Mating Behavior in the Freshwater Prawn, *Palaemon paucidens*. A Study of the Sex Pheromone and its Effect on Males[1)]

By

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(With 5 Figures and 2 Tables)

It is well established that the courtship in many insects is initiated by sex attractant from females. Investigations have been made on the nature of such chemical communicants, and the purification, identification, and even synthesis of sex pheromones have been carried out successfully with several lepidopterans, the honey bee and cockroaches (for reference, see Highnam and Hill, 1969).

Unfortunately, however, very little information is available on the sex pheromone in Crustacea, although its existence in some species seems most likely. Recently several authors reported the sex pheromones in decapod crustaceans, because the males displayed a sexual attraction to the premolt females of the large edible crab, *Portunus sanguinolentus* (Ryan, 1966), or to the newly molted females of the American lobster, *Homarus americanus* (McLeese, 1970; Atema and Engstrom, 1971). However, these authors have failed to show the site of origin and chemical nature of the pheromones.

A female freshwater prawn, *Palaemon paucidens*, with a fully mature ovary performs prior to copulation and egg-laying the "parturial" molt, which accompanying a series of morphological changes in the abdominal region of the prawn leads to the formation of the brooding chamber (Kamiguchi, 1971). By the onset of this particular molt, the males, otherwise staying calmly on the substratum most of the daytime, suddenly become agitated and start to move around in seeking copulation-ready females.

In the present paper, behavioral responses of the male prawns to the females at various physiological conditions are analyzed for the purpose of providing more substantial evidence of the existence of sex pheromone in this animal.

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Materials and Methods

All the freshwater prawns, *Palaemon paucidens*, used in this study were collected in a lake in the vicinity of Sapporo during May and June of 1970. In the laboratory the stock animals were maintained in aerated aquaria ($21 \times 36 \times 60$ cm) and were fed twice a week on pieces of boiled fish paste. The water was renewed once a week. For observation of the mating behavior, a female was placed at first singly in a glass vat (15 cm in diameter and 7 cm in height) and then, two to five males were introduced into the vat. In a series of observations male prawns were subjected to females at various stages of an intermolt cycle. Intermolt stage of each specimen was determined according to the method previously described by the present author (Kamiguchi, 1968).

Observations

Although the prawns are polygamous, they breed only once a year during late May and early June in Sapporo area. Prior to mating and subsequent egg-laying the females perform the parturial molt, which results in a series of structural changes such as enlargement of the pleura, growth of ovigerous hairs and the formation of brooding chamber in the abdominal region, but these characters are lost at the next "ordinary" molt after the completion of brooding.

Mating behavior of the male prawn is divided into three phases. Phase I, searching: as soon as the presence of a copulation-ready female is perceived by a male, he starts to search her whereabout, moving around busily on the substratum, stretching the chelae forward and actively shaking outer flagella of his antennules. It is not easy, however, to distinguish this behavior from ordinary food-searching behavior. Phase II, mounting: coming across the newly "parturial-molted" female, the male mounts on her back (Fig. 1, A). A predator as well as a scavenger, the prawn usually attacks a newly molted, soft-shelled prawn. But no attack is made to a "post-parturial molt" female by males. Such a female with the soft shell does not only retreat from an approaching male but allows him to mount on her carapace for copulation. Phase III, copulation (deposition of the spermatophore): the male in mounting posture moves down backwards to

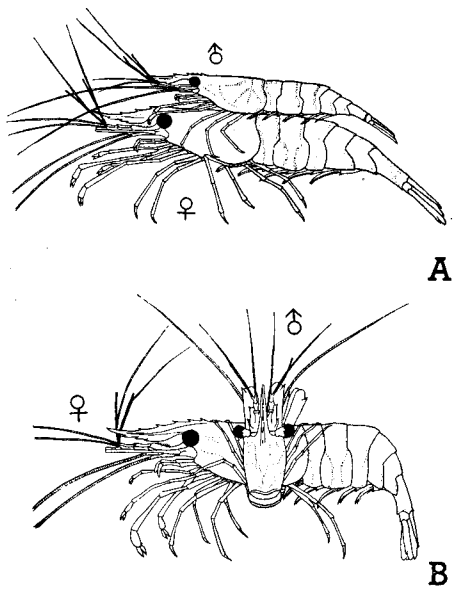

Fig. 1. Mating behavior of the freshwater prawn, *Palaemon paucidens*. A. Phase II, mounting; B. Phase III, copulation. For further explanation, see text.

Table 1. The number of copulation performed by twenty post-parturial molt females, each singly placed together with thirty mature males in an aquarium, as calculated from the number of spermatophores attached on females.

No. of matings (spermatophores)	0	1	2	3	4	5	6	7	8
No. of females	0	0	0	1	4	9	5	1	0

hang on female's thoracic sternum at the right angle turning over his back (Fig. 1, B), and deposits the spermatophore there. The time required for completing mounting and copulation altogether is about 5–6 seconds. As the prawn is polygamous, most females that are kept together with males in a large aquarium are seen to have more than one spermatophore. Actually, judging from the number of spermatophores attached on females, 75% of females copulated more than five times with different males (Table 1). A male is polygamous, too, and if a female he once copulated with in an aquarium is replaced by a newly molted female he is attracted to the newcomer and immediately copulates with her.

Copulatory behavior of the male in different intermolt stages

Sexually mature males (9–12 mm in carapace length) in the intermolt stages A, B, C, D_1 and D_{2-3}, respectively, were placed together with the females just completed the parturial molt. Results of the observation on fifty males in total number are summarized in Fig. 2. Males at stages C and D_1 had a better chance to copulate (90% and 80%, respectively) than those at stages A, B, and D_{2-3} (0%, 30% and 30%, respectively). This is in accordance with the data on the distribution of each intermolt stage of mature prawns collected shortly before and during the breeding season (Fig. 3). Of the animals collected on April 21 (about one month before the onset of breeding season), 86% of males and 93% of females were in stage D of an intermolt cycle, whereas in the animals collected on May 28 and checked on June 1 (at a beginning of the breeding season), number of the males in stage D decreased to 15% and those in stage C increased to 78% and not much change was observed in the number of females at stage D during the period. Judging from the damage and dirt of their shell the majority of females

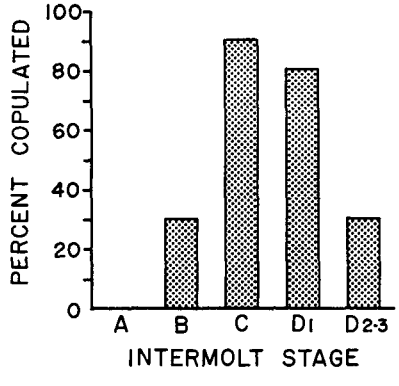

Fig. 2. The number of mating males at different stages of the intermolt cycle.

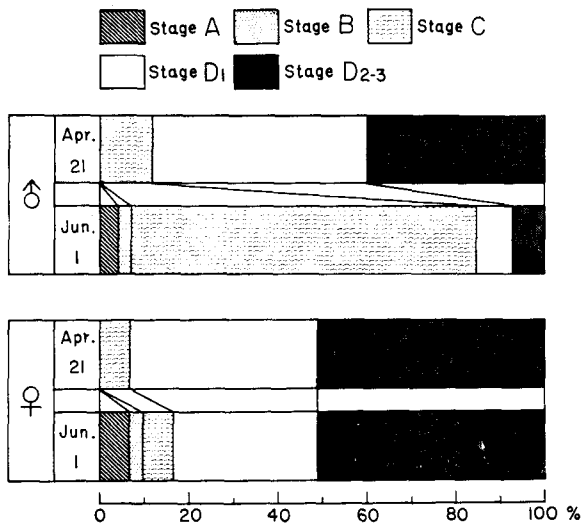

Fig. 3. The number of prawns at each intermolt stage (stages A, B, C, D_1 and D_{2-3}) in the populations collected a month before (April 21) and at the beginning (June 1) of the breeding season.

(83%) had not molted during the period from April 21 to June 1, whereas most males had molted at least once during the same period. These results seem to show that gametogenesis proceeds most actively in the males that have molted at least once and in the females that have not molted through the winter months until the beginning of breeding season.

Possible existence of sex attractant in Palaemon

A series of experiments was designed to know whether male *Palaemon* are really attracted to females through chemical means. On the basis of foregoing results, only the stage C males ranging 9–12 mm in carapace length were used in the following experiments. Five such males were placed in a container together with a female prawn at a certain stage of the intermolt cycle and copulatory response of the males was observed. Also the water of a container in which the parturial mot took place was secured and its effect on the behavioral response of the males was examined. Premolt (stage D_3) females with fully grown ovaries were placed singly in an individual glass vat and allowed to perform parturial molt in it, producing 800 ml of "post-parturial molt-water" (PPM-water). Fifteen minutes after the molt, the prawn was replaced by an experimental female of different physiological conditions; intermolt, premolt or postmolt (non-parturial) females. Then, five stage C males were added to the vat and their mating behavior was observed. Results of all of these experiments are summarized in Fig. 4.

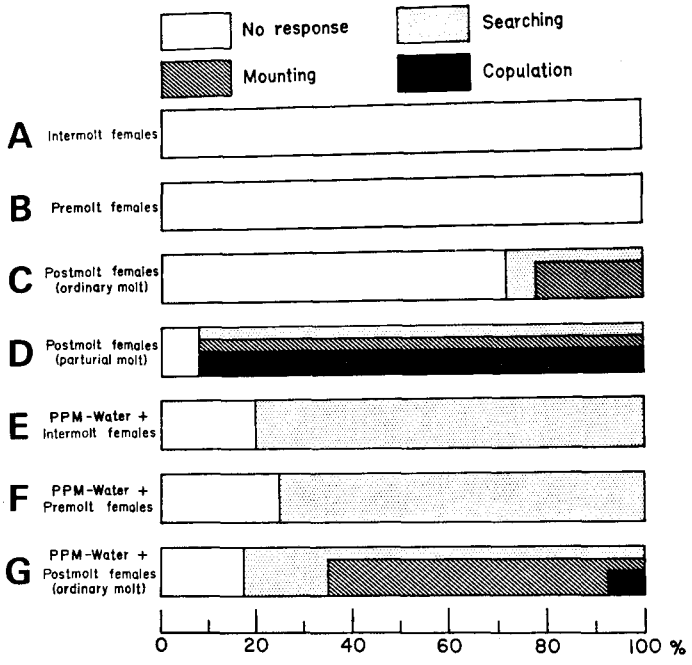

Fig. 4. Behavioral response of male *Palaemon* to females at different intermolt stages (A-D). The PPM-water, or the post-parturial molt-water, in which the copulation-ready females had molted, was also tested together with females at different intermolt stages (E-G). Each column represents the results of tests on fifty males.

No copulatory response was observed in males that were placed together with the intermolt (stage C) or premolt (stage D) females (Fig. 4, A and B). It is interesting to note that 14 out of 50 test males responded by showing searching behavior and 11 of them by mounting behavior to the females just after the ordinary molt (stage A), but none of them performed copulation (Fig. 4, C). To the females immediately after the parturial molt (stage A), on the contrary, forty-six out of 50 males responded by searching, mounting and copulation (Fig. 4, D). When such a female placed in a rubber-plugged glass tube (3 cm in diameter and 9 cm in length) was put into a vat containing males, no behavioral response was observed at all among the latter. These results clearly indicate that the searching behavior of males is induced by the sex attractant released by a female during and after the parturial molt. Presence of an intermolt (Fig. 4, E) or premolt (Fig. 4, F) females in PPM-water did not produce more than Phase I (searching) response in males. However, if a soft-shelled female that had just completed an ordinary, non-parturial, molt was placed in PPM-water together with males, the latter

responded not only by searching (82%) but by mounting (52%) or even copulation (8%) (Fig. 4, G). From these results it may be concluded that the soft shell of the female is another important factor for inciting males to mating behavior.

Time of effectiveness of the sex attractant

To estimate the time of effectiveness of the sex attractant, a number of males were put together with a female at various intervals after the parturial molt, and their mating behavior was observed. The results are shown in Fig. 5. Ninety-two percent of the males copulated with the females immediately after the parturial molt and 60% males copulated with the females 30 minutes later. Thereafter, the rate of copulation fell rapidly and only one-third of males mated with

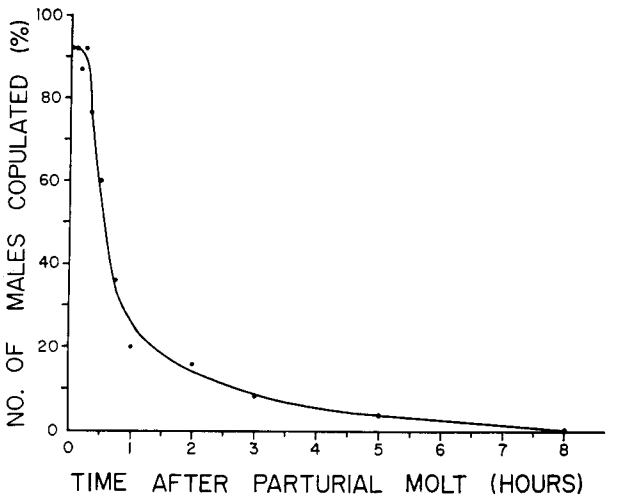

Fig. 5. The relation between the time lapse following the parturial molt of females and the number of copulations performed by males. Each point on the graph represents the average of the tests on twenty-five males, five at a time.

females 45 minutes after the molt and 16% or one-sixth of males to females at 2 post-molt hours. During this period males did not actively search around the female and some males copulated only when they came across the female by chance. Three to five hours after the parturial molt, only one or two out of 25 test males mated. No male mated with a female 8 hours after the parturial molt or later. These results suggest that the substance from mature female is effective as sex attractant only for a limited time, 30 minutes at most, after the parturial molt.

Discussion

In the fiddler crab, *Uca lactea*, females molt neither before nor after the copulation (Yamaguchi, 1971). However, in many crustaceans there seems a clear relationship between the molt and mating. Thus, a male king crab, *Paralithodes camtschatica*, "searches out a female that he senses is preparing to molt and claims her by grasping her front legs with his stout claws" (Idyll, 1971). Similar attraction of males to premolt females is reported of the large edible crab, *Portunus sanguinolentus* (Ryan, 1966). In the American lobster, *Homarus americanus*, on the other hand, sexual attraction is observed in the newly molted females (McLeese, 1970; Atema and Engstrom, 1971). In the present species, *Palaemon paucidens*, males showed sexual attraction only to a female that had just performed the parturial molt.

The mating behavior of male *Palaemon* is divided into three phases: searching, mounting and copulation. Phase I of the sexual response or the searching is seen when males are put into the PPM-water, "post-parturial molt-water", either with or without females. Therefore, it is concluded that the sexual attraction in this species is not caused by ordinary exuvial fluid but by sex pheromone released from females at this particular molt.

According to their mode of action, Wilson (1963) has categorized the pheromones into two groups, the "releasers" and the "primers". The releaser pheromone stimulates the recipient's central nervous system into producing an immediate change in behavior, whereas the primer pheromone alters a set of long-term physiological conditions so that the recipient's behavior can subsequently be influenced by specific accessory stimuli. The sex pheromone in general is said to belong to the releasers. In *Palaemon*, however, the sex attractant alone was not effective enough in arousing a series of mating behavior in males, and an aid of physical stimuli from female's soft shell was an important factor for completion of mating behavior in males. Moreover, presence of a soft-shelled female after an ordinary (non-parturial) molt in PPM-water produced a relatively high rate of responses (Phase I, 82% and Phase II, 52%) in males but very few males (8%) proceeded to Phase III. It remains, therefore, to be elucidated whether another "short-distance pheromone" is liberated from the post-parturial molt females which, through the substance from the sternal gland, have already attracted nearby male prawns close to her.

The sex pheromone in aquatic animals presents a new problem to their ecology. In lepidopterans such as the gypsy moth, transmission of the sex attractant in the air is partly facilitated by the wind (Wilson, 1963). For an aquatic animal living in the stream, the pheromone released by a female in water running too fast would be completely meaningless to nearby males. Although *Palaemon* is widely distributed in ponds, lakes, and streams throughout the year, soon after the quiescent winter months they occur most abundantly in running water. However, with the onset of breeding season in May they start to migrate

from running water to still water, and swarm inshore of the lakes and ponds or in creeks with stagnant water. Apparently the seasonal migration from running to still waters of *Palaemon* before the breeding season would provide a favorable condition for transmission from females and detection by males of the sex attractant, and subsequent successful matings. Fast loss of effectiveness (about 30 minutes following liberation) of the sex attractant is apparently favorable for the prawns to avoid confusion since in a population many females liberate the pheromone in a considerably short period of time.

Further, a circumstantial evidence in favor of the presence of sex pheromone in *Palaemon* is provided by the fact that the male has much developed sensory

Table 2. Comparison of the length of the inner branch of bifurcated outer flagellum of the antennule and of the number of its sensory hairs between the sexes.

Sex	*No. of animals used*	*Relative length of inner branch* $\left(\frac{\text{inner branch length (mm)}}{\text{carapace length (mm)}} \times 100\right)$	*Total number of sensory hairs on the inner branch*
♂	30	52.1±3.6	376.0±20.7
♀	30	31.8±3.0	197.2±15.8

(possibly chemoreceptive) hairs on the inner branch of the bifurcated outer flagellum of the antennule than the female (Table 2). Sexual dimorphism in non-reproductive (sensory) organs such as these hairs is assumed to be due to different mating behavior of the two sexes.

At present, little is known of the chemical nature of the pheromone. However, a histological study on the possible site of origin of such a substance has been carried out and will be published elsewhere (Kamiguchi, 1972).

Summary

1) Mating behavior in the freshwater prawn, *Palaemon paucidens*, was investigated with special reference to the response of males to the females at different physiological conditions.

2) Most of the large (9–12 mm in carapace length), sexually mature and copulation-ready males were at stages C or D_1 of an intermolt cycle during the breeding season. Those males, similarly large but at stages A, B, or D_{2-3}, had a poorer chance to copulate.

3) Males displayed sexual attraction to the female soon after the parturial molt. On the contrary, those females soon after the ordinary, non-parturial, molt failed to incite mating behavior in males.

4) The water of an aquarium in which the parturial molt had taken place was effective in inducing searching behavior in males even in the absence of female prawns.

5) It is concluded that in this species the sex pheromone from post-parturial molt females plays an important role for initiation of the mating behavior in males.

6) The soft-shelled condition of a postmolt female was found to be very important for the continuation of mating behavior in males.

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