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RAPID COMMUNICATION

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Parallel changes in agonistic and non-agonistic behaviors during dominance hierarchy formation in crayfish

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Abstract Agonistic and non-agonistic behaviors were studied before, during, and after the formation of social status in crayfish. Differences in the expression of a non-agonistic behavior by dominant and subordinate crayfish developed in parallel with the differences in agonistic behaviors that indicate the animals' social status. An increase in burrowing behavior marked the ascendancy of the dominant animal, while an immediate suppression of burrowing paralleled the inhibition of aggressive behavior in the new subordinate. The strikingly similar changes in both agonistic and non-agonistic behaviors following the decision on status suggest related underlying neural mechanisms.

Keywords Burrow · Crayfish · Dominants · Hierarchy · Subordinates

Introduction

The limited availability of necessary resources, such as food, shelter and space, provokes agonistic interactions among many social animals (Wilson 1975), including several species of crayfish. These interactions may lead to fighting and the formation of a dominance hierarchy that determines the order of access to both present and future resources. Agonistic elements of crayfish behavior displayed during fighting and formation of a dominance hierarchy have been intensively studied (Bovbjerg 1953; Lowe 1956; Copp 1986; Bruski and Dunham 1987; Figler et al. 1995b; Rutherford et al. 1996; Guiasu and Dunham 1997; Edwards et al. 1999; Issa et al. 1999; Tierney et al. 2000; Goessmann et al. 2001; Herberholz et al. 2001; Schapker et al. 2002). The later stages of initial encounters between well-matched crayfish are marked by an aggressive escalation that can include strikes and grappling with the claws, and bouts of offensive tail-flips. At some point, fighting is interrupted by an abrupt change in the agonistic behavior of one animal as it switches from aggressive to submissive behaviors. This switch identifies the new subordinate (Herberholz et al. 2001). Dominant and subordinate crayfish then show clear differences in their agonistic behavior, with the dominant animal displaying a dominant posture, initiating most attacks and claiming first access to most resources, while the subordinate displays a submissive posture, avoids or escapes from the dominant's attacks, and is left to claim unwanted resources.

One of the rewards of social dominance is access to a burrow that provides shelter, allows the occupant to claim a territory, to avoid predators, and promotes survival in temporary ponds (Bovbjerg 1970; Cobb 1971). *Procambarus clarkii* was classified as a *secondary* burrower that excavates relatively simple burrows (Gherardi 2002). When *P. clarkii* is introduced into an aquarium that contains a floor substrate (i.e., sand or gravel) it soon starts excavating a depression in the substrate as part of its territorial behavior (Figler et al. 2001). This burrowing behavior is neither sex specific nor related to sexual maturity. Fighting often takes place in, or in the vicinity of the depression indicating the defense of the territory by the resident (Figler et al. 2001).

We found that burrowing, a behavior that is not part of the behavioral repertoire used during fighting, is strongly affected by hierarchy formation in a manner very similar to agonistic behaviors.

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Materials and methods

Crayfish (*P. clarkii*) were obtained from a commercial supplier and then isolated in small water-filled plastic containers (8.5 cm×15 cm×8.5 cm) for at least 1.5 days and a maximum of 9 days before being used. They were fed ad libitum (not on the day of testing) and kept under a constant 12 h:12 h light-dark cycle. All experiments were performed at approximately the same time each day (between 0900 and 1200 hours).

In the *first experimental set*, eight male and eight female crayfish (size: 2.8–5 cm, measured from rostrum to telson) were used. They were combined in eight pairs of the same sex matched for size (differences <10%) and weight (differences <15%). One member of the pair was chosen at random and marked with a small dot of White-Out (BIC) on its carapace. Before being tested, all animals were found to be physically intact and at inter-molt stages by use of microscopic examination. None of the animals had molted within 2 days prior to the experiment or within 2 days after the experiment.

In the first trial of the set (I), the members of a pair were put in separate aquaria (15 cm×30 cm×20 cm) with gravel floors (Wilmar; height: 2.5 cm, crushed coral chips of 4–6 mm in size) and filled with distilled water (height: 10 cm) from the laboratory water supply. The walls were covered with white paper to prevent any visual distraction. Three-quarters of the aquarium received overhead illumination, leaving one-quarter in shade to create a preferred habitat. The behavior of both animals was taped for 30 min with a video camera (Hitachi, VK-C350) mounted on a tripod above the two aquaria and connected to a video-recorder (Panasonic, AG 7350).

In the second trial (II), the two animals were put together in another aquarium of identical dimensions and with substrate of the same type but filled with new water. They were observed for another 30 min and their behaviors were videotaped.

In the third trial (III), the animals were re-isolated by transferring them each into a new tank of identical dimensions and with substrate of the same type but filled with new water. Here again they were videotaped for 30 min.

In the *second experimental set*, twelve different animals (size: 2.7–3.1 cm, measured from rostrum to telson) of both sexes (8 males, 4 females) were used in three experimental trials similar to the ones described above (I–III, first experimental set), except that the animals were not paired during the second trial. They were instead put each in a new, separate tank so that each animal was tested in three different tanks and videotaped for 30 minutes each time.

The duration of each behavior (see below) was measured using single-frame analysis on a TV monitor (Panasonic, 2010-Y) and the internal clock of the video-recorder. All data are presented with means and standard deviation. Non-parametric statistical tests (SigmaStat 2.0) for dependent data (Wilcoxon Signed Rank test) were used for comparison with $P \le 0.05$ and $P \le 0.01$ indicated by one or two asterisks, respectively, in Table 1 and in Fig. 1.

The different non-agonistic behaviors observed were: 'locomotion,' defined as walking forward or backward for at least two consecutive steps; 'burrowing,' excavating a depression in the substrate by use of the claws and walking legs (a minimum of three coral chips moved); and 'no activity', resting, sometimes including movement of the appendages so as not to produce locomotion or burrowing. The remaining time (usually only a few seconds) was covered by activities not used in our analysis, including grazing and attempts to climb the aquarium walls. Agonistic behaviors observed included 'offensive locomotion' (chasing the opponent), and 'defensive locomotion' (fleeing the opponent), and an 'agonistic bout', defined as an encounter beginning with physical contact and ending with both animals being separated by a distance of one or more body lengths. Each bout was scored for aggressive and submissive elements employed by both animals: escape (i.e., tail-flip); retreat (i.e., walking away from opponent); approach (i.e., walking towards opponent); threat display (i.e., spread of claws); attack (i.e., dart towards opponent with open claws resulting in physical contact); claw grasping; and offensive tail-flip. The number of offensive tail-flips was underestimated throughout the study because they require a camera side view and high-speed videography for non-ambiguous detection (Herberholz et al. 2001). After reviewing and analyzing the experiments on video, scores from both animals were compared. The animals that consistently retreated and escaped after the hierarchy was decided were declared the losers (subordinates), the opponents were declared the winners (dominants). Subordinates in all pairs showed at least eight times as

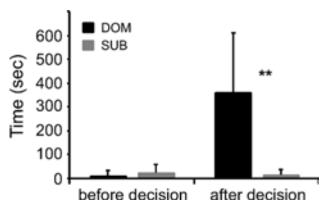


Fig. 1 Mean + SD for time spent in burrowing behavior by the emerging dominant (*black bars*) and subordinate (*gray bars*) crayfish before and after the decision on social status was reached. $^{**}P \le 0.01$

Table 1 Mean ± SD for time spent with agonistic and non-agonistic behaviors by dominant, subordinate and naïve crayfish before, during, and after hierarchy formation. Animals labeled as dominants and subordinates in A established their status in the subsequent experiment (B). Naïve animals were not compared statistically to dominants and subordinates. For further explanation see text

	Regular locomotion	Offensive locomotion	Defensive locomotion	Burrowing	No activity
A. Before hierarchy formation					
Dominants	884 ± 337 s	–	–	84 ± 190 s	785 ± 225 s
Subordinates	792 ± 320 s	–	–	96 ± 135 s	869 ± 314 s
Naive animals	716 ± 240 s	–	–	178 ± 216 s	888 ± 260 s
B. During hierarchy formation					
Dominants	386 ± 152 s	*153 ± 115 s	**1 ± 3 s	**365 ± 245 s	*891 ± 225 s
Subordinates	287 ± 179 s	*19 ± 14 s	**97 ± 64 s	**32 ± 41 s	*1295 ± 181 s
Naive animals	542 ± 230 s	–	–	266 ± 339 s	969 ± 347 s
C. After hierarchy formation					
Dominants	409 ± 325 s	–	–	424 ± 339 s	913 ± 413 s
Subordinates	277 ± 197 s	–	–	186 ± 233 s	1311 ± 345 s
Naive animals	252 ± 148 s	–	–	459 ± 527 s	1080 ± 505 s

$^{*}P \le 0.05$; $^{**}P \le 0.01$

many retreats and five times as many escapes as their opponents. Trial III was always analyzed before trial II so that the social status of the animals was unknown during the analysis.

Results

Behavior before, during, and after hierarchy formation

We found no significant differences in any of the analyzed behaviors for animals that would eventually (in the next experiment) emerge as dominants or subordinates (Table 1, part A). Both spent similar time walking around (exploring the habitat) and resting, although future dominants spent slightly more time in locomotion and less time in no activity than subordinates. Most of the remaining time was spent in burrowing, and again, no significant differences between the two groups are found.

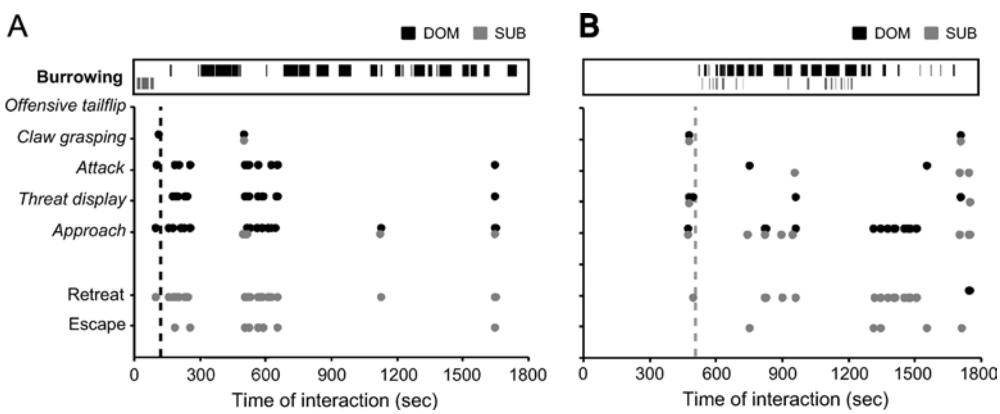
In seven of the eight pairs, social status was decided sometime between the first and fourth agonistic bout when an abrupt change from aggressive to submissive behaviors occurred in the new subordinate animals. This occurred within the first 5 min of pairing for most pairs (2.3 ± 2.8 min). In these animals, the new dominant displayed no submissive behaviors and the hierarchy was maintained throughout the period after it was established. In one pair, however, the apparent subordinate approached the dominant twice in the last two bouts before the recording time expired and caused it to retreat (see below).

During the period of hierarchy formation, animals of different social status differed significantly in the time spent in both agonistic and non-agonistic behaviors (Table 1, part B). Consistent with their new status, dominant animals spent more time in offensive locomotion, while subordinates spent more time in defensive locomotion. Although no difference was found with respect to non-agonistic locomotion, burrowing, a non-agonistic behavior, was strongly affected by formation of social status. The new dominant animals spent much more time burrowing (Table 1, part B) than the same animals did in the previous 30 min when they were alone (Table 1, part A). Moreover, the dominant animals also burrowed for significantly longer than the new subordinate animals, which spent less time with this activity when paired than they had when alone (Table 1, parts A and B). The subordinate animals also spent significantly more time in no activity than the dominants, which were engaged in burrowing when the subordinates were inactive. Dominant animals made burrows in both the lighted and shaded part of the aquarium, although they spent significantly more time in the shade than did the subordinates (dominant animals: 919 ± 486 s, subordinate animals: 488 ± 348; $P \le 0.05$).

Since the decision on social status is reached at different times in different pairs, it is of particular interest to examine the changes of burrowing behavior before and after the hierarchy was decided. Burrowing activity was nearly absent among the future dominants but increased dramatically once their status was clear (Fig. 1). The new dominants started digging very shortly after the decision was reached: five of them within 25 s after the decisive bout, two others chased the subordinate for approximately 4 min and started burrowing immediately afterwards. Only one newly established dominant started burrowing without any time-correlation to the previous decisive win (~13 min later). While all dominant animals burrowed after the status was decided, only three subordinates did. Initial burrowing activity of all subordinates was greater than dominants and decreased slightly following the status decision (Fig. 1). This cumulative measure may overestimate the burrowing activity of most subordinates. One subordinate, which had challenged its dominant partner towards the end of their 30-min interaction accounted for 69% (66 s of 96 s) of the total time spent burrowing by all subordinates following the dominance decision.

The close correlation between agonistic elements and burrowing behavior can be illustrated by combining the

Fig. 2A,B Patterns of agonistic behaviors and burrowing displayed by two different pairs of crayfish during 30 min of interaction. *Black and gray circles* (agonistic elements) and *bars* (burrowing) mark the behaviors of dominants and subordinates, respectively. **A** A pair in which the hierarchy was decided (*dashed vertical line*) after the first agonistic encounter. **B** A pair in which the subordinate challenged the dominant late in the experiment after a preliminary decision (*faint dashed vertical line*) had been reached earlier



fighting elements and the burrowing behavior in one graph. We have done this for two exemplary pairs, one in which the hierarchy was decided early and remained stable (Fig. 2A) and one in which the hierarchy may have been reversed at the end of the experiment (Fig. 2B).

In the first pair (Fig. 2A), the subordinate was active burrowing for 57 s before the first contact. The first agonistic bout occurred 103 s after the animals were introduced into the tank and it was decisive. After the hierarchy was formed, he subordinate animal showed very little aggressive behavior and much more submissive behavior. It did not burrow throughout the remaining time of the experiment. On the contrary, the dominant animal showed no submissive behavior but only aggressive behavior. It spent most of the time between the agonistic bouts excavating a depression in the substrate.

In the second pair (Fig. 2B), the first agonistic bout occurred 480 s into the experiment. Neither animal showed any burrowing behavior before the first encounter; the victor was identified as the new dominant. Both animals engaged in brief bouts of burrowing immediately afterward. The dominant animal then displayed long periods of burrowing, whereas the subordinate attempted short periods of burrowing throughout most of the remaining time. Clearly, its burrowing behavior was not as much suppressed as in other pairs although it did spend much less time with this activity than its opponent did. The dominant approached the subordinate several times, which retreated from each approach. During the final 5 min of the experiment, however, the subordinate challenged the dominant repeatedly and caused it to retreat twice in the final agonistic bout (symbols overlap). It appears that in this particular pair the hierarchy may not have been conclusively decided during the time of pairing, thereby causing less suppression of subordinate burrowing than seen in all other pairs. The ambiguity in status formation may be explained by the prolonged time that both animals spent in the aquarium before they first met. Both had enough time to adapt to their surroundings, which may have decreased their willingness to give up the claimed space.

After the animals were returned to isolation, the differences in the behavior of dominant and subordinate crayfish did not reach statistical significance (Table 1, part C). A non-significant trend, however, was observable: the subordinates were less active than the dominants, spending less time in locomotion and more time in no activity. They also spent less than half the time in burrowing than was spent by the dominant animals. Variability among individual members of both groups has probably prevented the difference from reaching statistical significance.

Behavior of socially naïve animals

To determine the effect of pairing and dominance hierarchy formation on the behavior of crayfish over time, we performed another experiment in which the time spent by isolated crayfish in non-agonistic behaviors was measured during three sequential 30-min periods spent in different aquaria. The results from this group and the previous group cannot be tested statistically (they represent a combination of dependent and independent data) but they allow for a revealing comparison (Table 1).

The time spent in locomotion was less in each subsequent trial period, whereas the time spent with burrowing and no activity increased (Table 1). Burrowing was performed without being actuated by a particular stimulus and happened spontaneously in these undisturbed crayfish. The animals were forced to give up their burrow every time they were transferred to a new aquarium, and they resumed and expanded the activity in the new environment. The reduction in locomotion together with the increase in burrowing and no activity lead to the conclusion that the animals acclimatized over time to the similar features of the aquaria to which they were transferred every 30 min.

Discussion

This study aimed to answer two questions: (1) whether crayfish would indicate a disposition to acquire a certain social status before that status was determined through agonistic interactions, and (2) whether the display of non-agonistic behaviors was affected by status formation. With respect to the first question, we found no significant differences in the analyzed behaviors of crayfish without agonistic experience that would later become dominant or subordinate. With respect to the second question, we found that the period spent burrowing was dramatically different in both subordinates and dominants after their social status was established. Burrowing, a behavior not associated with fighting, is inhibited in subordinate animals by only a few agonistic interactions and the inhibition persisted thereafter in the presence of the dominant animals. This inhibition is strikingly similar to the immediate and lasting inhibition of aggression in newly established subordinates and suggests that the inhibition of burrowing and the inhibition of aggression are controlled by the same or similar neural mechanisms. In the dominant animal, the determination of social status and the onset of burrowing are also correlated, strengthening the suggestion that burrowing and its inhibition are reflections of the dominant and subordinate states, respectively. The data obtained from socially naïve animals, however, show that burrowing behavior needs no agonistic stimulus but can be performed in the absence of a conspecific. The activity may receive enhancement as soon as dominant status is achieved.

Burrowing in subordinates is reduced after re-isolation when compared to dominant or naïve animals. Although not statistically significant, this reduction indicates that inhibition of burrowing outlasts the presence of the opponent and may be controlled by neural mechanisms that act immediately and persist without further reinforcement.

Shelters are of great importance for crayfish survival. Burrowing behavior and shelter use increase in the presence of a predator (Stein and Magnuson 1976; Garvey et al. 1994) and shelter possession significantly decreases the risk from predators (Söderbäck 1994). Burrows are a valuable resource; shelter occupants resist replacement by larger opponents more than those outside of shelters do (Figler et al. 1995a). Smaller crayfish living in burrows near where food was made regularly available were displaced by larger crayfish living farther away (Ranta and Lindström 1992). Under these circumstances, it seems imprudent for a subordinate animal to burrow a shelter in the vicinity of a dominant because the subordinate would constantly be competing with the dominant for limited resources such as food. Moreover, since shelter construction is an investment, it seems that creating a burrow that is likely to be lost to the dominant animal dissipates valuable energy. Therefore, inhibition of burrowing behavior near a dominant animal is advantageous when the subordinate is a recent loser to that animal. Once the subordinate is no longer near a dominant, its burrowing activity begins to recover, as was seen in the re-isolated subordinates after hierarchy formation. When an animal becomes dominant over its neighbors, however, it should start burrowing to take advantage of the benefits of the shelter.

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