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ANTENNULE ABLATION, SEX DISCRIMINATION, AND MATING BEHAVIOR IN THE CRAYFISH *PROCAMBARUS CLARKII*

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ABSTRACT

In order for the crayfish *Procambarus clarkii* to mate, each animal involved must identify the sex of the other. Crayfish are able to use chemoreception, mediated by the first antennae (antennules), as well as vision in sex identification. The relative importance of these two senses is not known; most work has centered on the use of the antennules. To assess the importance of antennules we studied mating in control pairs, in pairs where the females lacked antennules, in pairs where males lacked antennules, and in pairs where both lacked antennules. Ablation of antennules did not significantly affect the likelihood of mating or the delay before mating began. These findings demonstrate that *P. clarkii* can readily identify the sex of conspecifics without the use of their antennules. If the ability to identify sex had been impaired, one would expect mating to be less likely or to follow an unusually long delay. In addition, antennule ablation did not significantly affect the duration of mating. This stands in contrast to findings in the green crab *Carcinus maenas*, where the ablation of antennules in males leads to the substitution of multiple short matings for single long, continuous matings. It is concluded that *P. clarkii* can readily determine the sex of conspecifics without the use of the antennules and that the loss of antennules has no obvious effect on mating behavior.

A major chemosensory structure of decapod crustaceans is the lateral branch of the biramous first antennae (antennules). This appendage contains many aesthetasc sensilla which have a thin, porous cuticle and contain the chemosensory endings of large numbers of sensory cells (Ghiradella *et al.*, 1968; Tierney *et al.*, 1986; Laverack, 1988). In contrast, the chemosensory sensilla found on other regions of the body have a much thicker cuticle and are innervated by only a handful of chemosensory cells (Laverack, 1988). The importance of antennules is illustrated by findings which show that crayfish can use chemosensory information to determine the sex of a conspecific only if their antennules are intact (Ameyaw-Akumfi and Hazlett, 1975; Dunham and Oh, 1992). Other chemosensory sites cannot perform this function.

If antennules are indeed important for sex recognition, one would expect the loss of antennules to perturb mating behavior. Work on a number of crustaceans has consistently shown that antennule ablation or restriction interferes with normal courtship or mating (Christofferson, 1972; Gleeson, 1980; Cowan, 1991; Bamber and Naylor, 1996), while absence of vision does not (Gleeson, 1980; Snyder *et al.*, 1992). We performed antennule ablations in the red swamp crayfish *Procambarus clarkii* (Girard) to determine the importance of antennule chemoreception in mating behavior and, by inference, in sex discrimination. This species is capable of using both chemoreception and vision in sex discrimination (Ameyaw-Akumfi and Hazlett, 1975; Dunham and Oh, 1996).

MATERIALS AND METHODS

Reproductively active (form I) males and adult female *Procambarus clarkii* with carapace lengths ranging from 45–68 mm were obtained from Wauban Labs (Schriever, Louisiana) and maintained at room temperature in individual aquaria on a 14:10 h light-dark cycle. Three times per week animals were fed either carrot, potato, or chicken, and the water in the aquaria was replaced with filtered, dechlorinated, deionized tap water (Instapure Water Filter®, Teledyne Water Pik®, Fort Collins, Colorado). Crayfish were acclimated to these laboratory conditions for at least 14 d before being tested. Experiments were performed during June through August of 1997 and 1998. All crayfish had at least 3 ambulatory pereiopods on each side, 2 intact first pereiopods (large claws), 2 intact eyes, 2 antennae both of which were at least as long as the carapace, and 2 intact biramous antennules (prior to ablation). Since crayfish were kept separate from one another, their only opportunity to mate was while being tested. This ensured that at least a large number of them were highly motivated to mate.

One to 3 d before testing, crayfish were briefly removed from their aquaria and their carapace lengths were measured. If their antennules were to be ablated, then at this time both filaments of their antennules were excised as close to their bifurcation as possible. Ablation of antennules within 3 d of testing prevented 2 problems that can occur when longer recovery periods are allowed: antennules can regenerate (Cowan, 1991) and the sensitiv-

ity of chemoreceptor cells on other appendages can increase dramatically (Hazlett, 1971).

For testing, a pair of crayfish was placed in a circular glass bowl measuring 19 cm in diameter and filled with filtered, dechlorinated, deionized tap water to a depth of ~6 cm. Pairs were chosen so that their carapace lengths differed by no more than 3 mm. The bowl was surrounded by a circular piece of stiff white paper, 29 cm high, which prevented animals from both seeing out of their container and escaping from it. Confinement of crayfish to such a small space ensured that they could not wander about without encountering each other. Allowing them to wander would have resulted in highly variable delays before the onset of mating and, because the delay could exceed the duration of the test, and would have resulted in highly variable likelihoods of mating. Since the likelihood of mating and the delay until mating were used as indicators of sex discrimination, it was essential to obtain useful measures of both. Thus, these experimental conditions were chosen so that sex discrimination could be assessed accurately.

Each pair was videotaped for 1 h. If a pair were mating at the end of the hour, they were taped until the mating ended. Crayfish were considered to be mating if they were positioned with their ventral surfaces closely apposed for more than 2 min. While matings that lasted only a few minutes may not have allowed significant transfer of sperm, such short matings clearly constituted a kind of mating behavior. Individuals were assigned randomly to 1 of 4 groups of pairs: controls in which antennules of both animals were intact, female ablations with males intact, male ablations with females intact, and double ablations where both partners lacked antennules. Eleven pairs were studied in each group. No crayfish was used more than once. In each experiment the number of matings that occurred (0, 1, or 2), the length of time between the introduction of the second individual into the bowl and the beginning of the first mating (delay to mating), and the duration of the first mating were determined.

Results

Among the controls, four pairs did not mate, five pairs mated once, and two pairs mated twice (Fig. 1). Among the female ablations, four pairs did not mate, six pairs mated once, and one pair mated twice. Among the male ablations, five pairs did not mate, four pairs mated once, and two pairs mated twice. In the double ablations, seven pairs did not mate, one pair mated once, and three pairs mated twice. For a pair to mate twice the second mate must obviously begin within the hour-long observation time. Therefore, a pair was more likely to mate twice if the first mating occurred following a short delay rather than a long one, and if the first mating lasted only a short time. Because of these factors, we chose to ignore the second mating in statistical analyses. If we simply consider whether or not a pair mated, and then compare the four groups with a contingency table, we find that ablation did not significantly affect the likelihood of mating (χ^2 = 2.2, *d.f.* = 3, $P > 0.50$).

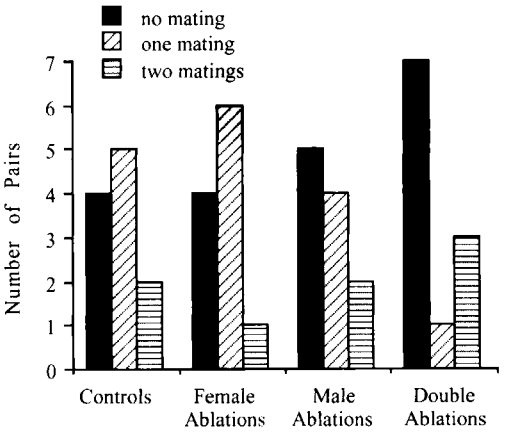

Fig. 1. Numbers of pairs of *Procambarus clarkii* that did not mate, mated once, and mated twice in control, female ablation, male ablation, and double ablation groups.

Despite the fact that the experimental chamber closely confined the animals, there was still considerable variability in the delay to mating. In one female ablation pair, this period lasted only 3.6 min, whereas in one control pair it lasted 53.0 min. Nevertheless, mean delays in the four groups were comparable (Fig. 2), and there was no significant difference between the four groups in delay to mating (1-way ANOVA, $F_{3,20} = 0.618$, $P > 0.25$).

Considerable variability was also found in the duration of the first mating. One control pair mated only 5.6 min, while one female ablation pair mated for 99.6 min. Several long matings also occurred in the male ablation group; one lasted 96.4 min. The long matings in the female and male ablations inflated both the means (Fig. 3) and variances of these groups. To determine whether there was significant heterogeneity of variance, we performed an analysis of Z-scores (Keppel, 1991). Variances differed significantly ($F_{3,20}$ = 3.36, $P < 0.05$). Because of this heterogeneity, the nonparametric Kruskal-Wallis test was used to analyze the data. There was no significant difference between the four groups in the duration of the first mating (H = 5.49, *d.f.* = 3, $P > 0.10$).

Discussion

In the double ablation group, mating occurred in only four out of eleven pairs, while in all other groups larger numbers of pairs mated (Fig. 1). At first this appears to suggest

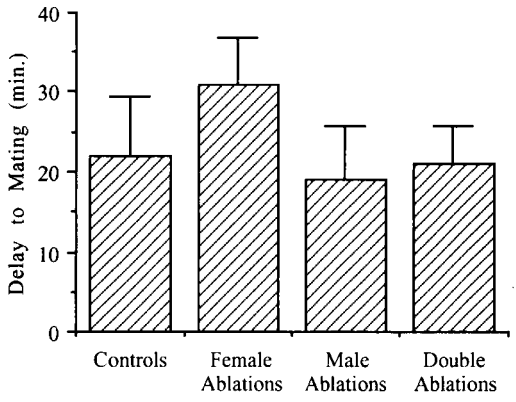

Fig. 2. Mean delay to mating in *Procambarus clarkii* in control, female ablation, male ablation, and double ablation groups. Bars show standard error.

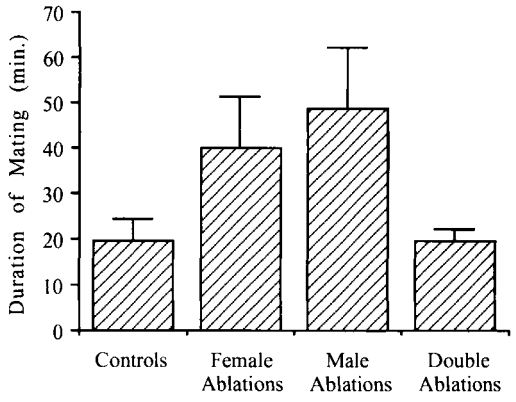

Fig. 3. Mean duration of the first mating in *Procambarus clarkii* in control, female ablation, male ablation, and double ablation groups. Bars show standard error.

that antennule ablation reduces the likelihood of mating. However, these results were far from being statistically significant ($P > 0.50$). We conclude that antennule ablation does not affect the likelihood of mating. This conclusion implies that antennule use is not necessary for sex discrimination in *P. clarkii*.

Antennule ablation does not affect the delay before the onset of mating. Even in pairs in which the antennules of both animals were ablated, the average delay to mating was nearly the same as in the control group (Fig. 2). If the ability of these animals to identify the sex of a conspecific were impaired but not eliminated by antennule ablation, one might expect that it would take longer for an animal to accomplish sex discrimination, and this in turn would delay mating. The finding that mating was not delayed implies that not only is antennule use not necessary for sex discrimination, but the loss of antennules does not measurably impair this ability, at least at close proximity. Crayfish must rely primarily on other appendages or senses for sex discrimination. While some studies have documented the use of chemoreception for sex discrimination in crayfish (Ameyaw-Akumfi and Hazlett, 1975; Hazlett, 1985; Dunham and Oh, 1996), other studies have failed to confirm this ability in *P. clarkii* (see Itagaki and Thorp, 1981) and to demonstrate it in *P. acutus* (see Thorp and Ammerman, 1978). Differences in species and experimental design may account for this discrepancy. Findings presented here, however, again call into question the importance of the use of antennules for sex discrimination in *P. clarkii*.

Whereas antennules are considered to be the major chemosensory site of crayfish, chemosensory sensilla are present in large numbers on other appendages as well (Bell, 1906; Derby, 1982). Therefore, it is possible that crayfish use nonantennule chemoreception for sex discrimination. Previous work, however, has demonstrated that, without antennules, crayfish cannot use chemoreception for sex discrimination (Ameyaw-Akumfi and Hazlett, 1975; Dunham and Oh, 1992); other chemosensory sites do not serve in this capacity. Still, these studies used different experimental conditions than those which we employed. Perhaps *P. clarkii* can use other chemosensory sensilla for sex discrimination if the crayfish are in close proximity to one another, as they were in this study.

Crayfish may also use vision for sex identification. This may seem unlikely, since these animals are most active at night (Penn, 1943), when their dimorphic features would not be conspicuous to other crayfish. Other crustacean species will mate when vision-deprived (Gleeson, 1980; Snyder *et al.*, 1992). However, *P. clarkii* can use vision in sex discrimination (Dunham and Oh, 1996), and precopulatory behavior appears to involve certain submissive postures such as "curled telson" (Ameyaw-Akumfi, 1981) that could be recognized visually. Postural cues may also be important in lobster sexual behavior where intersexual pairs share a shelter prior to mating. When a male lobster attempts to enter the shelter of another male, the two animals fight. When a female enters, she adopts a different posture that apparently reduces aggression on

the part of the resident male and he allows her to stay (Bushmann and Atema, 1997). Posture may be assessed not only by vision but also by touch. Extensive precopulatory touching has been described in *P. clarkii* (Ameyaw-Akumfi, 1981) and in the crayfish *Orconectes nais* (Faxon) (see Pippett, 1977).

In this study, we used likelihood of mating and the delay to mating as indicators of sex discrimination. Others have approached this topic differently. Typically investigators have monitored a number of behaviors such as meral spread, grooming, etc., and determined whether their frequency of occurrence could be affected differently by exposure to male or female conspecifics (Ameyaw-Akumfi and Hazlett, 1975; Hazlett, 1985; Snyder *et al.*, 1992, 1993; Dunham and Oh, 1996). One weakness to this approach is that, if one looks at many behaviors, one is forced to perform many statistical tests. This increases the probability that one will encounter a seemingly significant effect simply by chance, i.e., that one commits a type I error (Keppel, 1991). In this study, we performed only two statistical tests to assess sex discrimination, thus limiting the probability of a type I error.

The weakness of our approach is that it assumes that mating requires sex discrimination. In other species, this assumption has been challenged; rare homosexual matings have been reported and males bearing spermatophores have been collected from the field (Pearse, 1909; Mason, 1970). However, in *P. clarkii* evidence shows that sex discrimination is a necessary prerequisite for mating. Ameyaw-Akumfi (1981) found that male *P. clarkii* attempt to mate only with females, never with other males. Instead, intrasexual encounters are marked by aggression. Sex discrimination is also a prerequisite for mating in *Orconectes nais*, where Pippett (1977) found that males expressed precopulatory behavior toward other males only twice in 2,667 encounters. Recently, it was reported that female *P. clarkii* may attempt to mate with other females (Kasuya *et al.*, 1996), but this occurred when males were also present. Thus, if crayfish use chemical cues for sex identification, both female- and male-identifying cues may have been confused. In our experience, intrasexual encounters are aggressive, whereas intersexual encounters typically are not and often result in mating.

Our procedure was designed so that mating could be used to assess sex discrimination. This required a small container to limit variability in the likelihood of mating and delay to mating. Despite these unnatural conditions, seemingly normal mating behavior occurred. Mating proceeded as described by Ameyaw-Akumfi (1981) with one exception; in our study, initial encounters were usually not marked by aggression. *Procambarus clarkii* is thought to mate when they encounter each other by chance (Penn, 1943). They do not coordinate mating with molting, as found in other crustaceans. Our sudden placement of two animals together appears to have sufficiently mimicked the natural scenario for normal mating to occur.

Not only did normal mating occur in the unnatural conditions we imposed, but this behavior was not affected by antennule ablation in *P. clarkii*. In contrast, antennule ablation or restriction can have profound effects on the behavior of other crustaceans. In *Portunus sanguinolentus* (Herbst) (see Christofferson, 1972), the blue crab *Callinectes sapidus* Rathbun (see Gleeson, 1980), and the green crab *Carcinus maenas* (L.) (see Bamber and Naylor, 1996) antennule ablation or restriction inhibits male courtship behavior. In addition, antennule restriction in *C. maenas* causes animals to mate for several, relatively short, periods of time rather than for one long continuous period (Bamber and Naylor, 1996). In our study, we measured mating duration specifically to determine if the same occurs in *P. clarkii* as in *C. maenas*; it does not. In the lobster *Homarus americanus* H. Milne Edwards, ablation of male antennules has subtle behavioral effects, but the effects of ablating female antennules are more pronounced; females lacking antennules fail to cohabit with males for the period surrounding their molt. Instead, they molt and mate in the open where they are likely to be killed while soft and prone to cannibalism (Cowan, 1991). Mating behavior is a complex phenomenon in crustaceans and further studies are necessary to elucidate the roles of the various signals and senses involved.

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