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RESEARCH ARTICLE

Interlocking of chelae is a key factor for dominance hierarchy formation in crayfish

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SUMMARY

We characterized the role of chelae during agonistic encounters of the crayfish *Procambarus clarkii*. Physical asymmetries in body length, body mass and chelae size were directly related to dominance hierarchy formation. More than 80% of winning crayfish had longer body and chelae lengths, and winners were usually heavier in body mass, even if their differences were less than 3% compared with losing opponents. In mismatched pairings, large crayfish with short chelae were beaten by small crayfish with large chelae. Three physical elements of body length, body mass and chelae length equally affected the outcome of agonistic bouts. Chelae restriction, in which chelae were tightly closed using rubber bands, affected the outcome of agonistic bouts between large and small crayfish. Before chelae restriction, large crayfish won in all pairings. Following chelae restriction for at least 30 days from the first encounters, the winning rate of large crayfish that were previous winners decreased significantly in the second encounters against the same opponents that were previous losers. The handicap of chelae restriction significantly prolonged the time to formation of the winner–loser relationship. Individual fights escalated during agonistic bouts between large crayfish with one chela restricted and small crayfish with intact chelae, whereas the number of fights increased but the duration of individual fights did not increase in large crayfish with both chelae restricted. Furthermore, when the chelae of both large and small crayfish were disabled, the dominance order was frequently not formed during 30 min of agonistic bouts. Preventing chelae from interlocking prevented escalation of agonistic bouts. We show that interlocking of chelae acted as a key factor for the formation of dominance hierarchy.

Key words: crayfish, dominance hierarchy, agonistic behaviour, physical body size, chelae.

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INTRODUCTION

Status-dependent hierarchy formation is essential for territorial animals to maintain social stability. Dominant animals have increased opportunity to access good shelter, food and a mating partner, whereas subordinate animals can be rescued from severe injury or death. Dominance hierarchies are formed in various species of arthropods including crickets (Alexander, 1961), spiders (Aspey, 1977), cockroach (Ewing, 1974), lobsters (Fiedler, 1965), hermit crabs (Hazlett, 1968) and crayfish (Bovbjerg, 1953). Decapod crustaceans, in particular, are good models with which to characterize dominance order both physiologically and behaviourally (Fujimoto et al., 2011; Sato and Nagayama, 2012). Physical asymmetries have been shown to be adequate predictors of dominant–subordinate relationships. For example, carapace length (Pavey and Fielder, 1996), body length (Bovbjerg, 1956; Lowe, 1956; Rubenstein and Hazlett, 1974), chelae size (Garvey and Stein, 1993; Rutherfold et al., 1995; Barki et al., 1997; Sneddon et al., 1997; Gabbanini et al., 2006), body mass (Pavey and Fielder, 1996) and sex (Stein, 1976; Figler et al., 1995) have all been implicated as determining factors of the outcome of agonistic encounters. Larger animals usually win in agonistic encounters. Furthermore, extrinsic and intrinsic factors, such as habitat and social experience, also affect the outcome of agonistic bouts. For example, crayfish agonistic interactions are longer and more intense when the agonistic bout took place near a shelter than on or near food-resource habitats (Bergman and Moore, 2003), and residence owners are more likely to win than intruders (Ranta and Lindstrom, 1992; Peeke et al., 1995). The winner and loser effect is also important, with previous winners winning significantly more encounters than previous losers during their second fight (Goessmann et al., 2000; Daws et al., 2002; Seebacher and Wilson, 2007; Zulandt et al., 2008).

In many decapods, especially Astacidea, Anomura and Brachyura, the front pair of walking legs have enlarged pincers, the chelae. The chelae are unique, being versatile appendages for both offence and defence. The chelae are used as weapons during agonistic encounters, and display patterns of chelae, e.g. extension, grasping, lifting, scissoring, striking, thrust, embrace, nip and push, have an important role in agonistic and aggressive interactions (Mariappan et al., 2000). During growth, the body proportions are similar, with the cephalothorax and abdomen being approximately equal; however, the chelae show positive allometry (Bovbjerg, 1956; Aiken and Waddy, 1992). There is a positive correlation between the pinching force and body length, between the pinching force and body mass, and also between chelae size and chelae force (Claussen et al., 2008; Wilson et al., 2009). Rutherfold et al. (Rutherfold et al., 1995) showed that chela length was an important indicator of fighting ability in the crayfish *Orconectes rusticus*, as there was no significant difference in cephalothorax length but winners had significantly longer chelae than losers. Furthermore, larger chelae clearly result in greater dominance, but the pinching force of the chela had no bearing on dominance in slender crayfish *Cherax dispar* (Wilson et al., 2007). In the crab *Potamon fluviatile*, chela force is the main factor influencing the outcome of agonistic bouts only in females, but chela size, more than chela force, is used to settle agonistic bouts in males (Gabbanini et al., 2006). There is, however, a lack of clarity in the role of chelae interlocking during agonistic bouts of the crayfish *Procambarus clarkii*. In this study, we have characterized the relationship between body length, body mass and chelae size during agonistic encounters, and found that interlocking of the chelae was a key factor in forming dominance hierarchies.

MATERIALS AND METHODS

Animals

Adult male crayfish *Procambarus clarkii* (Girard 1852) of 6–9 cm in length, from rostrum to telson, were purchased from a commercial supplier and maintained individually in separate opaque containers of 19×33×15 cm (width×length×height) filled with water to a depth of 10 cm for at least 30 days. Each crayfish was fed equal amounts of small food pellets once a week and was last fed at least 5 days before pairings. Crayfish were maintained under a 12 h:12 h light:dark cycle. Experimental trials were carried out in a dimly lit laboratory at a room temperature of approximately 23°C.

Pairing

Two crayfish with a length difference between 1 and 15% were selected and paired in a new opaque container (26×38×24 cm width×length×height) filled with water to a depth of approximately 12 cm. The day before pairing, body length (from rostrum to telson), wet body mass and average length of the right and left chelae were measured. Prior to each trial, an opaque plastic barrier was placed in the centre of the tank separating it into two areas. A crayfish was placed on each side of this barrier and allowed to acclimate for at least 10 min before the divider was removed.

The agonistic bouts of the crayfish were recorded using a video camera (Victor GZ-MG330-S, Okoyama, Japan) mounted on a tripod above the container for 30 min. The behaviour of each crayfish was analyzed using a single frame measurement to construct an ethogram of each second. Behavioural acts that occurred during agonistic bouts were categorized as one of seven types: capture, fight, contact, approach, retreat, tailflip and neutral (Sato and Nagayama, 2012). Capture, fight, contact and approach were aggressive behavioural acts, whereas retreat and tailflip were submissive acts. Capture was defined as the act in which one crayfish held an opponent using its chelae. Fight was defined as the act in which both crayfish fought using their chelae. Contact was defined as the act in which both crayfish made physical contact without fighting, and approach was defined as the act in which one crayfish moved forward towards the other. Retreat was defined as the act in which one crayfish walked away from an approaching or attacking opponent, and tailflip was defined as the act in which one crayfish escaped from an approaching or attacking opponent using rapid tailflipping. Neutral was defined as other behavioural acts including pause, walking with no correlation to an opponent and no response. The winner–loser relationship was determined with several fights. Before determination of dominance status, the crayfish that initiated the approach was frequently beaten in the following bouts by their opponents. After establishment of dominance order, subordinate crayfish almost always showed a retreat or tailflip following the dominant's approach without fight. We determined the time of establishment of the winner–loser relationship when the subordinate crayfish showed a retreat or tailflip following the dominant's approach at least three times in succession (Sato and Nagayama, 2012).

Mismatched pairing

Juvenile crayfish that were hatched in our laboratory from commercially obtained females were collected at the third stage (after the second moult). They were isolated and reared to over 6 cm in length. At specific stages during growth, both chelae were removed to regenerate new, small chelae at the next moult. In some experiments, these animals with short chelae were paired with small crayfish (body size) with big chelae.

Chelae restriction

In some experiments, dominant and subordinate crayfish were separated from each other after their first encounter. To negate the effect of the result of first encounter, animals were re-isolated in separate tanks for more than 30 days. Then, they were paired again in a new container and tested once more. Two days before the second encounter, the right or left chela of the dominant animals, both chelae of dominant animals, or both chelae of both the dominant and subordinate animals were closed tightly using rubber bands around each chela. Crayfish were then paired again and, after the dominant–subordinate relationship was determined, they were re-isolated for at least 30 days. After the second re-isolation, the chelae restriction was released by removal of the rubber bands. The same animals were paired again for a third encounter with the exception of pairs in which either one or both animals moulted during isolation.

Statistical analyses were carried out using SigmaPlot v11 (Systat Software, Chicago, IL, USA) and R 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented as means ± s.e.m. unless otherwise indicated.

RESULTS

Body size and agonistic encounters of crayfish

After pairing of two crayfish in the fighting arena, they raised both chelae and attacked each other. The period of each fight was variable from several seconds to some minutes. The probability of fighting declined gradually over time and the relationship between dominant and subordinate was usually determined within 20 min after pairing. The subordinates were then usually observed to move away rapidly from the approach of dominants using mainly a retreat. In 43 pairs (Fig. 1), crayfish with a longer body length, from rostrum to telson (1.3–15.1% longer than small opponents), won in 37 pairings. The smaller crayfish (2.9–4.3% shorter than large opponents) won in three pairings, whereas in the remaining three pairings (length difference ranged between 2.4 and 8.1%) no winner found in a 30 min period. Heavier crayfish (1.0–28.8% heavier than small opponents) won in 40 out of 43 pairings whereas in the remaining three pairings (mass difference between 14.5 and 19.8%) no winner was found in a 30 min period. The crayfish that had longer chelae

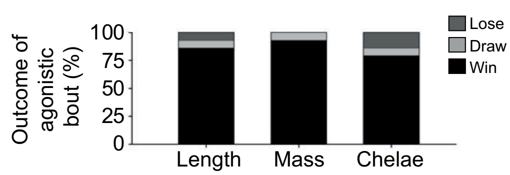

Fig. 1. Winning rate of larger crayfish during agonistic bouts against smaller crayfish based on 43 pairings. The percentages of outcomes of agonistic bouts of larger crayfish are plotted as wins (black), draws (light grey) and losses (dark grey). The left bar represents the results of animals of greater body length, the middle bar represents that of animals of greater wet mass and the right bar represents that of animals of greater chelae length.

(0.7–28.8% longer than small opponents) won in 34 pairings and lost in six pairings (2.5–16.2% longer chelae). In the remaining three pairings in which the chelae length difference was between 4.1 and 9.0%, no winner was found in a 30 min period. The winning percentage of large crayfish (in terms of body length) was 86.0%, that of heavy crayfish was 93.0% and that of crayfish with long chelae was 79.1%. Crayfish larger in terms of body length, body mass and chelae length were thus likely to win (binomial test, $P<0.001$). Furthermore, the probability of large animals winning was not statistically different among these three groups (Fisher's exact test, $P=0.166$).

Mismatched pairings

Body length and mass are highly correlated (Wilson et al., 2007), and the chelae are large structures that make up 35–50% of crayfish total mass (Stein, 1976). Furthermore, as shown in Fig. 2, length and mass of chelae were highly correlated ($r^2=0.925$, $P<0.001$).

Because crayfish routinely use their chelae for both intimidation and fighting (Wilson et al., 2007), large crayfish with short chelae were paired with small crayfish (1–9% difference in body length and 3–12% difference in wet mass) that had long chelae (7–25% difference). As control, normal crayfish with different body mass (3–12% difference) were paired ($N=17$). In all normal pairings (triangles in Fig. 3), heavy crayfish won irrespective of whether they had long or short chelae (from –16 to 9% difference). Sixteen out of 17 winners had a longer body length than losing opponents. In contrast, heavy and large crayfish won in one out of 10 pairings in mismatched pairings (circles in Fig. 3). In the remaining nine pairings, crayfish with long chelae became dominants despite their lighter body masses and shorter body lengths. The possibility of winning of heavy crayfish was significantly different between control and mismatched pairings (Fisher's exact test, $P<0.001$), suggesting that the length of chelae relative to body size was important in predicting the outcome of agonistic bouts.

In control pairings, dominant–subordinate relationships were formed within 75 to 1300 s (725.8±90.4 s). The time to form hierarchy in mismatched pairings was within 400 to 1100 s (696.8±78.9 s), similar to control pairings (Mann–Whitney rank sum test, $P=0.829$; Fig. 4A). The total number of fights during agonistic encounters of 30 min was 7.3±1.2 in control pairings and 6.8±0.8 in mismatched pairings (Fig. 4B). Furthermore, the total fight duration was 192.6±37.6 s in control pairings and 291.6±81.8 s in mismatched pairings (Fig. 4C). The number and duration of fights between control and mismatched pairings were not statistically different (Mann–Whitney rank sum test, $P=1.000$ and $P=0.353$, respectively;). As shown in Fig. 4D, the number of approaches of heavy animals in the control pairings was 26.7±2.5, whereas that of heavy animals in the mismatched pairings significantly decreased to 9.8±2.9 (Mann–Whitney rank sum test, $P<0.001$). In contrast, the number of submissive behavioural acts, i.e. retreats and tailflips, of heavy animals in the mismatched pairings significantly increased to 50.1±7.9 compared with that of heavy animals in the control pairings (3.8±0.5) (Mann–Whitney rank sum test, $P<0.001$; Fig. 4E).

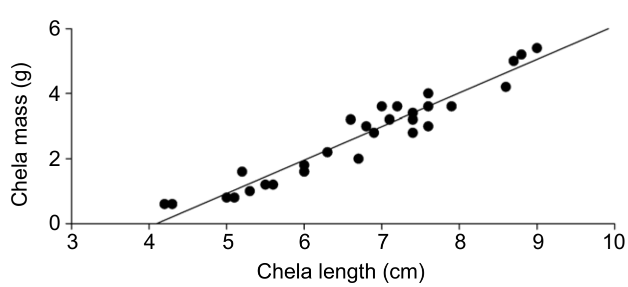

Fig. 2. The relationship between chela length and chela mass. There was a significant relationship ($y=-4.221+1.030x$, $r^2=0.925$, $P<0.001$), based on 32 chela from male crayfish between 6.5 and 8.4 cm in body length. These crayfish were not used for pairing experiments throughout this study.

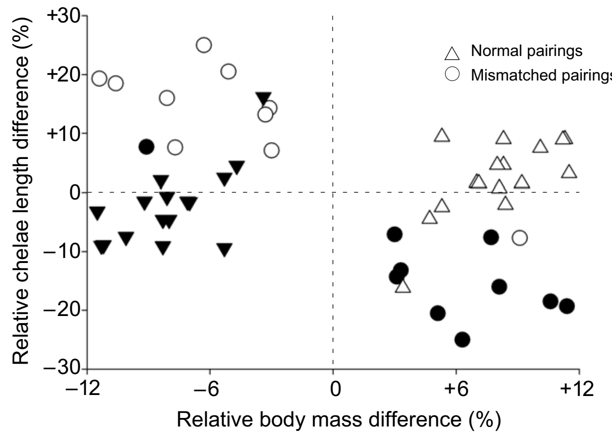

Fig. 3. The relationship between relative differences in body mass and chelae length for outcomes of agonistic bouts in normal pairings (triangles) and mismatched pairings (circles). Open symbols represent winners and filled symbols represent losers of the bouts.

Chelae restriction

In addition, we examined the effect of chelae interlocking during agonistic bouts using chelae restriction pairings. Before the restriction of chelae, large (from 1 to 12% difference) and heavy (from 1 to 29% difference) crayfish with long chelae (from 1 to 27% difference) were paired with small and light opponents with a short chelae length as a first encounter. Large crayfish won in all pairings ($N=36$). After the dominant–subordinate order was determined, crayfish were re-isolated for more than 30 days. Then they were paired again with the same opponents following three different treatments of chelae restriction. In the first treatment, one chela was restricted in large, previously dominant crayfish. Either the right or the left chela of the dominant crayfish was closed tightly using a rubber band before a second pairing with the previous loser. In 11 pairings, large crayfish won in seven pairings. Small, previously subordinate crayfish won in one pairing and in the remaining three pairings no winner was found in a 30 min period (Fig. 5). The winning probability of large crayfish with one chela restriction was not significantly different before and after chela restriction (McNemar's test, $P=0.125$), but they were not likely to win (binomial test, $P=0.549$). In the second treatment, both chelae were restricted in large, previously dominant crayfish. Both the right and left chelae of large crayfish were closed tightly using rubber bands before the second pairings with previous losers. In 15 pairings, large crayfish won on six occasions. Small, previously subordinate crayfish with intact chelae won in four pairings whereas in the remaining five pairings no winner was found in a 30 min period (Fig. 5). Large crayfish with both chelae restricted were not likely to win (binomial test, $P=0.607$) and their winning probability after chelae restriction significantly decreased from the first encounter (McNemar's test, $P=0.004$). In four new pairings, the base of both chelae of the large crayfish was surrounded by rubber bands as a dummy treatment. In this case, large crayfish could use chelae

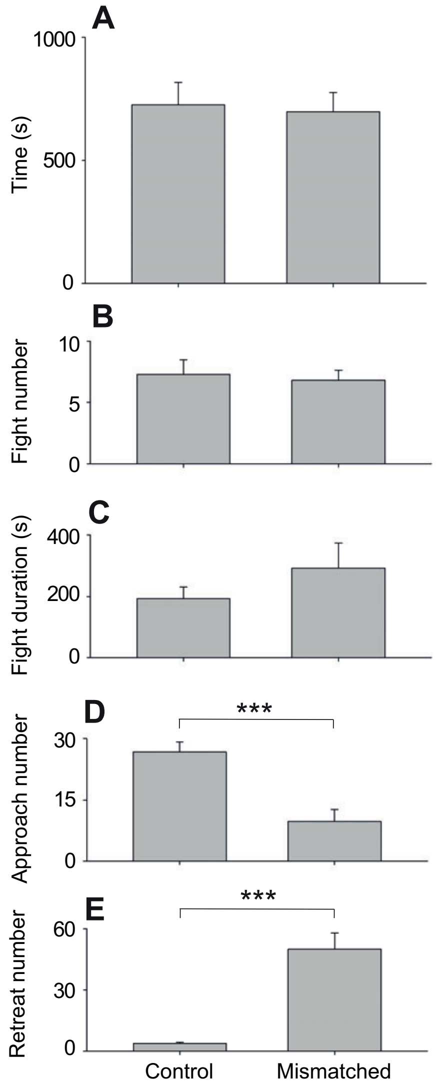

Fig. 4. Comparison of agonistic behavioural acts of heavier crayfish in both normal and mismatched pairings. (A) The time in which the dominant–subordinate relationship was determined. (B) The number of fights of pairings for 30 min agonistic bouts. (C) The total duration of fights of pairings for 30 min agonistic bouts. (D) The number of approaches of heavier animals for 30 min agonistic bouts. (E) The number of retreats and tailflips of heavier animals for 30 min agonistic bouts. Bars represent means ± s.e.m. from 15 pairs of crayfish in normal pairings as controls and 11 pairs of crayfish in mismatched pairings. Asterisks indicate that the number of responses differed significantly between normal and mismatched pairings (Mann–Whitney rank sum test, ***P<0.001).

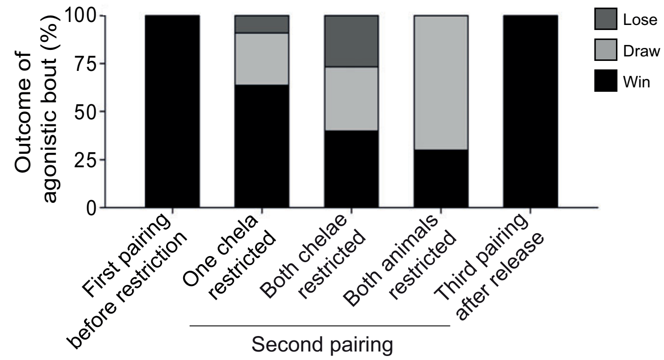

Fig. 5. Winning rate of larger crayfish during agonistic bouts against smaller crayfish before, during and after chelae restriction. The percentages of outcomes of agonistic bouts of larger crayfish are plotted as wins (black), draws (light grey) and losses (dark grey). The left bar represents the result of large animals in the first pairings before chelae restriction (N=36). Middle bars represent the results of large animals in the second pairings during chelae treatments (left: either the left or right chela was restricted in the large animals, N=11; centre: both left and right chelae were restricted in the larger animals, N=15; and right: both chelae of both large and small animals were restricted, N=10). Right bar represents the result of large animals in the third pairings after release from chelae restriction (N=16).

to interlock with opponents during agonistic bouts. These large crayfish always won against small opponents. In the third treatment, the chelae of both the large and small crayfish were restricted. Both the right and left chelae of the previous losing small crayfish as well as the previous winning large crayfish were closed tightly using rubber bands before second encounters. In 10 pairings, large crayfish won in three pairings. No small crayfish won, but in the remaining seven pairings no winner was found in a 30 min period (Fig. 5). Large, previously dominant crayfish with both chelae restricted were not likely to win (binomial test, P=0.344) and their winning probability was significantly different between the first and second encounters (McNemar's test, P=0.016). After the second pairings with chelae restriction, animals were re-isolated for more than 30 days following release from chelae restriction by removing the rubber bands. In 16 pairings, the large animals were paired again with the same small opponents for a third encounter. The large animals won in all pairings regardless of the results of the second encounters (Fig. 5).

Fig. 6 shows the time in which the dominant–subordinate relationship was determined in each pairing. In the first pairings before chelae restriction, dominance hierarchy was formed within 143 to 1680 s (907.9±69.7 s). The time to dominance formation in the second pairings with one chela restricted in large animals (N=8 out of 11 pairings) was within 626 to 1658 s (1314.5±137.3 s), that in the second pairings with both chelae restricted in large animals (N=10 out of 15 pairings) was within 530 to 1772 s (1214.0±113.4 s), and that in the second pairing with chelae restriction of both the large and small animals (N=3 out of 10 pairings) was within 1129 to 1502 s (1364.0±118.1 s). In the remaining second pairings of each treatment (N=1, 5 or 7, respectively), dominance hierarchy was not formed in a 30 min period. The time in which the dominant–subordinate relationship was determined in the third encounters after release of chelae restriction (N=16) was within 143 to 1127 s (752.9±72.2 s). There was no statistical difference (t-test, P=0.174) in the time between the first and the third encounters. Compared with the first and third encounters, the time for formation of dominance hierarchy in the second pairings with a treatment of chelae restriction was significantly longer (t-test, P<0.05 for the first *versus* second pairings with one chela restricted or with both chelae restricted, P<0.01 for the third *versus* second pairings with all three treatments), although there was no difference statistically among the second pairings of three different treatments of chelae restriction (t-test, P=0.577 for one chela *versus* both chelae restricted, P=0.842 for one chela *versus* both crayfish restricted, and P=0.512 for both chelae *versus* both crayfish restricted).

The total number of approaches during agonistic encounters of 30 min of the large crayfish and small opponents is plotted in Fig. 7A. In the first encounters before chelae restriction, the number of

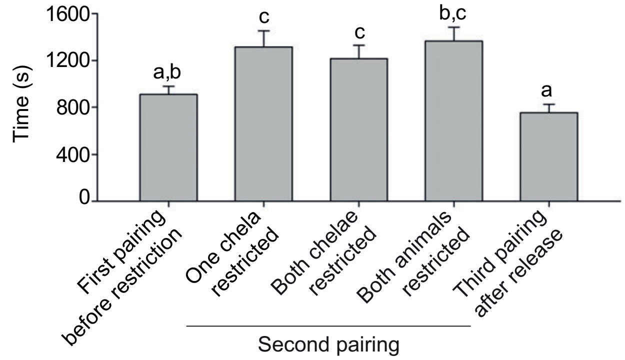

Fig. 6. The time in which the dominant–subordinate relationship was determined before, during and after treatment of chelae restriction. Bars represent means ± s.e.m. from 36 pairs of the crayfish in the first pairings, 11 pairs in the second pairings with one chela restricted in large crayfish, 15 pairs in the second pairings with both chelae restricted in large crayfish, 10 pairs in the second pairings with chelae restriction of both crayfish, and 16 pairs in the third pairings after release from chelae restriction. Letters above plots show statistical differences (*t*-test).

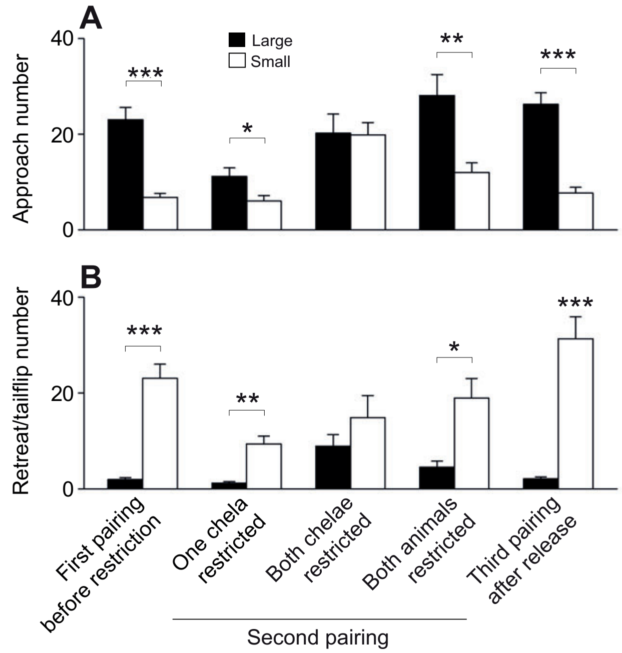

Fig. 7. Comparison of aggressive and submissive behavioural acts in both large and small crayfish before, during and after treatment of chelae restriction. (A) The number of approaches of large (filled bars) and small (open bars) animals for 30 min agonistic bouts. (B) The number of retreats and tailflips of large and small animals for 30 min agonistic bouts. Bars represent means ± s.e.m. from 36 pairs of the crayfish in the first pairings, 11 pairs in the second pairings with one chela restricted in large crayfish, 15 pairs in the second pairings with both chelae restricted in large crayfish, 10 pairs in the second pairings with chelae restriction in both crayfish, and 16 pairs in the third pairings after release from chelae restriction. Asterisks indicate that the number of responses differed significantly between large and small animals (*t*-test or Mann–Whitney rank sum test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

approaches of the large crayfish was 23.0±2.6 whereas that of small opponents was significantly lower (6.8±0.8; Mann–Whitney rank sum test, $P<0.001$). In the second pairings, the number of approaches in the large crayfish with one chela restricted was 11.8±1.8 whereas that of the opponents was 6.0±1.2. The number of approaches between them was statistically different (Mann–Whitney rank sum test, $P<0.05$). The number of approaches in the large animals with both chelae restricted was 20.2±4.0 whereas that of small opponents with intact chelae was 19.9±2.6. There was no statistical difference (Mann–Whitney rank sum test, $P=0.944$). The number of approaches in the large animals with both chelae restricted was 28.1±4.4 whereas that of small opponents with both chelae restricted was 12.0±2.0 that was significantly lower (Mann–Whitney rank sum test, $P<0.01$). In the third encounters after the release of chelae restriction, the number of approaches in the large animals was 26.3±2.4 whereas that of small opponents significantly decreased (Mann–Whitney rank sum test, $P<0.001$) to 7.7±1.2.

The total number of retreats and tailflips during agonistic encounters of 30 min of the large crayfish and small opponents is plotted in Fig. 7B. In the first encounter before chelae restriction, the number of retreats/tailflips of the large crayfish was 1.9±0.4 whereas that of small animals was 23.1±2.9. In the second pairings, the number of retreats/tailflips in the large crayfish with one chela restricted was 1.2±0.3 whereas that of small opponents was 9.4±1.6. The number of retreats/tailflips of the large animals with both chelae restricted was 8.9±2.4 whereas that of small opponents with intact chelae was 14.9±4.6. The number of retreats/tailflips in the large animals with chelae restricted was 4.5±1.3 whereas that of small opponents with chelae restricted was 18.9±4.1. In the third encounter after the release of chelae restriction, the number of retreats/tailflips of the large crayfish was 2.1±0.4 whereas that of small opponents was 31.3±4.5. There were significant differences in the number of retreats/tailflips between large and small crayfish (Mann–Whitney rank sum test, $P<0.001$ for the first and third pairings, $P<0.01$ for the second pairings with one chela restriction, $P<0.05$ for the second pairings with chelae restriction of both animals), with the exception of the second pairings between large crayfish with chelae restricted and small opponents that had intact chelae (Mann–Whitney rank sum test, $P=0.506$).

The total number of fights during agonistic encounters of 30 min was 6.6±0.7 in the first pairing before chelae restriction, 6.0±0.9 in the second pairings with one chela restricted, 15.1±2.5 in the second pairings with both chelae restricted, 11.3±1.7 in the second pairings with chelae restricted in both animals, and 6.7±0.9 in the third pairings after release from chelae restriction (Fig. 8A). In treatments with both chelae restricted, the number of fights increased significantly. The total duration of fights was 321.6±47.3 s in the first pairing before chelae restriction, 606.0±121.3 s in the second pairings with one chela restricted, 575.8±68.4 s in the second pairings with both chelae restricted, 408.5±53.5 s in the second pairings with chelae restriction of both animals, and 197.4±35.9 s in the third pairings after release from chelae restriction (Fig. 8B). Treatments of chelae restriction in the large crayfish increased the fight duration significantly. The mean duration of individual flight was 59.8±10.4 s in the first pairing, 115.5±25.3 s in the second pairings with one chela restricted, 46.9±6.9 s in the second pairings with both chelae restricted, 47.8±11.0 s in the second pairings with chelae restriction of both animals, and 27.9±3.7 s in the third pairings after release from chelae restriction. Individual fight duration between large animals with one chela restricted and small opponents with intact chelae was considerably longer than that of other pairings of the first, second and third pairings (Mann–Whitney rank sum test, $P<0.05$).

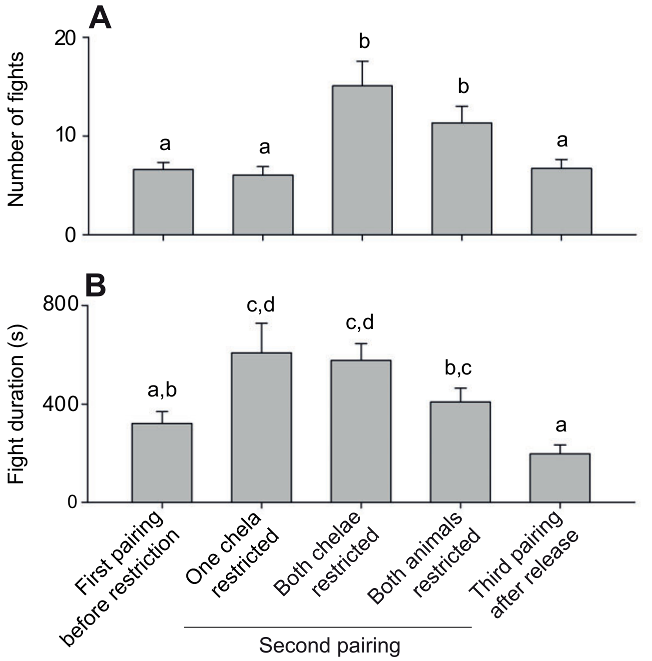

Fig. 8. Comparison of agonistic fights of pairings before, during and after treatment of chelae restriction. (A) The number of fights for 30 min agonistic bouts. (B) The total duration of fights for 30 min agonistic bouts. Bars represent means ± s.e.m. from 36 pairs of crayfish in the first pairings, 11 pairs in the second pairings with one chela restricted in large crayfish, 15 pairs in the second pairings with both chelae restricted in large crayfish, 10 pairs in the second pairings with chelae restriction of both crayfish, and 16 pairs in the third pairings after release from chelae restriction. Letters above plots show statistical differences (*t*-test or Mann–Whitney rank sum test).

Fight dynamics also changed between the first and second pairings. Before chelae restriction, crayfish used chelae to strike and to grasp their opponent's chela or walking leg. Chelae were also observed to cut opponent's antennae. Crayfish attempted to upset their opponent by lifting and twisting interlocking chelae. After chelae restriction, crayfish used chelae to strike and push their opponent. They attempted to press the opponent from the upper side.

DISCUSSION

Relative size is sufficient to predict the outcome of agonistic bouts

Many previous studies have indicated that physical asymmetries are adequate predictors of the dominant–subordinate relationship in crayfish (Bovbjerg, 1956; Lowe, 1956; Rubenstein and Hazlett, 1974; Garvey and Stein, 1993; Rutherfold et al., 1995; Pavey and Fielder, 1996). We have confirmed this in present study, which showed that the relative size of crayfish (in terms of body length, body mass as well as chelae size) was directly related to dominance hierarchy formation. More than 80% of winning crayfish had longer bodies and/or chelae length and winners were usually heavier in mass, even if their differences were less than 3% of losing opponents.

In shore crabs, winning crabs have heavier chelae with a greater surface area than losing opponents. Sneddon et al. (Sneddon et al., 1997) showed that weapon size is a more reliable predictor of the outcome of pairwise fights than body size. Barki et al. (Barki et al., 1997) also showed that cheliped size is used by male prawns in the assessment of fighting ability. Relatively small asymmetries in chelae size overrode large contradictory asymmetries in body size, and the large asymmetry in body length did not bias the probability of winning the contest toward the larger opponent, even when the opponents were symmetric in chelae size. Furthermore, Rutherfold et al. (Rutherfold et al., 1995) showed that chela length is a reliable predictor of relative fighting ability in the crayfish *Orconectes rusticus*. In the present study, however, six crayfish out of 43 pairings who had longer chelae (even more than 10% difference) were beaten by opponents with smaller chelae. In these cases, winning opponents were usually larger in terms of body length and body mass. The crayfish with large body lengths with heavy body masses and with long chelae lengths always won in this study. These three physical elements were equally important in predicting the outcome of agonistic bouts of crayfish. Mismatched pairing experiments supported this idea. Long and heavy crayfish usually won in normal pairings in which the chelae length difference with opponents was less than 10%. Longer and heavier crayfish were, however, beaten when they had very small chelae against opponents with short body lengths and light masses but with long chelae of more than 10% in difference.

The effect of chelae restriction

As well as agonistic interactions, chelae of decapods have a major role in defensive postures, copulation, prey manipulation, acquisition of shelters and courtship displays (Hazlett, 1962; Hazlett, 1972; Salmon et al., 1978; O'Neill and Cobb, 1979; Ameyaw-Akumfi, 1981; Nagayama et al., 1986; Mariappan et al., 2000). As a weapon, chelae length, more than pinching force, is thought to be the main factor influencing fighting ability in crayfish (Rutherfold et al., 1995; Wilson et al., 2007; Walter et al., 2011), though Seebacher and Wilson (Seebacher and Wilson, 2007) have indicated that greater chelae closing force significantly increases the chances of winning.

Our chelae restriction experiments showed that preventing chelae from opening and/or closing or decreasing their interlocking ability considerably affected the outcomes of agonistic bouts. Restriction of the right or left chela in the large animals who won in previous encounters decreased the winning probability in the second encounters with the same small opponents who had intact chelae. Restriction of both chelae in the large animals significantly decreased the winning probability in the second encounters with the same previous losing opponents who had intact chelae. Furthermore, when right and left chelae of both the large and small crayfish were restricted, their dominant–subordinate relationship frequently was not formed. These results suggest that chelae opening and closing strongly influenced outcome of fight. Because the length and mass of chelae were highly correlated, which suggested that large chelae had a greater mass of muscles, the pinching force of chelae affected the outcome of the agonistic encounter. Seebacher and Wilson (Seebacher and Wilson, 2007) performed similar chelae restriction experiments with the chelae of winner crayfish disabled by attaching the dactylus to the propodus with acrylamide glue. Previous winners with their chelae disabled after the first pairings won significantly more encounters than previous losers with intact chelae during their second fight 30 min or 24 h after their initial pairings. When they were matched against unknown opponents with similar body size, there were no significant differences in the number of victories between previous winners with chelae restriction and new opponents with intact chelae. This difference seemed to be explained by a winner effect, i.e. previous winners won significantly more encounters than previous losers during their second fight (Goessmann et al., 2000; Daws et al., 2002). Winner effects are known to last for 1–2 weeks (Hemsworth et al., 2007); therefore, in the present study there was no effect of previous experience in our experiments when the same large and small crayfish were paired again after at least 30 days from the previous encounters. A handicap that prevented chelae interlocking thus decreased the winning probability of crayfish that had a physical advantage of large body size. The finding that the dominant crayfish drop to low rank when one chela was removed after only 5 days with a group (Lowe, 1956) supports our conclusion.

The role of interlocking chelae

When two crayfish encountered each other, agonistic bouts escalated from chelae display to interlocking (Bruski and Dunham, 1987). As crayfish were facing each other, the chelae extended laterally and upwards. One of them stroked its opponent with a chela, and the chelae of both crayfish opened and interlocked with those of the opponent. With the chelae interlocked, the walking legs were fully extended to forcefully push the animals towards each other. They took hold of each other's chelae and attempted to unbalance each other. The winner–loser relationship was suddenly determined when subordinates moved away from opponents and subsequently subordinates avoided approaching dominants with either a retreat or a tailflip.

The time in which the winner–loser relationship was determined was significantly prolonged in the second encounters with chelae restriction even if only one chela was restricted. Furthermore, the number of outcomes in which the winner–loser relationship was not clearly determined increased significantly when the chelae of both large and small animals were restricted. The number of approaches and retreats would reflect a result of outcomes of agonistic bouts, as subordinates avoided the approach of dominants by means of retreat. The number of approaches and retreats between large and small crayfish in the first encounters before chelae restriction, as well as in the third encounters after the release from chelae restriction, was statistically different. Furthermore, there was a significant difference between large and small animals in the second pairings of the treatments with one chela restricted in large animals and with chelae restriction of both animals. The occurrence probabilities of approaches were not statistically different between large and small crayfish in the second pairings of the treatment with both chelae restricted in large crayfish. The number of retreats between large and small crayfish in the second encounters was also statistically indifferent. In a sense, dominance order might depend upon the reaction of subordinates. When one contestant gave up and disengaged, the dominant–subordinate relationship was determined. No action of interlocking from large opponents might motivate fighting of small crayfish. The number of fights in the second pairing between large crayfish with one chela restricted and small crayfish with intact chelae was not statistically different from that of their first pairing, but the duration of the fights of their second encounters was significantly longer than that of the first pairing. These results indicated that individual fights between the large crayfish with one chela restricted and the small crayfish with intact chelae escalated owing to the handicap of one chela restriction of the large crayfish. However, the number of the fights in the second pairings of the remaining two treatments of both chelae restriction of the large crayfish and chelae restriction of both large and small crayfish significantly increased, whereas the duration of individual fights was not statistically different from that of their first encounters. Disturbance of chelae interlocking behaviour prevented escalation of agonistic bouts, which resulted in a lack of formation of the dominance order. Thus, interlocking of chelae functioned as key factor for dominance hierarchy formation of the crayfish. At present, it is still unclear whether pinching force of interlocking chelae is an essential element for dominant hierarchy formation. Further experiments manipulating chelae force or fixing chelae in an open position would be needed to clarify this point.

Further interesting observations would be the reactions of small crayfish in the second encounters with large opponents with both chelae restricted. Small crayfish with intact chelae seemed to have a certain advantage over opponents as only small crayfish performed chelae interlocking. However, small crayfish with intact chelae were beaten frequently and disengaged. By contrast, no dominance hierarchy was determined in agonistic bouts against large opponents with chelae restriction when chelae of small crayfish were also restricted. At present, it is still unclear how subordinates decide to give up fighting. Further behavioural, physiological and pharmacological analyses would be indispensable to clarify the intrinsic state underlying the aggressive motivation of subordinate animals.

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