Chemical Communication in the Reproductive Isolation of the Crayfishes Orconectes propinquus and Orconectes virilis (Decapoda, Cambaridae)

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CHEMICAL COMMUNICATION IN THE REPRODUCTIVE ISOLATION OF THE CRAYFISHES *ORCONECTES PROPINQUUS* AND *ORCONECTES VIRILIS* (DECAPODA, CAMBARIDAE)

Ann Jane Tierney and D. W. Dunham

ABSTRACT

Laboratory experiments demonstrate that chemical cues are important in species recognition in the crayfishes *Orconectes propinquus* and *Orconectes virilis*. Males and females of both species can perceive the chemicals released from their own and the other species and are attracted only to the chemicals of conspecifics of the opposite sex.

Chemical cues are important in the social behavior of many decapod crustaceans, including crayfish. Little (1975, 1976) demonstrated that larval crayfish use chemical cues to distinguish brooding from nonbrooding females. Thorp and Ammerman (1978) showed that male crayfish recognized a "stress pheromone" released by agonistically interacting conspecifics. Most work on chemical communication in crustaceans has focused on the use of pheromones in reproductive behavior (Dunham, 1978). Sex pheromones have been reported in lobsters (Atema and Engstrom, 1971; Atema *et al.*, 1979; Dunham, 1979), crabs (Ryan, 1966; Eales, 1974; Gleeson, 1980), and shrimp (Kamiguchi, 1972). For crayfish Ameyaw-Akumfi and Hazlett (1975) demonstrated that male *Procambarus clarkii* use chemical cues to distinguish male from female conspecifics. Crayfish reacted aggressively when exposed to water from a tank containing a male conspecific, but showed submissive behavior to water from a tank containing a female.

In crabs (Ryan, 1966; Eales, 1974) sex pheromones are species specific and may contribute to reproductive isolation among sympatric species. Morphologically and behaviorally similar species of crayfishes commonly occur sympatrically without apparent hybridization (Fitzpatrick, 1967), suggesting that chemical cues may likewise be important reproductive isolating mechanisms in this group. We performed three experiments to investigate the use of chemical cues in species recognition in the crayfishes *Orconectes propinquus* and *O. virilis*. The first two experiments determined the response of crayfish to water conditioned by the presence of conspecifics or heterospecifics. The third experiment investigated the importance of chemical versus visual and tactile cues from a female in male behavior toward females of each species.

METHODS AND MATERIALS

We collected reproductively active males (Form 1, see Crocker and Barr, 1968) and mature females of both species from Lake Simcoe (Ontario County, Ontario) during July and August 1981. All animals were isolated for 48 h prior to testing, all were fed once a day, and each crayfish was used in only one experiment.

Experiment 1 used a chamber 18 cm wide × 50 cm long × 15 cm high, with a water inflow hole 3 mm in diameter in one corner of the chamber, 3 cm above the chamber floor (Fig. 1). An outflow hole, 3 mm in diameter, was located at the far end of the chamber, 10 cm above the floor. The chamber floor was covered evenly with black gravel and a shelter made from black plastic piping, 5 cm in diameter and 10 cm long, was directly below the outflow hole. Throughout testing, water from 38 l head tanks was siphoned through the chamber at a rate of 150 ml/min. Head tanks contained control water (dechlorinated tap water) or test water conditioned for 48 h by 4 or 5 stimulus crayfish.

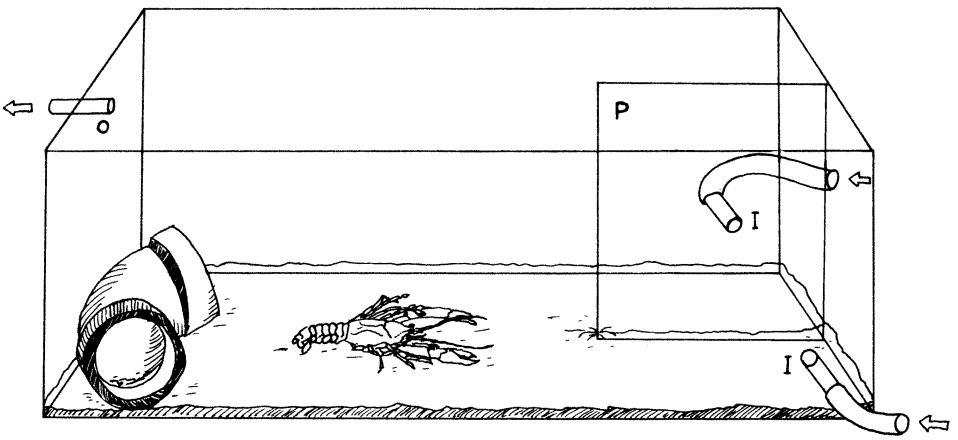

Fig. 1. Test chamber used in crayfish experiments. I = inflow holes, with tubing that delivered the water indicated by arrows. O = outflow hole with attached tubing. F = partition present in Experiment 2 only. Dimensions are given in the text.

In each test a crayfish was placed singly in the chamber and allowed to acclimatize for 30 min while control water flowed through the chamber. For the following 30 min either control water continued to enter the chamber or the tubing was switched to allow test water to flow through. During the latter period we used stopwatches to record the amount of time the test crayfish spent moving across the substrate, in maintenance activities (feeding, grooming), and motionless, both within 3 cm of the inflow hole and elsewhere in the chamber. These mutually exclusive categories described all of the animals' behavior.

For Experiment 2 the chamber was modified by adding a second inflow hole in the corner directly across from the first inflow hole (Fig. 1). A clear panel, 18 cm long × 15 cm high, set between the holes parallel to the long sides of the chamber, formed a two-choice maze. As before, crayfish were allowed 30 min to acclimatize to the chamber, then tested for 30 min. During testing conspecific conditioned water entered one inflow hole and heterospecific conditioned water entered the opposite hole. We recorded the amount of time the crayfish spent within 3 cm of each hole. Males were tested with female conditioned water from both species, females with male conditioned water from both species.

In the third experiment males were placed alone in a tank 52 cm long × 25 cm wide × 30 cm high containing 30 l of water which had previously been conditioned for 48 h by a single female. After a 30 min priming period a female was introduced to the male's tank and the pair were permitted to interact freely. Four groups of males were tested: in group 1 male *O. propinquus,* primed either with *O. propinquus* or *O. virilis* female conditioned water, interacted with female conspecifics; in group 2 similarly primed male *O. propinquus* interacted with female *O. virilis*. Groups 3 and 4 repeated these tests with male *O. virilis*. We recorded the following data for 30 min after female introduction: 1) latency of male's first turn toward female; 2) number of turns toward female; 3) number of approaches to within 1 cm of the female; and 4) time spent pursuing female and/or in attempted copulation with female.

Nonparametric tests were used in all analyses (Siegel, 1956).

Results and Discussion

The results of experiment 1 (Table 1) show that: 1) crayfish exposed to conditioned water spent more time near the inflow hole than did control animals (except male *O. propinquus* receiving female *O. virilis* water); 2) male and female *O. propinquus* spent more time near the inflow hole when receiving conspecific conditioned water than when receiving heterospecific conditioned water; 3) male and female *O. virilis* spent more time moving elsewhere in the chamber when receiving conspecific conditioned water than when receiving heterospecific con-

Table 1. Percent of total time crayfish (*Orconectes propinquus* and *O. virilis*) spent moving within 3 cm of inflow hole and elsewhere in the chamber in response to control and conditioned water. Remaining time was spent performing maintenance activities and motionless elsewhere in the chamber. Probabilities are from the Mann-Whitney U-test.

Test crayfish	n	Stimulus water	% time moving 3 cm from inflow hole		% time moving elsewhere	
♂ *propinquus*	12	control	11.15		64.00	
♂ *propinquus*	12	♀ *propinquus*	35.39**	††	51.26*	
♂ *propinquus*	12	♀ *virilis*	9.38		61.98	
♂ *virilis*	12	control	0.68		19.48	
♂ *virilis*	12	♀ *propinquus*	25.55**		21.18	
♂ *virilis*	12	♀ *virilis*	19.22**		45.79*	††
♀ *propinquus*	12	control	4.11		61.43	
♀ *propinquus*	12	♂ *propinquus*	16.19*	†	42.28*	
♀ *propinquus*	12	♂ *virilis*	8.33		40.84*	
♀ *virilis*	12	control	4.09		42.98	
♀ *virilis*	12	♂ *propinquus*	7.59		28.28	†
♀ *virilis*	12	♂ *virilis*	9.31*		54.39	

* Significantly different from group control at $p \leq 0.05$.
** Significantly different from group control at $p \leq 0.01$.
† Response to *propinquus* water significantly different from response to *virilis* water at $p \leq 0.05$.
†† Response to *propinquus* water significantly different from response to *virilis* water at $p < 0.01$.

ditioned water. Also, maintenance behavior was generally reduced in all crayfish exposed to test water from either species compared to those exposed to control water. This trend was significant in male *O. propinquus* exposed to female conspecific conditioned water ($p \leq 0.01$, Mann-Whitney U-test) and female *O. propinquus* exposed to male conditioned water from either species ($p \leq 0.05$, Mann-Whitney U-test). These observations show that crayfish react to chemicals from both species and distinguish conspecifics from the other species.

Experiment 2 demonstrated more clearly the ability of crayfish to discriminate between species. Individuals of both species and both sexes showed a significantly higher attraction to conspecific conditioned water than to heterospecific conditioned water (Table 2). Attraction was measured only by the amount of time spent near inflow holes as the crayfish showed no reliable qualitative differences in behavior toward different types of stimulus water.

In groups 1 and 2 of experiment 3 the latency of male *O. propinquus* response (first turn toward introduced females) was shorter in males primed with conspecific conditioned water than in those primed with heterospecific conditioned water (significant in group 1, Table 3). Also, within each group differently primed males

Table 2. Response of crayfish (*Orconectes propinquus* and *O. virilis*) in a two-choice maze to water conditioned by conspecifics and heterospecifics of the opposite sex. Probabilities are from the Wilcoxon matched-pairs signed-ranks test.

Test crayfish	n	% time 3 cm from *propinquus* inflow hole	% time 3 cm from *virilis* inflow hole	p
♂ *propinquus*	12	25.11	3.04	0.01
♀ *propinquus*	12	22.98	8.38	0.025
♂ *virilis*	12	4.38	25.75	0.005
♀ *virilis*	12	4.75	24.00	0.005

Table 3. Response of male crayfish (*Orconectes propinquus* and *O. virilis*) to conspecific and heterospecific females after chemical priming. Mean latency is expressed in minutes and seconds. Probabilities are from the Mann-Whitney U-test.

Group	Test male	n	Priming female	Introduced female	Mean latency	Number turns	Number approaches	% Time pursuing/ copulation
1	*propinquus*	10	*propinquus*	*propinquus*	51 s	78	115	36.15
	propinquus	10	*virilis*	*propinquus*	3 m 27 s*	35**	30***	35.09
2	*propinquus*	10	*propinquus*	*virilis*	2 m 27 s	46	62	11.55
	propinquus	10	*virilis*	*virilis*	4 m 32 s	32	44	7.15
3	*virilis*	10	*propinquus*	*propinquus*	5 m 13 s	28	24	7.83
	virilis	10	*virilis*	*propinquus*	1 m 41 s	21	74*	6.67
4	*virilis*	10	*propinquus*	*virilis*	10 m 57 s	16	16	19.48
	virilis	10	*virilis*	*virilis*	4 m 9 s	15	16	26.54

* Within group difference significant at $p \leq 0.05$.
** Within group difference significant at $p \leq 0.01$.
*** Within group difference significant at $p \leq 0.001$.

directed more turns and approaches to introduced females after conspecific priming (significant in group 1, Table 3). In group 2, males also showed a tendency to spend more time pursuing and copulating with *O. virilis* females after conspecific priming. However, a between group comparison indicates that, regardless of priming, males pursued and attempted to copulate more with *O. propinquus* than with *O. virilis* females ($p \leq 0.01$, Mann-Whitney U-test).

The latency of the male *O. virilis* (groups 3 and 4) response to introduced females showed a trend (nonsignificant) similar to that of male *O. propinquus*; it was shorter in males primed with conspecific conditioned water than in those primed with heterospecific conditioned water. In group 3, males also directed more approaches to *O. propinquus* females after experiencing conspecific priming (Table 3). A between group comparison shows differences between male *O. virilis* and *O. propinquus* response to introduced females. Male *O. virilis* generally responded sooner to female *O. propinquus* than to female *O. virilis* and directed more turns and approaches toward the former. This result is probably due to behavioral differences between the females. Female *O. propinquus* were more active than female *O. virilis* and consequently more conspicuous to males of both species. Male *O. virilis* did, however, show a tendency to spend more time pursuing and in attempted copulation with conspecific females.

Overall, the results suggest that conspecific chemical priming causes males to be more alert and to treat initially any introduced female with more interest. That males ultimately pursue and attempt to copulate most with conspecific females indicates that they can respond to additional information, probably visual and tactile (but conceivably immediate chemical cues), from introduced females.

Chemical and visual differences between the species do not provide infallible premating isolating mechanisms. In mixed laboratory populations occasional *O. propinquus* × *O. virilis* attempted copulations were observed. Such copulations were usually brief, terminated by the escape of the female. They apparently never resulted in successful sperm transfer. Hybrids between *O. propinquus* and *O. virilis* are unknown (Capelli and Capelli, 1980) indicating that heterospecific copulations do not occur in wild populations or that postmating isolating mechanisms also operate to separate the two species. Hybrids have been found, however, between *O. rusticus* and *O. propinquus* in Wisconsin (Capelli and Capelli, 1980)

and between *O. rusticus* and *O. limosus* in Massachusetts (Smith, 1981). In both cases hybridization occurred in areas where *O. rusticus* was recently introduced. Smith (1981) suggests that chemoethological isolating mechanisms may be relaxed in allopatric crayfish allowing such hybridization to occur when populations are artificially displaced.

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