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Chemosensory Tracking of Scent Trails by the Planktonic Shrimp *Acetes sibogae australis*

Abstract. *In the laboratory, planktonic shrimps* (Acetes sibogae) *precisely follow scent trails of food or paper soaked in meat extract,* L*-alanine,* L*-leucine, and* L*-methionine. In the ocean,* Acetes *may be able to follow scent trails as far as 20 meters to catch falling food. This demonstrates precise trail-following by pelagic animals.*

The importance of chemosensitivity in the marine environment has been emphasized, and highly sensitive chemoreceptive abilities have been described for benthic and pelagic animals (*1*); but almost no data are available for plankton. In the laboratory, copepods "search" for food or females (*2*); chemoreceptors have been described for several planktonic crustaceans (*3, 4*). The planktonic sergestid shrimp *Acetes sibogae australis* Colefax becomes increasingly active when stimulated by food scents, but when trials are run in appropriately designed aquariums this generalized searching behavior transforms into the most precise chemosensory trail-following response yet observed for any free-swimming aquatic animal including fishes (*1, 5*). This suggests that chemical perception in free-swimming organisms may be extremely effective ecologically and deserves additional attention.

Acetes sibogae australis were collected at night with light traps suspended about 2 m deep in the harbor off Townsville, Queensland, Australia, as well as in shallow water off the beach. In each unaerated aquarium (15 by 22 by 60 cm), 8 to 12 shrimps were kept and fed *Artemia*; water was changed every 2 days. Experiments were conducted in these aquariums. Each aquarium was illuminated from both sides by fluorescent light; the backs and bottoms of the tanks were painted black to make the transparent shrimps more visible. The fluorescent green dye used in the experiments became brightly visible under these conditions. The front pane was marked at 5-cm intervals to estimate swimming speed and distance. Experimental food stimuli consisted of diced banana prawns (*Penaeus merguiensis*) or freshly killed and crushed *Acetes* soaked in fluorescein dye and dropped into the quiet water of the tall aquariums. These sank slowly to the bottom, leaving erratic but unbroken thin trails of highly visible dye in the water. Small (2 mm^2) pieces of dyed blotting paper soaked with meat extract or amino acids also served as test stimuli. The shrimps' sensitivity to chemical trails decreased with successive tests, and the water in the tanks became murky from the dye, so that the water always was changed after four to five tests. In each series of tests, uncontaminated blotting paper soaked in dye was presented as a control at the start and end of the experiment.

In the absence of food stimuli, the shrimps swam in horizontal linear paths back and forth across the width of the aquarium (22 cm) at an average speed of 1.9 cm/sec. When dyed food was dropped into the aquarium, *Acetes* never deviated from the linear paths to avoid or seek out the trail. But, when a "patrolling" shrimp did by chance contact a trail head-on, its swimming behavior immediately changed to one of two distinctly different modes. The shrimp either would initiate a rapid horizontal circular "search pattern" in the vicinity of the trail or it would sharply reorient the body axis to the vertical trail and rapidly track the exact, but often erratic, path left by the falling food, swimming at approximately three times the normal speed (Fig. 1). (Average speed of 19 animals following food trails was 5.6 cm/sec.) Food particles and blotting paper sank to the bottom of the 60-cm-high aquarium in about 30 seconds, and, since *Acetes* swam down the twisting trail at about three times this speed, they often caught the particles in mid-water. The *Acetes* then ate the food but would discard impregnated blotting paper. If an *Acetes* lost the trail when the trail recurved at a sharply acute angle, it then swam a circular search pattern, usually relocated the trail, and again tracked the falling food. Some animals lost and relocated a trail five successive times as they traced it down through the water column.

Vision did not appear to play a role in trail-following. Both undyed meat and undyed blotting paper soaked in Millipore-filtered prawn juice elicited trail-following of the same precision and rapidity as did the visible scent trails. Furthermore, no shrimp among the thousands we watched appeared to deviate from its normal path to enter a visible dye trail with or without scent. Animals that had repeatedly responded to contact with visible scent trails never responded to contact with the visible but chemically clean control trails, which terminated as well as preceded each experiment.

An initial experiment recorded the behavioral response of *A. sibogae australis* to dyed pieces of shrimp. Of 63 animals that contacted the food trails, 54 responded; 39 of these followed the trail for at least 10 cm. These figures imply that the trail-following response was somewhat equivocal, but these data belie the regularity with which hundreds of *Acetes*, collected during a 3-month period, displayed the response. Experimental factors, such as chemical contamination from previous tests in the same water or reduced receptiveness of individual sergestids probably accounted for lower levels of response in the initial experiment. In a second experiment, the shrimps responded positively in 100 percent of their contacts with the trails, and in all experiments (with meat or meat extract) every trail contacted always elicited at least one tracking response.

Acetes sibogae australis rarely swam upward when following a vertical trail. Of the 54 animals responding in the initial experiment, one swam up a trail about 5 cm, then reversed and swam downward. In over 1000 subsequent tests, *Acetes* swam up a trail only six times. To determine whether *A. sibogae australis* generally swims down or follows a chemical gradient, we lowered dyed meat tied to a string near one side of the aquarium and then slowly pulled it up near the opposite side. Of ten animals that tracked an ascending trail, all swam down from the point of contact. Animals which followed the horizontal part of a trail near the bottom lost the trail when it ascended. It appears that *A. sibogae australis* does not follow a chemical gradient but instead has evolved a behavioral response adapted to catching falling material, such as wounded animals or discarded scraps that would leave a strong chemical trail in their descent.

Descending scent trails persisted a surprisingly long time in the aquariums. The dye trails remained discrete and relatively linear in our experimental tanks for up to 6 minutes. Thereafter they lost their integrity and formed a dye cloud. In six tests in tanks with no animals present, the dye cloud remained visible for about 50 minutes. With one to four animals in the tank the dye cloud was visible for 35 minutes; eight animals produced sufficient turbulence to completely disperse the visible dye in 19.5 minutes; 30 animals reduced this dispersal time to 15 minutes. Therefore animal-generated turbulence becomes significant in crowded aquariums. When the dye trail included meat extract or an attractive chemical, it was dispersed rapidly by turbulence from the wake of the tracking shrimp as it swam down the trail (Fig. 1).

The sensitivity of *Acetes sibogae australis* to food odors over time was tested. Observations (202) were made of individual shrimps contacting and either responding to or passing through a visible portion of the scent trail or scented dye cloud. During the first minute after presentation, 100 percent of the animals making contact exhibited a trail-following response to the stimulus. By 6 minutes, only 50 percent of the animals responded to the stimulus; the response was mixed, with some "trail-following" and some "searching." Thereafter, the dye trail dispersed rapidly because of thermal and animal-generated turbulence and responses after about 6 minutes were generally "searching" patterns. These searching patterns continued for up to 14 minutes after the introduction of the stimulus. Larger *A. sibogae australis* were approximately 3 cm long and swam at an average speed of 5.6 cm/sec, and discrete dye trails remained in the water column for up to 6 minutes. Therefore, in quiet waters of the ocean, *Acetes* potentially could track a perceived scent trail left by a falling particle of food as far as 20 m. Several factors, such as dispersion rates of different attractants or unexpected turbulence, obviously would affect this distance. Persistence of dyed scent trails could be measured by divers at sea (*6*) or by experiments in deep tanks. If discrete chemical trails are as important ecologically to pelagic animals as the response of *Acetes* suggests, the time scale for dispersion of chemicals and fine-scale patterns of natural turbulence in the sea need particular attention.

Table 1. Response of *Acetes sibogae australis* to chemical trails of ten amino acids (saturated solutions).

Compound	Contacts with trails	Responses*	
		Number	Percent
L-Methionine	25	21	84.0
L-Alanine	23	19	82.6
L-Leucine	24	18	75.0
L-Serine	15	5	33.3
L-Valine	20	6	30.0
L-Cysteine	20	5	25.0
L-Lysine	18	2	11.1
L-Isoleucine	12	1	8.3
L-Threonine	12	1	8.3
L-Histidine	13	1	7.7

*As defined in the text.

The sensitivity of *A. sibogae australis* to specific chemical stimuli also was tested with pieces of blotting paper soaked in saturated solutions of 17 different L-amino acids. No response occurred in contacts with scent trails of L-arginine, L-asparagine, L-aspartic acid, glycine, L-glutamic acid, L-phenylalanine, or L-tryptophan. Seven other amino acids elicited searching or tracking responses from some individuals and three more elicited responses from most *Acetes* tested, the percent response being only slightly less than the 86 percent response of the initial experiment when meat stimuli were used (Table 1). Serial dilutions of L-methionine (the most stimulatory) from $10^{-6}M$ to $10^{-1}M$ indicated a threshold for trail-following at $10^{-4}M$. The actual threshold is probably lower, since the chemical dilutes along the trail. Two other sulfur-containing compounds, sulfanilimide and 2-mercaptoethanol, did not elicit a response. Urea and glucose were nonstimulatory. Similar chemicals are effective as stimulants for other Crustacea (*7*).

Since the behavioral response of trail-following is so rapid and yet so precise, the anatomical location of the chemoreceptors involved was of interest. The sharp, discrete edges of the thin dye trails (± 2 to 5 mm in diameter) in our tanks aided in localizing probable receptor sites. For example, *Acetes* did not respond to a scent trail if they broke it with an antenna alone or touched it with the dorsal surface, the telson, or swimming legs. We suspected originally that the antennules were involved because from the initial horizontal swimming position the antennules first contact the trail, and, since the antennules of other crustaceans possess chemoreceptors (*8*), it appeared reasonable to suspect that these structures mediated the response. We excised antennules of ten specimens of *A. sibogae australis* and tested their behavioral response to dyed stimuli 18 hours later. Of 14 contacts with Millipore-filtered prawn extract, 13 positive responses were recorded. In four cases, the shrimp initiated rapid circular searching patterns, and in nine, they traced the trail, but never more than 5 cm. The antennules thus do not appear to be necessary for perceiving the trail, but they are involved in the response because tracking distances were short. Other receptor sites probably are involved as well. Ultrastructure analysis shows that *A. sibogae australis* antennae bear setae

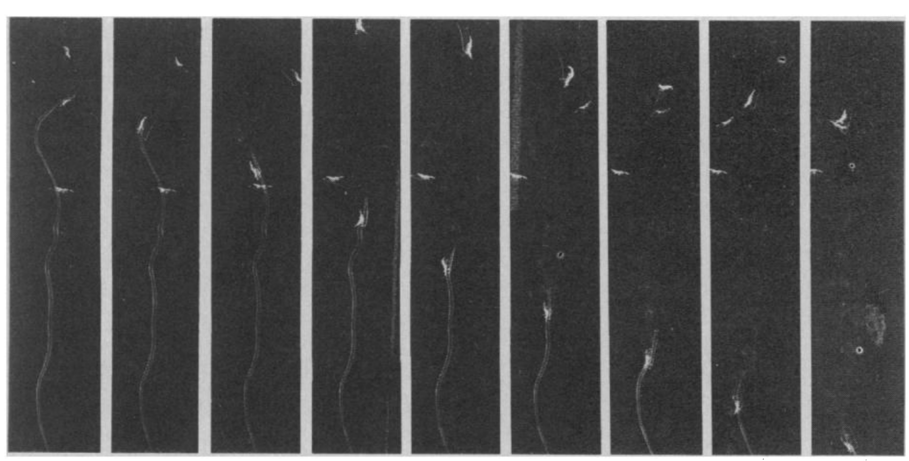

Fig. 1. *Acetes sibogae australis* tracks the scent trail of fluorescein-dyed banana prawn. In frame 1 (left) the center shrimp hit and followed the trail. The lowest shrimp, swimming horizontally, was behind trail and did not contact it. In subsequent frames other individuals reacted to the dissipating scent cloud. The intact trail in frame 1 was 45 cm long. Successive frames represent intervals of one-fourth of a second.

which probably are chemoreceptors (*4*), while some photographs of *Acetes* following trails show antennules raised above the trail which impinges on the mouth parts and proximal sections of the pereiopods.

Acetes manifests its fine-tracking response only in particular laboratory situations. In smaller aquariums than those used, the animals are too agitated to respond, and in cylindrical containers they aggregate against the side. Properly selected laboratory conditions probably will show that the ability to track scent trails is widespread throughout planktonic taxa. Copepods (*Calanopia elliptica*) and a reef lagoon mysid (as yet unidentified) both follow scent trails in our aquariums.

A field observation made during research in the Florida Current (*6*) was partly responsible for these experiments and indicates that scent trails occur naturally in the open sea. Diving to a depth of 15 m, we collected samples of a "remarkable amorphous material" covered with copepods. Upon surfacing we found that a boat tender had become seasick and regurgitated a partially digested meal, part of which was deposited in the water. The falling particles probably had produced discrete scent trails that quickly attracted surprisingly large numbers of copepods.

The genus *Acetes* lives in coastal waters (*9*), which often provide little or no visibility and where chemosensory cues should be extremely important. In the perpetual dark of the deep sea, scent trail-following may be an even more important behavioral response. Perhaps the deep sea, with its low turbulence, is laced with a complex array of attractive or repellant chemical trails. If so, scents may prove to be even more important for aquatic animals than they are for terrestrial organisms.

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Paradoxical Effects of Amphetamine on Preweanling and Postweanling Rats

Abstract. *In adult rats amphetamine acts as a strong behavioral stimulant leading to a marked increase in random, nondirected locomotor activity. In contrast we report that amphetamine administered to preweanling rats in the presence of an anesthetized adult rat produces no visible increase in motor activity. Instead, it appears to enhance the normal tendency of neonatal rats to approach and maintain contact with conspecifics. In postweanling rats amphetamine disrupts the tendency to aggregate and produces an increase in behavioral activity comparable to that seen in adult rats. These findings may constitute the basis for an animal model of minimal brain dysfunction hyperkinesis.*

Minimal brain dysfunction (MBD) is one of the most common clinical syndromes of childhood. It is characterized by high levels of motor activity, short attention span, nonresponsiveness to social influences, and lack of impulse control (*1*). One of the most unusual aspects of MBD is the calming effect that stimulants, especially amphetamine and methylphenidate, have on the hyperkinetic child. The mechanisms of action for this paradoxical calming effect are unknown. Numerous models of both MBD and the paradoxical calming effect of amphetamines have been put forth, but none have received general acceptance (*2*).

A widespread view, however, is that MBD represents a lag in some aspect of neurological development rather than a permanent impairment (*3*). The behavioral profile of MBD is reminiscent of the preschool child; moreover, the syndrome typically disappears as the child enters late adolescence. Unfortunately, it is not known whether amphetamine has a calming effect on preschool children during their period of normal hyperkinesis, and such research is now precluded for ethical reasons. Most altricial mammals, however, pass through comparable periods of hyperactivity during ontogenesis (*4*); the developing normal mammal may therefore serve as an animal model of hyperkinesis.

In this report we describe the effects of amphetamine on normal rats during various stages of development, when tested either in isolation or in their natural environment. In the nest, preweanling rats spend most of their time in close physical contact with siblings and the dam, and, given the opportunity, will orient toward, approach, and remain in contact with those conspecifics (*5*). In preweanling rats (15 days of age) the response to amphetamine is an enhancement of these approach and contact behaviors. Later in development (30 days of age) amphetamine disrupts the normal tendency of rats to aggregate and produces its characteristic increase in random, nondirected motor activity. These data suggest that stimulants serve to direct or "canalize" behavior during certain stages of normal development, thereby producing its apparent calming effect.

The basic experimental procedure was to compare the amount of locomotor activity elicited by various doses of amphetamine administered to 15- and 30-day-old rats tested either alone or in the presence of an adult, anesthetized male rat. Time spent in contact with the anesthetized male was also recorded. Previous research had shown that this preparation elicits vigorous approach and contact behavior in the preweanling rat (*6*).

The subjects were 160 albino rats of Sprague-Dawley descent, bred and raised in the Princeton University colony. Rats were randomly assigned to 20 equal groups in a factorial combination of age (15 or 30 days), dose (0, 0.25, 0.5, 1.0, or 2.0 mg of *d*-amphetamine sulfate per kilogram of body weight), and test condition (tested in isolation or in the presence of an anesthetized adult). All doses were calculated as the sulfate salt dissolved in isotonic saline. Control animals were injected with the saline vehicle only.

All observations were made in four polypropylene rat cages (25 by 46 cm), the floors of which were covered with dried beet pulp. Neither food nor water