maximum effect. Prior treatment with phentolamine ($1 \times 10^{-6} M$) or indomethacin (50 μg/ml) did not inhibit this effect (data not shown), suggesting that it was probably not mediated by the activation of the α-adrenergic receptor or the prostaglandin system. Furthermore, addition of verapamil ($1 \times 10^{-6} M$), which reversed completely the maximum KCl-induced contraction (Fig. 1F), was able to reverse only approximately 40 percent of the thrombin-induced contraction (Fig. 2B). These results suggest that an increase in intracellular calcium necessary for thrombin-induced vasoconstriction may not have been mediated entirely by way of the activation of voltage-dependent calcium channels.

These results indicate that ischemic damage following acute coronary occlusion occurs not only in myocardial cells but also in coronary vasculature. The functional alterations in coronary vascular reactivity to vasoactive agents, such as thrombin, after acute myocardial ischemia have not, to my knowledge, been reported previously. These results also suggest that similar ischemic damage with corresponding altered vascular responses may occur in the endothelium of the small intramyocardial resistance vessels as well as in the epicardial coronary vessels. The enhanced vasoconstrictory response to thrombin in coronary vessels with damaged endothelium may play an important role in the pathogenesis of coronary vasospasm and contribute to the development of coronary "no reflow" phenomenon during reperfusion of the previously ischemic myocardium.

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A Pheromone Influences Larval Development in the Nematode *Caenorhabditis elegans*

Abstract. *A* Caenorhabditis*-specific pheromone and the food supply influence both entry into and exit from a developmentally arrested juvenile stage called the dauer larva. The pheromone increases the frequency of dauer larva formation and inhibits recovery but does not affect adult behavior such as chemotaxis and egg laying. The fatty acid–like pheromone has been partially purified and characterized by a new bioassay. If similar developmental control mechanisms are used by parasitic nematodes, such mechanisms might be exploited to develop highly selective anthelmintic agents.*

Primer pheromones influence the physiology or development of a variety of metazoan organisms. In insects, honeybee queen pheromones influence the physiology of workers (*1*), and termite societies use pheromones as mediators in caste control (*2*). In rodents, primer pheromones have been shown to play an important role in reproductive strategies (*3*). The existence of sex attractants has been reported in several nematode species (*4*). We now report the existence of a nematode pheromone that controls development.

Under conditions of abundant food and low population density, *Caenorhabditis elegans* develops through four larval stages (L1 through L4), reaching the hermaphroditic adult stage 3 days after hatching (*5*). However, in response to starvation or overcrowding, development can be arrested at the second molt. The arrested stage, called the dauer larva (German, "enduring" larva) is a non-feeding form specialized for survival and dispersal (*6*), and such larvae may survive for months until they encounter food, at which time they molt and resume development (*7*). More than 20 genes influencing the developmental sequence leading to the dauer larva have been identified (*8*, *9*). Some mutants which fail to form dauer larvae are defective in chemotaxis and have morphologically abnormal afferent processes of specific sensory neurons (*10*). These genetic defects affect both entry into, and recovery from, the dauer larva stage.

We describe an environmental cue, a "crowding" pheromone, which triggers formation of dauer larvae in wild-type nematode cultures and prevents the dauer larvae from resuming development. Dauer larvae are found in starved cultures but may also be induced by high population density on enriched agar plates and in high-density liquid cultures before exhaustion of food. When placed

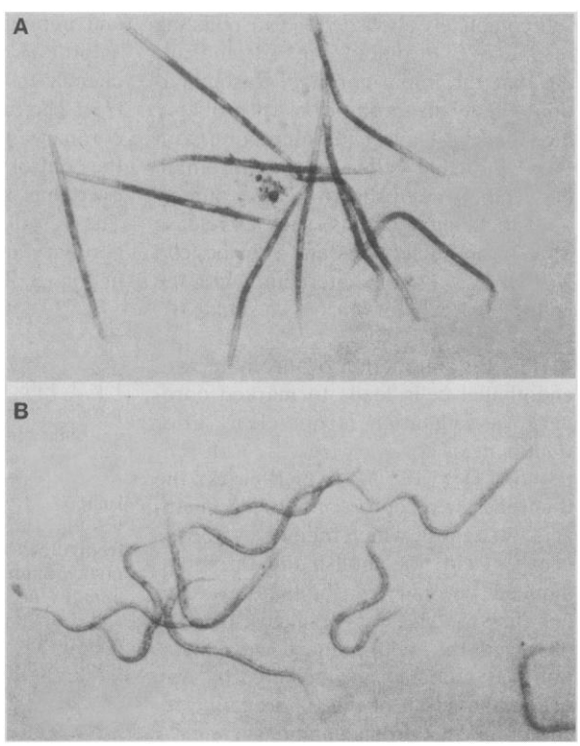

Fig. 1 (A) *Caenorhabditis elegans* nematode larvae incubated in microtiter wells containing a pheromone responsible for maintenance of the developmentally arrested dauer larva stage. (B) In the absence of pheromone, dauer larvae recover to resume development within 4 hours (× 70).

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in fresh media, the larvae quickly and synchronously recover and resume development (*6*).

We have developed a rapid bioassay for the crowding pheromone which is based on the inhibition of dauer larva recovery in the presence of a "food signal" (Table 1). The bioassay may be reliably scored after the larvae have incubated for 4 hours, and further incubation for at least 18 hours does not influence the results (Fig. 1). Thus, in the bioassay a recovery response is initiated either early or not at all. By testing a dilution series of a sample, the amount of pheromone can be determined and expressed in units of activity. The number of pheromone activity units per milliliter of sample is defined as the reciprocal of the final dilution that produces a borderline (plus-minus) response. In tests performed at threshold conditions, differences in the response of individual dauer larvae are revealed; some animals recover, but others do not. Approximately 200 units of pheromone activity are obtained per milliliter of starved liquid cultures of wild *C. elegans* (*11, 12*).

We also have the bioassay to partially characterize a food signal which is heat-stable, dialyzable, neutral, and hydrophilic. It stimulates dauer larva recovery and is found in bacterial cultures and in yeast extract.

The bioassay reveals the antagonistic effects of pheromone and food signal on dauer larva recovery (Table 1). A matrix of assay conditions, composed of a dilution series of both pheromone and food signal, shows that the relative proportion of the two signals, not their absolute concentrations, is important in the recovery of the dauer larva. As the concentration of both signals is decreased, the assay approaches the plus-minus results obtained with M9 buffer alone (not shown), indicating that the threshold of sensitivity has been reached. In the range of concentrations tested, the two signals show a one-to-one relation; if one signal is increased, the other signal also must be increased by the same factor to obtain a similar result. This linear relation is reproducible; however, we found quantitative variation in the responses of different dauer larva preparations.

The apparent ability of the animals to integrate the two environmental cues may maximize survival in the soil by allowing the worms to continue larval development only when sufficient food is available to support reproduction of the existing population. The relationship between population density and the amount of available food is a more appropriate environmental parameter than either measure alone, but the worms also respond to the individual signals. Only a portion of a dauer larva population recovers when suspended in fresh M9 buffer. All larvae recover with addition of the food signal, but none recovers if pheromone is added.

By testing extracts of media from synchronously grown cultures (*13, 14*), we determined that the pheromone is produced throughout the life cycle of *C. elegans*. It can be detected in roughly equivalent concentrations in both culture media and washed homogenized worms (*15*). Its release is not dependent on population density or food concentration, but it is not known whether it is actively secreted or excreted. The pheromone active on *C. elegans* is produced by both Bristol and Bergerac strains of this species and by *C. briggsae*, whether the strains are grown monoxenously on *Escherichia coli* or in axenic medium (*16*). No pheromone activity was detected in extracts from either type of medium before inoculation with nematodes, nor was it detected in cultures of *Panagrellus redivivus, Turbatrix aceti, Rhabditis oxycerca*, or two rhabditid nematode species that we isolated from Missouri soil. The Missouri isolates form dauer larvae when starved or crowded and, although they fail to produce a pheromone active on *C. elegans*, they do produce a pheromone active on their own species. Thus, pheromone-mediated control of dauer larva formation is not unique to *C. elegans*.

Physical data indicate that the *Caenorhabditis* pheromone is present in very small amounts, and that it may be a hydroxylated, short-chain fatty acid or a mixture of closely related compounds

Table 1. Competition between pheromone and food signal in the bioassay of dauer larva recovery. The matrix represents the standard bioassay modified by the fourfold serial dilution of both partially purified yeast extract and column-purified pheromone. Dilutions are numbered 1 (undiluted) through 11 (10^{-6} dilution). Test wells contained undiluted pheromone (100 unit/ml, or half the concentration found in starved culture media) or undiluted food signal, the equivalent of 0.5 percent (weight/volume) yeast extract. Standard bioassays were performed in 96-well microtiter plates in a total volume of 60 μl. Each well had 30 μl of sample in M9 (*19*) buffer (0.3 percent KH_2PO_4, 0.6 percent Na_2HPO_4, 0.5 percent NaCl, and 0.0025 percent $MgSO_27H_2O$). M9 buffer alone was used as a negative control, and a dilution series of supernatant fluid from a starved liquid culture served as a positive control. Each well then received 30 μl of 0.5 percent yeast extract in M9 buffer containing 20 ± 5 dauer larvae, which were isolated by layering 0.5 ml of an 8- to 10-day-old starved liquid culture of *C. elegans* strain N2 (dauer larvae formed on days 4 to 5) on 1 ml of 15 percent Ficoll, type 400, in 0.1*M* NaCl. The dauer larvae settled into the Ficoll after 5 to 10 minutes, and the upper layer and interface were removed by aspiration. The dauer larvae were then washed with distilled water three times by low-speed centrifugation. The assay was incubated at room temperature (23°C) for 4 to 12 hours and then scored. With a Wild M5 stereomicroscope, a sample was scored as positive for pheromone when less than 20 percent of the worms recovered and negative when more than 80 percent recovered; intermediate (or borderline) results were scored as plus/minus (+/−). Recovery was assessed by observation of pharyngeal pumping, movement, and appearance of the worms. To purify the pheromone, a starved liquid culture (1 liter) was concentrated by evaporation under reduced pressure and then centrifuged at 10,000*g* for 10 minutes. The supernatant fluid was collected and dried under a stream of air. The residue was extracted four times with $CHCl_3$/MeOH (2:1) and the extracts combined. The extracted pheromone was applied to a 7-ml DEAE-cellulose column in $CHCl_3$/MeOH (20:1), rinsed, and then eluted with $CHCl_3$/MeOH (1:1). The eluted fraction was then applied to a 3*g* silicic acid column in $CHCl_3$/MeOH (50:1), rinsed, and the pheromone was eluted with $CHCl_3$/MeOH (2:1). The solvent was evaporated, the oily residue was extracted three times with 2.5 ml of glass-distilled water, and the combined extracts were concentrated by evaporation to 1.5 ml. This column-purified pheromone contained 10,000 activity units per milliliter of sample. To purify the food signal, the neutral fraction from a 10 percent (weight/volume) aqueous solution of Bacto yeast extract (Difco) was obtained in three chromatographic steps: Dowex 1-X8 (formate form), Dowex 50W-X4 (H^+ form), and DEAE-cellulose (acetate form). After application of the sample, each column was washed with two volumes of water and the eluate was collected. The final eluate was concentrated to a volume of 10 ml at 60°C under a stream of air. This partially purified yeast extract contained 100 percent of the original food signal as determined by the pheromone bioassay.

Pheromone dilution series	Food signal dilution series										
	1	2	3	4	5	6	7	8	9	10	11
1	−	+/−	+	+	+	+	+	+	+	+	+
2	−	−	+/−	+	+	+	+	+	+	+	+
3	−	−	−	+/−	+	+	+	+	+	+	+
4	−	−	−	−	+/−	+	+	+	+	+	+
5	−	−	−	−	−	+/−	+/−	+	+	+	+
6	−	−	−	−	−	+/−	+/−	+/−	+	+	+
7	−	−	−	−	−	−	+/−	+/−	+/−	+	+
8	−	−	−	−	−	−	−	+/−	+/−	+/−	+

(*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.

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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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