match the method used in (*9*) so that the starting point of each clade was randomly chosen, subject to the constraint that there be no time intervals of zero diversity. To maintain consistency with the empirical analysis of (*3*), a single simulation run generated 90 random clades each allowed to "evolve" over 63 time steps, corresponding to 63 stratigraphic intervals. To maintain consistency with (*3*) and (*9*), each set of 90 random clades was analyzed by Q-mode factor analysis [see (*3*) for further explanation; K. G. Joreskog, J. E. Klovan, R. A. Reyment, *Geological Factor Analysis* (Elsevier, Amsterdam, 1976)] in which a cosine-theta similarity index was computed and the varimax technique of axis rotation was used.

11. Sepkoski (*3*) labeled factor 1 evolutionary fauna III and factor 3 evolutionary fauna I in order to put the evolutionary faunas in temporal sequence from earliest dominant fauna to most recently dominant fauna.
12. J. A. Kitchell and T. R. Carr, in *Phanerozoic Diversity Patterns*, J. W. Valentine, Ed. (Princeton Univ. Press, Princeton, NJ, 1985), pp. 277–309.
13. S. J. Gould, D. M. Raup, J. J. Sepkoski, Jr., T. J. M. Schopf, D. S. Simberloff, *Paleobiology* **3**, 23 (1977).
14. Because the *CG* statistic requires knowledge of the full diversity history of the clade, more than 1000 clades were actually produced; clades not extinct by the end of the simulations were excluded from the data set for all cases of Table 1, a restriction consistent with the procedure of Gould *et al.* (*4*).
15. A proportion of random clades are single lineage clades: only one 'lineage' is present throughout the clade's history. Since such clades are symmetrical by definition, their inclusion biases the results in favor of clade symmetry. Of multi-lineage clades, only 4.7% had $CG = 0.5$.

16. We thank D. C. Fisher for helpful discussions.

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Predator-Induced Trophic Shift of a Free-Living Ciliate: Parasitism of Mosquito Larvae by Their Prey

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Larvae of the treehole mosquito, *Aedes sierrensis*, release a waterborne factor that induces morphogenesis of one of their prey, the tetrahymenid ciliate *Lambornella clarki*. Induced free-living trophonts of *L. clarki* undergo a synchronous response in which cells divide and transform into parasitic cells (theronts) that encyst on larval predators. Parasitic ciliates penetrate the cuticle, enter the hemocoel, and ultimately kill their predator-host. In nature, this trophic shift can lead to predator extinction and dramatic changes in microbial populations. Facultative parasitism by this polymorphic ciliate may have evolved as an antipredator strategy. The experimentally inducible parasitic response of *L. clarki* provides a novel model for studying cellular morphogenesis of ciliated protozoa.

PREDATION IS A POTENT ECOLOGICAL force that has affected physiological, morphological, and behavioral traits of prey species through evolutionary time. The widespread occurrence of toxins, crypsis, mimicry, and nocturnalism may be attributed, to a considerable extent, to the evolutionary power of predation for shaping natural histories of prey species. Some animals have inducible antipredator defenses that are manifested only in the presence of predators, an ecologically prudent strategy that conserves an individual's resources until the threat of predation is imminent. Most induced defenses lower the value of prey by increasing a predator's handling time or by making the prey unpalatable. Such induced antipredator transformations are known for a variety of aquatic organisms including protozoa (*1*), rotifers (*2*), and cladocerans (*3*). In these interactions, prey respond to waterborne substances released from natural predators by developing spines, crests, capsules, or other protuberances that increase prey size and preclude or restrict predator consumption.

While searching for new biological control agents of larval mosquitoes, we discovered a more extreme induction defense in a protozoan living in water-filled treeholes of western North America: larvae of *Aedes sierrensis* (Diptera: Culicidae) induce transformation of one of their protozoan prey, *Lambornella clarki* (Ciliophora: Tetrahymenidae), into obligate parasites that attack the insect predator. Larval mosquitoes release a water-soluble factor that induces cell division and rapid morphological transformation of the free-living, pyriform morphs (trophonts) of *L. clarki*. Daughter cells of induced trophonts transform into spherical, astomatous cells (theronts) that form invasive cysts on cuticles of larval predators. These encysted morphs form small holes in the cuticle and enter the hemocoel where they multiply and ultimately kill their predator-host. Moribund and deceased hosts release numerous trophonts, some of which differentiate into theronts that attack surviving predators (Fig. 1). Using the ecological strategy of shifting trophic levels, trophonts avoid predation and parasitize their would-be predators. This shift allows for changes in the microbial community by reducing or eliminating populations of the dominant predator.

Aedes sierrensis and *L. clarki* are widespread inhabitants of treeholes on the west coast of North America (*4, 5*). Mosquitoes and ciliates survive during the summer dry season in desiccation-resistant eggs and cysts, respectively. Larval mosquitoes hatch within a few hours after treeholes fill with water during the fall rainy season; shortly thereafter, *L. clarki* and other protozoans appear in the water column (*5*). Larvae persist in diapause throughout the winter and feed by browsing substrates and filtering microorganisms from the water.

With the exception of newly hatched first instars (<48 hours old) which are too small to ingest trophonts, all larval instars of *A. sierrensis* feed on *L. clarki*, and filter feeding rates progressively increase with later instars. In nature, larval populations are often limited by food (*6*), and their feeding activities eliminate free-swimming *L. clarki* and other protozoans from treehole water (Fig. 2). When *A. sierrensis* and *L. clarki* co-occur in treeholes, trophonts are rare or absent, and ciliate populations primarily consist of endoparasitic morphs within the hemocoels of larvae. However, in the absence of mosquito predators *L. clarki* may reach densities

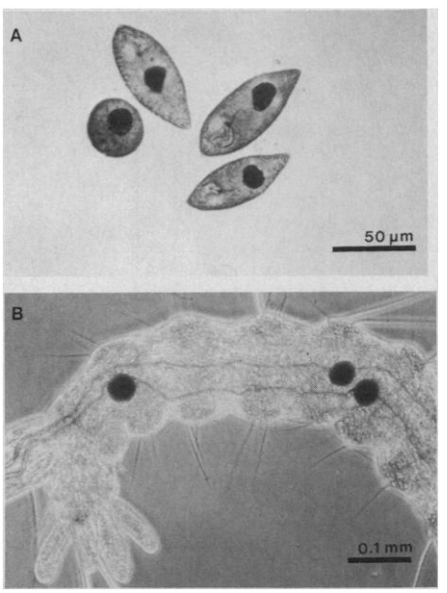

Fig. 1. Polymorphism in the ciliate *Lambornella clarki*. (**A**) Three free-living trophonts and one parasitic theront of *L. clarki*. Specimens stained by protargal. (**B**) Invasive cysts formed by theronts on the cuticle of a first instar *A. sierrensis*. Cysts are stained with amide black dye.

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in excess of 100 cells per milliliter as trophonts feeding on bacteria and other microorganisms. Such free-living populations persist indefinitely so long as treeholes hold water (*5, 7*).

We examined this predator-prey–host-parasite relationship in the laboratory using trophonts of *L. clarki* cultured in vitro in a cerophyl infusion (*8*). When we added larvae to trophont populations in artificial treeholes, invasive cuticular cysts first appeared on larvae approximately 40 hours after introduction (Fig. 3) (*9*). Most encystment occurred between 48 and 72 hours; by 90 hours trophonts were extinct, and parasitic ciliates had penetrated the cuticles of larvae. We demonstrated in another experiment the correlation between morphogenesis of free-living ciliates and predator introduction by staggered additions of *A. sierrensis* larvae. Larvae were added at 24-hour intervals to three ciliate populations from the same in vitro culture; both the rapid decline in trophont density and encystment of theronts were temporally correlated with larval additions (Fig. 4). Despite heavy predation pressure, 6 to 13% of the surviving ciliates transformed successfully and encysted on larval predators sampled at 48 and 72 hours after the first addition of larvae. No cysts were present in samples collected 24 hours after the first introduction of larvae.

To examine whether waterborne morphogenic agents mediate induction and transformation of *L. clarki*, we introduced trophonts to water that previously contained larval mosquitoes. Mosquito-conditioned water was produced by placing larvae with different feeding histories in a dilute solution of autoclaved, natural treehole water and deionized water (*10*); after 24 hours we filtered the water through a 10-μm Nitex mesh. Control water was similar, except that it was not conditioned by larval presence. We introduced aliquots of trophont cultures to control and treatment water and maintained these in environmental growth chambers at 11°C. After 48 hours, we added ten first instar larvae (<24 hours old) to each replicate; all replicates were returned to growth chambers for an additional 24 hours. Use of young first instars is a suitable test for transformed cells because they provide an encystment substrate for theronts, but do not feed on ciliates. By examining first instars after 24 hours of exposure, we eliminated induction of trophonts caused by addition of the first instars themselves since encystment of theronts occurs 24 to 72 hours after exposure to mosquitoes (Figs. 3 and 4). Results from three experiments (Table 1) show the presence of a morphogenic agent produced by larvae and contained in the mosquito-conditioned water. Encystment rates of ciliate populations exposed to water previously containing larval mosquitoes were 10 to 100 times greater than those in unconditioned controls. Encystment rates were not significantly affected by the feeding histories of larvae used during the conditioning period (Table 1). Thus, prey morphogenesis is triggered by the presence of predators alone and does not require actual predation of trophonts.

We examined cellular morphogenesis of trophonts in polystyrene Cell Wells (Corning) containing 0.10 ml of treatment or control water similar to that described above. Individual trophonts were isolated in each well and examined (×10 to ×40) at 4-

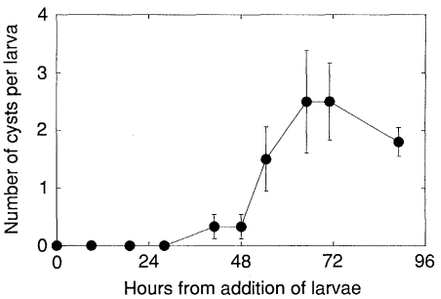

Fig. 3. Encystment of *L. clarki* on a population of second and third instar *A. sierrensis*. One hundred larvae were added to a 520-ml artificial treehole with a trophont density of 20 ciliates per milliliter at $t = 0$. Points are numbers (mean ± 1 SEM) of cuticular cysts on six larvae. Larvae were replaced to maintain a predator population of 100 until the first appearance of cysts (40 hours); after cysts appeared larvae were not replaced.

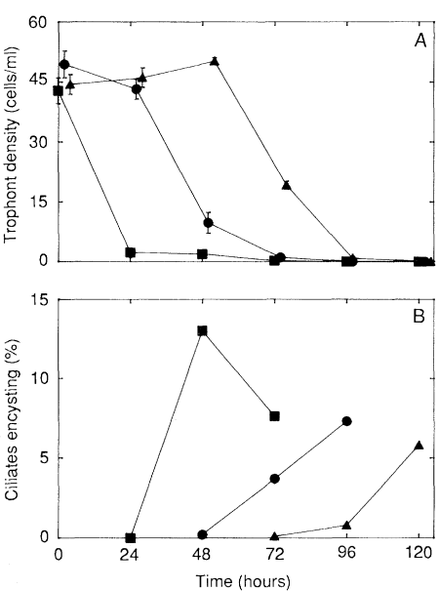

Fig. 4. (**A**) Predation of *L. clarki* trophonts by fourth instar larvae of *A. sierrensis* introduced at different times [$t = 0$ (squares), $t = 24$ hours (circles), and $t = 48$ hours (triangles)]. Six larvae were added to replicated 150-ml microcosms for each treatment. Points are means (± 1 SEM) of six replicates. (**B**) Encystment rates of transformed ciliates on larval predators. Percentages of encysting theronts were calculated by dividing the total number of cysts on all larvae in each treatment by the total number of trophonts estimated surviving in each treatment at the end of the previous 24-hour interval.

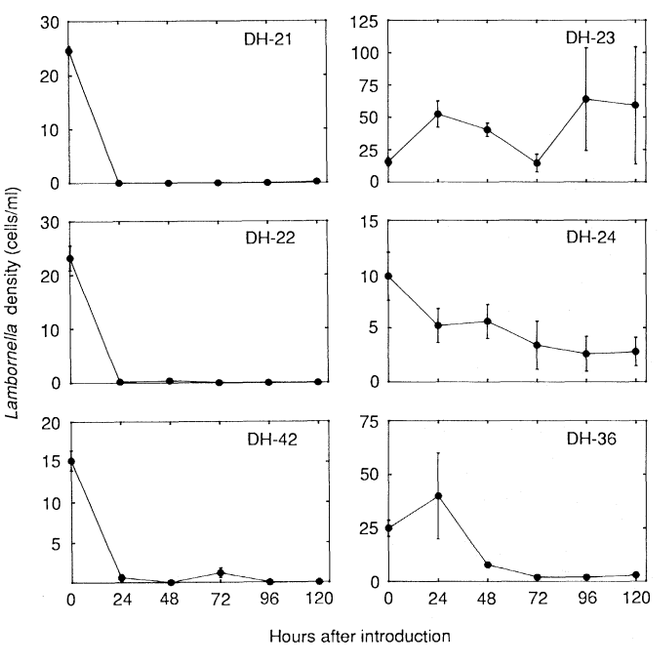

Fig. 2. Density changes in populations of *L. clarki* trophonts introduced into natural treeholes with (**left**) and without (**right**) resident larval populations of *A. sierrensis*. Prior to ciliate introductions, all treeholes were drained and the larvae removed by filtering. All holes were then flooded with deionized water and drained three times to remove remaining larvae. Each treehole was then refilled with its original water, and trophonts were added in a dilute cerophyl solution at $t = 0$. Resident larval populations were returned only to the three treeholes on the left. Ciliate populations were sampled by removing five 2-ml samples at 24-hour intervals. Samples were fixed with amide black, and *L. clarki* densities were estimated by removing 1-ml aliquots from each sample and counting cells with a Sedgewick-Rafter counting chamber. Each point is the mean of five samples (± 1 SEM). Ciliate densities in treeholes without mosquitoes (right) declined between 48 and 72 hours following death of theronts induced by natural treehole water that had previously contained mosquitoes.

hour intervals for 60 hours. Ciliates were categorized as trophonts, dividing cells, transitory cells, or theronts on the basis of morphology and swimming characteristics. Among the control population of 59 ciliates, 12 trophonts (20%) divided and 17 theronts were produced during the first 60 hours of observation (Fig. 5A). In contrast, in water previously exposed to predators, 37 of 58 trophonts (64%) divided and 64 theronts were generated (Fig. 5B). Cytokinesis of trophonts was evident as early as 8 hours after introduction into water conditioned by larvae. Theronts were produced from daughter cells of trophonts (89%) and from differentiation of trophonts directly (11%). All daughter cells of treatment trophonts differentiated into theronts and transitory cells whereas some divisions (25%) by control ciliates produced two trophonts. In the absence of larval hosts, theronts and transitory cells died within 24 hours of transformation indicating that such cells are irreversibly committed to parasitism.

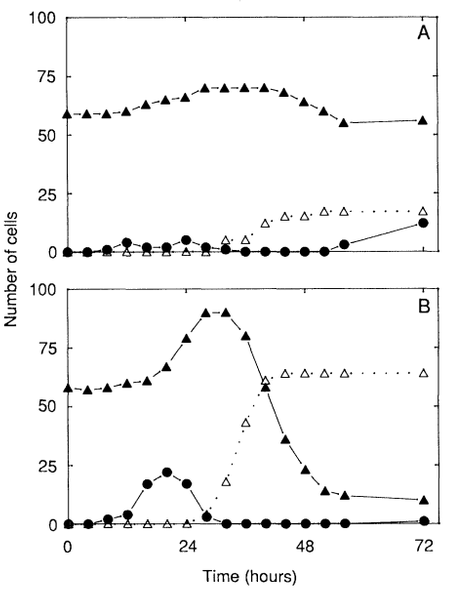

Fig. 5. Fate of individually isolated *L. clarki* trophonts exposed to (**A**) control water and (**B**) water previously containing *A. sierrensis* larvae. Symbols are as follows: total number of ciliates (closed triangles), dividing trophonts (closed circles), and the cumulative number of theronts produced (open triangles).

The predator-mediated induction of *L. clarki* trophonts exceeds simple morphological transformation; its ecological strategy of facultative parasitism incorporates an obligatory trophic shift by a free-living organism to a parasitic existence. This trophic shift may be an evolutionary adaptation to the intense predation pressure imposed by larval mosquito populations whose densities may exceed 1000 individuals per liter in the restricted confines of treeholes. *Aedes sierrensis* is usually the only culicid predator that occurs over the range of *L. clarki*, and sizes of larval populations vary within and among seasons. The rapid induction response of trophonts provides a flexible and effective antipredator defense that is responsive to resident predator populations. Successful attack on larval predators is further enhanced by trophont division which increases the number of theronts produced.

In nature, it is not uncommon for *L. clarki* to eliminate mosquitoes from treeholes. When this occurs, predation pressure on free-swimming protozoans including *L. clarki* is relaxed and microbial populations increase (*5, 7*). Thus, this predator-prey–host-parasite relationship may be important in determining the structure of treehole microbial communities as well as the number of emerging adult mosquitoes, an important concept for vector-transmitted pathogens. Finally, *L. clarki* provides an excellent model for the study of morphogenesis and cell cycle phenomena of tetrahymenid ciliates. Because division is incorporated into the induction response, the identification of the morphogen and its action could be useful in determining events that relate to cell cycle regulation or control.

Table 1. Formation of invasive cysts on first instar *A. sierrensis* larvae by theronts of *L. clarki* produced by exposure of trophonts to water previously containing mosquito larvae. Values are means (± 1 SEM) of six replicates of ten larvae. Conditioned water was produced using larvae that were: a, held without food for 72 hours and then fed *L. clarki* trophonts during the 24-hour conditioning period; b, held without food during the conditioning period; c, provided with food (ground, autoclaved rat laboratory food) for 72 hours and then fed trophonts during the 24-hour conditioning period; and d, provided with food for 72 hours and then held without food during the conditioning period. Data were analyzed by the Kruskal-Wallis test; treatment means within experiments that are followed by different letters are significantly different using multiple comparisons based on Bonferoni's inequality.

Experiment	Conditioning history	Cuticular cysts per larva		Significance	
		Control	Treatment	χ^2	P
1	a	0.03 ± 0.05^{f}	1.68 ± 1.54^{g}	8.31	0.003
2	a	0.75 ± 1.08^{h}	10.80 ± 5.59^{i}	12.32	0.002
	b		8.73 ± 3.05^{i}		
3	a	0.11 ± 0.10^{j}	6.02 ± 2.47^{jk}	15.31	0.004
	b		7.17 ± 2.07^{k}		
	c		7.20 ± 3.52^{k}		
	d		10.80 ± 7.64^{k}		

REFERENCES AND NOTES

1. H.-W. Kuhlmann and K. Heckmann, *Science* **227**, 1347 (1985).
2. J. J. Gilbert, *ibid.* **151**, 1234 (1966); in *The Evolution and Ecology of Zooplankton Communities*, W. C. Kerfoot, Ed. (University Press of New England, Hanover, NH, 1980), pp. 509–517; R. S. Stemberger and J. J. Gilbert, *Freshwater Biol.* **14**, 639 (1984).
3. W. J. O'Brien, D. Kettle, H. Reissen, *Ecology* **60**, 287 (1979); J. W. G. Grant and I. A. E. Bayly, *Limnol. Ocean.* **26**, 201 (1981); J. A. E. Stenson, *Ecology* **68**, 928 (1987).
4. J. O. Corliss and D. W. Coats, *Trans. Am. Micros. Soc.* **95**, 725 (1976); T. B. Clark and D. G. Brandl, *J. Invert. Pathol.* **23**, 341 (1976); D. E. Egerter and J. R. Anderson, *ibid.* **46**, 296 (1985).
5. J. O. Washburn and J. R. Anderson, *J. Invert. Pathol.* **48**, 296 (1986).
6. W. A. Hawley, *J. Anim. Ecol.* **54**, 955 (1985); in *Ecology of Mosquitoes: Proceedings of a Workshop*, L. P. Lounibos, J. R. Rey, J. H. Frank, Ed. (Florida Medical Entomology Laboratory, Vero Beach, 1985), pp. 167–184; J. O. Washburn, D. E. Egerter, G. A. Saunders, J. R. Anderson, *J. Med. Entomol.*, in press; J. O. Washburn, J. R. Anderson, D. R. Mercer, unpublished data.
7. Based on observations of ciliate and mosquito populations in approximately 180 natural treeholes in California over 4 years of study.
8. In vitro cultures were initiated using *L. clarki* released from infected *A. sierrensis* larvae. Ciliates were septically transferred to 16 mm by 150 mm test tubes containing 5 ml of autoclaved cerophyl medium. Media consisted of cerophyl extract and vitamins (stored at 5°C without Biotin) prepared with migration salt solution [A. T. Soldo and W. J. van Wagtendonk, *J. Protozool.* **14**, 497 (1967); A. T. Soldo and E. J. Merlin, *J. Protozool.* **19**, 519 (1972)]. This method provided for growth of bacteria and flagellates which could serve as food for *L. clarki* trophonts. Cultures were maintained by transferring (every 1 to 2 weeks) half of a mature culture to a tube containing 2.5 ml of fresh media. Cultures have been maintained by this method for 10 to 12 months. For experiments, trophonts were grown in batch cultures with 100 ml of media in 250-ml culture flasks. These cultures were inoculated with ciliates and media transferred from mature test tube cultures. Test tube and batch cultures were maintained in total darkness at 21°C. Ciliates concentrated with 10-μm Nitex were added to dilute, autoclaved treehole water to create artificial treeholes 30 to 520 ml in volume. All larval mosquitoes were from a laboratory strain of *A. sierrensis*. Experimental artificial treeholes were maintained in total darkness in growth chambers at 11°C. We estimated ciliate densities by mixing the contents of each replicate and removing one or more 1.5-ml samples. Samples were stained with 0.1 ml of amide black dye [A. T. Soldo and E. J. Merlin, *J. Protozool.* **19**, 519 (1972)]. Ciliate counts were obtained using a Sedgewick-Rafter counting chamber.
9. Larvae were removed at 24-hour intervals and replaced until the first cysts appeared; after that, larvae were removed but not replaced. Larvae were stained with amide black dye and examined under magnification (×100 to 400) for the presence of invasive cysts.
10. For induction experiments, larval densities in conditioning water ranged from 0.06 to 1.13 larvae per milliliter.

11. Supported by NIH grant AI20245 and by University of California Mosquito Research Funds, J.R.A. principal investigator. We thank J. Acanfora, D. Batzer, D. Egerter, C. Montllor, and L. Sanborn for technical assistance and discussion.

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