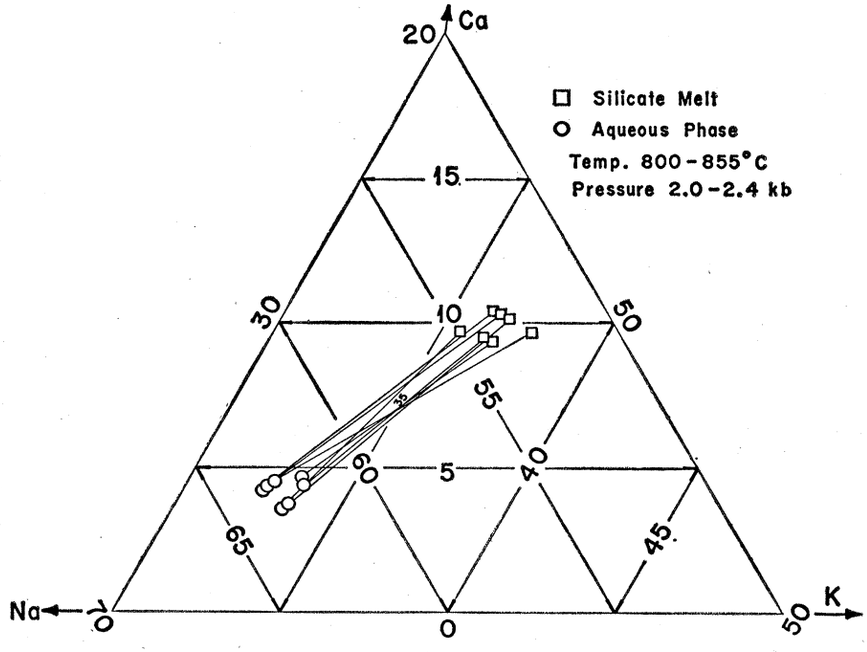

Fig. 3. The mole percent of potassium, sodium, and calcium in coexisting chloride solutions and granitic melts; the chloride concentration in the aqueous phase was between 2 and 4 mole/kg.

drothermal solutions tend to be depleted in calcium relative to the silicate melts with which they are in equilibrium. This is true to an even greater extent for magnesium. On the other hand, the concentration of chloride in the aqueous phase is between 10 and 100 times greater than that in the associated silicate melts. After the runs at 800° to 855°C and pressures of 2.0 to 2.4 kb were quenched, the *p*H of the 2- to 4-molar chloride solutions (initial *p*H 4.2) changed to between 1.5 and 2.0.

Our data define the initial ratio of potassium to sodium in hydrothermal solutions derived from siliceous magmas in a geologically interesting range of temperatures and pressures. During the reaction of such solutions with silicate wall rocks on cooling, the ratio of potassium to sodium tends to decrease and ultimately to approach 0.02, the value of m_K/m_{Na} in ocean water. Sawkins (*6*) and Rye (*7*) have shown that the hydrothermal solutions responsible for the deposits of lead-zinc ore at Providencia, Zacatecas, Mexico, were probably derived from the deeper levels of the granodiorite stock at Providencia-Concepción del Oro. The range of (m_K/m_{Na}) represented in two samples of fresh granodiorite analyzed by us and in three samples analyzed by Dittrich (*8*) was 0.56 to 0.86. Hydrothermal solutions derived from melts of such composition would have ratios of potassium to sodium between 0.41 and 0.64. Rye and Haffty (*9*) have found ratios of potassium to sodium between 0.09 and 0.43 in inclusion fluids from minerals of the Providencia ore bodies. The higher values are characteristic of the most concentrated (2.5*M* to 6*M*) chloride solutions. These findings are consistent with the hypothesis that the solutions were derived from the granodiorite magma, and that the more concentrated solutions have suffered only a small reduction in the ratio of potassium to sodium by reaction with the enclosing wall rocks.

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Sexual Pheromone in Some Fishes of the Genus Hypsoblennius Gill

Abstract. *A pheromone found in the males of some species of* Hypsoblennius *apparently facilitates male courtship by releasing sexual appetitive behavior and by increasing sexual receptivity.*

Nonvisual communication is common in sexual behavior (*1*). Pheromones in fishes have been reported to serve for species and individual recognition (*2–4*), and as stimuli involved in courtship behavior (*3, 5, 6*). Both chemical (*6, 7*) and auditory (*8*) signals have been implicated in the courtship behavior of blenniid fishes. This report describes a pheromone response that involves social facilitation of sexual behavior between males of some *Hypsoblennius* species. Most of the previously reported sexual pheromone responses in fishes involve recognition or intersexual communication (*3, 5, 6, 7*). In the three-spined stickleback, courtship of the male is enhanced by the odor of his own nest (*9*). This response is similar to the enhancement of sexual receptivity noted in *Hypsoblennius*, but no mention is made of the effects of this pheromone on other males in regard to sexual receptivity or sexual appetitive behavior.

The three species studied are territorial bottom fish found in shallow-water, much-broken habitats. *Hypsoblennius jenkinsi* (Jordan and Evermann), from California, is morphologically similar to its apparent counterpart in Peru, *H. robustus* (Hildebrand); *H. gentilis* (Girard), from California, is less similar and seemingly is a more primitive member of the species group (*10*). The sexual behavior of these three species is similar in most respects (*10*). When a female approaches a ripe male, he responds by displaying in a head-up posture from his refuge or dwelling place, and on further approach by the female, by quivering his body. The female enters the male's refuge, deposits her eggs, and leaves them to the care of the male. Sexually ripe males are frequently attracted to courting males, often from beyond the visual field.

Isolated 12-gallon (45-liter) aquariums were used. Both ends of each tank were constantly supplied with seawater through tubes that emptied just above the surface of the water. Glass lines for introduction of the stimulus emptied in either end with the tank's water supply in order to avoid collateral

Table 1. Summary of 90 trials with three species of the genus *Hypsoblennius*. The types of water that elicited a positive response are capitalized; types of water that failed to elicit a reaction are listed in lower case. The level of significance (P) is presented when the number of observations was greater than four.

Species tested	Types of test water donate by:		
	H. robustus	*H. jenkinsi*	*H. gentilis*
H. robustus	male-water female-water* low courtship HIGH COURTSHIP† MATING†	male-water female-water	male-water female-water MATING
H. jenkinsi		male-water female-water no courtship* HIGH COURTSHIP† MATING†	male-water female-water no courtship MATING
H. gentilis			male-water* female-water*
		mating	mating*

* $P > .3$. † $P < .01$.

stimuli during the introduction. Opaque plastic tubes were provided as refuges in the center of the tank. Observations were made through slits in a blind from a darkened observation chamber. Fish were isolated in the tanks for at least 1 week before testing.

The stimulus water was obtained from aquariums containing fish that were not to be tested, and was characterized as to sex, species, and the behavior of fish in the tank. A quantity of water was first drawn from the tank of an isolated, sexually ripe male and was designated "male-water." A female was introduced into this tank; water was then drawn from the tank and characterized according to the behavior of the male at that time, that is, "no courtship-water" (male not courting but female present), "low courtship-water" (head-up display but no quivering), "high courtship-water" (including head-up display and quivering), and "mating-water" (oviposition). "Female-water" was obtained from tanks containing isolated ripe females.

A 2.5-minute control record of an animal's activities was made on a manually activated event recorder. The water to be tested was then introduced and allowed to flow continually for 3 minutes at a rate of about 1 ml per second. Dyes were used initially to insure that a concentration gradient was formed at the end of the tank receiving the stimulus. A 2.5-minute test observation was begun 30 seconds after the start of the introduction period; at least 45 minutes were allowed to elapse before retesting. The duration of activity was measured with dial calipers on the event records to the nearest half-second. In each trial, the individual tested, the test stimulus, and the stimulus side of the tank were randomly chosen.

Initial observations suggested that sexually ripe males are attracted by water from a tank that contains an actively courting male. A ripe *H. robustus* male was conditioned by constant perfusion of conspecific "mating-water" in the left side of the tank for 1 day. When tested with "mating-water," he showed a preference for the left half of the tank during the control periods, and for the right half when the stimulus was presented there ($P < .05$ by analysis of variance, $N = 28$). The typical reaction included an apparent increase in swimming activity and movement toward the source of the stimulus.

In subsequent tests this reaction was used as an indication of a positive response. The time that the individual spent out of his refuge was divided into four categories—on bottom in nonstimulus half of the tank, flutter in nonstimulus half of tank, on bottom in stimulus half of tank, or flutter in stimulus half of tank. Fluttering was defined as any swimming during which no part of the body touches the bottom. In order to indicate a reaction to the pheromone, the categories were weighted 1 through 4, respectively. The total score for each observation period is the sum of the number of seconds of each activity, multiplied by its weight. Test scores were checked for significant difference from the control scores by the rank sum test (*11*). Tests on any one type of water were usually discontinued after four trials if no reaction was shown. In order to indicate attraction to the stimulus, the number of seconds of each activity during each control period was subtracted from that of the corresponding test period. All values for fluttering were doubled; values for the time spent "on bottom" and fluttering were summed for the two halves of the tank for each test, and then compared by the rank sum test.

The 90 trials summarized in Table 1 were done with ripe, nonparental males. In each case where a significant reaction was indicated, there was also a significant attraction to the stimulus source ($P < .01$). Additional tests on males that were guarding eggs, but still showing high courtship responses, revealed no reaction to conspecific "high courtship-water" or "mating-water." Also, in order to determine the validity of the test conditions for *H. gentilis*, individuals were tested with water that contained the odor of food (from juices squeezed from frozen edible shrimp) and they always showed a strong attraction ($P < .01$). Incidental observations suggested that the sexual receptivity of *H. jenkinsi* and *H. robustus* males may be enhanced after prolonged exposure to "high courtship-water" or "mating-water." This was indicated by an increase in the frequency and intensity of courtship released by an approaching female.

The conclusions were: (i) Ripe, nonparental *H. robustus* and *H. jenkinsi* males are attracted by a conspecific or *H. gentilis* pheromone that is present only during strong male courtship and mating. The strongest attraction is seemingly elicited by the conspecific pheromone. (ii) Females and egg-guarding males are not attracted by this pheromone under the experimental conditions.

The source of the pheromone was not studied, but it is known that ejaculation occurs during the same stage of courtship that the pheromone first appears (*10*). The secretory cells in the anal pads may be the source of an aromatic secretion (*6, 7*).

Appearance of the pheromone response corresponds with the increase in sexual appetitive behavior seen in ripened males when no receptive females are present. Egg-guarding males usually court and mate repeatedly but do not show sexual appetitive behavior that takes them away from the refuge, and they were not attracted by the pheromone. Thus the pheromone in species of *Hypsoblennius* is interpreted as effecting a social facilitation of courtship both by attracting ripe, nonparental males to a courting male and thus prospective mates, and possibly by enhancing the sexual receptivity of males. This response is probably of selective advantage to the species in that it in-

creases the number of heterosexual contacts that might lead to pair formation, and it promotes spawning synchrony. These findings point to the existence of sexual facilitating pheromones in fishes in addition to those affecting male-female interaction.

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Lysozyme Retention by Cockroach Periplaneta americana L.

Abstract. *Blood from the cockroach* Periplaneta americana *usually does not react with a suspension of* Micrococcus lysodeikticus. *When lysozyme is added to the mixture it causes an immediate reduction in the optical density at 450 nanometers which is soon followed by a strong rise. Blood from roaches injected with lysozyme causes a similar series of changes in the optical density of a suspension of* M. lysodeikticus, *an indication that lysozyme has been retained in the blood. It may persist for many days or weeks.*

The bacterial symbiont of the cockroach (*Periplaneta americana*) is intimately connected with the egg (*1*) and with mycetocytes of the fat body (*2*). This association suggests that it may have an influence on reproduction and growth, and indeed its removal proves to be injurious to these functions (*3*). It is by no means sure, however, whether such injury is due to the loss of the symbionts or to the sterilizing treatment by heat (*4*), antibiotics (*2*, *3*), or lysozyme (*3*).

Use of the first of these two has entailed considerable mortality. However, Malke (*3*) has sterilized the cockroach *Periplaneta americana* with egg-white lysozyme, 0.5 mg of which destroyed most of the bacteroids within a week and eventually completely disintegrated them. Such a dose is not lethal to the insect, and even the injection of 22 successive doses at 3-day intervals is not toxic (*3*). If this is so, it would aid in understanding the physiology of growth and reproduction in this and similar associations.

It might be feasible to detect the enzyme in 20 μl of blood, the volume expected from an individual male. We used egg-white lysozyme which was crystallized three times, dialyzed, and lyophilized; it had an activity of 30,000 sigma units (*5*) per milligram. To reduce coagulation of the blood, we used as diluting fluid 0.2 percent potassium oxalate solution (*6*) in 0.066*M* potassium phosphate buffer (*p*H 6.2) in the ratio of 4 : 1. Twenty microliters of blood were added to a reaction volume of 2.6 ml, except for the experiment shown in Fig. 1*b*, in which 50 μl of normal blood pooled from several insects was used to attain an appropriate optical density (O.D.). This increase in concentration of blood increased its tendency to clot. Reactions were conducted at room temperatures. The addition of 0.1 ml of a solution of lysozyme (0.1 mg/ml) to 2.5 ml of a suspension of *Micrococcus lysodeikticus*, having an $O.D._{450}$ of about 0.6, caused a pronounced and uninterrupted decrease in the absorbance until lysis was completed and the suspension became clear (Fig. 1*a*). The addition of the same amount of lysozyme to 50 μl of normal blood in 2.5 ml of potassium oxalate buffer showed no significant effect (Fig. 1*b*) (the slight decline in absorbance is attributed to developing coagulation and can be considerably reversed by inverting the container).

The addition of 20 μl of normal blood to 2.6 ml of a suspension of *M. lysodeikticus* generally shows no effect (Fig. 1*c*). However, some blood samples do react. This is shown after a delay of from 1 to 2 minutes by a strong rise in absorbance over a period of 6 to 8 minutes, to a value that may be two to three times the original absorbance to complete a sigmoid curve (Fig. 2*a*). On the other hand, the addition of 0.1 ml of lysozyme solution to a mixture of 2.5

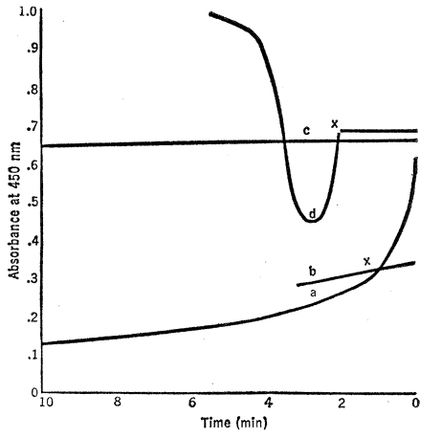

Fig. 1. (*a*) Action of lysozyme on suspension of *Micrococcus lysodeikticus*. (*b*) Action of lysozyme on normal blood. The lysozyme was added to the blood sample at time *X*, and produced no significant change in absorbance. The slight decrease in absorbance was due largely to coagulation of the blood which, in this sample of 50 μl, was greater than in the samples of 15 to 20 μl used in the other experiments. (*c*) Apparent lack of action of normal blood on *M. lysodeikticus*. (*d*) Action of lysozyme on a mixture of normal blood and *M. lysodeikticus*. Lysozyme added at time *X*.

ml of bacterial suspension and 20μl of normal blood caused an immediate and sharp reduction in absorption, followed in about 1 minute by an even greater rise (Fig. 1*d*). This evidently was the type of curve to expect from blood with high lysozyme activity. If the injected lysozyme were uniformly distributed throughout the insect, a 0.5-mg dose in an 800-mg male cockroach would yield approximately 0.06 mg of the enzyme per 100 ml of blood. Twenty microliters of such blood added to 2.6 ml of a suspension of *M. lysodeikticus* would thus

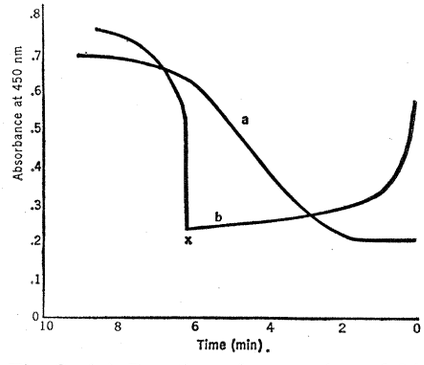

Fig. 2. (*a*) Reaction of suspension of *M. lysodeikticus* and blood from certain normal males. Note delayed rise in absorbance. (*b*) Immediate increase in absorbance and continuing rise after addition, at time *X*, of normal blood to a reacted mixture of lysozyme and *M. lysodeikticus*.