(*17*). The pheromone has a molecular weight of less than 1000 (as determined by ultrafiltration) and is insensitive to drying, autoclaving, and treatment with 1*N* acid or base for 1 hour at room temperature. Partial purification is achieved by organic extraction of dried, starved culture media followed by diethylaminoethyl-cellulose and silicic acid column chromatography (see Table 1). Pheromone purified to this extent is suitable for biological experiments. However, the pheromone can be further purified by silica gel thin-layer chromatography with solvent systems developed for the separation of prostaglandins (*18*). The extreme stability of the pheromone suggests that it is not a prostaglandin.

The pheromone is capable of inducing dauer larva formation even in the presence of abundant food, such as bacteria on a petri plate. Incorporation of purified pheromone into NGM agar medium (*19*) at 200 and 400 activity units per milliliter of medium induced 11 ± 3.2 percent (mean ± standard error) and 28 ± 6 percent dauer larvae, respectively. The presence of pheromone did not greatly affect the overall developmental rate of the worms which did not form dauer larvae. The only observed effect of the pheromone was to increase the frequency of dauer larva formation. Dauer larvae formed in the presence of bacteria and pheromone were larger and darker than starvation-induced dauer larvae and closely resemble those produced by mutants which form dauer larvae constitutively in the presence of food (*9*).

We studied the pheromone's effect on two types of *C. elegans* behavior, egg-laying and chemotaxis. Starved liquid culture medium induced adult hermaphrodites to retain eggs so that the rate of egg laying was reduced by 86 ± 3 percent in a 2-hour period, but this effect was not produced by partially purified pheromone even at approximately twice the concentration found in starved media. Thus, other unidentified components of starved media appear to modulate egg laying. The effect of pheromone on chemotaxis was assayed by use of sodium ion and cyclic adenosine monophosphate as attractants in orientation assays (*20*). In typical tests, 85 to 100 percent of 30 adult worms responded to the attractants. Neither incorporation of pheromone [organic extract of starved media (Table 1)] into the chemotaxis assay plates nor growth of worms with pheromone before testing affected their ability to respond to either of these two attractants. This shows that the pheromone does not interfere with chemoreception per se.

In summary, a fatty acid–like pheromone, produced constitutively by *C. elegans*, enhances dauer larva formation and inhibits recovery. The concentration of pheromone and availablity of food are apparently monitored by the chemosensory organs of the L2 and dauer larvae, and the integration of these opposing signals determines the course of larval development. Pheromone-mediated control of dauer larva formation is not unique to *C. elegans* since at least two other dauer-forming species (of unknown genus) produce a different pheromone. The stability and potency of the *C. elegans* pheromone suggest that if similar compounds are involved in control of parasitic nematode development, a highly specific anthelmintic agent could be developed. At present, the most effective nematocides (*21*) are neurotoxins which pose a potential health hazard to other animal species, including man.

JAMES W. GOLDEN
DONALD L. RIDDLE
Division of Biological Sciences, University of Missouri, Columbia 65211

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15. Unsynchronized populations of worms from both starved and nonstarved liquid cultures were washed three times with distilled water by low-speed centrifugation. The pelleted worms and an equal volume of distilled water were homogenized, on ice, by ultrasonic treatment. The homogenate was centrifuged at 15,000*g* for 10 minutes and the supernatant fluid was collected and tested. Worms from both starved and nonstarved cultures contained 200 units of pheromone per milliliter of culture.
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Aggressive Signal in "Courtship" Chirps of a Gregarious Cricket

Abstract. *Unlike other known species of crickets,* Amphiacusta maya *in Central America mates in groups. Experimentally silenced males experience reduced mating success, not owing to decreased receptivity by females, but owing to increased time spent fighting with other males that persistently interrupt silent courtships. Thus, the data indicate that "courtship" chirping functions as a warning to other males, rather than as a signal to females.*

In a discussion of sexual selection, Fisher (*1*) stated that "a sprightly bearing with fine feathers and triumphant song are quite as well adapted for war-propaganda as for courtship." His specific example was bird behavior, but his concept has general implications for many other organisms. It appears that female choice is frequently involved in the evolution of the conspicuous acoustic signals that precede mating (*2, 3*). In particular, the chirps of male crickets have been shown to attract females and to increase the probability that a female will mate with a male (*3, 4*). However, in the gregarious cricket *Amphiacusta maya* Hubbell (Gryllidae: Phalangopsinae) the function of chirping appears to be "war propaganda" rather than courtship.

Amphiacusta maya, in Central America, mates in groups. The groups, which

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consist of adults of both sexes plus subadult nymphs, form each dawn in large holes such as hollow trees (*5*). For a few hours after dawn, males fight one another and court females; both of these activities are accompanied by chirping. Chirping occupies about 33 percent of courtship time and is nearly continuous during aggressive interactions (*5*). The chirps produced during aggression and courtship are indistinguishable in their temporal and spectral characteristics (*5, 6*) so that the communicative significance of "courtship" or "aggressive" chirps depends on the context. We found that caged groups of laboratory-reared animals displayed behavior similar to that seen in the field and thus provided a readily manipulated model of the natural situation.

Caged groups of males in the laboratory formed dominance hierarchies through aggressive interactions (*7*). Dominance was correlated with mating success (*8*). The relative importance of acoustic signals in female choice and in intermale competition was evaluated by experimentally silencing certain males (*9*). Silencing a male could influence two processes in intermale competition, namely, the establishment of rank and the maintenance of rank.

We studied the effect of chirping on the establishment of rank by the following procedure. Three males were ranked in each cage on the basis of three separate observation sessions (*7*). Then new groups of three males, each of similar initial rank, were formed and placed in new cages. One of the males in each of the new groups was silenced, while the others were allowed to produce normal chirps (*9*). On the day after this manipulation, a silent male was dominant in just one of 19 experimental groups (cumulative binomial probability, .005). The dominance rankings established in these trials were maintained over two additional observation sessions per group. The mating success of silent males was low (*10*), as predicted by their inability to establish a high rank.

The effect of chirping on the maintenance of rank was studied by silencing a male after he had become dominant and then monitoring his subsequent aggressive interactions upon return to his original group. Silenced males did not drop in rank more frequently than dominant normal males in control groups (*11*). Thus silencing affected a male's ability to achieve initial dominance over his rivals, but not his ability to maintain already established dominance. Although silenced males were able to maintain established dominance rankings in a group, mating success after silencing was much reduced (13 of 16 copulations before silencing and 5 of 18 copulations after; $\chi^2 = 16.54$, d.f. = 1, $P < .001$).

Table 1. Comparisons of behavior before and after silencing the dominant male. N.S. not significant.

Item	Amount of time females walked* (minutes)	Uninterrupted copulation attempts by dominant male that failed†	Number of observations with at least one attempt to copulate	
			By dominant male‡	By subordinate male
Before	5.5 ± 2.4	24 of 32	23	19
After	4.5 ± 4.0	8 of 9	10	24
Significance	N.S.	N.S.	$P \leq .05$	$P \leq .05$

*Before silencing, there were 17 observations of six groups with six females. After silencing, there were 15 observations of the same six groups with six new females. Mann-Whitney U test. †These data derive from 30-minute samples. The copulation failures represent female rejection of the male; χ^2 test. ‡Forty-seven half-hour observation sessions before and 37 after silencing the dominant male; χ^2 test.

The reduction in mating success was not due to discrimination by females. In tests in which one male and one female were caged together, the likelihood of copulation and the latency to copulation were not affected by silencing the male (*5*). In the experiments with groups, the female's response to courtship was either to stand still or to walk, and walking was considered to be a rejection of courtship. We compared the total time that females walked (*7*) when all three males were normal and when the dominant male had been silenced. Females did not behave differently in these two situations (Table 1). When one female was in a cage with three males, the rate at which the silent male's uninterrupted copulation attempts failed was no higher than it had been before silencing (Table 1); this verifies that females did not discriminate against silent males. However, silent dominant males tried to copulate less frequently and subordinates tried to copulate more frequently than before (Table 1).

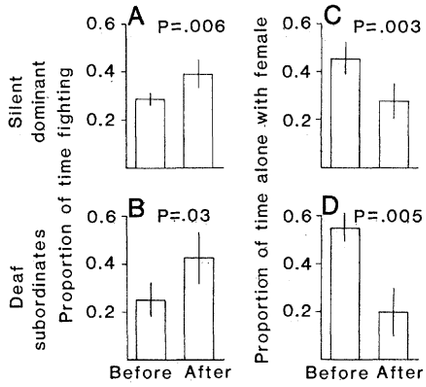

Fig. 1. Effects of rendering the dominant male inaudible (mean ± 2 × standard error). Values are the proportion of time devoted to a behavior in the interval before copulation, or if no copulation was observed, 30 minutes, whichever interval was shorter. (A) Proportion of time that the dominant male spent in fighting before and after he was silenced. (B) Proportion of time that the dominant male was fighting before and after the subordinates were deafened. (C) and (D) Proportion of time "alone with female" for the dominant male (C) before and after the dominant male was silenced and (D) before and after the subordinates were deafened; these time proportions include either antennation or courtship of the female. For (A) and (C) 18 groups were observed; for (B) and (D), 6 groups were observed. Mann-Whitney U tests were used throughout.

The changes in rates of copulation attempts may have been the result of increased interference by subordinates when a dominant male was silenced. A dominant male was considered to be "alone" with a female if he was the only male that was touching or courting her. Before silencing, 73 percent of the dominant males' attempts to copulate occurred when they were alone with a female, and the time alone was 43 percent of the total time that a dominant male was actually touching or courting a female. After the dominant male had been silenced, his time alone with the female dropped significantly (Fig. 1), although his total time touching or courting her did not change. A large part of the extra time that subordinate males spent near the female was devoted to fighting with the dominant male. After being silenced, dominant males fought more frequently, although they continued to win (Fig. 1).

The reduced mating success of silenced dominant males conceivably could have been due to some other disturbance related to our muting procedure, rather than to the silence of the dominant males. We tested this possibility by performing a reciprocal experiment in which subordinates were deafened, whereas the dominant males underwent a sham operation (*12*). These dominant males spent significantly more time fighting and less time alone with the female after the subordinates were deafened (Fig. 1). Thus, whether the dominant

male was silenced or the subordinate males were deafened, the results were very similar.

A dominant male that was audible to his subordinates devoted less time to fighting and more to courting and, consequently, had a higher rate of copulation. Our study, as well as more detailed studies of female behavior (*5*), indicates that male chirping does not affect female behavior directly. We conclude that acoustic signals influence male mating success because inaudible males are interrupted by other males more often during courtship than are audible males. The structural similarity in chirps produced during courtship and during aggression may reflect a convergence of function: no matter what the context, male chirps signal an aggressive warning to other males.

The chirps of many species of crickets have a different structure for each of several contexts (*13*). Most species of field crickets are solitary, and the males produce loud species-specific "calling" songs that attract females from a distance and possibly serve a role in the territorial spacing of neighboring males (*3*). The less intense "courtship" chirps of solitary males are audible to females in the immediate vicinity, but may not be detectable by other males (*5*); female field crickets discriminate against males that do not chirp during courtship (*4*). "Aggressive" chirps usually have yet another structure (*13*). A gregarious cricket such as *Amphiacusta maya* need not produce a calling song but is very likely to be interrupted during courtship. Thus a male in a gregarious species, at risk of constant interruptions and fights, produces "war propaganda" whenever he chirps.

CHRISTINE R. B. BOAKE*
ROBERT R. CAPRANICA
Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

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7. Three marked males were housed in each cage. One female was put in immediately before observations began. Most aggressive interactions occurred in the vicinity of the female, when a male was interrupted during courtship. The interactions varied in intensity from one male antennating the other and displacing him, to fights in which both males stood on their hind legs, hit each other with their front legs, tried to bite, and chirped continuously. All aggressive interactions [described in (*5*)] and all attempts to copulate were recorded for 0.5 hour. If the female had mated during this period, she was removed; otherwise, she was left in the cage for another 0.5 hour. The ranks of males were assigned on the basis of the number of aggressive interactions that each male won during each observation session. A cage was observed only once per day.
8. Fifty cages each containing three males were observed for one to four sessions each (*7*). If mating in this species occurs by chance alone, then each male should have achieved approximately 0.33 of the copulations in his cage. To test this hypothesis, one day on which just one copulation occurred was chosen at random for each cage, and the number of times that the dominant or a subordinate male copulated was compared with an expected value of the dominant male copulating in 0.33 of the cases and the subordinates in 0.67. Dominant males copulated in 39 cases and subordinates in 11 ($\chi^2 = 45$, d.f. = 1, $P << .001$).
9. Males were silenced by first anesthetizing them with CO_2 or N_2 or restraining them, then waxing the stridulatory file on the underside of the right wing. This allowed them to move their wings normally but they were unable to produce sound. Control males were anesthetized or restrained similarly and in the second experiment a drop of wax was put on their pronota.
10. Seven of 40 copulations in 57 observations were made by silent males.
11. Fisher test, not significant, 14 experimental and 9 control cages.
12. Subordinate males were deafened by tearing the tympana on their prothoracic legs. The dominant males were handled in similar fashion except that the analogous sites on their metathoracic legs were scratched rather than their prothoracic tympana. All of the males walked normally after this operation.
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* Send requests for reprints to Allee Laboratory of Animal Behavior, University of Chicago, 940 East 57 Street, Chicago, Ill. 60637.

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Intracellular Recordings from Cochlear Outer Hair Cells

Abstract. *Intracellular recordings were made from outer hair cells in the third turn of the guinea pig cochlea, and the electrical characteristics of the cells were compared to those of inner hair cells, supporting cells, and extracellular spaces from the same recording region. Outer hair cells have higher membrane potentials than do inner hair cells, but they produce smaller a-c receptor potentials. The frequency response characteristics of both types of hair cells are probably not significantly different. In the frequency region where tuning is optimal, both cell types produce depolarizing d-c receptor potentials, but outer hair cells also generate hyperpolarizing responses at low frequencies.*

The most advanced auditory organs have two morphologically distinct sensory receptors. Electrical characteristics of individual mammalian receptors have been described only for one type, the inner hair cell (*1*). We now describe results of a 4-year study of cochlear outer hair cells (*2*). Information from single outer hair cells may explain some central questions of cochlear physiology, such as the role of this receptor cell in hearing and the types of interaction, if any, that occur between outer and inner hair cells.

Anesthetized guinea pigs were maintained at a constant core temperature and their heart rates were monitored; in later experiments, exhaled CO_2 was also measured. To assess the normalcy of the ear, a wire electrode was placed in the scala tympani of the first cochlear turn, and tone-burst–generated compound action potentials were recorded. In preparing for the recording of hair cell potentials a fenestra (~ 0.3 by 0.5 mm) was made in the bone over the stria vascularis in the third turn of the cochlea. Microelectrodes were introduced through the stria and aimed at the organ of Corti. The cochlea was back-lighted with a fiber optics illuminator, so that the shadow of the organ of Corti could be seen through the fenestra. Attempts were made to insert the electrodes so that they would travel parallel with the reticular lamina (*3*) (Fig. 1A). Electrodes were driven by a motorized microdrive in increments of multiples of 2 μm (*4*). Only responses obtained with tone-burst stimuli are presented.

The continuous recording of electrode position and a characteristic sequence of d-c potential changes (Fig. 1B) help identify the location of the electrode tip within the organ of Corti. Cell types may be identified by a combination of recording depth, membrane potential, and response magnitude. In all supporting cell types the membrane potential is high (steady-state values range up to −100 mV) and electrical responses are smaller, at any frequency, than those mea-

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