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Report

Ritualized Submission and the Reduction of Aggression in an Invertebrate

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Summary

Ritualized behaviors that signify acceptance of a dominance relationship and reduce aggression between rivals are a common feature of vertebrate social behavior [1, 2]. Although some invertebrates, including crayfish [3], lobsters [4], and ants [5], display dominance postures, more complex dominance rituals and their effects on fitness have not been reported. We found that crayfish display such a complex ritual, when two males engaged in pseudocopulatory behavior to signify their dominance relationship. This was followed by a reduction in aggression and an increased likelihood of the subordinate's survival. Pseudocopulation was initiated by the eventual dominant and could be accepted or refused by the eventual subordinate. The frequency of aggressive behavior declined significantly during the first hour in all pairs that pseudocopulated but remained high in pairs that did not. Whereas all the subordinate members of pairs that pseudocopulated survived the initial 24 hr of pairing, half of subordinates that did not pseudocopulate were killed during that time. This differential mortality indicates that the reduction of aggression induced by the pseudocopulatory ritual directly enhances the differential survival of male crayfish that engage in this behavior.

Results and Discussion

Male crayfish have been seen to display pseudocopulatory behavior in the wild and in the laboratory and to leave a spermatophore deposited on the subordinate [6, 7]. However, the social significance of this behavior is not known. Here, we have observed pseudocopulation by pairs of adult male crayfish as each pair formed an initial dominance relationship in a laboratory aquarium. In 80% of the pairs tested (16 of 20 pairs), the emerging dominant attempted to mount the emerging subordinate in a manner similar to a male sexually mounting a female [8]. In 60% of pairs (12 pairs), the attempt led to a behavior characteristic of male-female mating ("pseudocopulation," Figure 1). In 20% (4 pairs), the subordinates refused all of the dominants' attempts and pseudocopulation failed to occur, and in the remaining 20%, no attempts were made.

Prior to a mounting attempt, the dominant often displayed typical male courtship behavior by approaching the subordinate from behind with a lowered posture and chelipeds (claws) held close to the body and lashing the subordinate with its antennae [7, 9]. The dominant then climbed up on the subordinate and used its chelipeds and walking legs to turn the subordinate over, ventral side up. When the subordinate accepted the dominant's approach and mount, it extended its abdomen and promoted its chelipeds and walking legs forward and parallel to its body in a supine posture characteristic of female mating behavior (Figure 1B). The subordinate became very passive as the dominant grasped the subordinate's chelipeds with its own, clasped the subordinate's cephalothorax with its walking legs, and extended its abdomen parallel to and facing the subordinate's extended abdomen. The dominant erected its gonopodia and thrust them rhythmically toward the subordinate's gonopodia, which remained motionless (see the Movies in the Supplemental Data available with this article online). Bouts of pseudocopulation lasted from 7 s to 9 min, 3 s (m ± SD: 1 min, 17 s ± 2 min, 9 s) in the 12 pairs observed, a significantly shorter period than the 30–90 min durations reported for male-female copulation [8]. Pseudocopulation ended in the same manner that females end bouts of copulation, when the subordinate slowly flexed its abdomen, dissociated from the dominant's grasp, and retreated.

The dominance relationship within each pair became apparent when the balance of one animal's behavior suddenly became aggressive (attacks, approaches, and offensive tailflips) and the other became defensive (escape tailflips and retreats) (Figure S1). Dominance relationships in pairs that pseudocopulated were established early (m ± SD: 8.8 ± 10 min) and remained stable over 5 days for 11 of 12 pairs (Figure 2). Relationships in three out of the eight pairs that did not pseudocopulate or failed to pseudocopulate were reversed during the first hour of interactions, and the dominance relationship for one pair was never established. The mean time of final formation of a dominance relationship for these animals was 23 ± 20 min (m ± SD). Although larger animals tended to dominate, the smaller animals dominated the larger ones (maximum body-size difference did not exceed 6%) in six out of the 20 pairs.

Bouts of pseudocopulation occurred both before (in four of 12 pairs) and after (in 11 of 12 pairs) the time of dominance formation (Figure 2). In six of 12 pairs, the first bout of pseudocopulation occurred during the first 15 min of interactions when the dominance relationship was being established and agonistic interactions were most intense [10]. The average percent time that pairs spent pseudocopulating remained constant over the first half hour of pairing and declined to low values over the second half hour (Figure 3A). All but one bout (n = 20) were observed during the first hour of interaction; the exception occurred 10 min into the second hour of pairing.

The frequency of aggressive and submissive behavior acts came to be different between pairs that did not

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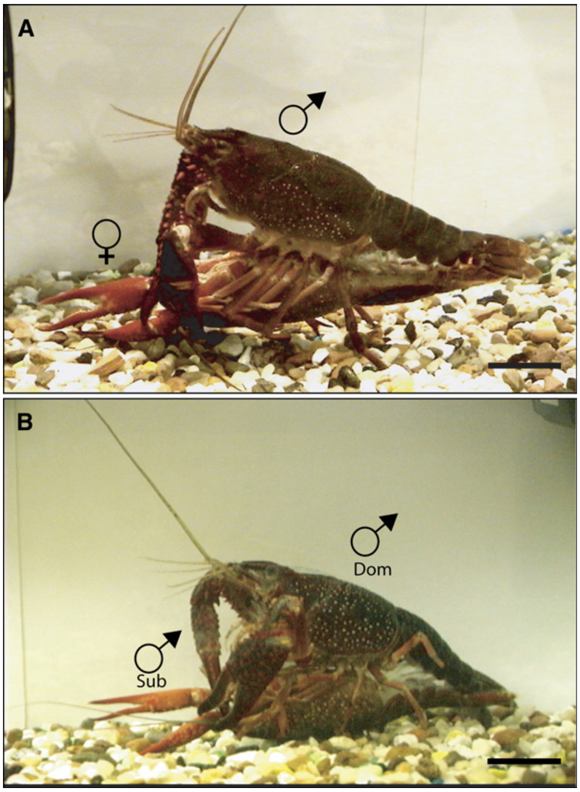

Figure 1. Pseudocopulation between Male Crayfish Resembles Copulation between Female and Male Crayfish

Male-female copulation (A) and male-male pseudocopulation (B) in crayfish (*Procambarus clarkii*). The scale bar represents 2 cm.

pseudocopulate or failed to pseudocopulate and those that did pseudocopulate (Figure 3). During the first 15 min of pairing, there was little difference in the agonistic behavior of the three groups. However, after the first 15 min, the pattern of fighting changed markedly (Figures 3B and 3C). For pairs that did not pseudocopulate or failed to pseudocopulate, the time spent fighting, the number of attacks and approaches per encounter by dominants, and the frequency of subordinate retreats remained unchanged over the first hour of pairing. However, for pseudocopulating pairs, the time spent fighting decreased significantly (Wilcoxon matched pairs test, two tailed; $p = 0.0005$), as did the number of attacks and approaches per encounter made by dominants (Wilcoxon matched pairs test, two tailed; $p = 0.0342$) (Figures 3B and 3C). Subordinates that pseudocopulated decreased the frequency of their retreats significantly over the course of the hour (Wilcoxon matched pairs test, two tailed; $p = 0.0068$) (Figure 3D). By the end of the first hour, each of these measures in the pseudocopulating animals had fallen significantly below the corresponding values recorded for both groups that did not pseudocopulate (Kruskal-Wallis one-way ANOVA test, two tailed; $p < 0.05$).

The difference in the frequency of aggressive behavior after the first hour led to significant differences in the mortality between pairs that pseudocopulated and those that did not or failed to pseudocopulate during the first 24 hr of interactions (Figure 4). Subordinates of pairs that did not or failed to pseudocopulate experienced much higher mortality (four of 8 killed, or 50%) than subordinates that pseudocopulated (0 of 12 killed, or 0%; total pairs = 20; n = 12 pseudocopulation, n = 8 no pseudocopulation; Fisher's exact test, two-tailed; $p = 0.0144$).

Analysis of the time-lapse recordings indicated that the dominants in pairs that did not or failed to pseudocopulate persisted in their aggressive behavior toward the subordinates, which repeatedly tried to retreat or escape. Four of these subordinates eventually slowed their movements, stopped escaping, and were killed, dismembered, and partially eaten during the first 24 hr of pairing. Two were killed toward the beginning of the first 24 hr together, and two others were killed at the end (Figure 2). The frequency of aggressive behavior was much less for pseudocopulating pairs. Although at times a dominant left its own shelter and approached and chased the subordinate out of its shelter, the lower aggressiveness of these dominants reduced the intensity of their social interactions.

The difference in aggression and mortality among the three groups diminished rapidly after the first day (Figures 3 and 4). One subordinate that pseudocopulated and one that did not pseudocopulate were killed on the fifth day of pairing (Figure 2) so that six of the 20 animals were killed. This 30% mortality over the 5-day experiment fell within the 20%–69.9% reported from various laboratory and field studies of crayfish population dynamics [11–14].

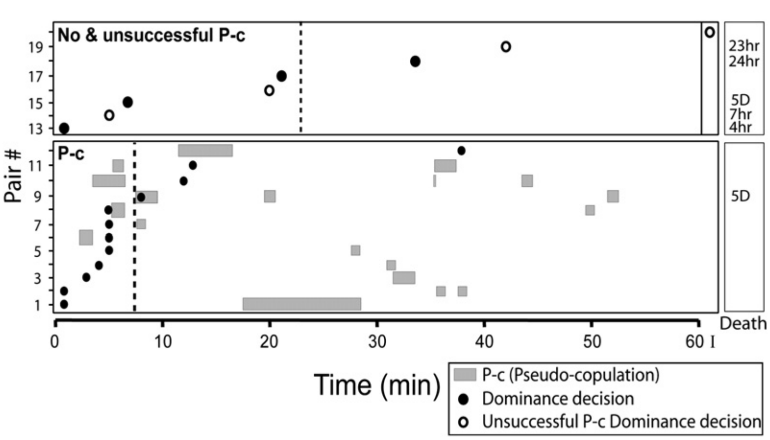

Figure 2. Times of Dominance-Relationship Formation and Pseudocopulation

Filled circles indicate the times of dominance formation of pairs that failed to pseudocopulate (top panel) and of pairs that pseudocopulated (bottom panel). The circle at time I (indefinite) in the top panel denotes the pair that failed to form a stable relationship. Filled gray squares in the bottom panel indicate the times of pseudocopulation of those pairs. The vertical dashed lines indicate the average time for the formation of the dominance relationship for all pairs. The right panel denotes the time when the subordinates were killed (hr = hours, D = day).

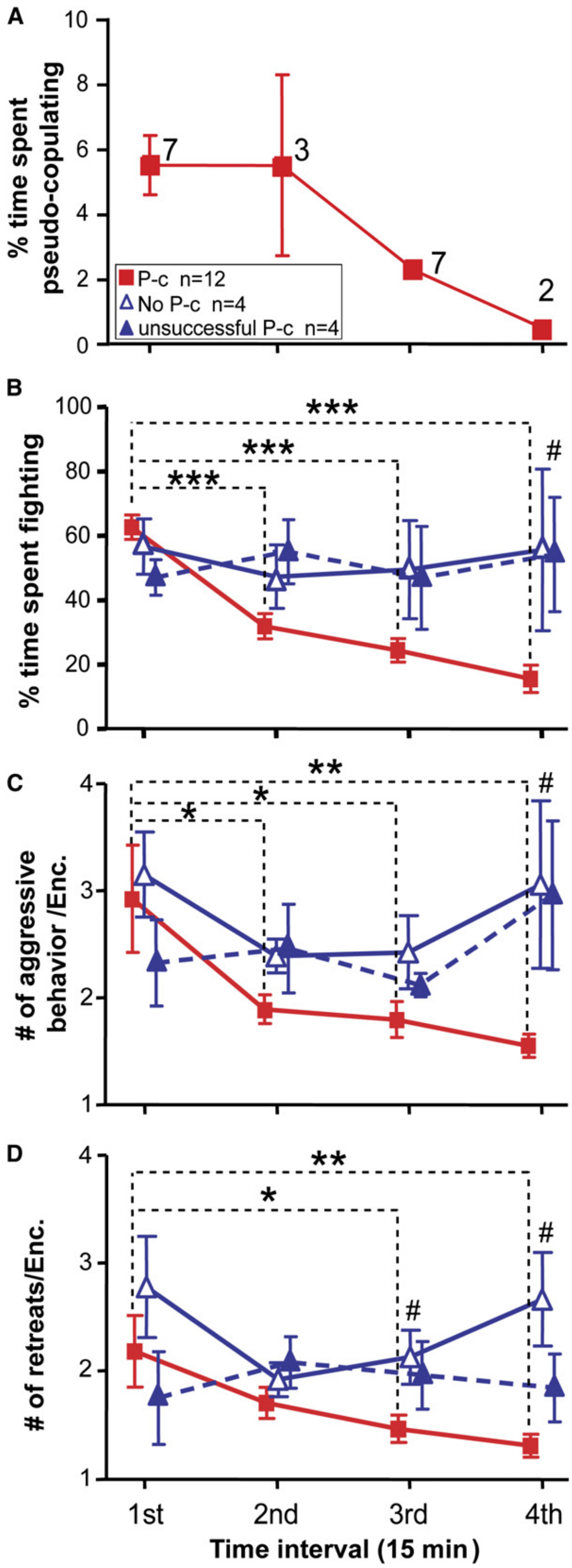

Figure 3. Changes in the Patterns of Pseudocopulation and Agonistic Behavior of Pseudocopulating and Non-pseudocopulating Crayfish Pairs during the First Hour of Pairing

(A) The total percent time (and SD) spent pseudocopulating in each quarter hour. The numbers next to each square give the total number of pseudocopulation bouts during that period.

(B) The percent time spent fighting. Pairs that pseudocopulated (red squares) significantly decreased their time fighting in the second 15 min period (asterisks), whereas pairs that did not (open triangles) or failed to pseudocopulate (closed triangles) spent about half their time fighting throughout the hour. Pound signs indicate significant differences between the pseudocopulating compared to the non-pseudocopulating pairs and ones that failed to pseudocopulate for each time period.

(C) Frequencies of aggressive behaviors per encounter for dominant animals. The number of aggressive behaviors (attacks, approaches, and offensive tailflips) were counted for each 15 min period and averaged over the pseudocopulating and nonpseudocopulating animals. Dominant animals in pseudocopulating pairs significantly decreased their aggression over the hour. The frequency of aggressive behavior was significantly different between the groups at the second and fourth 15 min time periods.

(D) The frequencies of retreats by pseudocopulating subordinates fell continuously over the first hour and came to differ significantly from those of the nonpseudocopulators in the third and fourth 15 min periods. Significance levels (*) or (#) = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.0005$.

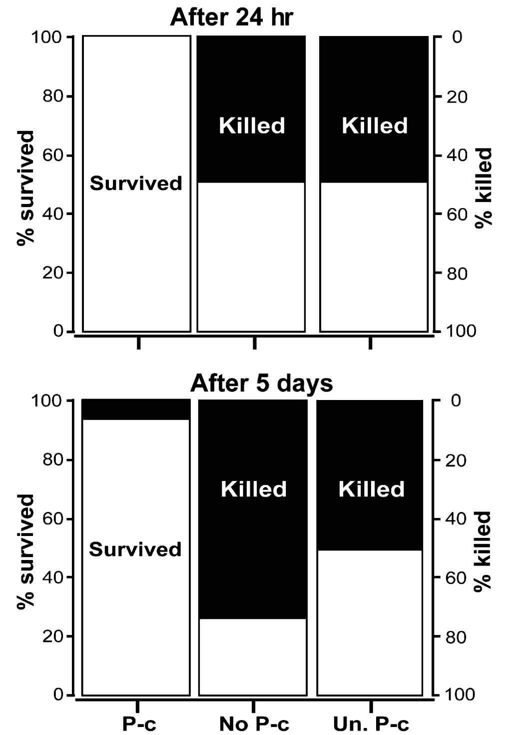

Figure 4. The Effect of Pseudocopulatory Behavior on the Survival of Subordinate Animals

All (12/12) pseudocopulating subordinates (P-c) survived 24 hr of continuous pairing, whereas half (4/8) of nonpseudocopulating subordinates were killed by their dominant partners during that time (two subordinates that did not pseudocopulate, No P-c; two subordinates that failed to pseudocopulate, Un. P-c). The difference in survival rate among the three groups is significantly different and is maintained after 5 days of pairing.

The slower decline in the frequency of aggressive behavior among pairs that did not or failed to pseudocopulate follows the time course of aggression seen among groups of juvenile crayfish as they formed a dominance hierarchy. A high frequency of aggression behavior during the first day of interaction led to deaths in one-third of the 32 groups of juvenile crayfish, which were too young to copulate or to pseudocopulate. The aggression was lower the next day and nearly disappeared over the next week as the frequency of agonistic encounters dropped by 90%. The dominance hierarchy in each group was still evident as the subordinates moved away at the approach of the dominant, thereby avoiding an attack [10].

Pseudocopulation in crayfish has evolved into a ritualized behavior that signifies establishment of a dominance relationship and a rapid reduction of aggression by the new dominant between male crayfish. Reduced aggression allows the subordinate to survive over the first 24 hr within the context of this dominance relationship. These effects are similar to those of copulation between male and female crayfish, and such copulation can also begin with an aggressive encounter and has been seen as an extension of male dominance behavior [3, 7]. Moreover, if the female refuses the male's attempts to mate, she can be killed [15].

Pseudocopulation appears to facilitate a stable dominance-relationship formation and to reduce aggression during agonistic interactions between male crayfish. When one animal assumes the dominant male role and the other assumes a female submissive role, pseudocopulation functions as a mutual honest signal of the opponents' relative social rank. Consequently, social dominance conflicts are resolved sooner leading to a reduction in both the time spent fighting and energy costs.

Although our results demonstrate the benefits of pseudocopulation between two male crayfish, its effects on the dynamics of social interactions of larger groups remains unknown. Pseudocopulation is observed to occur among groups of wild [6, 7] and captive crayfish of approximately equal ratios of males and females (F.A.I., unpublished data). However, its frequency and effects remain to be studied. Pseudocopulation within large groups may serve as a victory display for third-party observers as commonly found among many animal species (known as "eavesdropping"). This behavioral strategy allows group members to assess the strength and weakness of individuals through observation of their agonistic interactions and thus minimize direct social conflicts and associated energy costs [16, 17].

Pseudocopulation and copulation can be seen as two uses of the same behavior by crayfish to resolve dominance disputes with a minimum amount of aggression and to mate. Both uses appear to enhance the fitness of both members of each pair: Pseudocopulation increases the dominant's access to resources, whereas it reduces the chances of injury and death for both subordinate and dominant, and copulation contributes directly to the reproductive success of both the male and female.

Although ritualized courtship displays are common among invertebrates [1], similarly complex ritualized dominance displays are not common. Ritualized dominant and submissive postures, but not more complex behaviors like pseudocopulation, have been observed in lobsters [4], crickets [18, 19], ants [5], and wasps [20].

Among mammals, ritualized dominance displays, like pseudocopulation, occur among animals that form social groups. Pseudocopulation is one element in a set of ritualized submission behaviors used by various primate species to affirm dominance relationships and reduce aggression between rivals [21, 22].

The similarity in the form and function of pseudocopulation and copulation in crayfish and mammals is striking, given the very different body forms, brain structures, and lineages of these animals. This similarity allows these behaviors to provide an example of the convergent evolution of social and sexual behavior in animals across the animal kingdom.

Experimental Procedures

Animal Maintenance

Adult crayfish (*Procambarus clarkii*, 8–10 cm, form I) with intact limbs, antennae, and antennules were bought from a commercial supplier (Atchafalaya Biological Supply, Raceland, Louisiana) and housed individually in 20 l tanks each containing dechlorinated water, a filter (Duetto 100 submersible power filters), and an airstone for oxygenation. The animals were isolated for a minimum of 3 months on a 12/12 hr light/dark cycle and were fed shrimp pellets once a week.

Pair Formation

Fresh water was made 2 days prior to each experiment. The water was made from distilled water, and then fresh water aquarium salt was added to the water in accordance to the supplier's recommendations. This enabled us to ensure that the water would not contain contaminants and chlorine that might harm the animals. For 2 days, air was pumped into the water to ensure ambient oxygen levels before introduction of the animals into the tanks. Each tank contained its own filtration and oxygen system. We used Duetto 100 submersible power filters designed to handle 20 gallon tanks exceeding the requirements of the tanks used in this study of 5 gallons.

Between 8 and 10 a.m., pairs of individually marked crayfish were placed on either side of a transparent divider in the testing aquarium (20 liters, $20 \times 40 \times 27$ cm). The divider was removed after 15 min to allow the animals to interact. The animals were free to interact at all times during the 5 day pairing period. Two shelters were provided for each pair after the first 6 hr of interaction. A digital camcorder (Panasonic Corp.) recorded the agonistic interactions (attacks, approaches, defensive and offensive tailflips, and retreats) for each pair during the first hour of interaction. A separate time-lapse video system (Panasonic Corp. Time-Lapse AG-6730) recorded the first 24 hr of interactions. For the remaining 4 days, the pairs were observed for 30 min in the morning (crayfish peak activity time) and the number and type of aggressive and submissive behaviors were noted.

Dominance Indices and Formation

Data Analysis

Dominance between the two animals was determined based on the fraction of aggressive and submissive behavior each animal performed as described elsewhere [10]. The videotaped behavior was analyzed at each second and tabulated in an Excel spreadsheet. The dominance relationship between the two animals was accurately tracked at this time resolution (Figure S1).

The agonistic interactions of the animals were termed *encounters*. All the encounters were recorded chronologically for the five agonistic behaviors (attack, approach, offensive tailflips, retreat, and escape tailflips). Each behavior was defined as follows: an "attack" is an *aggressive physical contact* initiated by one animal toward another. An "approach" occurs when one animal moves towards another with no physical contact but a response is evoked from the other. "Offensive tailflips" are a series of rapid flexions of the abdomen while the animals are interlocked (and are used to test each other's strength). A "retreat" is an ambulatory movement away from an approaching or attacking animal. An "escape" is a rapid movement away from an aggressor produced by one or more tailflips (i.e., rapid flexions of the abdomen).

Each aggressive behavior (an attack, an approach, or an offensive tailflip) was assigned a value of +1, and each defensive behavior (an escape or a retreat) was assigned a value of −1. The dominance index for each animal was calculated as the sum of its values over each 2 min period of the first hour of pairing. A dominance transition was assumed to occur when one animal's index became positive and the other's index became negative for 5 min or longer.

Supplemental Data

Supplemental Data include one figure and two movies and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/22/2217/DC1/.

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