a factor that served a similar function—the Y chromosome was born. Its *SRY*-deficient partner became the X. The X and Y chromosomes most likely arose 200 to 300 million years ago, after mammals and the reptiles that lack sex chromosomes diverged.

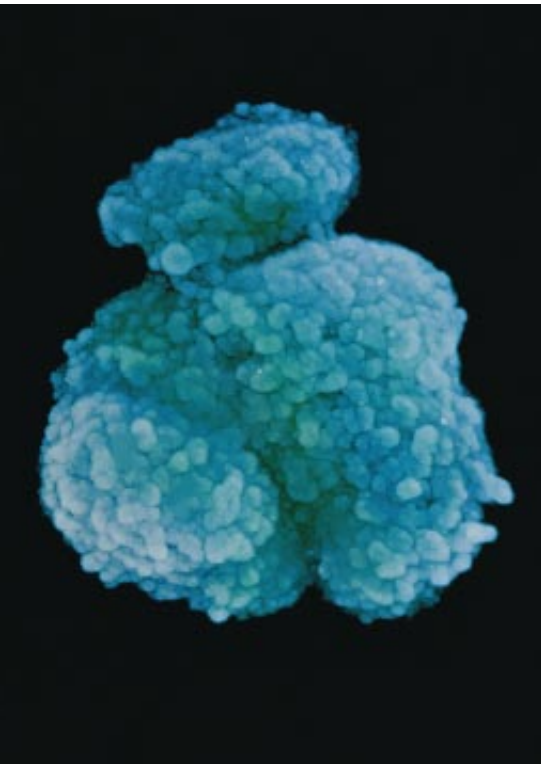
At first, the X and Y were basically identical except in the sex-determining region. But at some point the two chromosomes began to diverge. Evolutionary biologists believe that the X and Y went their separate ways when the two for some reason became unable to recombine—the process by which chromosome pairs line up and swap bits of DNA. Because recombination is essential for maintaining the integrity of DNA, the Y chromosome began to lose its genes. Genes on the X were maintained because X chromosomes could continue to recombine with one another in females. As genes on the Y degenerated, X inactivation emerged as a way of equalizing the dosage of X-linked genes—which lost their counterparts on the Y—between males and females. The X-homologous genes present on the Y today survived, most likely because they participate in the most basic cellular processes.

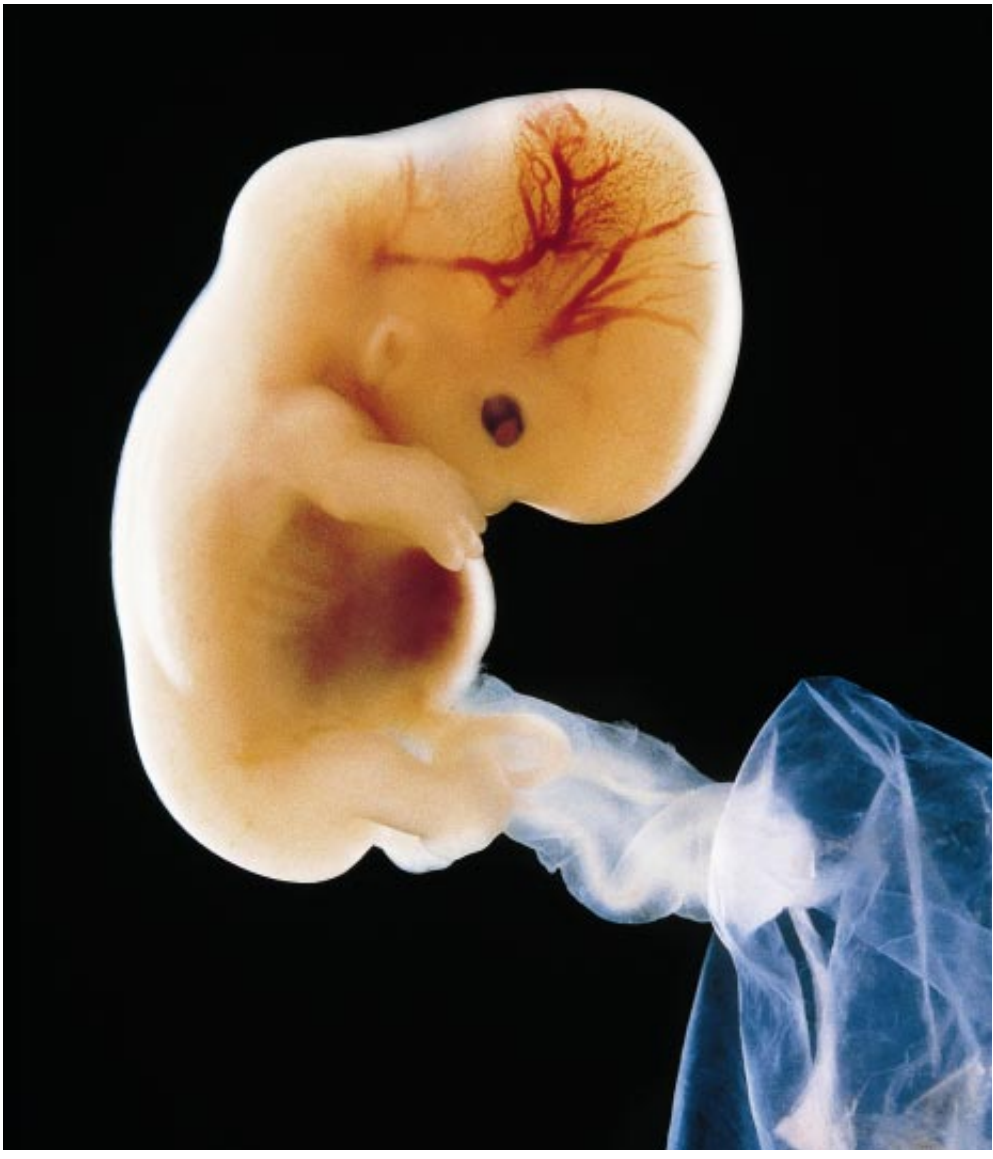
Less is known about the origin of the Y-specific genes, including those that may be involved in sperm production. Most of these genes have no homologues on the X chromosome. According to studies conducted in our laboratory, it appears that two of these Y-specific genes emerged on the Y chromosome much more recently, only 30 to 50 million years ago. In mammals that diverged from our primate ancestors before that time, the genes resided on other chromosomes, not on the X or the Y. These genes were then copied onto the Y chromosome in a creature that gave rise to humans and some of our primate relatives.

Why should these genes have sought to relocate on the Y chromosome? We think that these moves are not flukes. More likely, they represent a consistent trend in the evolution of the Y chromosome. There may be an advantage to storing genes that function only in males in the male-specific part of the genome. The possible advantage was first explored in 1931 by British geneticist Ronald A. Fisher. He suggested that if some genes benefit males but are inconsequential or even harmful to females, selective pressure might favor their sequestration in chromosomal regions present only in males, namely, the Y chromosome. The accumulation on the human Y of genes that function



GENE MAP of the human Y chromosome shows a large region that does not recombine during meiosis and the two small pseudoautosomal regions that recombine with portions of the X chromosome. The nonrecombining region is divided into 43 intervals defined by naturally occurring deletions. Researchers have identified genes in 17 intervals. The X-homologous genes, which have counterparts on the X chromosome, are shown above the map; the testis-specific genes, which are active only in the testes of males, are shown below. The phenotypes at the bottom of the map show the regions of the Y chromosome where deletions would cause sex reversal or defective sperm production.





***Y CHROMOSOME** is a bundle of tightly packed DNA, shown magnified 9,000 times in this scanning electron microscope image* **(left)**. *The chromosome's* **SRY** *gene encodes a protein that causes an embryo's gonads to develop into testes rather than ovaries. This sexual differentiation begins when the embryo is about six weeks old* **(right)**. *The embryo's testes then secrete testosterone and anti-Müllerian duct hormone, which in the following weeks stimulate the development of other male characteristics, such as the penis and scrotum* **(opposite page)**. *Without the* **SRY** *gene, the embryo would develop as a female.*

in sperm development may represent such an occurrence. Consistent with this notion, other species ranging from mice to fruit flies have also been shown to carry genes needed for male fertility on their Y chromosomes.

Such a setup could arguably benefit the species as a whole. But not everyone believes that the sex chromosomes always act in the best interest of the species. According to Lawrence Hurst of the University of Cambridge, sometimes a sex chromosome can harbor "selfish" genes that may enhance its probability of being transmitted to the next generation. A chromosome with such a selfish gene would then be passed along more than its fair share—that is, strictly half the time. Imagine, for example, that the X chromosome in an XY male acquires a selfish gene—say, one that encodes a toxin that selectively kills Y-bearing sperm. Most of this male's progeny will be female, as his X will be transmitted more often than his Y.

As this selfish X spreads, though, the sex balance of the population will be thrown off-kilter—a situation that can be harmful to the species as a whole. And the Y chromosome will be getting shortchanged. So to counter the effects of the selfish X, the Y would then evolve genes that can suppress the activity of the toxin—an antidote of some kind—as well as genes that will promote its own transmission. The result: a genetic arms race in which the X and Y try to outdo each other in their ability to gain entrance to the next generation.

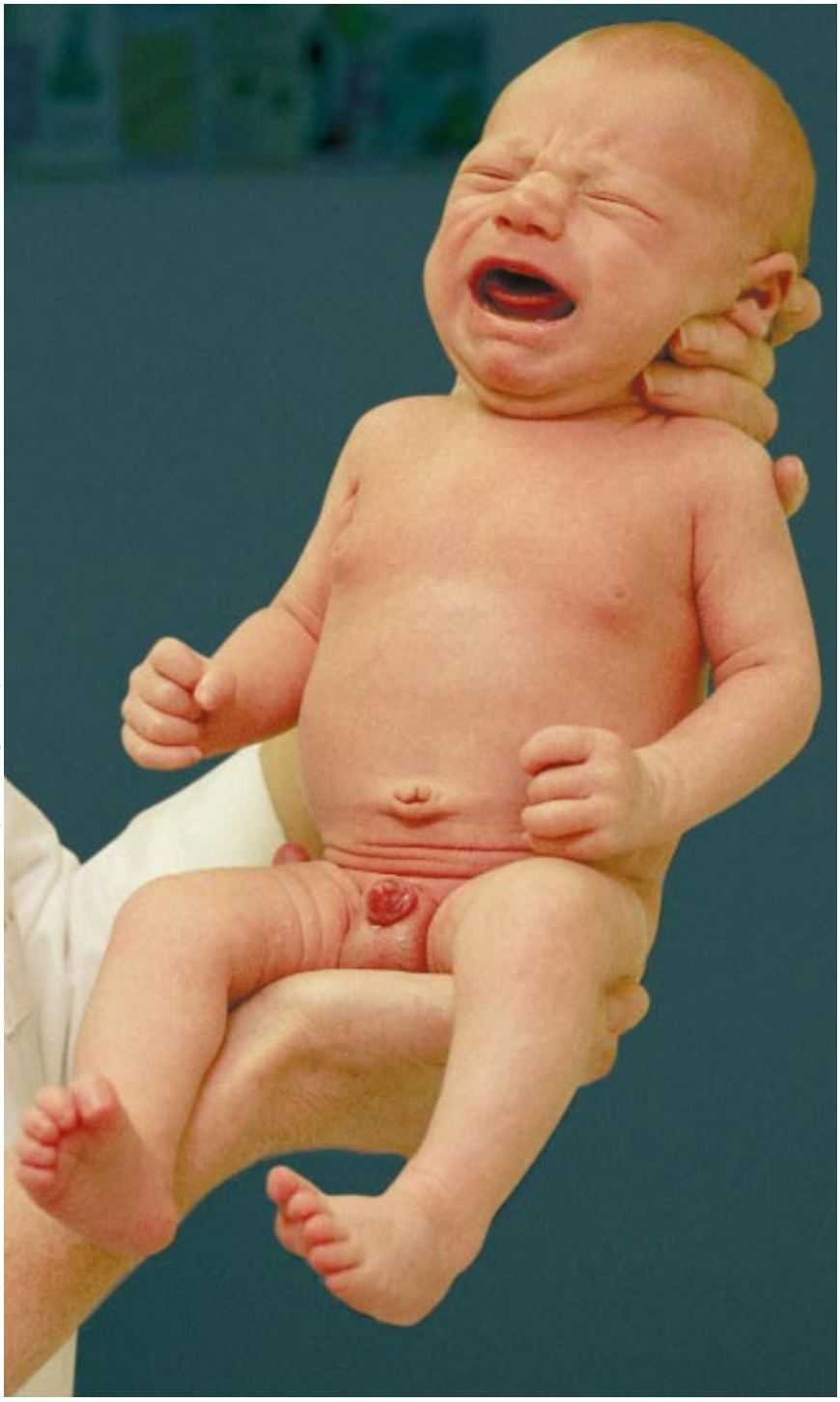
In the long run, the X and Y might still be transmitted at a 1 to 1 ratio, more or less, but the underlying mechanism would be one of genetic hostility rather than cooperation. The idea might not be that far-fetched. Such genetic antagonism is believed to occur in certain species of flies. In a particularly interesting study, researchers led by Gerald Wilkinson of the University of Maryland found that in stalk-eyed flies with a selfish X, the X chromosome was passed along about twice as often as the Y, resulting in more female than male offspring. In humans, slightly more boys are born than girls. Could this bias be the result of a slight imbalance in the war between the X and Y chromosomes? Although the theory is controversial and there is no solid evi-

dence for the existence of selfish genes on human sex chromosomes, the possibility remains intriguing.

Multipurpose Y

For scientists, the Y chromosome is good for more than just making males. It can be used as a marker for tracking human evolution, for example. Because the Y chromosome, unlike all the other human chromosomes, does not undergo recombination with a partner during meiosis, it is passed on nearly unchanged from father to son. Along the way, the Y chromosome may accumulate mutations, but these are also passed down along the paternal line. By grouping different human populations based on the relatedness of their Y chromosomes, evolutionary biologists have been able to trace human lineages. The Y chromosome has thus helped biologists construct an evolutionary tree that shows how humans originated and diverged from a single source. According to this now famous tree, our ancestors arose in Africa between 200,000 and 100,000 years ago and subsequently migrated and dispersed into Europe, Asia and the Americas. Several teams of researchers have also noted how similar the Y chromosome is in men from different ethnic groups. They have argued, based on this consistency, that human populations have dispersed more recently than we might previously have supposed.

The Y has also been used to help unravel human genealogies. It was a study of Y chromosomes that led researchers to conclude that Thomas Jefferson, the third president of the U.S., could have fathered the child of one of his slaves. So despite its small size and its relatively limited complement of genes, the Y chromosome has made—and continues to make—unique and far-reaching contributions to human biology. SA



BIOPHOTO ASSOCIATES/PHOTO RESEARCHERS, INC. (*chromosome*); NEIL HARDING *Tony Stone Images (embryo)*; OWEN FRANKEN *Corbis (infant)*

The Authors

BRUCE T. LAHN and KARIN JEGALIAN did their doctoral work in the laboratory of renowned geneticist David C. Page at the Whitehead Institute for Biomedical Research and the Massachusetts Institute of Technology. Both researchers earned their doctorates in biology in 1998—Lahn for identifying and classifying genes on the human Y chromosome and Jegalian for studying the evolution of X and Y. Now, as a postdoc, Lahn analyzes the Y-based fertility genes to determine their precise function. Jegalian went on to complete the science writing program at the University of California, Santa Cruz, and is now a freelance science writer in Cambridge, Mass.

Further Reading

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Information on the Whitehead Institute for Biomedical Research is available at http://www.wi.mit.edu/home.html on the World Wide Web.