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RESEARCH ARTICLE

Building a dishonest signal: the functional basis of unreliable signals of strength in males of the two-toned fiddler crab, *Uca vomeris*

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ABSTRACT

Males of many species use signals during aggressive contests to communicate their fighting capacity. These signals are usually reliable indicators of an individual's underlying quality; however, in several crustacean species, displays of weapons do not always accurately reflect the attribute being advertised. Male fiddler crabs possess one enlarged claw that is used to attract females and to intimidate opponents during territorial contests. After the loss of their major claw, males can regenerate a replacement claw that is similar in size but considerably weaker. As this inferior weapon can still be used to successfully intimidate rivals, it represents one of the clearest cases of unreliable signalling of strength during territorial contests. We investigated the functional mechanisms that govern signal reliability in the two-toned fiddler crab, *Uca vomeris*. Male *U. vomeris* exhibit both reliable and unreliable signals of strength via the expression of original and regenerated claw morphs. We examined the morphological, biomechanical and biochemical characteristics of original and regenerated claws to establish the best predictors of variation in claw strength. For a given claw size, regenerated claws have less muscle mass than original claws, and for a given muscle mass, regenerated claws were significantly weaker than original claws. The mechanical advantage was also lower in regenerated claws compared with original claws. However, the activity of three catabolic enzymes did not differ between claw types. We conclude that the structural and physiological predictors of force production influence the frequencies of reliable and unreliable signals of strength in *U. vomeris*. This study furthers our understanding of the proliferation of unreliable signals in natural populations.

KEY WORDS: Signal reliability, Claw regeneration, Enzyme activity, Muscle physiology, Claw biomechanics, Performance

INTRODUCTION

Males of many animal species use displays of weapons to signal aggressive intent and fighting capacity to opponents in order to avoid the potential costs of combat (Arnott and Elwood, 2010; Berglund et al., 1996; Kokko, 2013). The accuracy of the information transferred between opponents can govern the outcome of these aggressive displays (Searcy and Nowicki, 2005). Signals of potential strength can effectively change an opponent's behaviour as both competitors in a bout can assess the likelihood of combat success should the dispute escalate to physical contact (Bradbury and Vehrencamp, 2011; Hughes, 2000). Most signals reliably reflect the intrinsic attribute being advertised by the signaller, assuming a strong correlation between the perceived quality (e.g. signal size) and the actual underlying quality (e.g. strength, fighting ability, resource holding potential) (Számadó, 2011a,b). For example, dewlap (throat fan) size in many *Anolis* lizards is a reliable signal of strength (Lailvaux and Irschick, 2007). Male anoles frequently engage in prefight displays involving extension of their dewlaps, and while dewlap morphology is highly variable, size (perceived signal) is highly correlated with bite force (actual quality) (Lailvaux and Irschick, 2007). Theoretical models of signal reliability suggest that on average signals must be beneficial to both signaller and receiver in order to be evolutionarily stable, and signals should also transfer reliable information (Smith and Harper, 1995, 2003). However, this is not always the case and under certain conditions unreliable signals can pervade signalling systems and be maintained in frequencies greater than initially theorised (Számadó, 2000, 2008, 2011b). Unreliable signals do not transmit accurate information to receivers and occur when the perceived quality (via signal size) becomes decoupled from the actual underlying quality (Lailvaux et al., 2009). Empirical evidence of such signals has been identified in several species of crustaceans via this mismatch of signal size and underlying quality; for example, high variability of claw size to strength relationships in crayfish (Walter et al., 2011; Wilson et al., 2007), bluff displays in stomatopods (Steger and Caldwell, 1983) and discrete phenotypic claw morphs in fiddler crabs (Backwell et al., 2000). In fact, one of the best-studied examples of unreliable signalling has been described for the regenerated major claws of male fiddler crabs (e.g. Backwell et al., 2000; Bywater et al., 2014; Lailvaux et al., 2009; McLain et al., 2010).

Male fiddler crabs possess a greatly enlarged (major) claw that is used as a signal during both courtship displays and prefight assessment, and as a weapon during physical contests (Jordão and Oliveira, 2001; Lailvaux et al., 2009; Reaney et al., 2008). Claw size is considered to be the primary signal for male dominance and resource holding potential, and males with smaller claws retreat prior to physical contact more often than males with larger claws, and receive fewer visits from females (Lailvaux et al., 2009; McLain et al., 2010; Reaney et al., 2008). Following the loss of the major claw due to fighting or predator attacks, crabs can regenerate a replacement; however, males with a regenerated claw were found to be competitively inferior compared with original-clawed males (Backwell et al., 2000; Crane, 1975). Although regenerated (leptochelous) claws grow to similar sizes compared to the original (brachychelous) claws, they lack morphological features like dactyl teeth and tubercules and are comparatively weaker (see Backwell et al., 2000; Lailvaux et al., 2009; McLain et al., 2010). Claw regeneration among male fiddler crabs can be widespread, and

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between 10% and 45% of a crab population bear regenerated claws (Bywater and Wilson, 2012; Callander et al., 2012; McLain et al., 2010). Backwell et al. (2000) also established that the morphology of regenerated claws never develops to the original quality, even after multiple moult cycles. Fiddler crabs are unable to visually differentiate between the two claw morphs (Backwell et al., 2000; Lailvaux et al., 2009) and during the signalling stages of aggressive contests, individuals with weaker regenerated claws are equally successful at acquiring resources (Lailvaux et al., 2009). Yet, when combat does occur, individuals with regenerated claws are competitively inferior because claw strength, which is a critical determinant of fighting success, is lower for individuals with regenerated claws (McLain et al., 2010).

Unreliable signals of strength are common within the genus *Uca* and while modelling provides a theoretical understanding of how these signals evolve and remain in populations, the functional and mechanistic bases of these signals has been poorly examined. We theorised that the capacity to produce these phenotypes is likely to be constrained by an individual's underlying physiology. As such, the aim of the present study was to investigate the functional mechanisms underlying the development of unreliable signals of the two-toned fiddler crab, *Uca vomeris* McNeill 1920. We explored structural and physiological processes that may be driving this mismatch between claw size and strength in regenerated claws. Initially, we examined whether changes in morphology between claw types were biomechanically constraining force production. We predicted that alterations in shape should affect the leverage of the claw and thereby reduce the maximum strength achievable in regenerated claws. We also assessed whether muscle physiology differed between claw types. We predicted that the reduced muscle strength seen in regenerated claws might be a by-product of reduced metabolic capacity.

RESULTS

The size of the major claw did not significantly differ between claw types ($F_{1,104}$=2.57, t=1.60, P=0.13). However, claw shape was significantly different between original and regenerated claw types for any given claw size ($F_{3,99}$=200.1, t=−23.15, P<0.001) (Fig. 1A). Regenerated claws had longer dactyls and pollexes than original claws, but possessed a smaller manus area. Claw strength increased significantly with claw size ($F_{2,100}$=144.8, t=6.11, P<0.0001), but regenerated claws were weaker than original claws ($F_{2,100}$=144.8, t=−16.63, P<0.0001) (Fig. 1B). Claw size was also significantly associated with claw muscle mass, with larger claws possessing more muscle ($F_{3,99}$=136.5, t=16.73, P<0.0001) (Fig. 1C). However, the relationship between muscle mass and size differed significantly between claw types. Regenerated claws possessed significantly less muscle mass than original claws for any given size ($F_{3,99}$=136.5, t=−11.72, P<0.0001). Claw strength increased significantly with total muscle mass of the claw ($F_{2,100}$=132.5, t=5.36, P<0.0001); however, for a given muscle mass, regenerated claws were significantly weaker than original claws ($F_{2,100}$=132.5, t=−11.79, P<0.0001) (Fig. 2A).

Claw strength was also influenced by the velocity ratio of the claw. Regenerated claws had a small velocity ratio as they had longer but thinner dactyls, whereas original claws had a higher velocity ratio because of their shorter but wider dactyls ($F_{2,100}$=108.7, t=−3.452, P<0.0001) (Fig. 2B). Enzyme activity did not differ significantly between claw types for all enzymes tested (values reported are means±s.e.); lactate dehydrogenase (LDH, original=32.28±3.84 U ml^{-1}, regenerated=37.16±5.61 U ml^{-1}, $F_{1,19}$=0.43, P=0.52), citrate synthase (CS, original=1.40±0.14 U ml^{-1}, regenerated=1.24±0.15 U ml^{-1}, $F_{1,20}$=0.87, P=0.36) and cytochrome *c* oxidase (COX, original=0.90±0.16 units ml^{-1}, regenerated=0.92±0.13 units ml^{-1}, $F_{1,21}$=0.09, P=0.77).

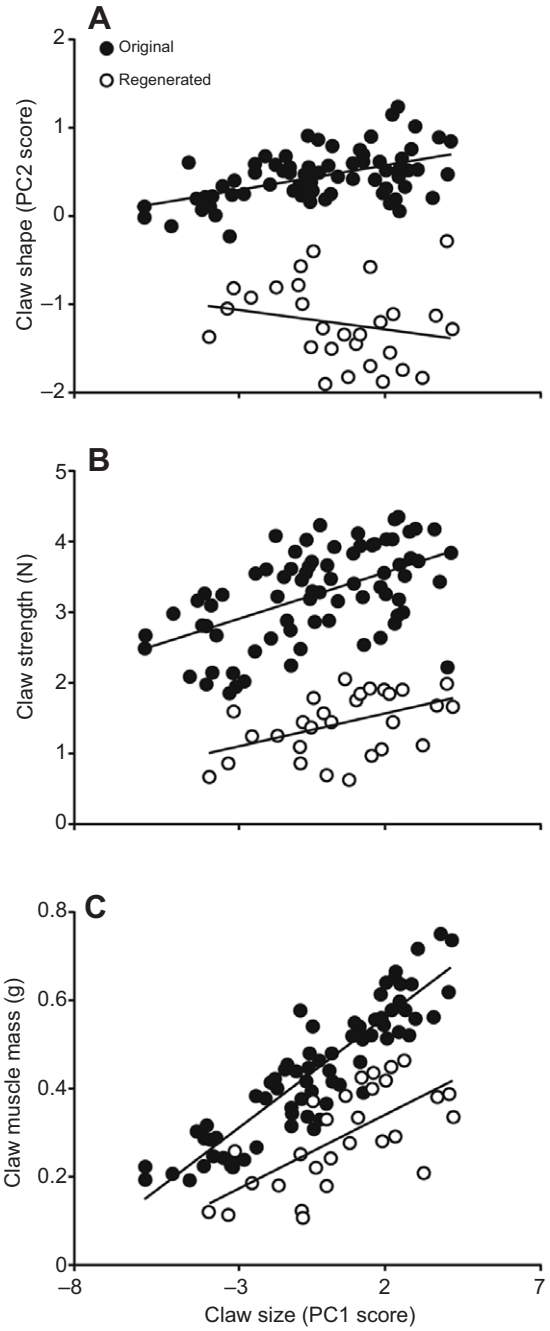

Fig. 1. The relationship between claw size and claw shape, strength and muscle mass for male *Uca vomeris*. (A) Claw shape (PC2), (B) claw strength and (C) claw muscle mass, plotted against claw size (PC1), for original and regenerated major claws. Claw shape in regenerated claws was significantly different between types ($F_{3,99}$=200.1, t=−23.15, P<0.001) whereby regenerated claws had longer dactyls than originals, but with a comparatively reduced manus area (negative PC2 values). Claw strength significantly increased with claw size ($F_{2,100}$=144.8, t=6.11, P<0.0001) yet differed between claw types ($F_{2,100}$=144.8, t=−16.63, P<0.0001), with regenerated claws being significantly weaker than original claws. Larger claws had more muscle ($F_{3,99}$=136.5, t=16.73, P<0.0001); however, the relationship between muscle mass and size was significantly different between types. Regenerated claws were significantly lighter than original claws for any given size ($F_{3,99}$=136.5, t=−11.72, P<0.0001).

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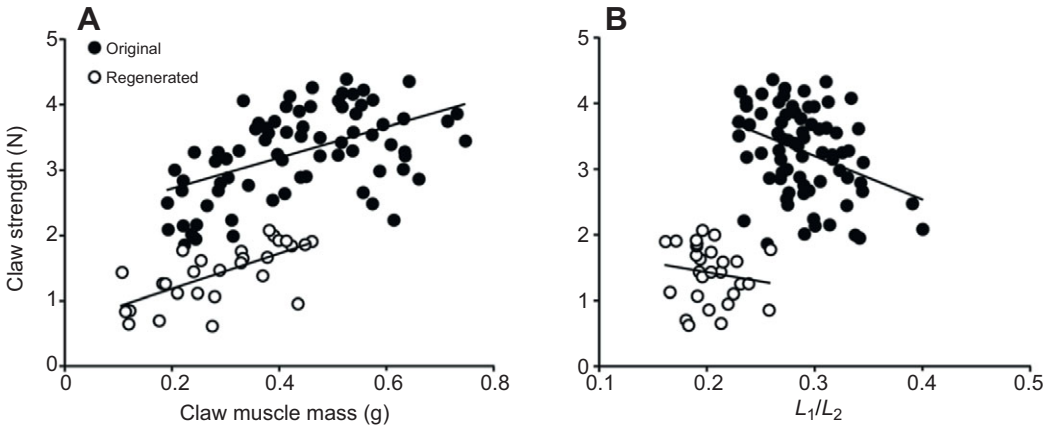

Fig. 2. The relationship between claw strength and claw muscle mass and velocity ratio for male *U. vomeris*. Claw strength plotted against (A) Claw muscle mass and (B) velocity ratio L_1/L_2, for original and regenerated major claws. Claw strength significantly increased with total muscle mass of the claw ($F_{2,100}$=132.5, t=5.36, P<0.0001); however, for a given muscle mass, regenerated claws were significantly weaker than original claws ($F_{2,100}$=132.5, t=−11.79, P<0.0001). Regenerated claws have relatively long but thin dactyls (small velocity ratio) and are significantly weaker than original claws, which have shorter but wider dactyls ($F_{2,100}$=108.7, t=−3.452, P<0.0001).

DISCUSSION

Functional processes influence the production of the unreliable claw morphs in male *U. vomeris*. We found that the mismatch between claw size and strength seen in regenerated major claws was affected, in part, by both the structure and physiology of the claw. The capacity to generate force is limited by the leverage system of the claw, and is a function of the claw's dimensions (mechanical advantage), the muscle cross-sectional area and muscle characteristics (Levinton and Judge, 1993; McLain et al., 2010). Like previous studies of claw morphs in *Uca mjobergi* (Reaney et al., 2008), we found that the regenerated claws of *U. vomeris* had a reduced manus area and longer dactyl length. Regenerated claws had significantly lower mechanical advantage than original claws, as a by-product of these changes in claw dimensions, which contributes to the lower closing forces observed.

Additionally, we identified that the differences in strength between claw morphs partially reflect the reduced muscle mass found in regenerated claws. Claw muscle mass fluctuates cyclically as a result of the tissue atrophy required for a crab to moult and grow (Ismail and Mykles, 1992; Skinner, 1966). During ecdysis, the major claw must undergo a large reduction in mass (up to 50% in *Uca pugnax*) in order to be withdrawn through a small joint at the base of the claw (Ismail and Mykles, 1992). Subsequent restoration of the muscle occurs once the new exoskeleton has formed. Muscle mass at any given time during this process is governed by the balance between the rates of protein synthesis and protein degradation (Mykles, 1997). When synthesis exceeds degradation, protein accumulates, and a net loss of protein is seen when degradation is up-regulated (Mykles, 1997). Muscle mass is also influenced by fluxing levels of growth hormones, which can regulate rates of protein synthesis and degradation, but also by capped numbers of myoblast satellite cells, which constrain the capacity for growth of new muscle tissue (Rai et al., 2014). Muscle atrophy and regeneration is a complex process that occurs on cellular and subcellular levels, so it is difficult to pinpoint the direct cause of muscle mass variation. In saying that, however, it is likely that tissue atrophy would affect the two claw types equally, and the variability we see in muscle mass and strength within each claw type may be partially attributable to an individual's moult stage at the time of testing.

Even when accounting for the differences in muscle mass between claw morphs, regenerated claws were still weaker than original claws. Because of the importance of underlying strength for combat success and given the unreliable nature of regenerated claws as signals of strength, we predicted that differences in muscle physiology might be constraining the development of strong claws. Measuring the enzyme activity of original and regenerated muscle tissue provided a means to estimate the metabolic capacity of the claw muscle. However, we found no differences in the enzyme activity of LDH, CS and COX between regenerated and original claw muscle, which suggests that the two claw types have a similar cellular metabolic capacity. This is also consistent with the observation that rates of oxygen consumption of muscle do not differ between claw types (Bywater et al., 2014). However, we would expect catabolic pathways to primarily regulate maximal oxygen consumption rate and not resting rate. Resting oxygen consumption rate is driven by ATP demand for protein synthesis and ATPase activity, and is not generally constrained by maximal enzyme activity (Horton et al., 2006; Seebacher and James, 2008; White and Kearney, 2013). The significant differences observed in whole-claw rates of oxygen consumption could be attributed to the reduced muscle mass found in the regenerated claws. As cell enzyme activity is often correlated with muscle fibre type (Mykles, 1988), we can infer that the two claw morphs have similar fibre composition. However, further histochemical and biochemical examination of the claw muscle is required to identify and eliminate fibre composition as a potential driver of variability in claw strength.

While there were no enzymatic differences between claw types, other muscle characteristics may be affecting force production. The contractile ability of muscle fibres is known to influence claw strength. A single muscle fibre consists of longitudinally packed myofibrils and the efficiency of muscle contractions relies upon the complementary movements of thick and thin myofilaments located within each myofibril (Campbell et al., 2006). These filaments occur as small contractile units, called sarcomeres, which repeat along the length of the myofibril. Taylor (2000) found that sarcomere length provides a reliable measure of the size of muscle contractile units in claw muscle and that resting sarcomere length was highly correlated to maximum muscle force. The ratio of thick to thin filaments, as well as filament density, also affects fibre

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contractile ability, and elimination of thin myofilaments during processes like atrophy leads to inefficient myofibril contractions, thus reducing the fibre's ability to generate force (Mykles, 1997; Mykles and Skinner, 1981, 1982). The arrangement of muscle fibres within the manus may also influence claw strength, as the bi-pennate muscle fibre arrangement found in crab claws increases the capacity to generate force compared with parallel-fibred muscles. Maximum force is dependent on the angle of pennation, and a change in the angle of fibre attachment and arrangement effect an equivalent change in mechanical advantage (Alexander, 1983). It would be ideal to examine muscle fibre arrangement in both claw types to see if regenerated claw fibres attach differently. Additionally, the level of muscle innervation also influences myofibril functionality and can differ between individual muscle fibres (Atwood and Bittner, 1971; Dewell and Belanger, 2008; Rai et al., 2014). Innervation is fundamental for initiating myofibril contraction and any outside factors that influence the nerve supply will also affect the contractile ability (Rai et al., 2014). It is possible that regenerated muscles are unable to contract efficiently as a by-product of myofibrillar development or have a reduced nerve supply, thereby constraining force production.

Furthering our knowledge of the structural and physiological predictors of force production, and the subsequent capacity to develop reliable signals, should allow for a greater understanding of the proliferation of unreliable signals in natural populations. We suggest that the functional constraints of major claw development, in part, influence the frequency of reliable and unreliable signals of strength in *U. vomeris*. However, while were able to empirically identify some of the functional predictors driving the variation in force production within the major claw, an explanation for why such a disparity remains between size and strength across claw types is largely theoretical. The regeneration of unreliable major claws in the fiddler crab is widespread, and given the importance of claw displays during signalling, and of underlying strength for combat success, it is important to understand how and why these unreliable signals develop. Claw size plays a large role in determining the success or failure of both mating and combat displays in many fiddler crab species, with larger-clawed individuals prevailing more often than not (Callander et al., 2013; Reaney, 2009; Reaney et al., 2008). It is thus unsurprising that an individual regenerating a claw would devote resources into developing a large claw quickly, whilst not investing in the metabolically costly muscle within. Yet, regardless of the explanations behind any physiological changes observed in the regenerated claw, it remains an inferior performer during combat (McLain et al., 2010). With this in mind, it is hard to reconcile the potential negatives of combat failure including loss of territory or mating opportunities, with the benefits an individual may gain from reduced muscle development via changes in either claw shape or muscle quality. An additional question that remains unanswered is why claw dimensions change during regrowth, particularly as these changes negatively impact on closing force, and it would be ideal to examine the internal and external growth patterns of the major claw throughout the regeneration process. Callander et al. (2013) suggest that it is sexual selection via male–male combat that could be driving shape variation in *U. mjobergi*, as females only responded to claw size and wave rate and did not select mates based on claw shape. If this is the case, claw development during regeneration should be balanced between sexual selection driven by female choice and sexual selection driven by male–male combat. In *U. vomeris* at least, claw development appears skewed by female choice given that claws are large but weak; however, evaluating this theory with any confidence is beyond the scope of our study. Many questions regarding the development of regenerated claws and the evolution of unreliable signals remain unanswered and open for further empirical and theoretical investigation.

MATERIALS AND METHODS

Animal collection and morphological measurements

We collected 104 male *U. vomeris* (75 original claws, 29 regenerated claws) for morphological and biomechanical analyses, from two mudflats in south-east Queensland, Australia, between October 2009 and March 2010. An additional 23 males (13 original claws, 10 regenerated claws) were collected for biochemical analyses. Crabs were collected by hand and housed in 40 l tubs (54×40×20 cm) containing a gravel substrate and shelter. Tubs were maintained at 23±1°C (mean±s.e.) with fewer than 10 individuals per container. All individuals were tested within 1 week of collection and then returned to their site of capture. We took morphological measurements of each individual including body mass; carapace width (anterolateral angle to anterolateral angle), length and depth; claw muscle mass; claw shape; and claw size. To measure claw size and shape, photographs were taken of each major claw using a digital camera (Sony, model DSC-W5), against a background of graph paper for calibration. Digital images were analysed using morphometric software (SigmaScan Pro5, Systat Software Inc., San Jose, CA, USA) and seven measurements were recorded for each claw (Fig. 3A) (see Bywater and Wilson, 2012). These measurements included: (1) width at heel, (2) width at dactyl/manus joint, (3) length of manus from

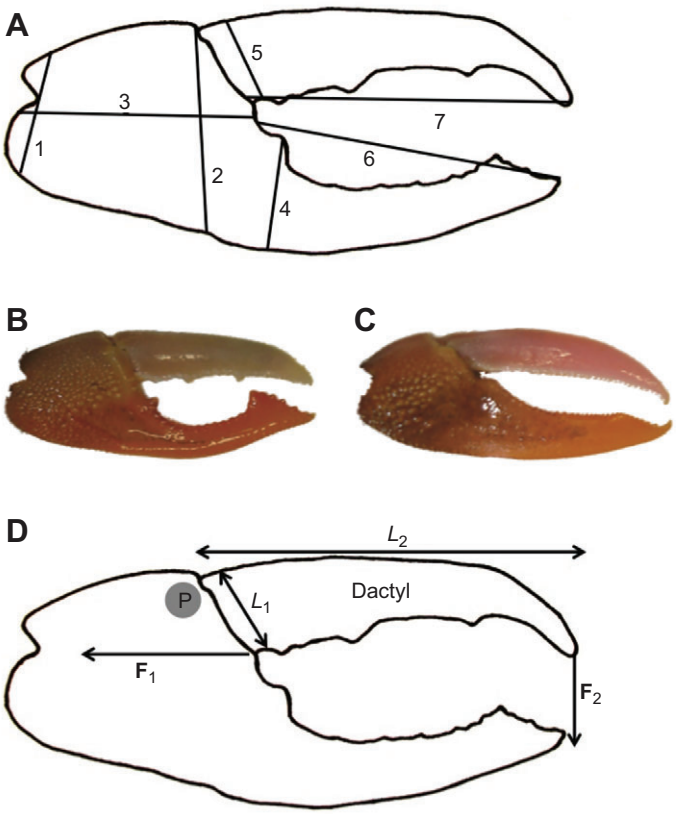

Fig. 3. Morphology of the major claw of male *U. vomeris*. (A) Diagram showing the seven measurements recorded to describe claw size and shape of the major claws of the two-toned fiddler crab (*U. vomeris*). (B,C) Example of the original (B) and regenerated (C) major claws. (D) Diagram of the forces applied during the closing of the major claw, where L_1 is dactyl height (in-lever), L_2 is dactyl length (out-lever), P is the pivot point for muscle attachment, $\mathbf{F}_1$ represents the force generated from muscle contraction and $\mathbf{F}_2$ represents the closing force along the dactyl. See Results (adapted from fig. 1.4 in Alexander, 1983).

Table 1. The principal component loadings of claw measurements

Claw measurement	PC1	PC2
Width at heel	−0.39	−0.20
Width at dactyl/manus joint	−0.40	−0.16
Length of manus from heel to joint	−0.37	−0.45
Width of pollex at dactyl joint	−0.38	0.35
Width of dactyl (at joint)	−0.37	−0.44
Length of pollex (tip to joint)	−0.37	0.47
Length of dactyl (tip to joint)	−0.37	0.44

Values represent the relative contribution of each of the seven claw measurements to the data variation explained by each principle component. PC1 represents claw size as all values have similar loadings in the same direction. PC2 represents claw shape and describes variation in claw proportions between the manus and dactyl/pollex.

heel to joint, (4) width of pollex at dactyl joint, (5) width of dactyl (at joint), (6) length of pollex (tip to joint) and (7) length of dactyl (tip to joint). We ran a principal components analysis (PCA) using the seven measurements recorded for all individuals combined to provide an overall measure of claw size (PC1) and shape (PC2). These measurements were selected based upon the co-variation and loadings generated for each principal component (Table 1), and only two principal components were retained for analyses based upon the generated scree diagram and 'elbow' method. PC1 was used as a measure of claw size as all vectors loaded in the same direction and 87% of data variation was explained within one component. PC2 accounted for an additional 10% of variation in the data and denoted claw shape, as manus size negatively co-varied with dactyl length. Original and regenerated claws in *U. vomeris* were identified via differences in claw morphology and the presence or absence of claw tubercles as per Lailvaux et al. (2009) and Backwell et al. (2000) (Fig. 3B,C).

Maximum claw strength of the major claw was measured for all males using a custom-built force transducer which records the flexion of metal plates via a strain gauge (for further details, see Bywater and Wilson, 2012). Each crab was encouraged to close the tip of its claw on the transducer plate at least five times, then rested for 5 min before repeating the procedure. The greatest claw closing force recorded for each individual was taken as their maximum claw strength. To avoid the claw slipping on the metal plate, and to ensure maximum repeatability, a small piece of thin cloth tape was adhered to the top as a target. The tape did not affect force recordings and was included in all calibrations. The transducer was calibrated daily using known weights and output data were converted from millivolts (mV) into newtons (N) for analyses. Claw strength was square-root transformed to achieve normality (Quinn and Keough, 2002) and transformed values were used in all analyses.

Biomechanical analyses

We performed an examination of the biomechanical constraints on force production. The claw is a simple lever system; force is applied via the contraction of claw muscle ($\mathbf{F}_1$), which is transmitted through the in-lever (dactyl height, L_1) to the out-lever (dactyl length, L_2) via the dactyl pivot (muscle attachment), to produce a closing force along the dactyl ($\mathbf{F}_2$) (Fig. 3D). When L_2 is longer than L_1, the lever reduces the overall input force, thus claws produce less force when the dactyls are longer. We calculated the velocity ratio (VR; L_1/L_2) at the dactyl tip for original and regenerated claws as an approximation of their mechanical advantage (MA; $\mathbf{F}_2/\mathbf{F}_1$). MA represents the ability of a claw to produce force efficiently. We assume the dactyl pivot point to be relatively frictionless; thus, MA is nearly equal to VR (Alexander, 1983). VRs were calculated by dividing dactyl height by dactyl length as per Warner and Jones (1976).

Biochemical analyses

Metabolic enzyme assays were performed on 23 individuals (13 original claws, 10 regenerated claws). After morphological and force measurements were collected, the major claw was removed by squeezing the base of the claw near the body, causing the crab to self-autotomize their claw. Muscle tissue samples (~0.05 g) were then dissected out, placed in cryo-Eppendorf tubes and immediately transferred into liquid nitrogen. Samples were maintained at −80°C for up to 3 weeks until testing occurred. We measured maximal activities of LDH, which catalyses the conversion of pyruvate to lactate, thereby releasing ATP in the absence of oxygen. In addition, we measured the activities of two mitochondrial enzymes, CS and COX, which control flux through the citric acid cycle and the electron transport chain, respectively. Muscle tissue was homogenised in nine volumes of cold extraction buffer (pH 7.5) consisting of 50 mmol l^{-1} imidazole, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} EDTA, 0.1% Triton and 1 mmol l^{-1} glutathione, and samples were kept on ice during processing. The homogenate was diluted 1:100 for LDH assays. Enzyme activities were measured using a UV/visible spectrophotometer (Ultrospec 2100pro, GE Healthcare, USA) equipped with a temperature-controlled cuvette holder. All assays were performed at 25°C and carried out in duplicate as per the methods outlined in Seebacher et al. (2003).

Statistical analyses

All statistical analyses were performed using R (version 2.12.2) or JMP (version 8). Statistical significance was taken at the level of $P<0.05$. ANCOVA were used to compare the differences between claw types for morphological and biomechanical analyses, with claw size, claw muscle mass or VR as covariates. ANOVA were used to compare the activity of enzymes between claw types.

Statistics were derived from the data in supplementary material Table S1.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

C.L.B., R.S.W. and F.S. conceived and designed the experiments, C.L.B. collected and analysed the data, and C.L.B., R.S.W. and F.S. wrote the manuscript.

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Supplementary material

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