Corals Chemically Cue Mutualistic Fishes to Remove Competing Seaweeds

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slope was negative, linear, and very strong (Fig. 3, $r^2 = 0.87$).

The interpretation of this finding rests upon understanding the causes of fawn mortality. If fawn mortality has a largely environmental cause, then our hypothesis that environmental mortality can affect the Bateman gradient is supported. In our population and across western North America, evidence points to coyotes (*Canis latrans*) as the primary cause. On the NBR and elsewhere, rates of fawn survival are directly related to rates of coyote removal practiced by state and federal agency personnel (*8*). Additionally, fawn survival in Yellowstone National Park is predicted by local wolf density and winter snowpack, two factors that reduce local coyote density (*9*). Finally, in the NBR population, fawn survival increases with maternal age, although the magnitude of maternal expenditure does not (*6*). With age, females appear to gradually improve the complex behavior of the hiding strategy, the mechanism to conceal fawns from predators during the first 3 to 4 weeks of life (*10*). Environmental characteristics that may affect the rate of coyote predation on pronghorn fawns include the density and litter sizes of territorial coyote pairs; the density of floaters; the densities of alternative prey, such as rodents of the genus *Microtus*; and the magnitude of spring precipitation, which can influence rodent densities as well as the quality of pronghorn milk and the concomitant change in fawn growth rates (*11*).

In all years of our study, the result of the fall rut was substantial variance in male mating success. However, mating success translated directly into reproductive success only when the rate of coyote predation was relatively low. When the rate was higher, fawn mortality eliminated most incipient variation in male reproductive success. Long-term studies show that the intensity and the direction of natural selection fluctuate with environmental conditions (*12*) and that the target of sexual selection varies with the nature of female mate choice (*13*). We now show that the maximum possible rate of evolutionary change under sexual selection varies with predator-driven offspring mortality. Bateman was a pioneer in the study of sexual selection (*14*) who established important principles that continue to guide empirical work. However, our study shows that single point estimators of the Bateman principles may be misleading and that ecological forces can modulate the potential for sexual selection. Sexual selection and natural selection are entangled.

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Supplementary Materials

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Corals in the genus *Acropora* generate much of the structural complexity upon which coral reefs depend, but they are susceptible to damage from toxic seaweeds. *Acropora nasuta* minimizes this damage by chemically cuing symbiotic goby fishes (*Gobidon histrio* or *Paragobidon enchinocephalus*) to remove the toxic seaweed *Chlorodesmis fastigiata*. Within minutes of seaweed contact, or contact from only seaweed chemical extract, the coral releases an odor that recruits gobies to trim the seaweed and dramatically reduce coral damage that would otherwise occur. In turn, chemically defended gobies become more toxic after consumption of this noxious alga. Mutualistic gobies and corals appear to represent a marine parallel to terrestrial ant-plants, in that the host provides shelter and food in return for protection from natural enemies.

Coral reefs are in global decline, with seaweeds commonly replacing corals. Coral cover has decreased by ~80% in the Caribbean (*1*) and by ~50% along the Great Barrier Reef (*2*). Drivers of decline are debated, but all major stresses—including overfishing of herbivores, pollution, ocean heating, acidification, and disease (*3*, *4*)—suppress corals, enhance seaweeds, and result in greater seaweed-coral competition.

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For reefs to flourish, rapidly growing, branching corals such as Acroporids are critical because they create much of the topographic complexity upon which other species depend (*4*, *5*). Other species, such as herbivorous fishes, then enhance reef resilience by grazing on competing algae and facilitating the colonization and growth of corals after disturbances (*3*, *4*, *6*). In the Caribbean, when two dominant *Acropora* species declined, structural complexity was lost across the entire region with likely effects on fishes, fisheries, biodiversity, coastal protection from wave damage, and ecosystem function in general (*7*, *8*).

Reef-scale herbivory facilitates coral growth and maintenance by removing competitively superior seaweeds (*3*, *4*, *9*, *10*), as exemplified by herbivore-rich reefs and marine protected areas that are higher in coral and lower in macrophyte cover, whereas overfished reefs with fewer herbivores have fewer corals and more macroalgae (*3*, *9*, *10*). However, individual corals are damaged only by adjacent seaweeds. Thus, critical aspects of competition occur at coral edges, a spatial scale over which corals might exert influence. Recent studies of seaweed-coral competition emphasize effects of seaweed allelopathy (*11*, *12*) (chemical suppression of competitors), seaweeds vectoring coral diseases (*13*, *14*), and near-contact creating anoxic zones or enhancing detrimental microbes on corals (*14*, *15*). These mechanisms all require close contact for seaweeds to damage corals. Thus, millimeter- to centimeter-scale differences in proximity may cause large differences in coral health (*11*, *12*, *15*). Just as mutualist ants on *Acacia* trees protect their host by removing nearby competitors (*16*), we reasoned that the goby or pomacentrid fishes that shelter in many Acroporid corals (*17*) might play a similar function and remove seaweed competitors from coral edges.

We therefore focused on the common coral *Acropora nasuta* and asked the following: (i) whether commensal fishes sheltering in *Acropora* suppressed an allelopathic seaweed competitor, (ii) whether different commensal fish species varied in the protection they provided the coral, (iii) whether the interaction was affected by a specialist crab that lives only in the allelopathic sea-

weed (*18*), (iv) if the coral chemically cued the fishes to remove toxic seaweed at sites of contact, and (v) whether mutualistic fish that consume the toxic seaweed become more toxic to generalist predatory fishes.

We assessed how coral-dwelling fishes affected seaweed-coral interactions in the field (*19*) by placing the allelopathic seaweed (*12*) *Chlorodesmis fastigiata* versus a control for shading and abrasion (an algal mimic made of nylon line) in contact with *A. nasuta* colonies occupied by four different commensal fishes ($n = 20$ corals per fish species). We then evaluated coral health at the coral-algal or coral-control area of contact using pulse-amplitude modulated (PAM) fluorometry to assess coral photophysiology as a proxy for coral health (*12*, *20*). In corals occupied by the gobies *Gobiodon histrio* or *Paragobiodon echinocephalus*, *C. fastigiata* abundance declined by 30% over 3 days and the damaging effect of *C. fastigiata* on *A. nasuta* declined by 70 to 80% compared with *A. nasuta* colonies lacking gobies (Table 1 and Fig. 1). In contrast, the control had minimal effect (Fig. 1). The alga's specialist crab (*18*), *Cyphyra rotundifrons*, had no effect on these interactions. *C. fastigiata* was found in the gut of 17 of 20 *G. histrio* and 0 of 20 *P. echinocephalus* from corals that were contacting *C. fastigiata* ($\chi^2 = 29.57$, df = 1, $P < 0.001$); thus, *G. histrio* consumed *C. fastigiata*, whereas *P. echinocephalus* removed the seaweed but did not consume it. Guts of *G. histrio* or *P. echinocephalus* occupying corals not contacting *C. fastigiata* ($n = 20$ each) were devoid of *C. fastigiata*. Given that the allelopathic compounds from *C. fastigiata* are hydrophobic and the alga must contact the coral for these to be transferred (*11*, *12*), gobiid removal of *C. fastigiata* filaments contacting the coral should lessen or prevent coral damage, which is what we found in our field experiments (Fig. 1). For this interaction to be broadly important, goby occupancy of *A. nasuta* would need to be frequent. We assessed this by running eight haphazardly placed 30- × 2-m transects across the reef and evaluating goby occupancy of all *A. nasuta* located in these transects. Gobies occurred in 81 ± 16% (mean ± 1 SD) of the 207 colonies assessed. An assessment in Australia also indicated common co-occurrence, with 1593 *Gobiodon* individuals occurring in the 1373 colonies of 11 Acropora species evaluated (*17*).

Table 1. Effects of coral-associated gobies and an alga-associated crab on abundance (volumetric displacement) of the alga *C. fastigiata* after 3 days of exposure (± SEM) ($N = 20$).

	Algal abundance (ml) before and after exposure to			
	G. histrio		*P. echinocephalus*	
Treatment	Initial	Post	Initial	Post
Empty	1.49 ± 0.03	1.48 ± 0.03	1.48 ± 0.07	1.48 ± 0.04
Crabs only	1.41 ± 0.04	1.39 ± 0.04	1.42 ± 0.03	1.39 ± 0.03
Gobies only	1.50 ± 0.04	0.99 ± 0.05*	1.45 ± 0.03	1.04 ± 0.02*
Crabs and gobies	1.40 ± 0.03	0.97 ± 0.06*	1.41 ± 0.04	0.99 ± 0.03*

*Significant ($P < 0.05$) loss of the alga.

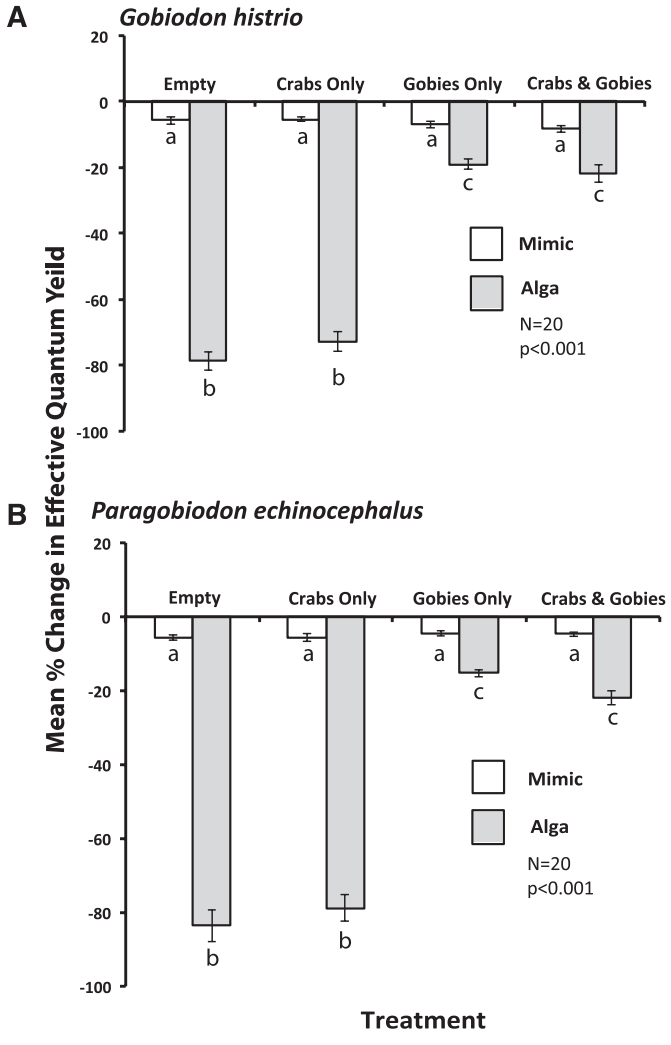

Fig. 1. Effects of gobies (**A**) *G. histrio* and (**B**) *P. echinocephalus* on algal-coral interactions. Mean (± 1 SE) percent difference in effective quantum yield of the coral holobiont when exposed to the seaweed *C. fastigiata* or an inert mimic compared to the control location on the coral that was exposed to no treatment. *P* value is from a split-plot analysis of variance (ANOVA) (arcsine-transformed data). Letters designate significant groupings.

Because G. histrio produces a toxic skin secretion, whereas *P. echinocephalus* does not (*21*), we tested the effect (*22*) of *G. histro* mucus on two model predators that consume a variety of invertebrates and small fishes to see if potency of *G. histro* secretions increased after consumption of *C. fastigiata*. We placed mucus from one disturbed *G. histrio* into 300 ml of seawater with the cardinal fishes *Ostorhinchus nigrofasciatus* ($n = 20$) and *Nectamia similis* ($n = 10$). The mucus of both control and *C. fastigiata*–exposed *G. histrio* produced significant effects; however, secretions of *G. histrio* from coral heads contacting *C. fastigiata* caused predators to lose equilibrium (falling forward or sideways) more than twice as fast (66 ± 4 and 65 ± 8 s, respectively) as mucus of *G. histrio* from corals without *C. fastigiata* (143 ± 9 and 162 ± 11 s, respectively; $P < 0.001$ for each species, *t* test). The toxins producing the effects are unknown, but thin-layer chromatograms of extracts from *C. fastigiata* and from *G. histrio* mucus did not show secondary metabolites from *C. fastigiata* in *G. histrio* mucus.

In contrast to the advantage that both gobies provide coral by removing *C. fastigiata*, the coral-sheltering damselfishes *Dascyllus aruanus* or *Chromis viridis* provided no advantage (fig. S1). All *D. aruanus* and *C. viridis* abandoned *C. fastigiata*–treated *A. nasuta* within 24 to 48 hours; none abandoned colonies treated with the mimic alone ($P < 0.001$ for each species, $n = 20$, Fisher's exact test). Initial presence or absence of fish had no effect on photosynthesis of the coral holobiont when in contact with *C. fastigiata* (*D. aruanus* $F_{1,38} = 2.221$, $P = 0.144$; *C. viridis* $F_{1,38} = 2.234$, $P = 0.147$). By day 3 of the experiment, contact with *C. fastigiata* had damaged corals and suppressed effective quantum yield by ~80%, whether or not damselfishes had been

present. In contrast, algal mimics suppressed photosynthesis by only ~5% ($F_{1,38}$ = 2280.66, P < 0.001 for both fish species), which indicated the primacy of chemical, as opposed to physical, effects.

To determine whether fish were responding to chemical cues from the seaweed or the coral, we used 60-ml syringes to pull in situ seawater from: among the filaments of *C. fastigiata* alone, the *C. fastigiata–A. nasuta* contact area with *C. fastigiata* still present, the *C. fastigiata–A. nasuta* contact area after removing *C. fastigiata* 20 min earlier (allowing loss of algal odor but retention of odor from the damaged coral), and the water column well away from the benthos (as a control) and then slowly released these odors into corals containing *G. histrio*. Olfactory cues from *C. fastigiata* alone generated no response by the goby. In contrast, odors from the coral-algal contact point or from the stressed coral alone caused 17 and 19, respectively, of the goby pairs in 20 separate *A. nasuta* colonies to move toward the odor source. Thus, the goby responds to chemical cues from the host coral, not to cues from the seaweed (Fig. 2, Υ = 559.12, df = 2, P < 0.001; G test,).

The same experiment conducted with odors from *Acropora millepora* produced no responses from *G. histrio* living in *A. nasuta* (Fig. 2). Thus, gobies responded to cues from their host species, but not to odors from a closely related coral, even one that *G. histrio* sometimes occupies.

Because *G. histrio* effectively defended its host and was the most common goby in *A. nasuta*, we conducted assays evaluating how rapidly *A. nasuta* cued its goby symbionts and whether the coral would signal in response to the seaweed's chemistry alone. *C. fastigiata* damages *Acropora* species via hydrophobic compounds including acetylated diterpenes (*12*). We obtained the hydrophobic crude extract from *C. fastigiata* via extraction in methanol followed by partitioning between water and ethyl acetate, removed the solvent in vacuo, redissolved the ethyl acetate partition in ether, coated this lipid-soluble extract onto algal mimics at natural volumetric concentration (*12*), and placed extract-treated mimics against *A. nasuta*–harboring *G. histrio*. Control mimics treated with the same solvent but without the algal extract also were placed against the coral. Gobies rapidly moved to the site of contact between the extract-treated mimic and the coral (Fig. 3). Fifteen minutes after contact, 70% of the 20 goby pairs were beneath the treated mimics, this increased to 95% by 30 min; movement to the control ranged from 0 to 10% (P = 0.001 at 30 min; Kolmogorov-Smirnov test). Patterns in Fig. 3 indicate that the coral signaled in response to *C. fastigiata* compounds alone and that the signal was produced within 5 to 15 min of coral exposure.

Thus, the gobies serve as bodyguards for host corals, and the coral chemically cues gobies to attract them to the site of coral-algal contact where they begin removing the alga within minutes of seaweed contact (or contact by the seaweed's hydrophobic extract alone). Gobies are not attracted to cues from *C. fastigiata* alone nor to cues from related corals in contact with *C. fastigiata*; they respond only to odors from their host species. Symbiotic gobies that spend their adult life in a single coral played this protective role; damselfishes did not. Just as terrestrial plants release volatile signals that attract predators of herbivores (*23*), *A. nasuta* releases chemicals that cue symbiotic gobies to remove a competing, allelopathic seaweed. We could find no previous example of a species chemically cuing consumers to remove its competitors.

As corals have declined and seaweed cover has increased on reefs over recent decades, understanding seaweed-coral competition has become more important (*3*, *24*). Because Acroporid corals are major builders of topographic complexity on coral reefs, they play critical roles as foundation species (*5*, *7*), creating critical habitat that is associated with the diversification of numerous lineages of reef fishes (*25*). Coral-dwelling gobies facilitate persistence of these corals despite increased competition from seaweeds. These small, inconspicuous fishes may have effects considerably larger than their mass would predict.

The coral-goby relation appears similar to terrestrial ant-plant symbioses as exemplified by ants and *Acacia* trees (*16*). Ants receive food and shelter from their host *Acacia* and protect the host from competitors and consumers. Symbiotic gobies have a similar relationship with Acropid corals. Several gobies consume coral tissue (5 of the 19 species of *Gobiodon* are corallivores), but no species feeds exclusively on coral (*26*, *27*). Most Acroporid coral colonies host at least one pair of gobiids (*17*), these fish remain in the same coral colony for most of their adult life, and death of host corals is commonly correlated with goby population decline (*17*). Gobies consume coral

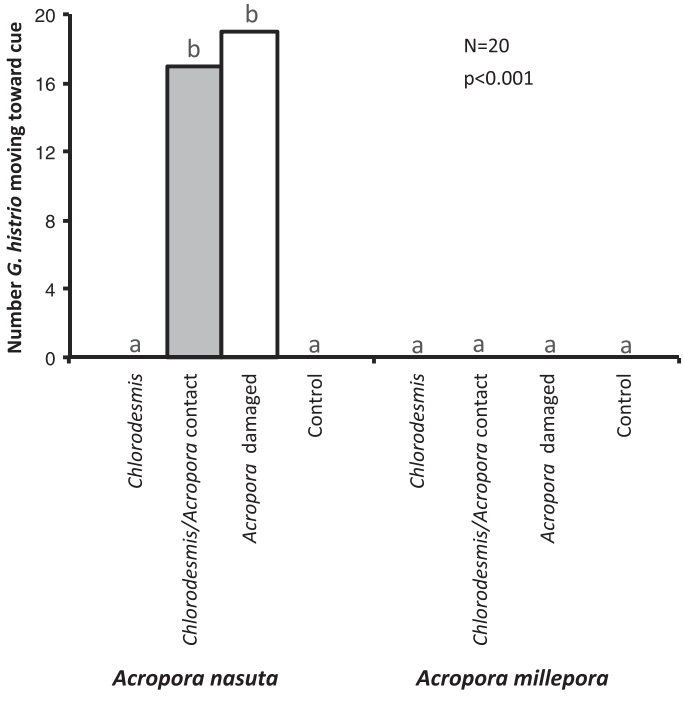

Fig. 2. Response of the goby *G. histrio* to chemical cues from: the alga *C. fastigiata* alone, *C. fastigiata* in contact with the coral *A. nasuta*, or the damaged coral that had been in contact with *C. fastigiata* but with the *C. fastigiata* removed 20 min before the odor was collected. Right side of graph is the same experiment with the same types of cues from *Acropora millepora* introduced to gobies living in *A. nasuta*. *P* value from a *G* test. Letters indicate significant groupings.

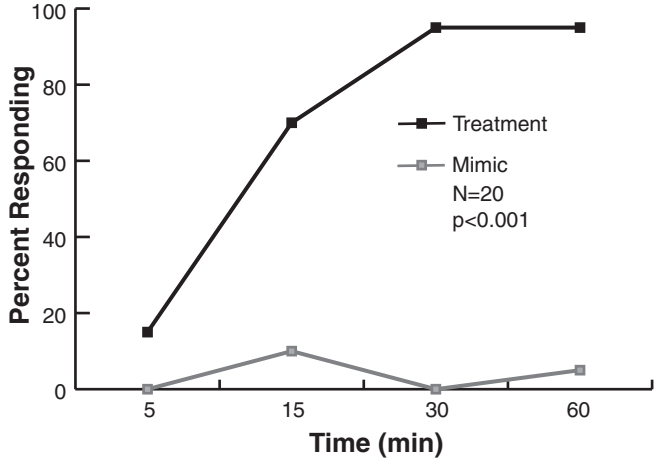

Fig. 3. Response of *G. histrio* to *A. nasuta* in contact with algal mimics treated with natural concentrations of the lipid-soluble extract from *C. fastigiata* (treatment) versus control algal mimics treated with solvent only and placed against the coral host. *P* value from a Kolmogorov-Smirnov test of the 30-min data.

tissue [they also consume copepods (*26*) and algae growing against the coral base (*27*)]. Thus, like ants on *Acacia*, they receive shelter and food from their host, which they protect from a damaging competitor.

Both gobies we investigated protected their host by removing *C. fastigiata*; however, only *G. histrio* consumed the alga, which contains metabolites that deter feeding by numerous reef herbivores (*18*, *28*). These findings may explain why *P. echinocephalus* removes, but does not consume, algal tissue in contact with its host coral. Consumption of this chemically noxious alga may benefit *G. histrio* by making its skin secretions more noxious to predators. However, metabolites from *C. fastigiata* are unlikely to be a primary source of skin toxins because *G. histrio* not exposed to *C. fastigiata* were also toxic, just less so.

As reefs continue to convert from coral to macroalgal dominance, there is increasing need to understand interactions that enhance coral resilience or suppress seaweed impacts on corals. Symbiotic gobies play a key role in defending Acroporid corals from an allelopathic alga, with chemical signals and cues mediating responses of both the coral (Fig. 3) and fish (Figs. 2 and 3). A worrisome recent discovery is that chemically mediated behaviors, such as these, that often are critical to reef function can be disrupted or even reversed (i.e., attraction to predator odors) by changes in ocean pH (*29*, *30*). With ocean acidification (*24*), critical aspects of chemical communication in the sea may be destabilized, with the attendant loss of key processes underlying reef resilience.

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Supplementary Materials

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Fig. S1
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A Core Metabolic Enzyme Mediates Resistance to Phosphine Gas

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Phosphine is a small redox-active gas that is used to protect global grain reserves, which are threatened by the emergence of phosphine resistance in pest insects. We find that polymorphisms responsible for genetic resistance cluster around the redox-active catalytic disulfide or the dimerization interface of dihydrolipoamide dehydrogenase (DLD) in insects (*Rhyzopertha dominica* and *Tribolium castaneum*) and nematodes (*Caenorhabditis elegans*). DLD is a core metabolic enzyme representing a new class of resistance factor for a redox-active metabolic toxin. It participates in four key steps of core metabolism, and metabolite profiles indicate that phosphine exposure in mutant and wild-type animals affects these steps differently. Mutation of DLD in *C. elegans* increases arsenite sensitivity. This specific vulnerability may be exploited to control phosphine-resistant insects and safeguard food security.

Extensive use of phosphine has selected for pest insects that are highly resistant (*1*–*3*), but a suitable replacement fumigant does not exist. The nematode *Caenorhabditis elegans* is also vulnerable to phosphine. We previously isolated *phosphine-resistant* (*pre*) *C. elegans* strains by ethylmethane sulfonate mutagenesis (*4*). Four independent mutants were found to survive a phosphine dose that killed 100% of wild-type N2 nematodes (Fig. 1A) and to have resistance factors >4 based on median lethal concentration (LC_{50}) values at 20°C (Fig. 1B and fig. S1) (*5*–*10*). Complementation analysis revealed that the four alleles define two complementation groups (fig. S2): *pre-7* (alleles *wr1, wr2*, and *wr3*) and *pre-33* (allele *wr4*). We localized the *C. elegans pre-7* locus to a 96-kb region on chromosome II (*10*, *11*) (fig. S3A). Genomic DNA rescue experiments revealed that a wild-type, but not a *wr3* mutant copy of one of the genes in the interval, *alh-6*, restored phosphine sensitivity to *wr3* mutants (Fig. 2A and fig. S4A). *C. elegans* subjected to RNA interference (RNAi) of *alh-6* acquired phosphine resistance (Fig. 2A and fig. S5A). Sequence analysis revealed a unique point mutation in the coding sequence of the *alh-6* gene in each of the three *pre-7* alleles (Fig. 2A and table S1).

For the *pre-33(wr4)* mutant, crossing with the strain CB4856 and mapping of phosphine-resistant F_5 *C. elegans* (*10*) confined the resistance locus to a 2-Mb interval on chromosome IV (fig. S3B), and then a 475-kb region, revealing it to be a C to T transition in the *dld-1* gene (table

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