The Journal of Experimental Biology 209, 1355-1363
Published by The Company of Biologists 2006
doi:10.1242/jeb.02126

The effects of social experience on the behavioral response to unexpected touch in crayfish

Cha-Kyong Song[1], Jens Herberholz[2] and Donald H. Edwards[1,*]

[1]*Department of Biology, Georgia State University, Atlanta, GA 30303, USA and* [2]*Department of Psychology, University of Maryland, College Park, MD 20742, USA*

*Author for correspondence (e-mail: dedwards@gsu.edu)

Accepted 26 January 2006

Summary

Crayfish fight and form a dominance hierarchy characterized by a pattern of repeated agonistic interactions between animals with a consistent outcome of winner and loser. Once a dominance hierarchy is established, dominant animals display an elevated posture with both claws held laterally and forward, whereas subordinate animals display a more prone posture with both claws extended forward and down. Dominant animals behave aggressively towards the subordinate opponent, often approaching and attacking, whereas subordinate animals behave submissively by tailflipping and retreating. To evaluate whether the differences in social behavior are accompanied by differences in responses to non-social stimuli, we exposed socially naïve and experienced crayfish (*Procambarus clarkii*) to an unexpected touch in different social conditions. Socially naïve animals turned to confront the source of a unilateral touch with raised claws and elevated posture. Dominant animals also turned to face the stimulus source with raised claws and elevated posture, both when tested alone and in the presence of a subordinate opponent. Subordinate animals displayed this orienting response only while separated from their dominant partners. When paired with their dominant partners, subordinates avoided the stimulus source by walking rapidly forwards or backwards. When the subordinate animals were later tested again, first while semi-separated from the dominant and later while fully separated, they displayed a mixed pattern of avoidance and orienting responses. These results indicate that the behavioral responses of subordinate crayfish to touch depend on their social status, their current social conditions and their recent social history.

Key words: *Procambarus clarkii*, Crustacea, dominance hierarchy, behavior, touch, social condition.

Introduction

Dominance hierarchies in crayfish populations, as in other social animals, are characterized by differences in individual behavior that span the range from the aggressive displays of the most dominant to the submissive and avoidance responses of the most subordinate. Dominant crayfish often display an elevated stance, move about freely and compete aggressively for available resources, whereas subordinate crayfish adopt a more flat posture, move to avoid contact with dominant animals and give way to dominants in competitive interactions (Hayes, 1975; Livingstone et al., 1980; Bruski and Dunham, 1987; Krasne et al., 1997; Figler et al., 1999).

Dominance status affects non-social behaviors as well as social behaviors. Herberholz et al. found that burrow digging increased among new social dominants and was inhibited in new social subordinates (Herberholz et al., 2003). Similarly, an unexpected touch of the tailfan elicited an avoidance reaction from low-ranked or small crayfish and an immediate, oriented and aggressive reaction from high-ranked or large crayfish (Bovbjerg, 1953; Nagayama et al., 1986). Under the threat of unexpected attack, dominant and subordinate crayfish rely on different types of escape circuitries that produce the escape response, the tailflip (Krasne et al., 1997).

To study the effect of social context on the crayfish's response to unexpected touch more completely, we examined the behavioral responses of socially inexperienced and experienced crayfish in three different social conditions: while socially isolated for a prolonged period, while briefly isolated from a dominant or subordinate partner and while in the presence of a dominant or subordinate partner. We found that the behavioral responses of a crayfish to an unexpected touch depended on both its social status and on the social context at the time of the touch. Part of this study was previously presented in abstract form (Song et al., 2000).

Materials and methods

Animal preparation

Adult crayfish (*Procambarus clarkii* Girard), of wet body mass 4–8 g, were used in this study. Animals were purchased from a local supplier (Atchafalaya Biological Supply, Raceland, LA, USA) and divided into groups of 8–12 in communal tanks (76 l) until use. Pairs of same-sex animals, mismatched by wet body mass (>5%), were transferred to 9 l tanks (20×25×40 cm, W×H×L). An opaque plastic divider placed halfway along the long axis of the aquarium prevented the animals from seeing or touching each other but failed to isolate them chemically. The bottom of each tank was covered with gravel and the tanks were filled with continuously aerated dechlorinated tapwater. Animals were each fed with five shrimp pellets three times a week and maintained on a 12 h:12 h light:dark cycle.

Preparation of socially 'inexperienced' and experienced animals

Twenty-eight animals were isolated in their divided aquaria for 4 weeks without disturbance to eliminate the influences of previous social history (Yeh et al., 1996; Yeh et al., 1997) (Fig. 1A). Eight animals ('Isolates' in Fig. 1) remained isolated thereafter with their opaque dividers intact (Fig. 1B), while members of 10 pairs ('Social Group' in Fig. 1) were then allowed to interact and form a dominance hierarchy by removing their dividers for 30 min day^{-1} for two weeks. One of the isolated animals died during molting and two subordinate animals died or were severely injured during pairing, leaving seven isolates and eight pairs for analysis. The eight pairs were monitored continuously during their daily 30 min periods of interaction. Six pairs retained the same dominant–subordinate relationship throughout the period of pairing; however, two pairs experienced status reversal. Status reversals did not occur during the period of experiments. Social status was determined during each 30 min period of interaction by scoring the numbers of attacks, approaches, retreats and tailflips (escapes) of both members of a pair. The animal that made a higher dominance score (attack, +2; approach, +1; retreat, –1; tailflip, –2) was identified as the dominant, and its opponent as the subordinate. Dominance score (D) was calculated for all pairs during the pairing period:

$$D = [100\,(2Att + App - Ret - 2Esc)] \,/\, (2Att + App + Ret + 2Esc)\,,$$

where Att is the number of attacks, App is the number of approaches, Ret is the number of retreats and Esc is the number of escapes.

The animals were 'semi-separated' between daily interaction sessions by an open plastic square lattice (the plastic strips were 9 mm thick, 20 cm deep, and the square openings were 15×15 mm wide) (Fig. 1C). This open divider allowed the paired animals to see, touch and smell one another but prevented vigorous fighting. For the eight pairs in Social Group, an opaque divider replaced the open divider at the end of the two weeks of pairing to establish isolated conditions for subsequent experiments.

Experiments on Day 1

On the next morning, all animals were tested over a 3-h period (Fig. 1D). Isolates and paired animals remained isolated by a closed divider during testing. A minimum of five manual touches was delivered in alternation to each side of the first abdominal segment with a fine brush by an experimenter working under dim red light and ignorant of the social status or experience of the animal undergoing tests. Each touch stimulus was delivered to minimize disturbances of the water, which would alert the animal to the approach of the brush, so that the animal was unexpectedly touched. Responses were recorded on videotape (Panasonic, WV-BP500; 30 frames s^{-1}) for later analysis. The interval between touches was 10 min. Tests were performed when the animals were stationary and not interacting. Following the experiment, the opaque dividers were replaced to separate the dominant and subordinate for approximately 15 h overnight (Fig. 1E).

Experiments on Day 2

The opaque dividers between the pairs were removed to allow the dominant and subordinate animals to interact freely (Fig. 1F). After 30 min of interaction, these animals were tested for 2–3 h while together, whereas the Isolates were tested while remaining isolated (Fig. 1G). After the initial tests, open dividers were placed between the dominant and subordinate animals for 30 min to minimize physical contact between them while permitting chemical signaling (Fig. 1H). The animals were then tested with the open dividers still in place (Fig. 1I). After these tests, the open dividers were replaced with opaque dividers to re-isolate the paired animals for 30 min (Fig. 1J). The animals were then tested for a final time while isolated (Fig. 1K). Each set of tests took 2–3 h to perform.

Quantification of behavioral responses

Video images containing the positions of an animal before and after each manual touch (stimulus) were captured using Scion image (Scion Image, NIH) (Fig. 2). Behavioral responses were examined and categorized into 'Orienting', 'Avoidance' and 'No Response' groups. Orienting responses were movements immediately following the touch that reoriented the animal to face the stimulus source (Fig. 2A). The category includes three behaviors: a turn towards the stimulus source, a brief walk backward while turning to face the stimulus source, or a short backward jump with a brief abdominal flexion to face the stimulus source. Avoidance responses were movements away from the stimulus source. These included five behavioral responses: the animal rapidly walked forward away from the site of the touch (Fig. 2B), it walked backward away, it walked sideways away, it rotated the body axis away from the stimulus source without walking or it tailflipped away. The No Response behavior included all those in which the animal remained in the same position. Movements or responses that were initiated two seconds or more after the manual touch were not considered.

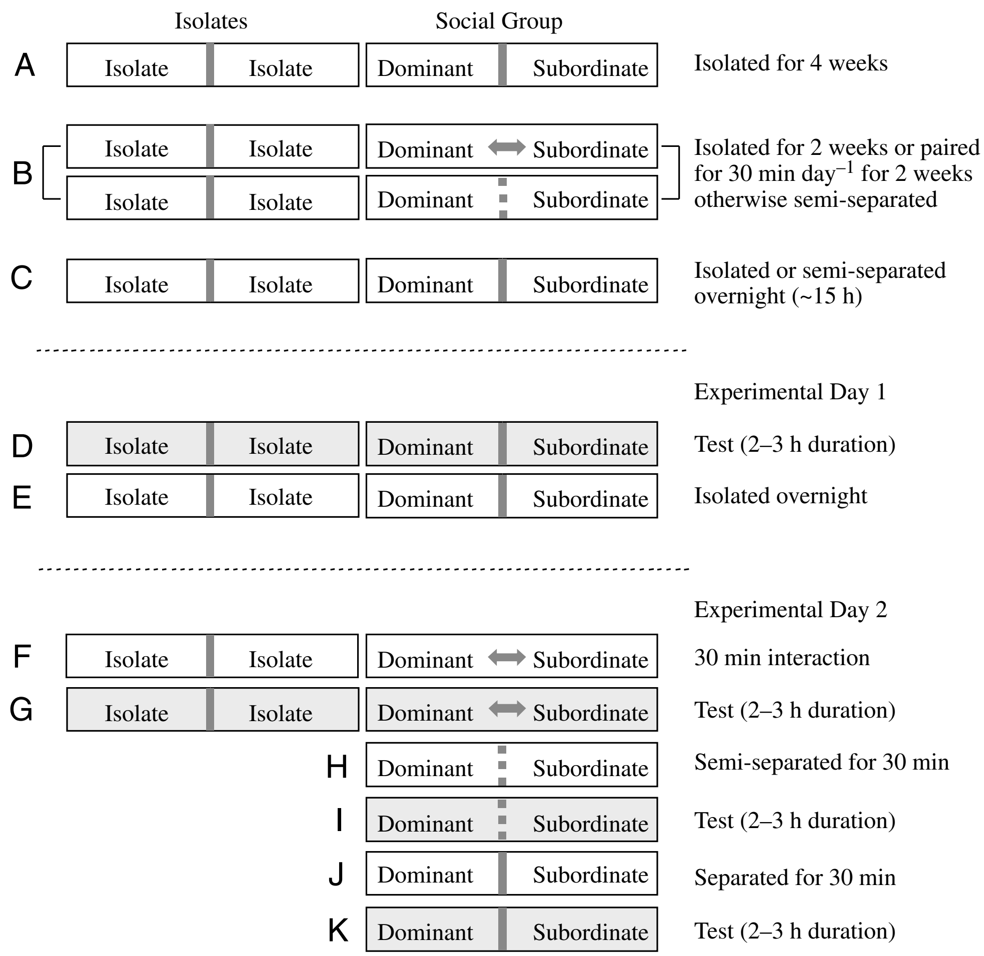

Fig. 1. A time chart of animal preparation and experiments. Pairs of animals share aquaria (rectangular boxes), where they can be isolated from each other by an opaque barrier (gray vertical bar), kept apart by an open divider that allowed pairs to smell, see and touch each other but not to fight (broken gray bar), or interact freely (gray double-headed arrow). The sequence of treatments is shown vertically, in rows labeled from A to K. Tests are indicated by light coloring of the rectangles. Animals were divided into two groups, Isolates and Social Group. (A) All animals were separated from an aquarium partner by a closed divider (i.e. isolated) for four weeks. (B) (two rows) Isolates were separated for two more weeks and thereafter through the tests. Social pairs were allowed to interact freely and form dominance relationships for 30 min day^{-1}; at other times they were kept apart by an open divider. Dominance status was determined during each 30 min of interaction by scoring the numbers of aggressive and submissive behaviors performed by each member of a pair (see equation in Materials and methods and Fig. 3). (C) At the end of the two weeks, the social pairs were re-isolated overnight (~15 h) to prevent further agonistic interactions. (D) On experimental Day 1, Isolates and the socially paired animals were tested while separated. (E) Isolates and socially paired animals remained separated overnight. (F) On Day 2, Isolates remained separated while social pairs were allowed to interact freely with their partners for 30 min. (G) All animals were tested, Isolates while separated and social pairs while in the presence of their partner. (H) Social pairs were kept apart by an open divider for 30 min and then (I) tested over a 2–3 h period. (J) Social pairs were then re-isolated by a closed divider for 30 min and then (K) tested again over 2–3 h while isolated.

A quantitative description of the movements was obtained by marking the position of the stimulus probe at the time of the manual touch (P) and the positions of the animal's head (H) and the center of body mass (C) after the manual touch (Fig. 2C,D). Using these marks, the head to probe distance value (HP distance value=HP distance/body length) and the angle γ (the angle between the body axis and the HP vector; see Fig. 2C,D) were obtained. The HP distance decreased when an animal moved towards the stimulus probe (Fig. 2C), whereas it increased when an animal moved away from the stimulus probe (Fig. 2D). The angle γ tended to decrease either when the animal turned to orient towards the probe (Fig. 2Ci) or moved a body length or more backward to avoid the probe (Fig. 2Di). The angle γ was large when an animal did not move, turned toward the stimulus probe and moved forward, beyond the probe position (Fig. 2Cii), or moved forward (Fig. 2Dii).

Analysis of behavioral responses to touch

The behavioral response patterns of the isolate, dominant and subordinate animals were compared using the Kruskal–Wallis test (nonparametric one-way ANOVA). When

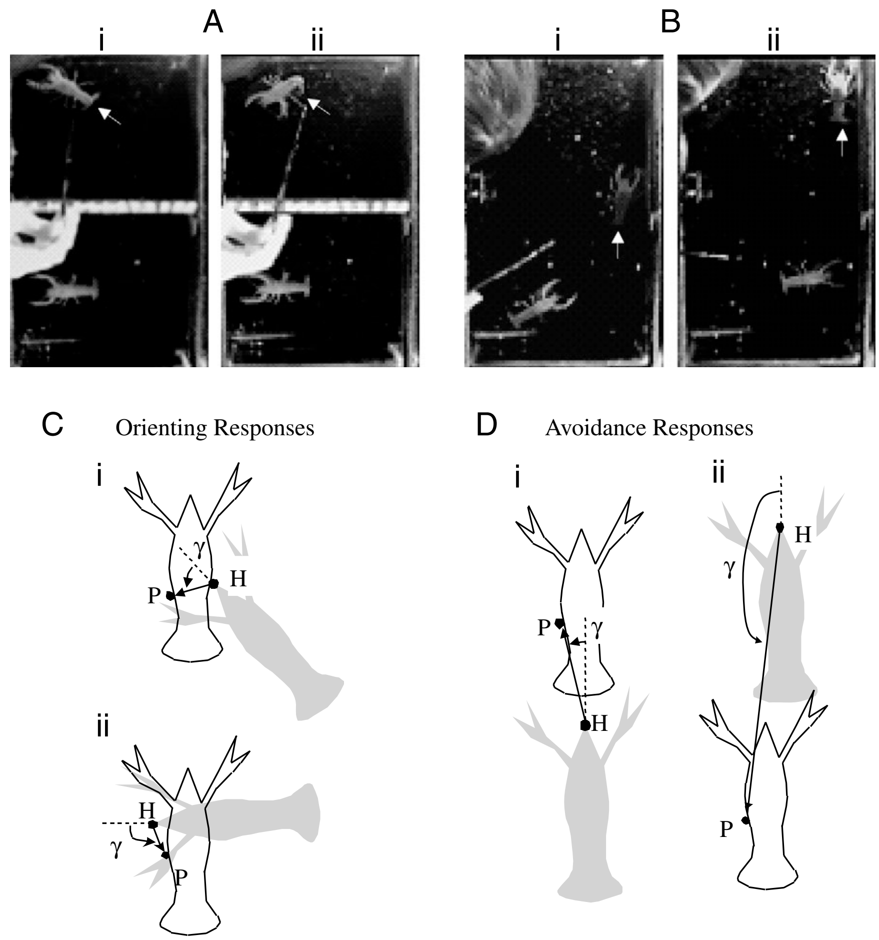

Fig. 2. Experimental tests and measures. (A) Response of a subordinate crayfish separated from its dominant partner by an opaque divider. (Ai) Before the stimulus touch. (Aii) After the touch; the animal has turned to face the source of the touch. (B) Response of a subordinate in the presence of its dominant partner. (Bi) Before the stimulus touch. (Bii) After the touch, the animal moved forward away from the touch site. (C,D) Diagrams describing measurements of the HP distance and the angle γ values in orienting and avoidance responses. The open crayfish outline shows the position of the animal before stimulus; the gray crayfish shows the position of the animal after stimulus. The HP distance is the distance from the head (H) to the point of probe contact (P). γ is the angle between the body axis and the HP line segment. (C) Orienting responses. (Ci) Both γ and HP are small when an animal pivots backwards to face the probe. (Cii) γ is large and HP is small when an animal turns and advances past the probe. (D) Avoidance responses. (Di) γ becomes small and HP becomes large when an animal walks backwards away from the probe. (Dii) γ and HP both become large when an animal walks forward away from the probe.

an overall significant difference was found in the behavioral response patterns of the three social types of animals, the Mann–Whitney test (nonparametric two-tailed *t*-test) was used to identify which type of animals differed. The Friedman test was used to examine the overall difference among the response patterns of the dominant and subordinate animals in the four consecutive social conditions; separated, paired, semi-separated and separated. The Wilcoxon signed rank test was used for pair-wise comparison only when there was an overall significant difference throughout the four different behavioral response patterns.

Results

Social hierarchy formation induced by daily pairing

Eight pairs of animals that were allowed to interact for 30 min day^{-1} for two weeks formed dominant–subordinate relationships as indicated by their scores in a dominance index (attack, +2; approach, +1; retreat, –1; tailflip, –2). Six out of the eight pairs of animals in Social Group formed stable dominant–subordinate relationships (Fig. 3), while status reversals occurred in two pairs (pairs 2 and 3 in Fig. 3), after one animal in each pair molted. No status reversals took place during the experiments.

Initial responses to unexpected touch

Isolates were tested while isolated; members of pairs in Social Group were tested when separated from their partners by an opaque divider (Fig. 1D). Unexpected lateral touch stimuli were delivered to animals in both groups under dim red light so that the animals could not see the approach of the brush. The response patterns of the Isolates and dominant and subordinate members of Social Group were similar. The predominant response was to orient towards the stimulus: average frequencies fell between 68 and 80% for the three social classes (Isolates, dominants and subordinates of Social Group; Fig. 4A). The frequencies of Avoidance and No Responses were approximately equal in each class, and no statistical difference was found between the three classes' response patterns (Kruskal–Wallis test, P=0.4570 for Orienting Responses, P=0.7133 for Avoidance Responses, P=0.6163 for No Responses).

Tests while paired, Day 2

The Isolate animals and the animal pairs of Social Group spent the next 15 h still separated (Fig. 1E). Isolate animals remained isolated, while animal pairs in Social Group were allowed to interact freely for 30 min under dim red light (Fig. 1F), after which they were tested again (Fig. 1G). The

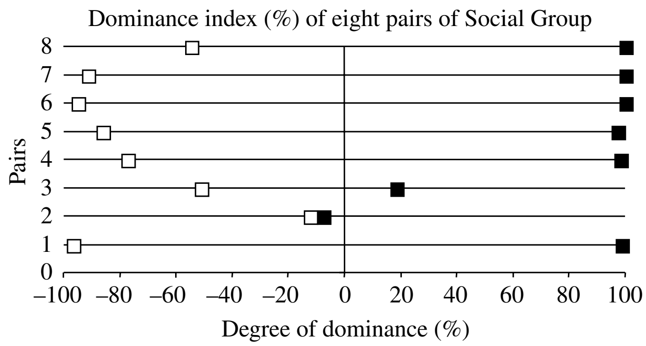

Fig. 3. Dominance index of eight pairs of crayfish recorded during the pairing period (Fig. 1B). All aggressive and submissive behaviors that were recorded during the daily 30 min pairing were scored (attack, +2; approach, +1; retreat, –1; tailflip, –2) and presented as a percentage to show the degree of dominance between each dominant–subordinate pair (see equation in Materials and methods). The degree of dominance was higher in all dominant animals (filled squares) than in subordinate animals (open squares). The dominant animals in pairs 2 and 3 were dominant for shorter periods (2 days and 8 days, respectively, at the end of 14 days) because of status reversals.

average frequencies of the Orienting Responses of the Isolates and the dominants and subordinates of Social Group significantly differed (Kruskal–Wallis test, P=0.0008). The Isolates and the dominants (who were free to interact with the subordinate partners) displayed the Orienting Responses in similar frequencies to each other (Fig. 4B; Mann–Whitney test, P=0.6126) and to those of the previous day (Fig. 4A). More than 70% of their responses were to orient towards the stimulus; most of the remaining stimuli evoked No Response (Fig. 4B). By contrast, the subordinate animals behaved very differently from both dominants and Isolates, displaying Avoidance Responses to more than 76% of the stimuli and Orienting Responses to only 19% (Fig. 4B). The average frequencies of the Orienting Responses displayed by the subordinates were significantly lower than those of the Isolates (Mann–Whitney test, P=0.0012) and dominants (Mann–Whitney test, P=0.0002). Conversely, the average frequencies of the Avoidance Responses of the Isolates, dominants and subordinates of Social Group significantly differed (Kruskal–Wallis test, P=0.0003), because the subordinates displayed the Avoidance Responses significantly more than the Isolates (Mann–Whitney test, P=0.0003) and dominants (Mann–Whitney test, P=0.0002). The behavioral responses of the subordinates were also different from those of the day before, when the subordinates were separated from their dominant partners (Fig. 4E; Friedman test, P=0.0004 for an overall significant difference; Wilcoxon test, P=0.0078 for pair-wise comparison). This difference indicates that the behavioral responses of the subordinate animals were significantly affected by the presence of the dominant animals.

Test on social pairs separated by an open divider, Day 2

To determine whether the change in the subordinate animals' responses depended on being able to interact freely with the dominants, the animals were tested again after being separated for 30 min by an open divider, again under dim red light. During this period, the subordinate animals usually stayed away from the open divider, whereas the dominants would often approach it. Both animals, especially the subordinates, often moved along the surrounding walls, touching them with their claws. When the subordinates touched the holes of the open divider, the dominants often tried to attack them, but the plastic mesh prevented serious physical interactions from occurring. The responses of the dominants were as before, with nearly two-thirds being the Orienting Responses (Fig. 4C). However, the subordinate animals displayed the Orienting and the Avoidance Responses at similar frequencies (Fig. 4C), a pattern that newly emerged in subordinates after pairing experience. Moreover, the frequencies of the Orienting Responses of the subordinate animals in a semi-separated condition were significantly different from each of the response frequencies recorded earlier (Fig. 4E), first when the subordinates were isolated on Day 1 (Wilcoxon test, P=0.0313), and then when they were paired on Day 2 (Wilcoxon test, P=0.0156). Similarly, the Avoidance Responses of the subordinate animals in a semi-separated condition were significantly reduced compared with those recorded earlier when they were paired on Day 2 (Wilcoxon test, P=0.0156).

Tests on re-isolated social pairs, Day 2

To determine whether the subordinate animals' change in behavior depended on the near presence of the dominant, as communicated through the open divider, we tested the animals again when they were completely separated by an opaque divider. The behavior patterns of both the subordinate and dominant animals (Fig. 4D) were little changed from when the animals were separated by the open divider (Fig. 4C). The behavioral responses of the subordinates were neither significantly different from those of dominants (Fig. 4D; Mann–Whitney test, P=0.2345 for Orienting Responses, P=0.3282 for Avoidance Responses) nor different from those recorded earlier when the subordinates were in a semi-separated condition (Fig. 4E; Wilcoxon test, P=0.5625 for Orienting Responses, P=0.8125 for Avoidance Responses). However, the subordinates when isolated on Day 2 (Fig. 4D) displayed the Orienting Responses less frequently compared with when they were isolated on Day 1 (Fig. 4E; Wilcoxon test, P=0.0313) but more frequently compared with when they were paired on Day 2 (Fig. 4E; Wilcoxon test, P=0.0156). These results indicate that both the presence of the dominant animal and the lingering effects of their interactions during the test period changed the behavioral response of the subordinate animals to unexpected touch.

There was no difference in the behavioral response of the dominant animals in the four different agonistic conditions tested (Friedman test, P=0.0979 for Orienting Responses, P=0.09042 for Avoidance Responses, P=0.2992 for No Responses), indicating that the behavioral responses of the

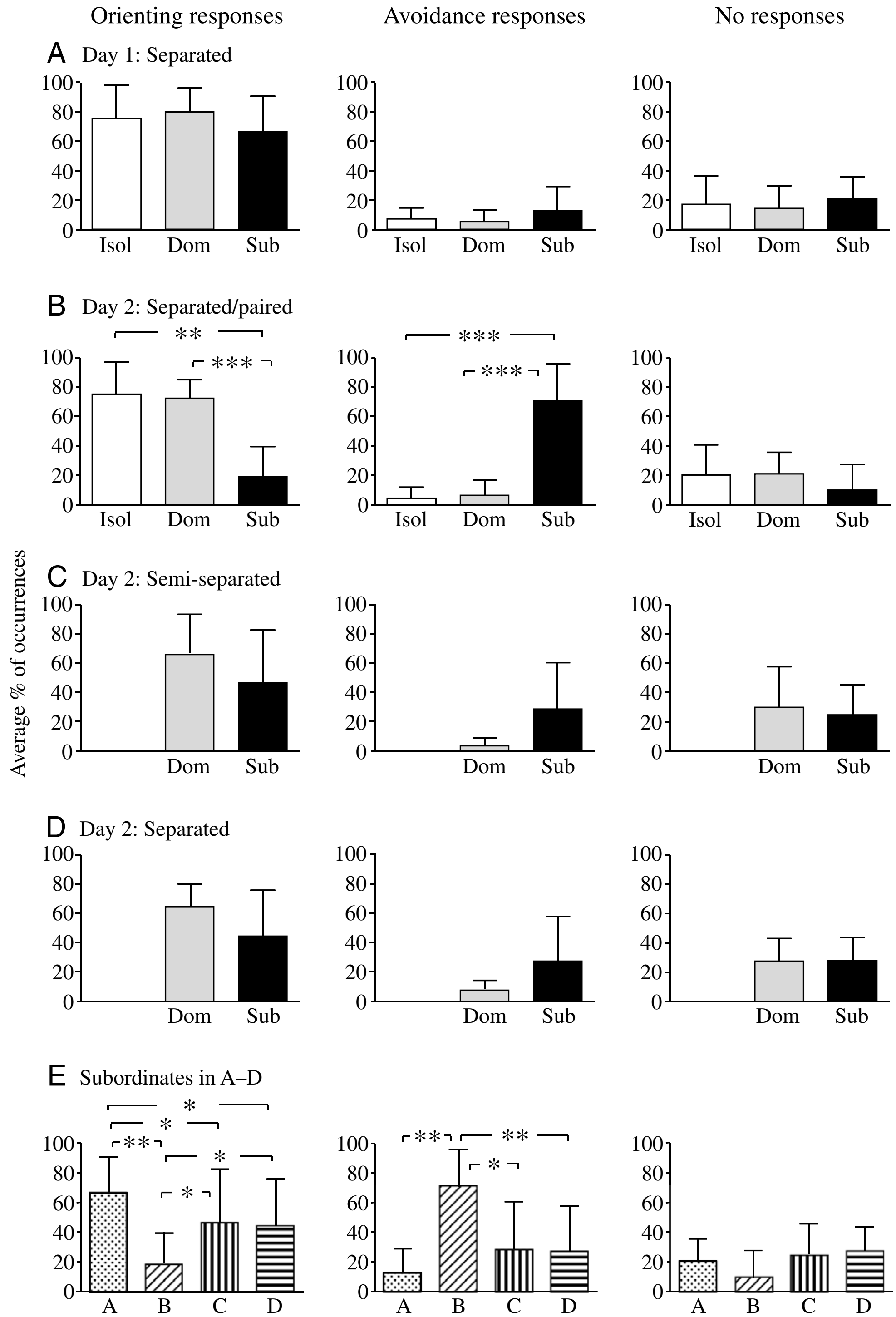

Fig. 4. Sequential comparison of the behavioral responses to an unexpected manual touch in socially inexperienced and experienced crayfish. (A) Average response frequencies of Orienting, Avoidance and No Response behavioral responses of Isolates, dominants and subordinates on Day 1 while separated from partners by an opaque barrier (condition as in Fig. 1D). The three behavioral responses of the Isolates and social pairs did not differ significantly (Kruskal–Wallis test, *P*=0.4570 for Orienting Responses, *P*=0.7133 for Avoidance Responses, *P*=0.6163 for No Responses). (B) Average response frequencies of the same animals on Day 2 when the dominant and subordinate crayfish were paired with their partners (condition as in Fig. 1G). The Orienting and the Avoidance responses of dominants and Isolates were significantly different from those of subordinates (for Orienting Responses, overall difference, *P*=0.0008; Isolates *vs* subordinates, *P*=0.0012; dominants *vs* subordinates, *P*=0.0002; for Avoidance Responses, overall difference, *P*=0.0003; Isolates *vs* subordinates, *P*=0.0003; dominants *vs* subordinates, *P*=0.0002) but were not different from each other (for Orienting Responses, *P*=0.6126; for Avoidance Responses, *P*=0.7789). The No Responses were not different in Isolates, dominants and subordinates (overall difference, *P*=0.2069). (C) Behavioral responses later on Day 2 when the dominants and subordinates were tested while separated by an open divider (condition as in Fig. 1I). The dominants' responses were not different from those of the subordinates (for Orienting Responses, *P*=0.2786; for Avoidance Responses, *P*=0.1605; for No Responses, *P*=0.7984). (D) Dominant and subordinate responses after re-isolation (condition as in Fig. 1K) are not significantly different (for Orienting Responses, *P*=0.2345; for Avoidance Responses, *P*=0.3282; for No Responses, *P*=0.9591). (E) The behavioral responses of the subordinates in the four consecutive social conditions (shown in A–D) significantly changed after pairing experience (Friedman test, *P*=0.0004 for both Orienting and Avoidance Responses). The subordinate animals when isolated on Day 1 displayed the Orienting Responses significantly more often than when paired on Day 2 (Wilcoxon test, *P*=0.0078), when semi-separated on Day 2 (*P*=0.0313) and when isolated on Day 2 (*P*=0.0313). The subordinate animals when paired on Day 2 displayed the Orienting Responses significantly less often than when semi-separated on Day 2 (*P*=0.0156) and when isolated on Day 2 (*P*=0.0156). The average frequency of the orienting response when semi-separated on Day 2 did not differ from those when isolated on Day 2 (*P*=0.5625). The subordinate animals when paired on Day 2 displayed the Avoidance Responses significantly more often than when isolated on Day 1 (*P*=0.0078), when separated by the open divider on Day 2 (*P*=0.0156), and when isolated on Day 2 (*P*=0.0078). The average frequency of No Responses did not change in the four social conditions examined (overall difference, *P*=0.0660). *$P<0.05$, **$P<0.01$, ***$P<0.001$. All values are means ± s.d.

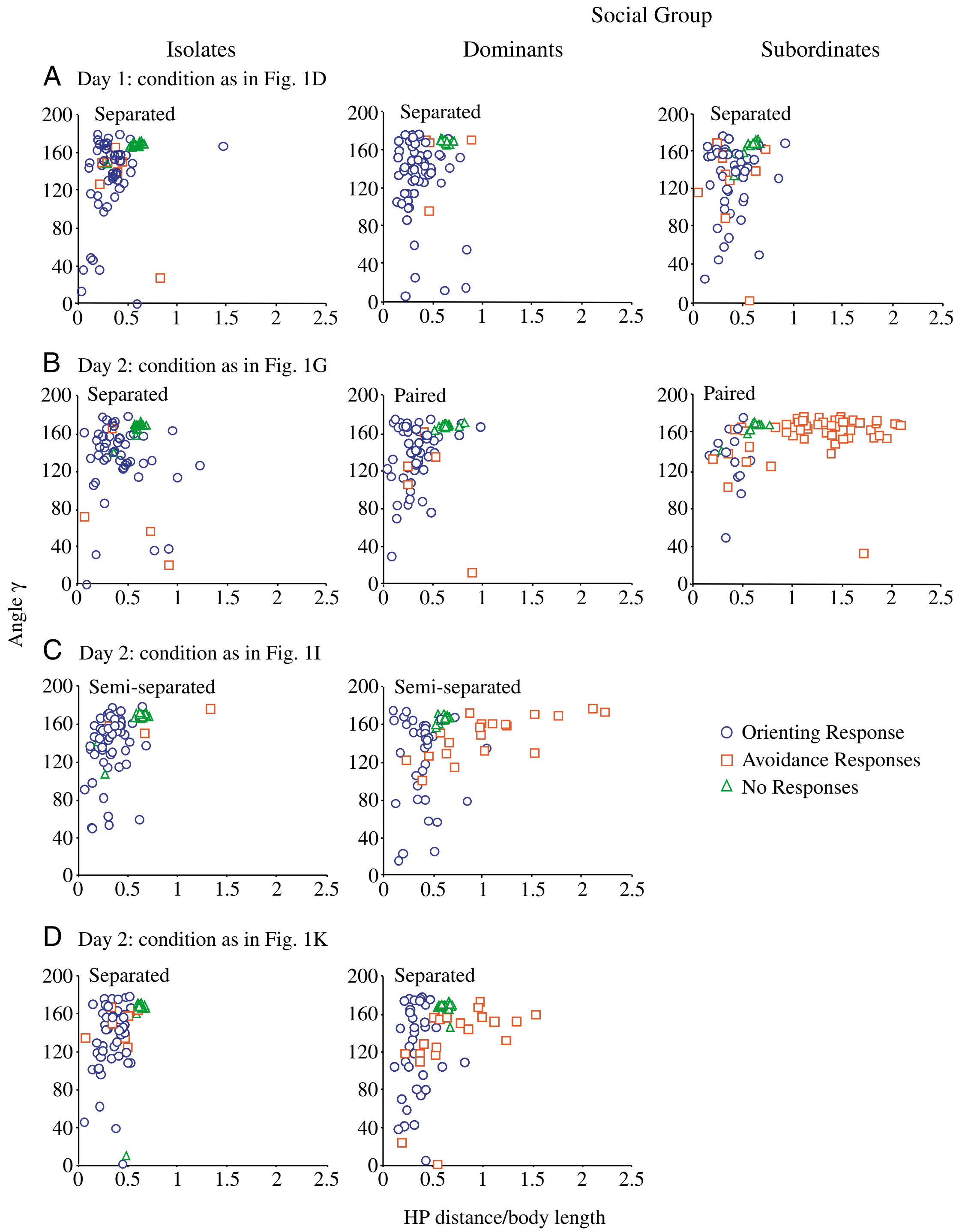

Fig. 5. The distribution of the HP distances and the γ angles of the Orienting (blue circle) Avoidance (red square) and No (green triangle) Responses of all animals tested. (A–D) Isolate and dominant animals displayed Orienting Responses. While separated (A,B_{isol},D_{dom}), while paired (B_{dom}) and while separated by an open divider (C_{dom}), most isolate and dominant animals displayed Orienting Responses, although some Avoidance Responses and No Responses were also produced. Note that the Avoidance Responses produced by the isolate and the dominant animals have a small HP distance value (<1). (C) Subordinate animals displayed Orienting Responses when separated but displayed Avoidance Responses when interacting with dominant opponents. The subordinate animals produced Orienting Responses while separated on Day 1 (A_{sub}) similar to the isolate and the dominant animals. While paired (B_{sub}), the subordinate animals showed Avoidance Responses. Note that the Avoidance Responses produced by the subordinates have a large HP distance value (1<HP distance value<2.5). In a semi-separated condition (C_{sub}), some subordinate animals showed Orienting Responses whereas other subordinate animals continued to show Avoidance Responses and large HP values. While separated on Day 2 (D_{sub}), most subordinate animals showed Orienting Responses, while three subordinate animals produced Avoidance Responses with large HP values (>1).

dominant animals were not dependent on the presence of their subordinate partners. Thus, it is either the opportunity to interact with the dominant or the interactions themselves that led to a shift in the response patterns of the subordinate animals from Orienting to Avoidance.

Quantification of the behavioral responses to manual touch

To visualize the difference between the Orienting and the Avoidance responses of the socially experienced and inexperienced animals, the positions of each animal before and after the stimulus were quantified (see Materials and methods for details). Two related measures were used: the distance between the final head position and the site of the probe's touch on the animal's side, in body lengths (HP), and the angle γ between the animal's body axis and the direction from the body center to the site of the probe contact.

No Responses were characterized by low HP values and γ angles between 90 and 180°, whereas Orienting Responses were associated with similar HP values and a broader range of angles, between 0 and 180° (Fig. 5). Avoidance Responses displayed much larger HP values and γ angles between 90 and 180°.

Isolate, dominant and subordinate animals displayed similar responses on Day 1 when tested while isolated (Fig. 4A). Their patterns of movement were also similar, clustering around low HP values and γ angles between 90 and 180° (Fig. 5A). A few animals of each social type displayed Avoidance or Orienting Responses characterized by low γ values. The movement pattern of the subordinates reflected the change in their responses when paired on Day 2 (Fig. 4B). Subordinates displayed Avoidance Responses (red squares) with much larger HP values, extending to more than three body lengths (Fig. 5B). Most Avoidance Responses also had γ angles near 180°, indicating that the animal had moved forward, away from the probe. A smaller number had low γ values, indicating that the animal moved backward. When the animals were semi-separated by an open divider, the responses of the social dominants did not change significantly (Figs 4C, 5C), whereas the Avoidance responses of the subordinates were again characterized by large HP values and large γ angles, indicating forward movement away from the probe. Separation of the subordinates brought partial recovery of the original movement patterns; the HP distribution of Avoidance Responses was reduced from its highest values but still extended to greater values than when the subordinates were tested initially (Fig. 5D).

Discussion

The importance of social context in determining the subordinate response to unexpected touch

Both the nervous system and behavior of crayfish depend on the animal's social status. Serotonergic modulation of the excitability of the command neurons for escape and the threshold for escape behavior depend on the social status of crayfish (Yeh et al., 1996; Yeh et al., 1997; Krasne et al., 1997; Teshiba et al., 2001), and crayfish display agonistic behaviors typical of their social status (Goessmann et al., 2000; Herberholz et al., 2001). Dominant and subordinate animals also differ in their willingness to engage in other non-social behaviors, such as shelter construction, in the presence of a social partner (Herberholz et al., 2003). Subordinate animals are inhibited from digging in the presence of a dominant partner, while the dominant's burrowing activity is increased. The inhibition was conditional upon the near presence of the dominant but also appeared to linger after the subordinate had been isolated.

Previous reports indicated that the response of a crayfish to an unexpected touch depended on the animal's size (Nagayama et al., 1986) or dominance status (Bovbjerg, 1953). The types of escape reflex circuitries to be activated were dependent on social status of crayfish under the threat of unexpected attack, while the excitability of the lateral giant escape reflex was independent of social status when the threat was removed (Krasne et al., 1997). Here, we have found that a crayfish's response to an unexpected touch depends on the animal's social context as well as its social status. The response of a social subordinate in the presence of its dominant partner differed from its response when alone. When kept apart from their partner by a closed barrier, the social status or experience of the animal had no effect on its responses, which were identical to those of socially isolated animals. Isolates and separated dominants and subordinates all turned to confront the source of the unexpected touch (Figs 4A, 5A,B). However, when subordinates were tested while in partial contact with their dominant partners (i.e. semi-separated), their patterns of responses differed from when tested alone (Figs 4C,E, 5C). These differences increased dramatically when the subordinates were tested in the presence of their dominant partner: instead of turning to confront the unexpected touch, they moved away (Figs 4B,E, 5B). It is apparent, therefore, that as in the case of burrowing behavior (Herberholz et al., 2003), the social context of being subordinate in the presence of the dominant partner determined the change in the subordinate's response to the stimulus.

Subsequent tests showed that the change in the subordinate's response brought about by being tested while paired persisted to a small degree for some hours (Fig. 4E). Tests while semi-separated (Figs 4C, 5C) and while alone (Figs 4D, 5D) revealed that the subordinates' behavior was not restored to that of the earlier isolated condition (Figs 4A, 5A) five hours after the tests while paired.

These results suggest that the effect of the paired context in which the unexpected touch was received lingers for some hours after the event. This effect may be a state change, similar to fear, or it may be a memory (i.e. a specific association) of having received the earlier unexpected touches in the presence of the dominant. When the subordinates were tested while semi-separated from their dominant partners, this state or memory may have been strengthened by olfactory stimuli from the dominant. When the subordinates were finally tested while isolated from their dominant partners, the response pattern showed some influence of the earlier paired context in which the animals were tested.

The persistence of the change in the subordinate's behavior when the dominant was absent is similar to the lingering inhibitory effect of the dominant on the subordinate crayfish's burrowing activity (Herberholz et al., 2003). In both these cases, it is not clear whether this persistence represents a specific memory of a more generalized change in behavior, like fear. Crayfish, like other crustaceans, can learn specific associations (Krasne, 1973). Several studies have demonstrated the ability of crayfish to learn to recognize crayfish in the process of dominance hierarchy formation and maintenance (Bovbjerg, 1953) – for individual recognition (Lowe, 1956), recognition of aggressive state (Copp, 1986) and status recognition (Zulandt-Schneider et al., 1999) – although what is recognized and when the animal learns are not completely understood.

The complete neural mechanisms of the different behavioral responses to unexpected lateral touch are unknown, but some elements have been identified. Bilateral pairs of serotonergic neurons in the abdomen and thorax of crayfish receive both excitatory and inhibitory inputs in response to a lateral touch of the rostral portion of the abdomen, but the mix of excitation and inhibition depends on the social status of the animal (Drummond et al., 2002). The ipsilateral 5-hydroxytryptamine (5-HT) neurons of Isolate and dominant crayfish were excited by a unilateral touch while the contralateral 5-HT neurons were inhibited. The same neurons in subordinate crayfish were symmetrically excited in several crayfish and inhibited in as many others; asymmetric responses did not occur. These 5-HT neurons have been found to modulate walking leg reflexes, which also display differences between dominant and subordinate animals (F. A. Issa, D. H. Cattaert and D. H. Edwards, unpublished observations). It is tempting to link the asymmetric neuronal responses of Isolate and dominant crayfish to their orienting responses described here, and the symmetric excitatory or inhibitory neuronal responses of subordinate crayfish to their symmetric avoidance responses. Future studies will determine whether these correlations between neuronal responses and behavior are indicative of underlying causal mechanisms.

This study was supported by a National Science Foundation grant IBN-9726819 to D.H.E. and by the Department of Biology, Georgia State University. We thank Dr J. Drummond for helpful comments on the designs of experiments, Dr C. D. Derby for help with the statistics, Dr S. Versteeg for help with quantification of data, and Mr F. A. Issa for help with animal preparation. We also thank Mr F. A. Issa, and Ms L. M. Johnstone for critical readings of the manuscript.

References

Bovbjerg, R. V. (1953). Dominance order in the crayfish *Orconectes virilis* (Hagen). *Physiol. Zool.* **26**, 173-178.

Bruski, C. A. and Dunham, D. W. (1987). The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. I. An analysis of bout dynamics. *Behaviour* **103**, 83-107.

Copp, N. H. (1986). Dominance hierarchies in the crayfish *Procambarus clarkii* (Girard, 1852) and the question of learned individual recognition (Decapoda, Atacidea). *Crustaceana* **51**, 9-23.

Drummond, J., Issa, F. A., Song, C.-K., Herberholz, J., Yeh, S.-R. and Edwards, D. H. (2002). Neural mechanisms of dominance hierarchies in crayfish. In *The Crustacean Nervous System* (ed. K. Wiese), pp.124-135. Berlin: Springer-Verlag.

Figler, M. H., Cheverton, H. M. and Blank, G. S. (1999). Shelter competition in juvenile red swamp crayfish (*Procambarus clarkii*): the influences of sex differences, relative size, and prior residence. *Aquaculture* **178**, 63-75.

Goessmann, C., Hemelrijk, C. and Huber, R. (2000). The formation and maintenance of crayfish hierarchies: behavioral and self-structuring properties. *Behav. Ecol. Sociobiol.* **48**, 418-428.

Hayes, W. A., II (1975). Behavioral components of social interactions in the crayfish *Procambarus gracilis* (Bundy) (Decapoda, Cambaridae). *Proc. Okla. Acad. Sci.* **55**, 1-5.

Herberholz, J., Issa, F. A. and Edwards, D. H. (2001). Patterns of neural circuit activation and behavior during dominance hierarchy formation in freely behaving crayfish. *J. Neurosci.* **21**, 2759-2767.

Herberholz, J., Sen, M. M. and Edwards, D. H. (2003). Parallel changes in agonistic and non-agonistic behaviors during dominance hierarchy formation in crayfish. *J. Comp. Physiol. A* **189**, 321-325.

Krasne, F. B. (1973). Learning in Crustacea. In *Invertebrate Learning* (ed. W. C. Corning, J. A. Dyal and A. O. D. Willows), pp. 49-130. New York, London: Plenum Press.

Krasne, F. B., Shamsian, A. and Kulkarni, R. (1997). Altered excitability of the crayfish lateral giant escape reflex during agonistic encounters. *J. Neurosci.* **17**, 709-716.

Livingstone, M. S., Harris-Warrick, R. M. and Kravitz, E. A. (1980). Serotonin and octopamine produce opposite postures in lobsters. *Science* **208**, 76-79.

Lowe, M. E. (1956). Dominance-subordinance relationships in the crayfish *Cambarellus shufeldtii*. *Tulane Stud. Zool.* **4**, 135-170.

Nagayama, T., Takahata, M. and Hisada, M. (1986). Behavioral transition of crayfish avoidance reaction in response to uropod stimulation. *Exp. Biol.* **46**, 75-82.

Song, C.-K., Herberholz, J., Drummond, J. and Edwards, D. H. (2000). Social experience changes the behavioral response to unexpected touch in crayfish. *Soc. Neurosci. Abstr.* **26**, 174.

Teshiba, T., Shamsian, A., Yashar, B., Yeh, S.-R., Edwards, D. H. and Krasne, F. B. (2001). Dual and opposing modulatory effects of serotonin on crayfish lateral escape command neurons. *J. Neurosci.* **21**, 4523-4529.

Yeh, S.-R., Fricke, R. A. and Edwards, D. H. (1996). The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science* **271**, 366-369.

Yeh, S.-R., Musolf, B. E. and Edwards, D. H. (1997). Neuronal adaptations to changes in the social dominance status of crayfish. *J. Neurosci.* **17**, 697-708.

Zulandt-Schneider, R. A., Schneider, R. W. S. and Moore, P. A. (1999). Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. *J. Chem. Ecol.* **25**, 781-794.