

THE COST OF RELIABLE SIGNALING: EXPERIMENTAL EVIDENCE FOR PREDICTABLE VARIATION AMONG MALES IN A COST-BENEFIT TRADE-OFF BETWEEN SEXUALLY SELECTED TRAITS

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Claw size of male fiddler crabs, *Uca perplexa* appears to be a target of female choice that increases the likelihood a female will initially approach a male. Here we show that a behavioral display trait, the maximum height that the tip of the claw reaches during a courtship wave, is a strong correlate of the subsequent likelihood that a female will visit a male's burrow (which is a prerequisite for a burrow mating). We experimentally manipulated claw mass, to test whether there is a trade-off between claw mass and wave height. Males with a metal weight added to their claw showed a large reduction in wave height, whereas control males (plastic added) showed no net change in wave height. There is therefore a trade-off between these two sexually selected traits (claw size and wave display). More importantly, the greater the initial wave height the smaller the subsequent decline in wave height. Assuming that variation in wave height is an index of quality, this variation in the cost-benefit trade-off is consistent with the requirements of a signaling system that conforms to the handicap principle when fitness is the multiplicative product of different fitness components. We conclude by discussing the ongoing difficulties in testing the handicap principle.

KEY WORDS: Courtship display, female choice, fiddler crab, handicap principle, sexual selection.

Considerable research is now directed at quantifying whether male sexual signals provide information about the bearer's genetic quality (i.e., breeding value for fitness; Hunt et al. 2004; Kotiaho and Puurtinen 2007), and whether female choice for indirect benefits has driven the evolution of male ornamentation (Tomkins et al. 2004; Kokko et al. 2006). Recent growth in such studies has, however, not been matched by an increase in studies of the proximate mechanisms that maintain the reliability of sexual signals. Indeed, we still have a poor understanding of the validity of the proposed mechanisms. This includes several that are widely accepted such as the hypothesized testosterone-driven trade-off between signaling and immune function (e.g., see the

meta-analyses of Roberts et al. 2004 and Boonekamp et al. 2008). We have even less information about individual variation in the costs that signals impose on males and how this relates to signaler quality. Assumptions about differential costs of signaling are central to many theoretical models (e.g., Grafen 1990) but Kotiaho (2001) only identified two studies that had experimentally manipulated sexual signals to test for differential costs of increased expression in different classes of males (Møller and de Lope 1994; Kotiaho 2000). It should be noted, however, that Møller (1991) and Møller et al. (1995) also provided indirect tests by modifying a trait (wing loading) that ameliorates the cost of a sexual display. Since then, there have been only a few more experimental studies.

In short, even when we detect a correlation between the expression of a sexual trait and the underlying benefit females seek, it is rarely known why the signal remains reliable. This question is relevant regardless of whether female choice is for genetic or material benefits. It is surprising, given the huge theoretical interest, that so few studies have experimentally manipulate sexual signals to measure the relative support for different processes that might explain honest signaling.

There are three main explanations for a reliable correlation between a signal and the quality it conveys (reviews: Maynard Smith and Harper 2003; Searcy and Nowicki 2005). First, a “common interest” between signaler and receiver that allows for minimal cost signaling (i.e., no more cost than needed to ensure the signal is perceived; Guilford and Dawkins 1991) (e.g., Maynard Smith 1991; Johnstone and Grafen 1992; Bergstrom and Lachmann 1998). This scenario is, however, unlikely to apply to sexual signals due to sexual conflict over mating. Second, that the signal is an “unfakeable” index of quality. Here the expression of an index is causally related to the quality signaled (e.g., the highest point a tiger can scratch on a tree conveys information about its body size). This makes it unlikely that sexual signals are indices of genetic quality unless a single trait, such as body size, is a causal and reliable predictor of breeding value for fitness. One might argue that a sexual signal is a reliable index of a component of genetic quality, such as athletic ability (as do Maynard Smith and Harper 2003, p60), but this does not make evolutionary sense when there are genetic trade-offs between fitness components. Females only obtain an indirect benefit from mate choice if net fitness is signaled (Hunt et al. 2004). More generally, to state that a signal cannot be faked is to assume that there are currently physical constraints on the evolution of cheating (e.g., limb elongation would allow tigers to reach higher, but might increase the risk of injury while hunting). The study of the proximate mechanism maintaining honesty is therefore the identification of constraints, which is essentially shorthand for costs of development (Searcy and Nowicki 2005, p. 216). The third and most widely accepted explanation for sexual signals being reliable invokes the “handicap principle” (Zahavi 1975; Grafen 1990). Emphasis is given to condition-dependent expression of signals, the high cost of signaling and individual variation in the ability to bear costs.

A general statement of the “handicap principle” is that a signal can reliably convey information about quality if the ratio of the fitness cost to benefit of a given signal is lower for a higher quality signaler (Maynard Smith and Harper 2003, p. 31). If a female’s response to a given sexual advertisement is the same regardless of a male’s quality (i.e., all males have the same benefit function) then the same signal must be less costly for a high-quality male. This is usually phrased in terms of a sporting analogy, “the handicap,” whereby better quality individuals can afford to “waste” more resources and still outperform their rivals. Grafen (1990) formalized

the argument in a mathematical model where: (1) bigger signals are more costly; and (2) the marginal cost of signaling is a decreasing function of signaler quality. This second requirement has long been read to mean that in honest signaling systems the marginal costs of a given increase in the size of a signal are lower for high-quality males, who can therefore produce bigger signals (Getty 2006). The available tests of the handicap principle all predict this outcome. For example, Møller and de Lope (1994) showed that experimental tail extension in barn swallows had a more detrimental effect on the survival of short-tailed males (who must be of low quality if tail length signals quality) than long-tailed males, and therefore concluded that the handicap principle was operating (see also Saino and Møller 1996). Similarly, because models show that a handicap evolves more readily when signal expression is condition-dependent (Zahavi 1977; Iwasa et al. 1991) it has been argued that another key test of the handicap principle is to demonstrate that the marginal cost of a signal is smaller for males in good condition. So, for example, Kotiaho (2000) and Hoeffler et al. (2008) used experimental diets to manipulate the body condition of male wolf spiders and altered investment in courtship signaling by varying the number of females encountered. Both studies then predicted that increased courtship would have a more detrimental effect on survival for males in poor condition.

Unfortunately, Grafen’s model is implicitly one where fitness is an additive function of viability and reproductive output. Biologically speaking this is difficult to interpret for a sexual signal: one cannot subtract male mating success from viability because they are in different currencies. For sexual signals it is more intuitive to treat fitness as a multiplicative function of viability and mating success (i.e., mating rate per day times days alive, where both terms are a function of signal size). This means that the benefit of a given signal is higher for a male with greater viability because he has “more time” to accrue the benefit (Getty 1998). The consequence of this shift is that the prediction that high-quality males pay lower marginal or higher absolute costs disappears. There can be reliable signaling systems in which high quality males pay greater marginal costs. Getty (2006) recently reviewed this issue and provided an illustrative example (Box 2, Fig. 1A) (see also Getty 1998, 2002). Inexplicably, however, his insights are underappreciated as one still finds newly published studies that state that honest signaling requires lower marginal costs for high-quality males (e.g., Vanhooydonck et al. 2007). As with many areas in evolutionary ecology, once a restrictive assumption is removed unidirectional predictions no longer apply. The empirical goal then shifts from performing seemingly definitive tests that distinguish between false dichotomies, to quantifying general trends. (For a comparable case study of sexual dimorphism in immune function compare Rolff [2002] with Stoehr and Kokko [2006]).



Figure 1. An experimental male with a piece of metal glued to the lower side of the pollex of the major claw.

Getty (1998) stated that a critical test of the handicap hypothesis “should establish that signalers of different quality are on a rising fitness ridge because of different cost-benefit trade-offs” (where fitness is plotted against quality and signal size). If one assumes that a sexual signaling system conforms to a handicap model, this reduces to the prediction that experimental manipulation of a male’s signal will (1) decrease his fitness; and (2) that the change in costs and benefits will differ depending on male quality (which can be inferred from signal expression given the starting premise). Given the marginal costs of signaling could be higher or lower for high-quality males it will be necessary to conduct sufficient studies to see whether any general trends emerge. If so, these could provide deeper insight into the design of signals (e.g., whether power-efficiency trade-offs where high-quality males have greater marginal costs are rare).

Here we conducted an experiment to determine how male quality, which we assume to be a correlate of signal attractiveness, is related to the cost of increased investment in a sexual signal in the fiddler crab *Uca perplexa*. Male fiddler crabs (genus: *Uca*) wave a greatly enlarged major claw to attract females to mate. The major claw can reach 50% of a crab’s total weight (Crane 1975). It appears to be costly to move the claw as there is evidence that male *U. perplexa* who engage in social activities in which the claw is used (waving and fighting) have different blood lactate levels than those engaged in nonsocial activities such as feeding where the claw is merely carried (Matsumasa and Murai 2005). More generally, there is likely to be a substantial energetic cost to bearing a heavy claw. Male *Uca pugilator* with an intact major claw have lower endurance than those that have autotomized their major claw (Allen and Levinton 2007).

Male attractiveness in fiddler crabs is usually a function of both claw size and waving behavior (e.g., Backwell and Passmore 1996; Backwell et al. 1999; Murai and Backwell 2006). In *U. perplexa* when a mate-searching females approach a male, who performs a high-intensity waving display in which he unflexes his major claw and raises and lowers it several times. The fiddler crab’s visual system is acutely sensitive to objects above

the visual horizon (Land and Layne 1995), and there is evidence that the maximum height of the claw above the surface during a wave increases a male’s attractiveness to females in *U. perplexa* (Murai and Backwell 2006).

Our aim was to determine whether the weight of the major claw imposes a cost on males, and whether the resultant cost-benefit trade-off varies among individuals. Studies that experimentally manipulate sexual signals to investigate individual differences have used a range of measures of costliness. These include: survivorship (Møller and de Lope 1994; Kotiaho 2000; Lindström et al. 2006; Hoefler et al. 2008), foraging efficiency (Møller 1989), and immune function (Saino and Møller 1996). Saino et al. (2003) also explored the potential cost that arises when there are trade-offs between different components of attractiveness. Here we specifically tested whether a byproduct of increased investment into claw size (namely greater claw weight) has a negative effect on male waving behavior (specifically maximum wave height); and whether this detrimental effect on attractiveness, which reduces the benefit of a large claw, varies among males in a predictable fashion.

Methods

We studied *U. perplexa* on an open area (10 × 15 m) of intertidal mudflat on the Okukubi River, Okinawa, Japan from May to August in 2001–2002 and 2005–2008. These crabs live in individual burrows in mixed sex colonies and are surface active for 6–8 h around the diurnal low tide. Mate-searching females vacate their own burrows and move across the surface in search of a mate. In our study, we only used adult crabs that fell within the size range of mated individuals reported by Nakasone and Murai (1998). All males were brachychelous and we excluded any male whose major claw had been regenerated following loss (see Backwell et al. 2000).

CORRELATIONAL EVIDENCE FOR FEMALE CHOICE BASED ON WAVE HEIGHT

First, we individually followed 68 mate-searching females as they moved through the population. For each female, we captured the first male that she approached. We ensured that she approached sufficiently close to this male that he responded by directing a courtship waving display toward her. This occurs only when the female is about 9 cm from the male (Nakasone and Murai 1998). A female will bypass many males while moving through the colony. After the approach, the female either “visited” the male ($n = 34$), which we defined as occurring when she fully entered the burrow of the displaying male, or “passed” the male and did not enter his burrow ($n = 34$). We measured each male’s carapace width and major claw length. Second, we setup a video camera with a 20× lens on a tripod so that the lens was 20 cm above the sediment

and about 1.5 m in front of a focal male. We then waited until a mate-searching female was in the vicinity and began to film if the female seemed likely to approach the male. In this way, we managed to successfully film the entire sequence of a female approach and the associated male waving from its onset until the female either entered the male's burrow ($n = 32$) or passed the focal male after he had actively directed waves at her ($n = 22$). Each male–female pair provided one recording. Active courtship waving occurs for a relatively short period (<4 s) from the time that the female starts to approach a male until he descends into his burrow (Murai and Backwell 2006). We subsequently individually numbered each frame of the video for frame-by-frame analysis. We only analyzed active courtship waves, rather than those given when a female temporarily stops moving (see Murai and Backwell 2006). The main variable of interest was the maximum height of the tip of the dactyl above the sediment surface (hereafter “wave height”).

To gain a mating, a male must ensure that a mate-searching female does three things; (step 1) approaches rather than bypasses him; (step 2) enters and inspects his burrow rather than turning away after initially approaching him; and (step 3) remains in the burrow to mate rather than vacating the burrow and moving on to another male. Visiting a male's burrow is therefore a prerequisite for burrow mating (Backwell and Passmore 1996; Murai and Backwell 2005). Failure to induce a female to visit the burrow must decrease a male's average mating success. Mating occurs when a male mates with a female prior to egg extrusion. The available data suggest that the sperm of the last male to mate is used to fertilize the eggs (P. Backwell, L. Reaney, and C. Linde, unpubl. ms.). Thus, a male's success in obtaining burrow matings is likely to be a major determinant of his lifetime fertilization success.

EXPERIMENTAL MANIPULATION OF CLAW MASS

Early in the ~ 14 -day-long mating cycle, we individually marked medium-sized males (carapace width: 13–17 mm) with small paint spots on the carapace and then returned them to their own burrow. After a settling-in period of 1 h, we began to scan the area around the marked male for mate-searching females. When a mate-searching female approached the male, we filmed his waving display until the interaction ended when the female either moved away or entered the male's burrow. We continued to observe each marked male for two to three days, filming his waving display each time a mate-searching female approached him. The video camera was positioned as described previously.

On day 3 or 4, we recaptured the male and attached either a piece of metal or plastic to his major claw. The metal was 1-mm-diameter wire folded up and glued to the pollex of the claw (see Fig. 1). We adjusted the length (hence mass) of the wire according to the size of the male, so that the wire increased the total claw

mass to $\sim 66\%$ of the total crab's mass. This is the maximum relative claw mass for male *U. perplexa* (M. Murai, unpubl. data). Males with a 14–15 mm carapace had 72 mg added to their claw (12 mm of wire), 15–16 mm males had 75 mg added (12.5 mm of wire), and 16–17 mm males had 78 mg added (13 mm of wire). In the control treatment, we folded in half a piece of plastic that was 0.5×2 mm in cross-section and 6 mm long and attached it to the claw in the same position as metal was attached to experimental treatment males.

The following day, we relocated marked males. For two to three days, we set up a camera, waited until a mate-searching female approached, and then filmed their waving displays as described above. Collecting sufficient data were a major challenge because many males were not approached by females while we had a camera set up; and some males were not seen again because they had died, moved out of the study area or molted, or lost their markings. In total, we successfully filmed waving sequences for 13 experimental treatment males with metal weights and 13 control males with plastic. We analyzed 2–13 wave sequences per male, each of which involved a different female approaching the male (2.77 ± 2.21 females per male before the treatment and 2.73 ± 1.61 females per male after the treatment, mean \pm SD). A wave sequence consisted of one to nine waves. We used these data to calculate the mean wave height before and after the manipulation for each male. Finally, when the experiment ended we removed the metal or plastic attachment from the claw without injuring the male.

STATISTICAL ANALYSIS

In the first part of the observational study, we compared the size of passed and visited males using analysis of variance (ANOVA) with male type (passed or visited) as a fixed factor. In the second part, we compared mean wave height between visited and passed males in a general linear model with male claw length and male type as predictor variables.

For the experimental study, we initially ran two-sample *t*-tests to test whether claw size, carapace width, or premanipulation mean wave height differed between males assigned to the control and experimental treatment groups. We confirmed that our measure of wave height was repeatable by running one-way ANOVAs with male identity as a random factor and calculating the intraclass correlation coefficient (r_I). We ran separate analyses for waves before and after the experimental manipulation. After the manipulation, we also tested for repeatability within each treatment type. Repeatability was high so, for simplicity, we used mean wave height in the subsequent analyses. (Subsequent analyses using each wave as a datapoint and including male identity as a random factor yield the same conclusions).

To test for an effect of the experimental treatment on mean wave height, we ran a linear mixed model in SPSS 14.0 (SPSS

Inc, Chicago, IL) with male identity as a random factor (because we made before and after measurements from the same male). To account for variation in mean wave height, we initially fitted a full model that included the fixed terms: treatment (plastic or metal added), time (before or after treatment), male claw length, and all two-way and three-way interaction terms. We then used a model simplification process starting with higher order interactions and removing nonsignificant terms, until the final model only contained significant terms (Crawley 2002). *P*-values for excluded main effects are presented as the values obtained when they are individually included in the final model. This first model showed that treatment had a strong influence on wave height (see Results). We then ran a second general linear model in which we tested for a difference in the relationship between wave height before and after treatment between the treatment types, again including male claw size as a covariate, and using a model simplification approach. Finally, because there was a significant difference, we ran a separate linear regression for each treatment type. Bonferroni correction did not affect the significance of results at the 0.05 level. Unless otherwise stated data are presented as mean \pm SE, and statistical tests are two-tailed with $\alpha = 0.05$.

Results

EVIDENCE FOR FEMALE CHOICE BASED ON WAVE HEIGHT

Males that the mate-searching female approached but then passed did not differ significantly in size from those that were visited (passed: 14.29 ± 0.20 mm carapace width, 24.84 ± 0.56 mm major claw length; visited males: 14.18 ± 0.24 mm carapace width, 24.67 ± 0.71 mm major claw length; $F_{1,66} = 0.13$, $P = 0.73$ for carapace width; $F_{1,66} = 0.03$, $P = 0.86$ for claw length; $n = 34, 34$). Larger males had a wave height that was greater than that of smaller males ($F_{1,51} = 13.02$, $P = 0.001$), but the effect of claw size on wave height did not differ significantly between passed and visited males ($F_{1,50} = 0.001$, $P = 0.97$) (Fig. 2). Importantly, however, after controlling for the effect of claw size, wave height differed significantly between males that the female passed and those that she visited. Males that were passed raised their claws to a height of 26.92 ± 4.16 mm, ($n = 22$), whereas visited males raised their claws to 30.38 ± 4.39 mm ($n = 32$) ($F_{1,51} = 12.91$, $P < 0.001$). This suggests that wave height, or a correlate thereof other than claw or body size (because these were controlled for statistically), is the target of female choice and determines whether or not a female visits a male's burrow.

THE EFFECT OF WEIGHT ADDITION

Our measurement of wave height prior to the experimental treatment was highly repeatable among the 26 males ($F_{25,46} = 11.02$, $P < 0.001$, $r_I = 0.83$). It was also highly repeatable after the

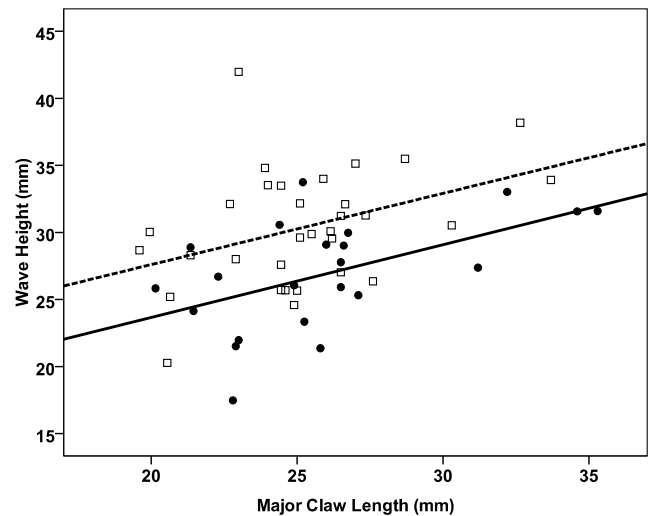


Figure 2. The least-squares regression of absolute height of the tip of the major claw above the sediment surface ("wave height") on claw length for visited and passed males ($n = 32, 22$) (open squares and dotted line, visited males; closed circles and solid line, passed males).

experimental manipulation ($F_{25,45} = 6.71$, $P < 0.001$, $r_I = 0.74$), even within each treatment type (metal addition: $F_{12,17} = 5.31$, $P = 0.001$, $r_I = 0.68$; plastic addition: $F_{12,28} = 8.40$, $P < 0.001$, $r_I = 0.79$).

Prior to the experimental manipulation there was no significant difference between males assigned to the metal and plastic addition groups in mean wave height ($t_{24} = 1.19$, $P = 0.244$), claw size ($t_{24} = 0.748$, $P = 0.462$), or carapace width ($t_{24} = 0.143$, $P = 0.888$). Taking into account the fact that wave height increased weakly with claw size ($F_{1,23} = 4.56$, $P = 0.044$), the experimental manipulation has a clear effect because there was a significant interaction between treatment type and whether the measurement was taken before or after the addition of the metal or plastic ($F_{1,24} = 5.82$, $P = 0.024$) (Table 1). No other interactions were significant (time \times type \times claw size: $F_{1,22} = 1.01$, $P = 0.33$; time \times claw size: $F_{1,23} = 2.18$, $P = 0.15$; type \times claw size: $F_{1,22} = 0.04$, $P = 0.85$). The relationship between wave height before and after the experimental manipulation differed significantly between the two treatments ($F_{1,24} = 11.05$, $P = 0.003$). In this case, claw size was excluded from the final model because it

Table 1. Final terms in a linear mixed model for mean wave height, with male identity as a random factor. The original model included all two-way and three-way interactions (see text).

Source	df	<i>F</i>	<i>P</i>
Treatment	1,23	0.02	0.883
Time	1,23	5.94	0.023
Male claw length	1,24	4.56	0.044
Treatment \times time	1,23	5.82	0.024

Table 2. Final terms in a general linear model for mean wave height after the experimental treatment. The original model included male claw length and all two-way and three-way interactions between terms (see text).

Source	df	F	P
Treatment	1,22	13.93	0.001
Mean height before	1,22	206.27	<0.001
Mean height before × Treatment	1,22	11.05	0.003

did not have a significant effect on wave height ($F_{1,21} = 0.369$, $P = 0.550$) (Table 2). Its inclusion did not change the main conclusion. It should be noted that the effect of claw size on wave height is considerably weaker than reported in the observational study because we confined our experiment to similar-sized males.

Given the interaction, we ran separate regressions for each treatment (Fig. 3). For control males to whom we attached plastic, the regression had a slope of 1.021 ± 0.043 , which did not differ significantly from a value of unity ($t_{11} = 0.49$, $P = 0.634$) and an intercept of -0.644 ± 1.284 , which did not differ from zero ($t_{11} = 0.502$, $P = 0.626$). There was therefore no detectable change in wave height after plastic was added. In contrast, for metal addition males the regression had a slope of 1.637 ± 0.201 , which differed significantly from a value of 1 ($t_{11} = 3.169$, $P = 0.009$) and an intercept of -22.051 ± 6.355 , which was significantly less than zero ($t_{11} = 3.470$, $P = 0.005$). Inspection of the 95% confidence intervals for the regression for the control treatment showed that the seven males with the lowest mean wave heights prior to the

metal addition, had significantly lower than expected mean wave height after the addition (i.e., below the 95% CI for the control). In contrast, five of the six males with the highest wave heights prior to the metal addition had wave heights the same as or greater than those expected based on the control regression after the addition (Fig. 3). Addition of a metal weight therefore had a more detrimental effect on males with a below average original wave height. Inclusion of male claw size in the models did not change this conclusion.

We added metal weights designed to increase claw mass to 66% of body mass. It could, however, be argued that (1) the metal weights used for different size class males did not accurately reflect claw mass allometry or (2) the difference in mass had a direct effect. However, when we reran the analysis excluding two males, so that all 11 males in the metal addition treatment were in the 14–15 mm size class and therefore has the same weight added (72 mg) we obtained the same results. In sum, the relationship in Figure 3 is not an artifact of smaller males receiving relatively heavier weights.

Discussion

CLAW SIZE AND WAVE HEIGHT ARE SEXUALLY SELECTED TRAITS

Male fiddler crabs repeatedly raise their greatly enlarged major claw in a courtship display to induce females to enter their burrow to mate. Claw size and wave display are therefore both likely to be targets of sexual selection. In our study of *U. perplexa*, there was no size difference between males that females visited and males that they approached but did not visit. Visited and nonvisited males did, however, differ in their waving display. Visited males raised their claw significantly higher above the surface than males that were passed. Although wave height increases with claw size, the difference in mean wave height between visited and passed males cannot be attributed to the effect of male claw size on wave height because there was no difference in mean claw size between the two groups of males (and the difference in wave height was apparent even if we statistically controlled for claw size). These two findings corroborate those of Murai and Backwell (2006), who also showed that visited males do not differ in size from passed males but had a greater mean wave height. This suggests that wave height, or a close correlate thereof such as the longer time taken to complete a higher wave, is a cue used during female choice. Variation in wave height that cannot be attributed to claw size occurs because males differ in the extent to which they flex their legs to raise their entire body off the ground while waving (Murai and Backwell 2006).

The similarity in size between visited and passed males should not be interpreted as evidence that females do not pay attention to male claw size. Mate choice in fiddler crabs consists

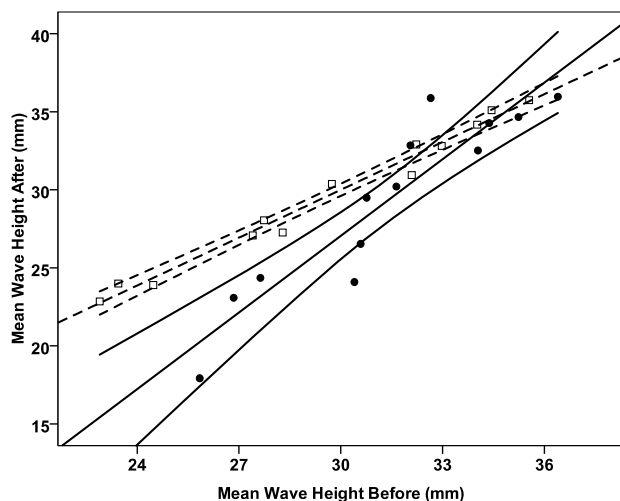


Figure 3. The relationship between mean wave height before and after experimental treatment for the 13 control (plastic addition) males (open squares) and 13 experimental (metal addition) males (closed circles). The lines are least squares regression with 95% confidence intervals. The steeper line is for experimental males. The line for control males does not differ significantly from a line of equality with a zero intercept (see text).

of a sequence of choice events, one of which is the initial decision of a mate-sampling female to approach or bypass a male. Thereafter she decided whether or not to enter his burrow (visit or pass) and, finally, whether or not to remain there to mate (e.g., Backwell and Passmore 1996; Christy and Backwell 2006). Previous studies of other species of *Uca*, including close relatives of *U. perplexa*, show that females are more likely to enter the burrows of males that are larger than the population average (e.g., Backwell and Passmore 1996; Reaney and Backwell 2007). More importantly, in *U. perplexa*, females mate in burrows with males that are significantly larger than those with whom they surface mate (Nakasone and Murai 1998). This means that mate-searching females are more likely to approach larger males when seeking out a burrow mating. (Surface mating is a secondary mating tactic whereby males approach neighboring females and induce them to mate, often without engaging in full courtship waving). Claw size is the most obvious cue of male size available to females, and recent work on the closely related species *Uca mjoebergi* has used robotic crabs to show, through controlled experiments, that females prefer larger claws (R. Milner, M. Jennions, and P. Backwell, unpubl. ms.).

Male claw size in *U. perplexa* is a component of male attractiveness that affects the first stage of female choice: whether or not to approach a male. Wave height (or a close correlate) affects the second stage of female choice: whether or not to “visit” a male and inspect his burrow. Males must therefore invest into both components of signaling to obtain a mate. Controlling for claw size, the considerable variation in mean wave height among males (see Fig. 2) reveals that some males do not invest as heavily as others in waving. If producing a wave of greater height is more costly, this suggests that males differ in the cost-benefit trade-off between claw size and wave height.

THE COST OF LARGER CLAWS AND THE COST-BENEFIT TRADE-OFF BETWEEN WAVE HEIGHT AND CLAW MASS

There is an obvious proximate explanation for a trade-off between claw size and wave height: a larger claw is heavier and it must take more energy to lift it into a fully vertical position and to raise the body further from the surface while so doing. It is possible to produce a “fake” claw that is lighter than a normal claw, but there are heavy costs to doing so because of its reduced effectiveness as a weapon (e.g., regenerated claws in *Uca annulipes*: Backwell et al. 2000). One potential cost of greater claw size therefore arises through sexual selection due to female choice: all else being equal, a larger, heavier claw lowers the attractiveness of the resultant waving display because it reduces wave height decreasing the likelihood that a female will visit a male’s burrow. To test this, we experimentally increased the weight of the major claw by attaching a piece of metal. A comparison of the mean wave height

of control and experimental treatment males showed that there was a clear negative effect of greater claw weight on mean wave height. Eight of 13 experimental treatment males had a mean wave height that fell below the 95% confidence interval of the control males, and the intercept of the regression of mean wave height after the manipulation on that before was significantly less than zero indicating a net decline in wave height. We could therefore show that one cost of an increase in claw size, which has the sexually selected beneficial effect of elevating the rate at which males are approached by females, is a decline in the subsequent ability of a male to induce a female to enter his burrow. Kotiaho (2001) has argued that many studies report indirect costs of sexual signal elaboration, whose link with actual fitness is tenuous (e.g., a small increase in energy expenditure). In *U. perplexa*, however, a strong correlation between wave height and the likelihood that a female enters a male’s burrow (this study, Murai and Backwell 2006) offers strong circumstantial evidence that increased claw weight will reduce a major component of fitness, namely mating success.

Most studies that experimentally manipulate sexual signals look for a trade-off with a naturally selected trait such as foraging efficiency (e.g., Evans and Hatchwell 1992; Møller 1989) or survival (e.g., Lindström et al. 2006; Hoefler et al. 2008). In general, experimental studies that show a trade-off between two sexually selected male traits tend to involve characters selected through male–male competition, such as weapon and testes size in dung beetles (Simmons and Emlen 2006), or a trade-off between investment in sexual attractiveness and social dominance (e.g., Moore and Moore 1999). There are fewer experimental studies that have looked for trade-offs between multiple signals used for female choice. For example, in barn swallows, tail length and certain song parameters are both targets of female choice. Experimental elongation of the tail did not, however, affect male singing activity or complexity (Saino et al. 2003). Interestingly, however, tail elongation did increase production of a song component associated with male–male interactions, implying that one cost of tail elongation is mediated through social interactions, as hypothesized for badges of status (for a recent example see Nakagawa et al. 2008). Pryke and Andersson (2005) showed that in red-collared widowbirds, *Euplectes ardens*, tail-length shortening resulted in more time in courtship when compared to courtship by control males. Despite this, however, control males attracted more females to their territory, which means that tail length did not trade-off with display rate in a way that reduced fitness (mating success) over the examined tail length range.

TESTING THE HANDICAP PRINCIPLE

Getty (1998) stated that a critical test of the handicap hypothesis is to demonstrate that signalers of different quality have different cost-benefit trade-offs. If we assume that male wave height is

correlated with a relevant aspect of male quality (which is plausible given the strong evidence for a female preference for greater wave height), we expect to see an effect of premanipulation wave height on the relative cost that greater claw mass imposes. Indeed, males with an initially large mean wave height paid a very low cost when claw mass was increased because they showed almost no change in mean wave height. In contrast, males with an initially small mean wave height showed a large decline in wave height (Fig. 3). This finding is generally consistent with the oft-stated, but surprisingly rarely tested, prediction for an additive model of the handicap principle that better-quality males pay a smaller marginal cost for an increase in signaling. As Getty (1998) and others have noted, however, this is not a general prediction of a handicap model when fitness is a multiplicative function of different fitness components. For sexual signals, these fitness components are usually viability and mating success (often called fecundity in the theoretical models, e.g., Grafen 1990). In our case, the fitness components in question are “the ability to attract females to approach” and “the ability to then induce females to enter a burrow so that mating can occur.” It is clear that a multiplicative model is the most appropriate one to use for a sequential process of mate choice. Regardless, our finding is indicative of variation among males in the cost-benefit trade-off that can be predicted based on their original wave height.

There are some important caveats to interpretation of our results. First, there are almost certainly other costs of increasing claw size that we did not examine. These include the production costs of a bigger claw (as opposed to simply carrying a heavier one), effects on other aspects of waving that are attractive [e.g., *U. perplexa* wave synchronously (Backwell et al. 1999) which seems to favor males that produce leading waves (Reaney et al. 2008)], and, perhaps most importantly, any negative effects of a larger claw on male survivorship. We do not know if these costs are higher or lower for males that produce higher waves. Second, we have assumed that wave height is a signal of a male quality beneficial to females. This type of assumption is almost always necessary in signaling studies because we rarely know what information a signal is reliable about (Searcy and Nowicki 2005, p. 213). If we are to test the handicap principle, we have to presuppose that a preferred signal is correlated with quality. We are therefore not providing a specific test of whether claw size or wave height is a handicap that signals male quality, only that the system fulfils the requirement of a handicap-based system for variation among signalers in a cost-benefit trade-off. Third, phenotypic plasticity means that males in poor condition can sometimes commit even more resources to a sexual signal than males in good condition. For example, Candolin (1999) showed that male stickleback experimentally manipulated to be in poorer body condition actually produced a redder breeding coloration than males in good condition. This “terminal investment” meant that, at least in the

short-term, they were more attractive to females. By extension it is possible, although arguably improbable, that male *U. perplexa* with high waves are actually in poorer condition and even of lower quality, so that the increased cost of waving a heavier claw amplifies their propensity to “over-invest” in maintaining a greater mean wave height. This problem, although often swept under the carpet, is inherent to any study in which sexual traits are experimentally manipulated because there is an implicit assumption that phenotypically plastic responses produce patterns identical to those that arise from genetic variation in condition/quality among individuals.

In sum, there is abundant evidence that sexual signals are expressed in a condition-dependent manner (Tomkins et al. 2004). The fact that male *U. perplexa* with an initially greater wave height are less adversely affected by an increase in claw mass (lower marginal cost) is consistent with those males having greater energy reserves (i.e., being in better condition) that can be used to maintain a greater mean wave height. As we have noted though, alternate explanations are possible. More generally, we have shown that males vary in the cost-benefit trade-off between the two sexually selected traits of claw weight (size) and wave display, which provides the basic conditions necessary for the evolution of a reliable signaling system. There are surprisingly few studies that experimentally manipulate traits subject to female choice and then investigate predictors of variation among males in the costs (if any) that this imposes. The extent to which higher quality males pay lower rather than higher marginal costs therefore remains an open empirical question until more data become available.

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LITERATURE CITED

- Allen, B. J., and J. S. Levinton. 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler crab. *Funct. Ecol.* 21:154–161.
- Backwell, P. R. Y., and N. I. Passmore. 1996. Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca annulipes*. *Behav. Ecol. Sociobiol.* 38:407–416.
- Backwell, P. R. Y., M. D. Jennions, J. H. Christy, and N. I. Passmore. 1999. Female choice in the synchronously waving fiddler crab *Uca annulipes*. *Ethology* 105:415–421.
- Backwell, P. R. Y., J. H. Christy, S. R. Telford, M. D. Jennions, and N. I. Passmore. 2000. Dishonest signaling in a fiddler crab. *Proc. R. Soc. Lond. B* 267:719–724.
- Bergstrom, C. T., and M. Lachmann. 1998. Signaling among relatives. III. Talk is cheap. *Proc. Natl. Acad. Sci. USA* 95:5100–5105.
- Boonekamp, J. J., A. H. F. Ros, and S. Verhulst. 2008. Immune activation suppresses plasma testosterone level: a meta-analysis. *Biol. Lett.* 4:741–744.
- Candolin, U. 1999. The relationship between signal quality and physical condition: is sexual signalling honest in the three-spined stickleback? *Anim. Behav.* 58:1261–1267.

- Christy, J. H., and P. R. Y. Backwell. 2006. No preference for exaggerated courtship signals in a sensory trap. *Anim. Behav.* 71:1239–1246.
- Crane, J. 1975. Fiddler crabs of the world. *Ocypodidae*; genus *Uca*. Princeton Univ. Press, Princeton, NJ.
- Crawley, M. J. 2002. Statistical computing: an introduction to data-analysis using S-Plus. Wiley, Chichester.
- Evans, M. R., and B. J. Hatchwell. 1992. An experimental study of male adornment in the scarlet-tufted malachite sunbird .2. The role of the elongated tail in mate choice and experimental evidence for a handicap. *Behav. Ecol. Sociobiol.* 29:421–427.
- Getty, T. 1998. Handicap signalling: when fecundity and viability do not add up. *Anim. Behav.* 56:127–130.
- . 2002. Signaling health versus parasites. *Am. Nat.* 159:363–371.
- . 2006. Sexually selected signals are not similar to sports handicaps. *Trends Ecol. Evol.* 21:83–88.
- Grafen, A. 1990. Biological signals as handicaps. *J. Theor. Biol.* 144:517–546.
- Guilford, T., and M. S. Dawkins. 1991. Receiver psychology and the evolution of animal signals. *Anim. Behav.* 42:1–14.
- Hoefler, C. D., M. H. Persons, and A. L. Rypstra. 2008. Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory. *Behav. Ecol.* 19:974–979.
- Hunt, J., L. F. Bussiere, M. D. Jennions, and R. Brooks. 2004. What is genetic quality? *Trends Ecol. Evol.* 19:329–333.
- Iwasa, Y., A. Pomiankowski, and S. Nee. 1991. The evolution of costly mate preferences .2. The handicap principle. *Evolution* 45:1431–1442.
- Johnstone, R. A., and A. Grafen. 1992. The continuous Sir Philip Sidney game: a simple model of biological signaling. *J. Theor. Biol.* 156:215–234.
- Kokko, H., M. D. Jennions, and R. Brooks. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Syst.* 37:43–66.
- Kotiaho, J. S. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav. Ecol. Sociobiol.* 48:188–194.
- . 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol. Rev.* 76:365–376.
- Kotiaho, J. S., and M. Puurtinen. 2007. Mate choice for indirect genetic benefits: scrutiny of the current paradigm. *Funct. Ecol.* 21:638–644.
- Land, M., and J. Layne. 1995. The visual control of behavior in fiddler crabs. 1. Resolution, thresholds and the role of the horizon. *J. Comp. Physiol. A* 177:81–90.
- Lindström, L., J. J. Ahtiainen, J. Mappes, J. S. Kotiaho, A. Lyytinen, and R. V. Alatalo. 2006. Negatively condition dependent predation cost of a positively condition dependent sexual signalling. *J. Evol. Biol.* 19:649–656.
- Matsumasa, M., and M. Murai. 2005. Changes in blood glucose and lactate levels of male fiddler crabs: effects of aggression and claw waving. *Anim. Behav.* 69:569–577.
- Maynard Smith, J. 1991. Honest signaling the Philip Sydney game. *Anim. Behav.* 42:1034–1035.
- Maynard Smith, J., and D. Harper. 2003. *Animal signals*. Oxford Univ. Press, Oxford.
- Møller, A. P. 1989. Viability costs of male tail ornaments in a swallow. *Nature* 339:132–135.
- . 1991. Morphological adaptations to song flight in skylarks. *Behav. Ecol. Sociobiol.* 25:309–314.
- Møller, A. P., and F. de Lope. 1994. Differential costs of a secondary sexual character: an experimental test of the handicap principle. *Evolution* 48:1676–1683.
- Møller, A. P., M. Linden, J. J. Soler, M. Soler, and J. Moreno. 1995. Morphological adaptations to an extreme sexual display, stone-carrying in the black wheatear *Oenanthe leucura*. *Behav. Ecol.* 6:368–375.
- Moore, A. J., and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. *Proc. R. Soc. Lond. B* 266:711–716.
- Murai, M., and P. R. Y. Backwell. 2005. More signaling for earlier mating: conspicuous male claw waving in the fiddler crab, *Uca perplexa*. *Anim. Behav.* 70:1093–1097.
- . 2006. A conspicuous courtship signal in the fiddler crab *Uca perplexa*: female choice based on display structure. *Behav. Ecol. Sociobiol.* 60:736–741.
- Nakagawa, S., J. W. Lee, B. K. Woodward, B. J. Hatchwell, and T. Burke. 2008. Differential selection according to the degree of cheating in a status signal. *Biol. Lett.* 4:667–669.
- Nakasone, Y., and M. Murai. 1998. Mating behavior of *Uca lactea perplexa* (Decapoda : Ocypodidae). *J. Crustacean Biol.* 18:70–77.
- Pryke, S. R., and S. Andersson. 2005. Experimental evidence for female choice and energetic costs of male tail elongation in red-collared widowbirds. *Biol. J. Linn. Soc.* 86:35–43.
- Reaney, L. T., and P. R. Y. Backwell. 2007. Temporal constraints and female preference for burrow width in the fiddler crab, *Uca mjoebergi*. *Behav. Ecol. Sociobiol.* 61:1515–1521.
- Reaney, L. T., R. A. Sims, S. W. M. Sims, M. D. Jennions, and P. R. Y. Backwell. 2008. Experiments with robots explain synchronized courtship in fiddler crabs. *Curr. Biol.* 18:R62–R63.
- Roberts, M. L., K. L. Buchanan, and M. R. Evans. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.* 68:227–239.
- Rolff, J. 2002. Bateman's principle and immunity. *Proc. R. Soc. Lond. B* 269:867–872.
- Saino, N., and A. P. Møller. 1996. Sexual ornamentation and immunocompetence in the barn swallow. *Behav. Ecol.* 7:227–232.
- Saino, N., M. Romano, R. Sacchi, P. Ninni, P. Galeotti, and A. P. Møller. 2003. Do male barn swallows (*Hirundo rustica*) experience a trade-off between the expression of multiple sexual signals? *Behav. Ecol. Sociobiol.* 54:465–471.
- Searcy, W. A., and A. Nowicki. 2005. *The Evolution of Animal Communication*. Princeton Univ. Press, Princeton, NJ.
- Simmons, L. W., and D. J. Emlen. 2006. Evolutionary trade-off between weapons and testes. *Proc. Natl. Acad. Sci. USA.* 103:16346–16351.
- Stoehr, A. M., and H. Kokko. 2006. Sexual dimorphism in immunocompetence: what does life-history theory predict? *Behav. Ecol.* 17:751–756.
- Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. *Trends Ecol. Evol.* 19:323–328.
- Vanhooydonck, B., R. Van Damme, A. Herrel, and D. J. Irschick. 2007. A performance based approach to distinguish indices from handicaps in sexual selection studies. *Funct. Ecol.* 21:645–652.
- Zahavi, A. 1975. Mate selection: selection for a handicap. *J. Theor. Biol.* 53:205–214.
- . 1977. Cost of honesty (further remarks on handicap principle). *J. Theor. Biol.* 67:603–605.

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