
**THE LIFE OF FIDDLER CRABS:
MATE CHOICE, MATE ATTRACTION AND
TERRITORIALITY**



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DECLARATION

The research presented within this thesis is my original work. I am senior author and principle contributor to all nine chapters, which have been published or are currently *in press*. The three additional published manuscripts within the Appendices are ones that I co-authored but are unrelated to the topic of this thesis. For each of these manuscripts I contributed significantly to the data collection process and the development of ideas. All Chapters except Chapter 8 are co-authored with Patricia Backwell and Michael Jennions, who contributed to the design of each project and the development of ideas. Chapters 1 and 9 were co-authored with Tanya Detto and Chapter 5 was co-authored with Isobel Booksmythe. For each of these chapters Tanya Detto and Isobel Booksmythe contributed to the data collection process and the development of ideas. No part of the main body of this thesis has previously been submitted as part of a degree.



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ABSTRACT

From violence to romance, fiddler crabs are an ideal study system to explore questions and predictions important to behavioural ecology and sexual selection. Fiddler crabs of the genus *Uca* are members of the Ocypodid family of brachyuran crabs. These highly social crabs occur in dense mixed sex colonies, and display pronounced sexual dimorphism. Males are endowed with one greatly enlarged claw that is used during courtship, as well as a weapon during aggressive interactions. Females lack this claw and instead have two small feeding claws. Fiddler crabs are highly territorial and display both male contest competition and female mate choice. The following nine chapters of this thesis explore the behavioural ecology of fiddler crabs.

Chapter 1 looks at seasonal shifts in female mating preferences for claw size using custom-built robotic crabs. Here I propose that a change in preference for claw size is due to seasonal difference in the value of larger burrows, which strongly influences burrow temperature and thus larval development rate (Milner et al. 2010a).

Chapter 2 explores non-independent mate choice and the role of stimulus enhancement in female mate choice. By conducting both natural mate choice experiments and mate choice experiments using custom built robotic crabs I show that the decision of one female to approach a group of males increases the probability that another female will approach and visit a male from the same group (Milner et al. 2011a).

In Chapter 3 I show that male fiddler crabs eavesdrop on the courtship displays of nearby courting males to detect mate-searching females and that males appear to adjust their wave rate according to the information available (Milner et al. 2010b).

Chapter 4 tests whether courting males adjust their wave rate, and therefore the cost of courtship, to the current level of competition. Here, I show that display rate increases as competition increases and that when competition is removed, males reduce their display rate by 30 per cent (Milner et al. 2011b).

Chapter 5 explores how well-armed males and weaponless female fiddler crabs obtain and defend territories. By conducting replacement experiments and collecting observational data, I show that females acquire burrows by seeking out empty ones,

while males acquire burrows by evicting male and female burrow owners (Milner et al. 2010c).

In Chapter 6 I show that males defend their female neighbours against male intruders. I also show that females sometimes mate with their immediate neighbour. Therefore males who ensure that they retain a female neighbour could benefit through increased opportunities for future reproductive success (Milner et al. 2010d).

Chapters 7 and 8 explore the selective pressures imposed by the formation of territorial coalitions. Within these two chapters I show that territory-seeking males choose whom to fight based partly on the size of the potential opponent's nearest neighbour (Milner et al. 2011c; Milner *in press*)

Finally in Chapter 9 I provide evidence of cannibalism and predation in a fiddler crab as well as the first evidence of fiddler crabs hunting shrimp and insects (Milner et al. 2010e).

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INTRODUCTION: THE LIFE OF FIDDLER CRABS

Fiddler crabs of the genus *Uca* are members of the Ocypodid family of brachyuran crabs. They display pronounced sexual dimorphism. Females are small, usually dull-coloured and have two small feeding claws. Males are larger, often brightly coloured and have a single greatly enlarged claw (up to 50% of total body weight) that is used during courtship, and as a weapon during antagonistic interactions (Crane 1975). They occur in dense mixed sex and mixed age colonies on tropical and subtropical intertidal mudflats. Both males and females live in burrows that are vital to survival and reproduction as they provide shelter from tidal inundation, predation and desiccation (Koga *et al.* 2001), as well as a site for larval incubation (Backwell & Passmore 1996). Burrows are therefore extremely valuable and each adult defends both its burrow and the small area of sediment around the burrow. The area of sediment around the burrow is a source of food as well as a platform for male mating displays (Crane 1975). Fiddler crabs primarily feed on detritus, bacteria and other small particles of organic material deposited on the sediment when covered by the tide (Dye & Lasiak 1986).

Breeding biology

Mating in fiddler crabs occurs at distinct times (usually over a few days) during each semi-lunar cycle, so that larval release is timed to coincide with high spring tides that provide optimal transport of larvae (Christy 1978; Morgan and Christy 1995). In many species, including the two I studied, *U. annulipes* and *U. mjoebergi*, receptive females leave their territories during the mating period and wander through the population in search of a mate (Backwell *et al.* 1999). Courting males stand at their burrow entrance and wave their one greatly enlarged claw to attract females. When a female passes close by, the males in the area will often approach and cluster around her. Males tend to produce waves that are in close synchrony with each other [Backwell *et al.* 1998]. Waving is extremely costly, in terms of energy, time and increased conspicuousness to predators (Jennions & Backwell 1998; Ribeiro *et al.* 2003; Matsumasa & Murai 2005). Females preferentially approach males with faster wave rates, greater wave leadership (slightly earlier waves during a synchronous bout) and larger claws [Backwell & Passmore 1996; Backwell *et al.* 1998; Backwell *et al.* 1999; Reaney 2009]. Final mate choice is based on burrow quality, so after approaching a male, the female will inspect the male's burrow. Mating and incubation occur within the male's burrow. Following

mating a male will usually forfeit his burrow to the female so that it can be used as the site of incubation.

Fighting

Fights occur over territory ownership, either with burrowless, wandering intruders or resident neighbours (Morrell et al. 2005). Males that lose their burrow, either through being evicted by another male or forfeiting it to a female for incubation following mating, must quickly find a new burrow. To do so, they must fight and evict a resident male or female crab, or find an unoccupied burrow, or dig a new burrow (Jennions & Backwell 1996). Fights between males begin with opponents lining up and pushing their major claws against each other, and can escalate to a grapple where the claws are interlocked. Fighting in fiddler crabs is costly and can result in wasted time, claw loss, injury, and even death (Jennions & Backwell 1996). Claw size largely determines fight outcome, however, in order to counteract an ownership advantage (Fayed et al. 2008), territory-seeking males tend to choose rivals that are slightly smaller than themselves (Jennions and Backwell 1996).

Unusually for a territorial species, resident males have also been shown to help their neighbours to fight intruders. These neighbour coalitions are thought to occur when the costs of helping a neighbour are outweighed by the benefit of not having to re-establish territory boundaries with a new, stronger neighbour (Getty 1987; Mesterton-Gibbons & Sherratt 2009). Such territorial coalitions have only been shown in rock pipits (*Arthus petrosus*) and three species of fiddler crab (Elfstrom 1997; Backwell and Jennions 2004; Booksmythe et al. 2010; Detto et al. 2010).

Benefits of studying fiddler crabs

Fiddler crabs occur in large numbers at high densities and can be easily observed from a short distance away. They are highly social and display a broad range of complex behaviours that can be observed over relatively short periods of time. We have an excellent understanding of fiddler crab biology (including their visual system, breeding, feeding and sociobiology), allowing us to explore questions and predictions relevant to major theoretical question in behavioural ecology. In addition, fiddler crabs communicate within networks of several signalers and receivers making them the ideal

study system to explore questions related to animal communication theory (Pope 2005). Lastly, the fiddler crab's social environment can be easily manipulated, either through tethering individuals, replacement experiments, the use of barriers to block the view of specific individuals and the use of robotic mimics. By using robotic mimics (robotic crabs) we are able to conduct experiments in the field, while controlling variables, such as social environment, claw size, wave rate, wave leadership, inter-male spacing and group size, that would otherwise be unachievable.

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CHAPTER 1: EXPERIMENTAL EVIDENCE FOR A SEASONAL SHIFT IN THE STRENGTH OF A FEMALE MATING PREFERENCE

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ABSTRACT

The costs and benefits of mate choice can vary both spatially and temporally. Phenotypic plasticity in mate choice, which could be due to changes in choice criteria (e.g. acceptance thresholds) or shifts in underlying mating preferences (i.e. relative values assigned to different males) can therefore increase reproductive success. We examined shifts in female mating preference for male claw size between two seasons (winter and summer) in the fiddler crab *Uca mjoebergi*. We used custom-built robotic crabs in two-stimulus mate choice experiments conducted in the field. Females showed a consistent preference for larger claw size, but this was significantly weaker during winter. This seasonal difference was observed for two successive years. We propose that the change in female preference for claw size is due to a seasonal difference in the value of a larger burrow, which strongly influences burrow temperature. Burrow temperature largely determines larval development rate and larvae release in fiddler crabs is constrained to a narrow time window where survival is optimal. Females appear to alter their preference for burrow width, which is signalled by claw size, between winter and summer potentially allowing them to optimise developmental time of larvae and/or to reduce mate search costs.

Keywords: female choice; fiddler crabs; mating preference; phenotypic plasticity; sexual selection; *Uca mjoebergi*.

INTRODUCTION

Variation in female mating preferences at both the individual and population level will influence the rate and direction of male sexual ornament evolution through sexual selection (Widemo and Sæther 1999). One source of variation is that the costs and benefits of expressing mating preferences can vary both spatially and temporally (Jennions and Petrie 1997). If female mating preferences are fixed, suboptimal mate choice decisions can therefore occur under some conditions (Qvarnström 2001). Consequently, there should be selection for females to facultatively adjust how they choose a mate.

Phenotypic plasticity in mating preferences and/or mate choice criteria will increase female fitness if the costs of plasticity are less than the increase in mean reproductive success that can be gained by shifting away from a fixed preference (Jennions and Petrie 1997). For example, in the collared flycatcher *Ficedula albicollis*, mate choice trials show that a female mating preference for forehead patch size (a trait also sexually selected through its effects on male-male competition) varies seasonally (Qvarnström et al. 2000). Females only prefer males with larger patches towards the end of the breeding season. The level of parental care largely determines breeding success and males with larger patches modify their behavior late in the breeding season to allocate more resources towards parental care. In contrast, early in the season males with larger patches allocate more resources towards pre-mating activities, such as seeking out extra-pair copulations (Qvarnström 1999). Females that mate with males with large patches late in the season therefore have increased reproductive success due to greater male parental care (Qvarnström et al. 2000). This is one of a very limited number of field-based studies that supports the notion that mating preferences are not static traits and that, furthermore, this behavioral plasticity is adaptive (Reaney and Backwell 2007a; Chaine and Lyon, 2008).

It is worth noting that most studies investigating plasticity in mate choice show variation in choosiness (e.g. a change in the average phenotype of males that mate due to shifts in mating thresholds), rather than directly testing for a change in mating preferences (i.e. which males females prefer when they are offered a simultaneous choice of mates so that mate sampling costs do not influence choice decisions) (e.g. Lynch et al. 2005; Borg et al. 2006). Studies that directly examine shifts in mating

preferences tend to be laboratory based, which makes it difficult to determine whether experimentally induced changes in mate choice are adaptive in the field (e.g. Pfennig 2007; Heubel and Schlupp 2008).

Temporal constraints in marine species

In many intertidal species, the tidal cycle determines the optimal time for mating and larval release. Reproductive success is strongly related to both these activities as there is only a small window of opportunity for them to occur (Morgan and Christy 1995). In most intertidal species, reproduction is timed to ensure successful dispersal of larvae that are at the appropriate developmental stage (Christy 1978). Many of these species release larvae on nocturnal, maximum-amplitude high tides, when larvae are least likely to be seen by predators, and most likely to be transported away from coastal areas into the open sea (Morgan and Christy 1995).

Mating in fiddler crabs occurs at distinct times (usually over a few days) during each semi-lunar cycle (Christy 1978). Females that mate outside this time risk reduced offspring survivorship by releasing larvae during unfavourable tides if they wait until larvae are at the correct stage of development (Morgan and Christy 1995); or, if they release larvae at the optimal time, by releasing larvae that are not at the appropriate developmental stage. Although the timing of larval release is largely determined by the time of mating, temperature also influences the rate of larval development (Yamaguchi 2001). Females might therefore be able to speed up or retard larval development by selecting an incubation environment that is, respectively, warmer or cooler than average (deRivera 2005).

In the fiddler crab *Uca mjoebergi*, mating and incubation takes place within a male's burrow (Crane 1975). Ambient air temperature and burrow size largely determine incubation temperature (Christy 1987; Yamaguchi 2001). Wider burrows hold a greater volume of air and are likely to allow fresher, cooler air to circulate into the burrow and are therefore less affected by high ambient temperatures. In *U. mjoebergi*, for example, with every 1mm increase in burrow width there is a 0.27°C drop in burrow temperature relative to air temperature (Reaney and Backwell 2007a). Burrow width is strongly correlated with claw size, which, in turn, tends to be positively correlated with male attractiveness (Reaney and Backwell 2007a; Reaney 2008). In a correlational study

Reaney and Backwell (2007a) showed that female choice based on claw size appeared to change over a semi-lunar mating cycle. Females breeding early in the cycle appeared to preferentially mate with large clawed males who occupy large burrows, while towards the end of the cycle they prefer small-clawed males who occupy small burrows. They argued that females can adjust the rate at which larvae develop to ensure the timing of larval maturation is optimal with regards to larval release during spring tide, and that they do this by decreasing their preference for male size/burrow width over the mating cycle (deRivera 2005; Reaney and Backwell 2007a).

Here we ask whether the phenotypic correlation between male size and attractiveness seen within a cycle is also observed over a larger seasonal time scale. However, we take an experimental approach to test whether any such shift in mate choice is due to changes in mating preferences rather than other factors that might vary seasonally. Like most tropical species of fiddler crabs, reproduction in *U. mjoebergi* occurs year round (Crane 1975), but average air temperature is not constant over the year. We predict that this will affect female mating preferences because larval development time is determined by burrow size/temperature (Yamaguchi 2001; deRivera 2005). Males with large claws have large burrows, which are relatively cooler than small burrows owned by smaller clawed males (Reaney and Backwell 2007a). We therefore predict that for females to release larvae at the optimal time during the summer, when air temperature is highest, they should strongly prefer large clawed males, while during winter when air temperature is lowest, they will show a weaker preference and might even prefer smaller clawed males. We therefore tested whether female mating preference changes between the two seasons.

METHODS

Study species and site

Uca mjoebergi is a small fiddler crab (carapace width up to 20 mm) that inhabits intertidal mudflats in northern Australia. They occur in dense, mixed sex colonies and both sexes defend territories that are centred on a burrow (Reaney and Backwell 2007b). Males have one greatly enlarged major claw, which they wave at females during courtship. During the six day mating period, receptive females leave their

territories and sample a series of courting males before eventually selecting a mate. During this process a female does one of three things: approaches a male and his burrow but then bypasses him and continues sampling; approaches a male, enters and inspects his burrow and then continues sampling; or approaches a male, enters his burrow and mates with him (Reaney and Backwell 2007a). Following copulation and oviposition, the male leaves his burrow, while the female remains to incubate the eggs. The female stays in the burrow until she is ready to release larvae at the next high-amplitude spring tide.

Our study was undertaken at East Point Reserve, Darwin, Australia. The site lies in the highest section of the intertidal zone and is only inundated by the highest amplitude spring tides (Reaney and Backwell 2007a). Females therefore have about a four day period in which it is possible to release larvae. We only ran mate choice trials over the first three days of the six day mating period during neap tide. This was done to control for an already documented weaker female preference for larger clawed males later in the mating period (Reaney and Backwell 2007a).

Average monthly temperature

Half-hourly temperature readings for Darwin Airport from July 2007 to December 2008 were obtained from the Australian Bureau of Meteorology. The average monthly temperatures for July through to December were then calculated.

Burrow diameter measurements

To establish whether crab size and burrow size is correlated we used dial calipers to measure carapace width, major claw length, and burrow diameter to the nearest 0.1mm for 55 randomly selected males.

Size preference experiments

A series of two-choice experiments were performed using a custom-built robotic crab system. In brief, each robotic crab produces a claw movement engineered to resemble closely that of a courting male *U. mjoebergi*. The system used consisted of a control box and two identical crab units. Each crab unit had a motor housed in a plastic container

that controlled the movement of a rigid metal arm. Wave rate was set at 8.4 waves/min (close to the population average), and both claws waved in synchrony to avoid a leadership effect (Reaney et al., 2008). Replica claws were temporarily attached to the robotic crab arm. Three experiments were performed using three different claw combination pairs: (1) 14.6 vs 19mm, (2) 14.6 vs 21.2mm, and (3) 19 vs 23mm. Female choice for each combination of claw sizes was tested 4 times across two years (September 2007, November/December 2007, July 2008, and December 2008) (for *N* see Table 1). From here on, July and September will be referred to as ‘winter’ and November and December as ‘summer’.

Mate choice experiments were performed in a cleared area of mudflat *in situ* that contained the two robotic crabs placed 5cm apart and 20cm from the female release point. All conspecific and heterospecific crabs within 2 m of the choice arena were removed and released elsewhere on the mudflat. Naturally occurring mate searching females, identified when they were observed sampling the burrow of a courting male, were captured on the mudflat and placed in a container with water to prevent dehydration. A female was then placed under a clear plastic container at the release site. The robotic crabs were activated and the female left under the container for at least three wave cycles. The container was then lifted using a remotely triggered lever. We scored a positive choice if a female moved in a direct line and stopped at the base of a waving robotic unit (for video footage see Reaney et al. 2008). A trial was discarded if the female ran immediately after being released or did not make a choice within 3 min. Each female was used in one trial per experiment. Female size in winter and summer 2007 did not significantly differ ($9.60 \pm 0.74\text{mm}$ versus $9.42 \pm 0.92\text{mm}$, $t_{88} = 0.985$, $P = 0.327$). However, pooled across all experiments, tested females were smaller in winter than summer ($9.01 \pm 1.09\text{mm}$ versus $9.58 \pm 0.87\text{mm}$, $t_{141} = -3.653$, $P < 0.001$). There was, however, no effect of female size on their claw size preference (see Results). To test this, we conducted t-tests comparing the size of females that chose the larger versus the smaller claw in each of the 12 experiments (see Table 1). We then converted all 12 experimental outcomes to the effect size r , using the formula $r = Z/n^{1/2}$ (where Z was obtained from the P -value for the experiment and n is the sample size) (Rosenberg et al. 2000). We then calculated the mean effect size in a random effect meta-analysis for the Fisher-transformed r values to test whether it differed from zero (Rosenberg et al. 2000).

The lack of a within-season female size effect suggests that the seasonal difference in female size is unlikely to explain any seasonal difference in female choice.

Furthermore, due to the presence of a major claw in males, large females are easily able to fit down the burrows of relatively small males (e.g. a female with a carapace width of 11.5mm can easily fit down the burrow of a male with a carapace width of 10mm and major claw length of 14.6mm). Therefore any observed seasonal change in preference cannot be explained by a seasonal change in the range of males that females are able to mate with due to burrow width constraints.

For all two-choice experiments any potential side-bias was eliminated by alternating the presentation of test stimuli between sides across trials. There was no detectable side bias (left versus right: 248:247; $N=495$, Binomial test, $P>0.999$).

Female preferences were first tested with binomial tests (two tailed) with $\alpha = 0.05$. To compare the strength of the female preference for claw size between the two seasons we converted all experimental outcomes to the effect size r . We then calculated the mean effect size in each season and tested for a significant difference in Fisher-transformed r between the two seasons by running a random effects model with season as a grouping factor in Metawin 2.0 and testing whether Q_b was significantly larger than expected (Rosenberg et al. 2000).

RESULTS

The monthly air temperature in summer (November – December) was 1.72 (2007; $t_{2372}=17.14$, $P<0.001$) to 5.14°C (2008; $t_{2026}=42.65$, $P<0.001$) hotter than in winter (July – September) (Figure 1).

Both claw length and carapace width were strongly correlated with burrow diameter ($r=0.92$ and 0.90 , $n=55$).

For each individual experiment during winter, females showed no significant preference for claw size ($P = 0.081$ to 0.736), while during summer females showed a significant preference for larger claws in all experiments (all $P<0.015$) (Table 1).

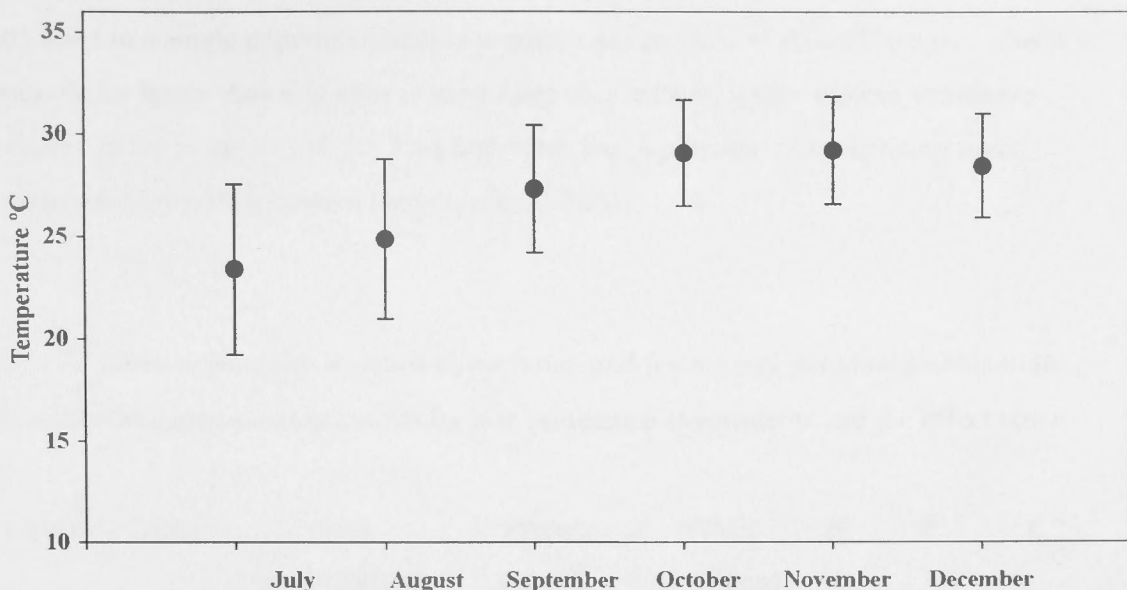


Figure 1. Average monthly air temperature for Darwin Airport (2007-2008). Mean $\pm$ sd.

When pooling data across all 12 experiments, females showed a significant preference for claw size as the mean effect was significantly greater than zero. The strength of the effect of claw size on female mating preference was significantly greater in summer than winter (meta-analysis: $Q_b = 17.76$, $df = 1$, $P = 0.003$, $r_{winter} = 0.176$, $r_{summer} = 0.728$). In both seasons, however, the mean effect was significantly greater than zero (Figure 2).

There was no significant difference in the size of females choosing the larger over the smaller claw in any of the 12 experiments (t-tests, all $P > 0.13$, $n = 35$ to 50). The mean effect of female size on choice of the larger claw was not significant given $r = 0.043$ (95% CI: -0.02 to 0.097). The positive value of r indicates that, if anything, larger females more often chose the smaller claw, which makes the seasonal trend highly unlikely to be explained by the seasonal difference in female size.

The strength of the female mating preference for male claw size in *Uca mjoebergi* changes between seasons. The female preference for larger clawed males in winter was significantly weaker than that in summer, when the mean temperature was 1.7 to 5.1°C hotter. Although none of the results for individual experiments undertaken in winter indicated a significant mating preference based on claw size, a meta-analysis showed that the mean effect size for the strength of the female preference for claw size in winter was significantly greater than zero ($r_{winter} = 0.176$). This is indicative of a weak size-

based preference. To have an 80% likelihood of detecting a significant preference at the 0.05 level in a single experiment would require a sample size of about 250 trials. This is a sample far larger than that seen in most mate choice trials, where median values are probably in the range $n = 30-50$. This highlights the importance of interpreting non-significant results with caution (Jennions et al. 2001).

Table 1. Mean $\pm$ standard deviation temperature and female size per claw combination per season and binomial test results for size preference experiments and the effect size r .

season	year	claw combinations (mm)	average temp °C	female size (mm)	N	P	r
winter	2007	14.6 vs 19.0	27.02 $\pm$ 3.34	9.60 $\pm$ 0.74	40	0.636	0.075
		14.6 vs 21.2	27.02 $\pm$ 3.34	9.61 $\pm$ 0.89	40	0.081	0.319
		19.0 vs 23.0	27.02 $\pm$ 3.34	9.29 $\pm$ 0.86	40	0.430	0.125
	2008	14.6 vs 19.0	23.87 $\pm$ 4.30	8.35 $\pm$ 1.05	35	0.175	0.229
		14.6 vs 21.2	23.87 $\pm$ 4.30	8.35 $\pm$ 1.05	35	0.175	0.229
		19.0 vs 23.0	23.87 $\pm$ 4.30	8.35 $\pm$ 1.05	35	0.736	0.057
summer	2007	14.6 vs 19.0	28.74 $\pm$ 2.67	9.42 $\pm$ 0.92	50	0.001	0.465
		14.6 vs 21.2	28.74 $\pm$ 2.67	9.38 $\pm$ 0.81	50	0.000	0.792
		19.0 vs 23.0	28.74 $\pm$ 2.67	9.38 $\pm$ 0.82	50	0.015	0.344
	2008	14.6 vs 19.0	29.01 $\pm$ 2.53	9.79 $\pm$ 0.77	40	0.000	0.763
		14.6 vs 21.2	29.01 $\pm$ 2.53	9.79 $\pm$ 0.77	40	0.000	0.647
		19.0 vs 23.0	29.01 $\pm$ 2.53	9.79 $\pm$ 0.77	40	0.000	0.592

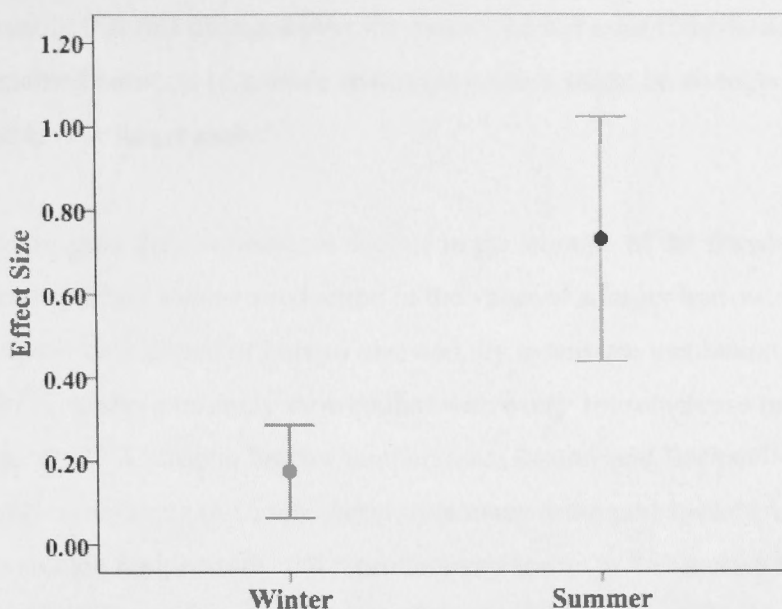


Figure 2. Strength of mating preference in winter compared to summer. Mean and 95% confidence intervals for effect size r ($n = 6$ per season).

DISCUSSION

Female *Uca mjoebergi* show a seasonal change in the strength of a mating preference for male claw size. During hotter months, the strength of female mating preference for larger clawed males was significantly stronger than during cooler months. We propose that the change in female preference for claw size is due to a seasonal difference in the value of a larger burrow, which strongly influences burrow temperature and therefore incubation temperature.

Reaney and Backwell (2007a) showed that strong temporal constraints appear to cause females to modify their preference for burrow width over a two-week mating cycle to optimise larval developmental time. They observed a decline in the mean size of mated males over the mating period and attributed this to large males having cool burrows that slow larval development and small males having warm burrows that elevate larval development. We suggest that the same process is occurring seasonally due to a similar change in temperature, albeit over a longer time scale, which selects for a temporal shift in mating preferences. It should be noted, however, that Reaney and Backwell (2007a) only observed the pattern of size-based male mating success and, while unlikely, it is

possible that this changed over the mating period even if the female mating preference remained constant (e.g. male-male competition might be stronger earlier in the cycle and favour larger males).

We propose that the seasonal decline in the strength of the female preference for claw size has arisen due to a reduction in the value of a larger burrow. Claw size provides a long distance signal of burrow size and, by extension, incubation temperature (deRivera 2005). A previous study showed that with every 1mm increase in burrow width, there was a 0.27°C drop in burrow temperature (Reaney and Backwell 2007a). Larval development rate in *U. mjoebergi*, like many arthropod species, is largely determined by incubation temperature, and reproductive success in this species is strongly influenced by the timing of larval release (e.g. Wear 1974; Christy 1978; Moriyasu and Lanteigne 1998; Yamaguchi 2001; deRivera 2005). If females are to successfully release larvae on time, they need to adjust their preference for burrow size as ambient temperature changes. In summer, when average air temperature is substantially higher, females should show a greater preference for large males with bigger burrows so as to select an incubation environment that is cool enough to prevent overly rapid development of larvae and a decline in female reproductive success (Christy 1983, Reaney and Backwell 2007a).

Reduced direct benefits are one potential cause of a weaker preference for claw size during winter. Smaller, warmer burrows during winter are probably less important than a larger, cooler burrow during summer because it is easier for a female to increase than decrease incubation temperature. This asymmetry occurs because, although incubation generally takes place at the bottom of a burrow, females could move up the burrow towards the entrance to increase the incubation temperature (Christy 2003). In contrast, females are limited in their ability to decrease incubation temperature as the burrows have a finite depth that is restricted by the water table and complex spatial variation in soil structure (Christy 1987). This has been illustrated in *U. mjoebergi*, where burrow depth varies very little between males that differ greatly in size (Reaney and Backwell 2007a). Therefore, during winter but not summer, females could position themselves precisely in the burrow cavity to accelerate embryo development rates and compensate for a drop in ambient air temperature or any error in the timing of oviposition (Christy 2003). One might then expect small males to dig wider burrows during summer, but

there are probably high costs to this due to greater risks of predation or eviction when there is a mismatch between male and burrow size.

One key question is why females show any preference for large clawed males during winter? Although the direct “thermal” benefit of mating with a large clawed male is reduced during winter, there are still potential indirect benefits. For example, across the year large clawed males still have greater mean mating success than small clawed males. The population variation in male size is largely a product of variation in male age as growth is indeterminate. This makes it less likely, but not impossible, for a female preference to be partly favoured by indirect genetic benefits (i.e. production of more attractive sons) if there is also heritable age-independent variation in claw size (Andersson 1994; Kokko et al. 2006). These benefits might be enough to maintain a weak female mating preference when sampling costs are low, (i.e. search time, predation risk and desiccation risk) (Smith and Miller 1973; Backwell and Passmore 1996). This was the case in our experimental study as females were offered a simultaneous choice of males. If, however, we had been observing naturally occurring sequential mate choice it is less clear whether we would have detected the same temporal shifts in male size-based mating success due to the higher sampling costs.

Although we have not directly shown that incubation duration changes with burrow size and/or season, the documented effect of temperature on crustacean larval development is strong. At present we suggest that the best explanation for our results is that female mate choice preferences shift due to changes in temperature. Nevertheless, further data is required to substantiate this argument: ideally we need to quantify the relationship between incubation duration and burrow size in winter versus summer. We can also test the prediction that, all else being equal, the decreased benefits of being selective in winter should result in shorter mate searching forays.

In summary, we have shown seasonality in the strength of female mate preference and by extension a reduction in selection on ornament size during part of the year. Only a handful of studies have shown such seasonal switches in mate selection (collared flycatchers: Qvarnström et al. 2000; two-spotted goby: Borg et al. 2006; field cricket: Velez and Brockmann 2006; sailfin mollies: Heubel and Schlupp 2008). Although this seasonal plasticity indicates there can be high flexibility in mating decisions, the adaptive link between a change in mating preference and the benefits accruing to

females from mating with a specific male phenotype often remains uncertain. In *U. mjoeborgi*, however, we have identified a change in mating preference that appears to occur at two scales (seasonally and within a biweekly tidal cycle) both of which can be accounted for by the same adaptive explanation based on burrow temperature.

Understanding the cause and effect of phenotypic plasticity in mating preferences is of huge importance (Jennions and Petrie 1997). Spatial and temporal variation in mating preferences has numerous implications for the process of sexual selection, evolution of mate choice, and the evolutionary dynamics of preferences and exaggerated traits (Chaine and Lyon 2008). Furthermore, this phenotypic plasticity could provide a mechanism by which multiple male ornaments arise and are maintained, and genetic variation for male traits preserved (Ellner 1996). Social and physical environments are rarely stable and flexibility in mating preferences might allow females to select males that best suit their current needs. Such plasticity is likely to be common. We predict that closer inspection will show that species that live in highly variable environments that change predictably (e.g. those with strong seasonality or strong altitudinal clines) and/or have prolonged mating seasons will display adaptive phenotypic plasticity in mating preferences. This is especially likely when mate choice confers direct benefits. When there are only indirect, genetic benefits to mate choice, however, the situation is more complicated because gene-environment interactions make it difficult to use the current phenotype of a male (i.e. his attractiveness) to predict the fitness of offspring who might develop in another environment (Kokko and Huebel 2008).

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CHAPTER 2: NON-INDEPENDENT MATE CHOICE IN A FIDDLER CRAB: A CASE OF STIMULUS ENHANCEMENT

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ABSTRACT

Non-independent mate selection occurs when the choice behavior of a female is altered by the interactions between other females and males. In the fiddler crab *Uca mjoebergi*, males court mate-searching females by waving their one greatly enlarged claw. When a female approaches a male, he initiates high intensity waving. We conducted one natural mate choice experiment and two mate choice experiments using custom-built robotic crabs. We show that the decision of one female to approach a group of males increases the probability that another female will approach and visit a male from the same group. We suggest that this behavior is best explained by the ‘stimulus enhancement’ hypothesis, where the presence of a female near a group of males makes them more likely to be detected by other females due to an increase in male display rate.

Keywords: Fiddler crabs, Mate choice copying, Non-independent mate choice, Stimulus enhancement, *Uca mjoebergi*

INTRODUCTION

Determining the factors that generate variation in male mating success is fundamental to our understanding of sexual selection (Andersson 1994). Non-independent mate choice, where the choice of one female influences that of another, can potentially increase intra-population variation in male mating success with fewer males gaining a greater proportion of matings. This has important consequences for the strength and direction of sexual selection on male traits (Westneat et al. 2000). Most studies of non-independent mate choice are laboratory based and focus almost exclusively on ‘mate-choice copying’ where the likelihood that a female chooses a male increases after she has observed him being chosen by another female (Dugatkin 1992; Witte and Massmann 2003; Godin et al. 2005; Brown and Fawcett 2005; Mery et al. 2009; Yorzinski and Platt 2010). In an early study Dugatkin (1992) showed that female guppies (*Poecilia reticulata*) could remember the identity of males they had previously seen in association with another female and that females found these male more attractive than males they had not been previously seen with a female.

Far fewer studies have looked at another form of non-independent mate choice: stimulus enhancement. Here, the presence of a female near a male or group of males increases male visibility, and possibly attractiveness, to other females (Westneat et al. 2000). Courtship and copulation often consists of conspicuous displays (Andersson 1994) that attract the attention of other mate searching females. This increases the likelihood that these females will approach an area and mate with the already courting males, thus resulting in non-independent mate choice (Westneat et al. 2000). Alternatively, females might chose to actively avoid a male or group of males who she has recently seen courting (e.g. to reduce the risk of mating with a sperm depleted male). To date, no study has specifically tested this hypothesis, and the prevalence of this form of non-independent mate choice is unknown.

In the fiddler crab *Uca mjoebergi*, males have one greatly enlarged claw, which they wave at approaching mate-searching females in a conspicuous courtship display (Milner et al. 2008, 2010a). During the mating period, receptive females leave their territories and wander through the population, visiting a series of males before eventually selecting a mate (Reaney and Backwell 2007). Males form a cluster around the female and tend to wave in synchrony (Backwell et al. 1998). Female choice is based partly on wave

rate, wave leadership and claw size (Backwell et al. 1999; Reaney and Backwell 2007; Milner et al. 2010b). Once a female selects a male, she enters his burrow, remains inside and mates with him. Occasionally, however, she mates with him on the surface and then finds another burrow in which to incubate her eggs (unpublished data, Slatyer R). Regardless of whether mating is in the burrow or on the surface, the conspicuous courtship display could potentially attract the attention of mate-searching females that are not the immediate target of the displaying male/s. This would increase the likelihood that one of the previously courting males or an immediate neighbor obtains a mate.

In this study, we used a natural mate choice experiment to determine whether the presence of a female in a cluster of naturally waving males increased the probability of a second female approaching these males. We then ran a choice experiment using two robotic crabs, one with a female tethered next to it, to determine whether the presence of a female by a male, without any effect on male waving, might increase the likelihood that he attracts a second mate-searching female. We documented the waving behavior of males that were courting a nearby female and males that were not courting a female. We used this information in the final robotic crab choice experiments: we gave a female the choice between two groups of robotic crabs, neither of whom had a female tethered near it. One group waved in a pattern that mimicked the natural waving of a cluster of males that were courting a nearby female and the other waved in a pattern that mimicked the natural waving behavior of a non-courting male cluster.

METHODS

The study was conducted from September to December 2009. *U. mjoebergi* is a small fiddler crab (carapace width < 20mm) that inhabits inter-tidal mudflats in northern Australia (Reading and Backwell 2007; Fayed et al. 2008). They occur in dense, mixed sex colonies (37 ± 17 crabs m^{-2} ; R. Slatyer, L. T. Reaney and P. R. Y. Backwell, unpublished data) and during the mating period, receptive females leave their territories and wander through the population, visiting the burrows of a series of courting males before eventually selecting a mate (Reaney and Backwell 2007). During this period, multiple mate-searching females are commonly seen in close proximity (R. Milner, personal observation).

Documentation of courting and non-courting waving

We videoed 50 groups of naturally occurring males either with or without a female tethered in the centre of the cluster ($n=25, 25$). All other females were removed from the area. A 20 second segment of the highest waving activity from each video was then analyzed and we documented: the total number of waves given by each male in the cluster; the number of waves that were given in synchrony with other males from the cluster; the spatial separation between each male and his nearest neighbor.

Natural mate choice experiment

We selected two identically sized (2-4) groups of males with approximately the same claw lengths (i.e. $<5\text{mm}$ difference between comparative individuals between groups; size matching was performed by eye). The groups were 50-60cm apart and all other crabs within a 50cm radius of the two groups were temporarily confined in their burrow after we sealed burrows with a sand plug. A female crab was then tethered with a 2cm piece of cotton glued to her carapace that was attached to a nail. The nail was embedded in the sediment so that the female was in the middle of one group of males (courting group). The second group of males did not have a female tethered among them (non-courting group). The two groups were $<60\text{ cm}$ apart so they were equally disturbed by the presence of the experimentor. After an acclimation period of two minutes for the males, a second female was placed under an opaque plastic container midway between the two groups. Once all males in each group were visible the container was lifted using a remotely triggered lever. We scored a positive choice if the female moved in a direct line to one of the males in either group and touched or entered the entrance of his burrow. A trial was discarded if the female ran immediately after being released or did not make a choice within 3 min ($N=10$). In total, 20 females made a positive choice.

Female presence effect experiment

We used a custom built robotic crab claw (see Reaney et al. 2008; henceforth ‘robotic crab’) in which each robotic crab produces a claw movement engineered to resemble the wave of a courting male *U. mjoebergi*. The system consisted of a control box and two identical crab units. Each crab unit had a motor housed in a plastic container that controlled the movement of a rigid metal arm. An identical replica claw was attached to

each robotic crab arm. All claws were the same size (17mm). The experiments were run in a cleared area of mudflat within the population. All crabs within 2m of the choice arena were first removed and released elsewhere on the mudflat.

To test whether the presence of a female near a male influences the mate choice decision of another female, we gave 53 focal females a choice between two identical robotic crabs, one of which had a female tethered 5 cm away (to simulate a female visiting a male). The two robotic crabs were placed 10 cm apart and 20 cm from the female release point (Fig. 1). The wave rate was set at 8.4 waves/min, which falls within *U. mjoebergi*'s natural courtship wave rate range and has successfully been used in past studies (see Booksmythe et al. 2008; Milner et al. 2008; Milner et al. 2010b). The females were placed under a clear plastic container at the release site and left for at least three wave cycles. The container was then lifted using a remotely triggered lever. We scored a positive choice if the female moved in a direct line and stopped at the base of a waving robotic unit (for video footage see Reaney et al. 2008). We discarded three trials because the female ran immediately after being released or did not make a choice within 3 min.

Effect of elevated courtship experiment

We used data from the 'Documentation of courting and non-courting waving' to create a more natural mate choice experiment in which females were given the choice between a cluster of four robotic crabs that waved in a pattern that either mimicked the natural waving behavior of a courting cluster or a non-courting cluster of males. Neither cluster of robotic crabs had a female tethered near it (for justification see Results). The four robotic crabs that mimicked courting males waved in synchrony at 22 waves/min and were placed 8cm apart (Fig. 2). The four robotic crab cluster that mimicked non-courting males waved at 6 waves/min in alternation with each other and were placed 12cm apart. The two cluster were opposite each other and each was 30cm from the female release point (to justify values see results for natural non-courtship waving; Fig 2). In total we released 33 females and their choice was documented as previously described for the 'female presence effect experiment'. In total 30 female made a choice.

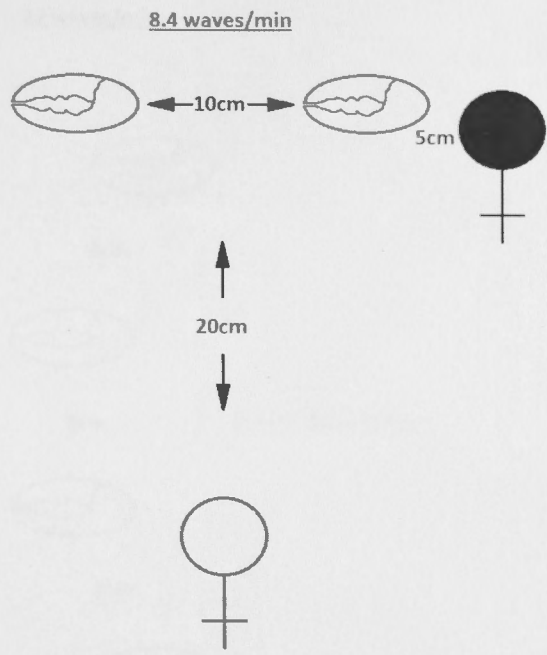


Fig. 1 Diagram showing choice arena for ‘Female presence effect experiment’ (‘model’ female absent versus model female present). Robotic crab: ; female release point: ; tethered ‘model’ female:

In all experiments, we used naturally mate-searching females that had been observed to visit at least one male before being captured and tested. For all mate-choice experiments any potential side-bias was controlled for by alternating the presentation of test stimuli between sides across trials. There was also no detectable side bias (female presence effect experiment: left vs. right: 23: 27; $n=50$, binomial test, $P=0.67$; effect of elevated courtship experiment: left vs. right: 17:13; $n=30$, binomial test, $P=0.59$).

Female preferences were tested with binomial tests (two tailed) with $\alpha=0.05$. To be conservative, two-tailed testes were used because we had no strong prior predictions for female preferences.

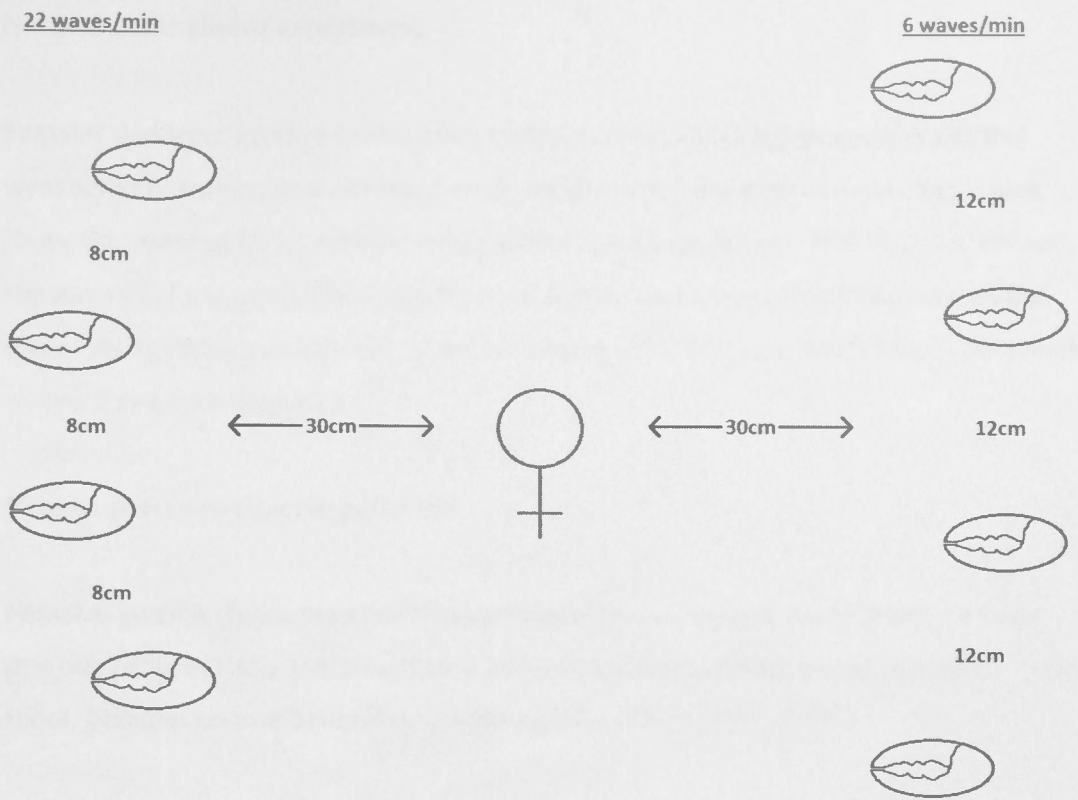


Fig. 2 Diagram showing choice arena for ‘Effect of elevated courtship experiment’ (courting versus non-courting). Robotic crab: ; female release point: 

RESULTS

Documentation of courting and non-courting waving

The natural waving behavior of courting and non-courting males differed. Courting males waved at a rate of 27.1 ± 6.1 waves/min ($n=52$ courting males/25 clusters) and 89% of the waves given were in synchrony with other males from the cluster. Courting males clustered tightly because they approached the tethered female so that the mean distance between male neighbors reduced to 8.1 ± 3.0 cm ($n=60$ courting males/25 clusters). Non-courting males waved at a much slower rate (3.8 ± 4.4 waves/min, $n=52$ non-courting males/25 clusters) and were far less synchronous (11.5% of waves overlapped those of other males in the group). The non-courting males did not cluster and the mean distance between males was 14.0 ± 6.3 cm ($n=67$ non-courting males/25 clusters).

Natural mate choice experiment

Females that were given a choice between a naturally occurring group of males that were actively waving at a tethered female and a control group of non-courting males, chose the courting males significantly more often (Binomial test; $P < 0.001$). In 90% of the successful trials ($n = 18$ of 20), the focal female moved in a direct line and visited one of the courting males, while in the remaining 10% of the successful trials the female visited a non-courting male.

Female presence effect experiment

Females given a choice between two waving robotic crabs that were identical except that one of them had a female tethered 5cm away showed no preference for either male robot. Females choose both robotic crabs equally (25:25; $P = 1$, $n = 50$).

Effect of elevated courtship experiment

We used the values from the natural waving data to create a choice experiment in which females could choose between two groups of robotic crabs that either mimicked the natural waving behavior of a courting or non-courting group of males. Females were significantly more likely to choose a robotic crab from the group that mimicked courting males (24 of 30; Binomial test, $P = 0.001$, $n = 30$).

DISCUSSION

We have shown that non-independent female mate choice occurs in the field in the fiddler crab *Uca mjobergi*. Females preferentially approached a group of males that was actively courting a female over a group that was not courting a female. This result could be due to direct ‘female copying’ in which a female is attracted to a male or group of male because she sees another female near him/them (Godin et al. 2005). Alternatively, it could be due to ‘stimulus enhancement’ in which a female is attracted to a courting male or group of courting males because their displays make them more conspicuous (Westneat et al. 2000). The results of our first mate choice experiment suggest that female copying is unlikely to play a role. The presence of a female near to a robotic

crab did not influence her choice of robotic crab. The wave rate used in this experiment was lower than that of males actively courting a female when she is at close range. It is, however, still more than twice the rate of non-courting males, and within the range of natural wave rates seen when a female is approaching a group of males. It was clearly a sufficiently high wave rate to attract females to approach the waving robotic crabs (only 3 of 53 females failed to approach a 'male'). We are therefore confident that our negative result reflects the absence of an effect on female choice of seeing a female near a male, rather than it being an artifact of females perceiving neither male as being sexually active and therefore indifferent to the presence of another female.

The second mate choice experiment suggests that stimulus enhancement can account for the non-independent choices of females. Courting males wave faster, in greater synchrony and in close proximity to each other than non-courting males. Females that were given a choice between two groups of robotic crabs that mimicked the natural waving behavior of either a courting or non-courting group of males, were significantly more likely to approach a robotic crab from the courting group.

In *U. mjobergi* the decision of a female to mate with a male is unlikely to directly result in another female mating with the same male. This is because most matings take place within the male's burrow and it can presumably only house a single breeding female (Reading and Backwell 2007). In the rare case where mating on the surface occurs (Slatyer unpublished data) it is unlikely that a female would wait to mate with the courting male suggesting that mate choice copying is an unlikely component of this mating system. Instead, non-independent mate choice is most likely to arise because the elevated rate of waving elicited by one female enhances the likelihood of other males in the group subsequently attracting another female who sees this heightened level of courtship. It should be noted, however, that the effect of 'stimulus enhancement' on female mate choice in *U. mjobergi* is likely to be brief as males begin to slow their wave rate soon after a female either enters a male's burrow to mate or leaves the area (R. Milner, personal observation), presumably because waving is costly (Matsumasa and Murai 2005). Nevertheless, the presence of a female does increase the probability that another female in the near vicinity will approach the same group of courting males (multiple mate-searching females are commonly seen in close proximity; R. Milner, personal observation) and, by extension, increase the mating probability for these males. This will ultimately lead to non-independent female mate choice decisions that could

significantly affect the evolution of sexually selected male traits (Gibson and Höglund 1992; Westneat et al. 2000).

Past studies of non-independent mate choice have either ignored or deliberately excluded any influence of stimulus enhancement because they were specifically designed to test for mate choice copying. For example, in a number of studies on guppies, the model female was removed before the focal female was allowed to choose (Dugatkin 1992; Dugatkin and Godin 1992; Briggs et al. 1996; Brooks 1996; LaFleur et al. 1997; Brooks 1999; Vukomanovic and Rodd 2007). This had the effect of eliminating any role for stimulus enhancement in the mate choice decision of the focal female (unless she had a memory of the male assigned the model female as one who courted at a higher rate than the control male, but did not attribute this to the presence of the model female). Given that most studies that investigate non-independent mate choice indirectly control for stimulus enhancement, we still know very little about its prevalence or importance in mate choice. In communally signaling species where a male can not readily mate with two females over a short interval, the extent to which stimulus enhancement is a factor that determines whether it is adaptive to retain neighbors that might increase a focal male's likelihood of acquiring a mate and/or whether selection favors males adjusting their courtship levels in relation to those of their neighbors remains poorly studied.

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CHAPTER 3: EAVESDROPPING IN CRABS: AN AGENCY FOR LADY DETECTION

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ABSTRACT

Although conspicuous courtship displays are an effective way of attracting the attention of receptive females, they could provide valuable information to rival males on the location of these females. In fiddler crabs, males that see a receptive female then wave their single, greatly enlarged claw in a highly conspicuous courtship display. We test whether other males use this courtship display to alert them to the presence of receptive females that they can not directly see. We show that male fiddler crabs (*Uca mjoebergi*) eavesdrop on the courtship displays of nearby males to detect mate-searching females. This allows males to begin waving before a female becomes visible. Furthermore, males appear to adjust their waving according to the information available: eavesdropping males wave 12 times faster than non-courting males but only 1.7 times slower than males in full visual contact with the female.

Keywords: animal communication; eavesdropping; Fiddler crabs; mate attraction; *Uca mjoebergi*

INTRODUCTION

Animal communication generally occurs within a complex social environment (McGregor 2005) with both target and non-target receivers (McGregor & Peake 2000). The use of signal information by non-target receivers is termed ‘eavesdropping’ (Peake 2005). By eavesdropping, individuals are able to effectively acquire information that might benefit them. To date, most studies concerning eavesdropping have focused on male-male aggressive interactions in either a territorial or mate choice context. For example, in green swordtails (*Xiphophorus helleri*), males that observed contests between two other males were less likely to initiate a contest with the observed winner (Earley & Dugatkin 2002). Similarly, in fighting fish (*Betta splendens*), females that eavesdropped on aggressive interactions between males subsequently spent more time with the winner of the encounter (Doutrelant & McGregor 2000). Far fewer studies have investigated the importance of eavesdropping in the context of mate attraction. One such example comes from the Australian bushcricket (*Elephantodeta nobilis*) where females give response calls to male advertisement calls, forming calling duets. In this case, unattractive males are attracted to these duets and occasionally successfully intercept the receptive female (Bailey & Field 2000). Similarly, in the Broadley’s painted reed frog (*Hyperolius marmoratus broadleyi*), males will increase their own call rate in response to the increased call effort of males that have detected a female (Grafe 2005).

Male reproductive success is usually limited by female availability and the difficulty and time associated with locating a mate (Alcock 2005). Males should therefore be finely attuned to any cues or signals that provide information on the location of potential mates, including cues from rival males’ courtship displays (Roberts *et al.* 2006). In fiddler crabs, males attract mate-searching females by waving their one greatly enlarged claw in a highly conspicuous display. Receptive females approach males based on wave rate, wave leadership and claw size (Reaney & Backwell 2009; Milner *et al.* 2010). The fact that males produce a conspicuous waving display once a receptive female is detected suggests that other males could potentially use this information to alert them to the presence of a mate-searching female. That is, males could use other males as ‘female detectors’, reducing the time taken to locate potential mates and effectively increasing the distance at which they detect, and therefore respond to, receptive females. Ultimately this should increase a male’s probability of

successfully attracting a mate. In this study we aim to determine whether male fiddler crabs (*Uca mjoebergi*) eavesdrop on the courtship displays of nearby males to detect mate-searching females.

METHODS

We studied *Uca mjoebergi* on mudflats in East Point Reserve, Darwin, Australia from September to November 2009. *U. mjoebergi* is a small fiddler crab (carapace width <20mm) that inhabits intertidal mudflats across northern Australia. They occur in dense, mixed sex colonies (37 ± 17 crabs/m², unpublished data) and both sexes defend individual territories that are centered on a burrow (Reaney & Backwell 2007a). During the mating period, receptive females leave their territories and wander through the population in search of prospective mates. They visit several males (2.2 ± 1.6 males) and can travel meters (50.2 ± 73.4 cm) while mate searching (Reaney & Backwell 2007b). Once a female selects a male, she enters his burrow and the pair mate. Following copulation and oviposition, the male abandons the burrow, while the female remains to incubate the eggs.

We conducted an experiment with a repeated-measures design with four treatments that manipulated the social environment of a focal male. We then measured his wave rate under each treatment. For the first treatment, we located a naturally occurring group of courting males and removed any wandering females within 50cm of the group. A group of males consisted of at least four neighbouring males that were all waving at the same female at the time of initial sighting. We then randomly designated a focal male from the group and, after a three minute waiting period, measured his wave rate. This was measured by counting the number of waves produced over a 30 second period.

For the second treatment, we placed a small piece of plywood (2.5 x 4.5cm) vertically into the mud approximately 13cm in front of the focal male. This prevented the male from seeing the area directly behind the barrier. After a three minute waiting period, we again measured the number of waves the male produced in a 30 second period. This is unlikely to have increased male wariness as wave rate actually increased slightly (see Results).

For the third treatment, we collected a mate searching female from another area of the population and tethered her on a short leash immediately behind the plywood visual barrier. She was not visible to the focal male, even if he moved to the edge of his territory. To tether her, we glued her carapace to a short (1–2 cm) length of cotton thread that was tied to a nail pushed into the mud. While the focal male could not see the tethered female, the vertical partition was placed so that all the other males in the group could see the female. After a three minute waiting period, we again measured the number of waves given by the focal male in a 30sec period.

For the fourth treatment, we removed the plywood barrier from treatment 3, making the tethered female visible to the focal male, waited for three minutes and again measured the number of waves produced by the focal male in a 30sec period. In this treatment, the tethered female was clearly visible to the focal male and to all the other males in the group.

We tested 50 different groups of males. The same male was designated the focal male for all four treatments. We tested for an overall treatment effect using Friedman's test. We then used Wilcoxon signed-rank tests to compare wave rate between pairs of treatments. All tests were two-tailed with a Bonferroni correction to account for multiple testing so that $\alpha_{\text{critical}} = 0.05/6 = 0.0083$. Data are presented as medians and upper and lower quartiles.

Carry over and Order Effects:

It was important to wait three minutes between successive treatments to minimize potential 'carry over' effects where male wave rate continues to be directly affected by the previous treatment. Any such effects should lead to an increase in wave rate when a male is stimulated to wave in the preceding treatment (e.g. when he sees a female). When designing our study we realised that 'carry over' effects were more likely to affect our results if we first presented the treatment predicted to be most stimulating (female visible). For this reason, we always presented the treatments in the same order: no female; visual barrier; visual barrier with female behind it; visible female. This does, however, mean that we have potentially introduced a confounding order effect (the order of the trials affects the results). On biological grounds, we argue that it is very unlikely that males increase their wave rates in subsequent treatments as a direct

response to the preceding treatments, or because there is a general increase in wave rate with time of day. There is an indirect way to test this. If a general temporal effect influenced our results, then we would expect it to be unidirectional: the wave rate should increase or decrease with each subsequent treatment. We did not find this: the wave rate stayed the same or dropped between the first and second treatment, and then increased substantially between the second and third, and increased less strongly between the third and fourth treatments (see Results).

RESULTS

The wave rate differed among the four treatments (Friedman's test, $\chi^2 = 140.4$, $df=3$, $P < 0.00001$). The median wave rate of the focal male in the first treatment (no female, no barrier) was 1 wave/minute (upper-lower quartile: 0-4). In the second treatment, when the barrier was added, the focal male's wave rate was 0 waves/minute (0-4). In the third treatment, when a female was placed behind the barrier and was therefore not visible to the focal male but clearly visible to the rest of the courting group, the focal male's wave rate was 12 waves/minute (10-14; $n = 50$). In the final treatment, when the female was visible to the focal male, his wave rate was 20 waves/minute (16-28) (all $n = 50$).

Individual male wave rates are shown in Fig. 1. There was a significant difference between all pairwise comparisons of wave rate (all $P < 0.001$, $Z = 6.17$ except treatment 3 versus 4 where $P < 0.001$, $Z = 5.90$), except for a non-significant difference between the wave rate in treatments 1 and 2 once multiple testing was taken into account ($P = 0.026$, $Z = 2.23$).

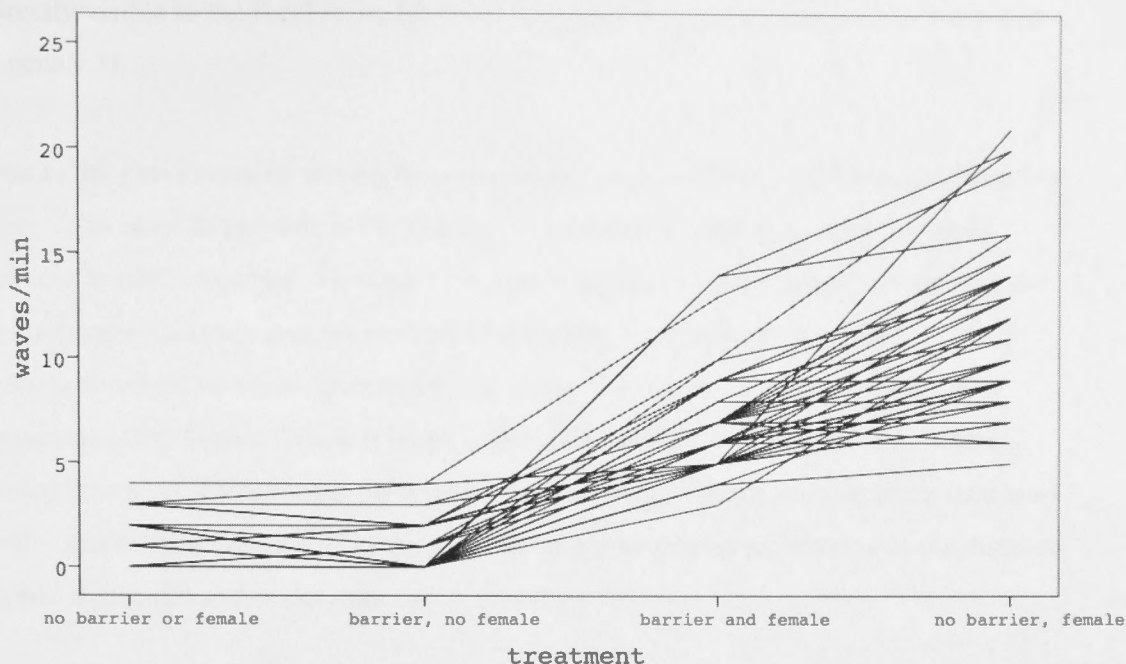


Fig. 1. Individual male wave rate across four treatments.

DISCUSSION

We have presented strong evidence that male fiddler crabs (*Uca mjoebergi*) eavesdrop on the courtship displays of nearby males to detect mate-searching females.

Furthermore, we found that males adjust their response according to the source of the information received. Directly seeing a female led to a higher wave rate than when inferring her presence from the behaviour of surrounding males.

Taking into account multiple testing, and our caveat about order effects, there was no difference in the wave rate of a male when a barrier was added (treatment 1 versus 2), so the barrier itself did not affect male wave rate. The small decrease of 1 wave/minute in the focal males' wave rates between the initial and subsequent measurement (i.e. when all females had been removed from the area versus approximately five minutes later when the barrier was in place) is probably attributable to a small carry over effect. That is, males in the first treatment most likely waved at a slightly higher rate because they had more recently seen a mate-searching female. However, when a male could not directly see a female (due to the presence of the barrier) but could see neighbouring males courting, he waved significantly faster than when there were no females present (treatment 3 versus 2). In addition, once the barrier was removed so that the female was

directly visible to the focal male, his wave rate again increased significantly (treatment 4 versus 3).

Due to the importance of waving for mate attraction in fiddler crabs, detecting receptive females as early as possible is likely to be of great importance in determining male success at mate attraction. Through eavesdropping, males are effectively increasing the distance at which they can detect receptive females, thereby increasing their conspicuousness and consequently elevating their likelihood of being sampled by a mate-searching female. While it might seem maladaptive for a male to wave before seeing a female, if this means the female cannot see him, the greater conspicuousness of males due to their enlarged claw means there is a probably an asymmetry in the distance at which females and males detect each other.

The use of socially acquired information has been shown in at least one other context in fiddler crabs. In *U. pugilator* fiddler crabs retreat into their burrows when they detect their neighbours fleeing, even if they are unable to see the stimulus that induced their neighbours' responses (Wong *et al.* 2005). Furthermore, in *U. tangeri* it has been suggested that males might use other males as female detectors (Pope 2005), but this data remains unpublished.

While the use of rival males' signals as a cue to detect receptive females is likely to be a widespread phenomenon, very few studies have investigated this possibility (*but see* Bailey & Field 2000; Grafe 2005; Roberts *et al.* 2006). Here we provide a rare example of males eavesdropping on rival males' courtship displays to detect receptive females. To the best of our knowledge this is the first such evidence from a visual communication system.

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CHAPTER 4: KEEPING UP APPEARANCES: MALE FIDDLER CRABS WAVE FASTER IN A CROWD

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ABSTRACT

Courtship displays are often energetically and temporally costly, as well as highly conspicuous to predators. Selection should therefore favour signalling tactics that minimize courtship costs while maintaining or increasing signal attractiveness. In fiddler crabs, males court females by waving their one greatly enlarged claw in a highly conspicuous and costly display. Here we investigate whether courting males adjust their wave rate, and therefore the cost of courtship, to the current level of competition. We show that display rate increases as competition increases and that when competition is removed, males reduce their display rate by 30%. These results suggest that male fiddler crabs actively reduce the cost of courtship by adjusting their wave rate in response to the immediate level of competition.

Keywords: animal communication; courtship competition; Fiddler crabs; mate attraction; *Uca annulipes*

INTRODUCTION

Mate choice often takes place within a complex social environment, where numerous individuals simultaneously compete for the attention of a mate (e.g. choruses and leks) [1]. Information exchange during these communication events is rarely confined to that between a signaller and receiver [2]. Instead individuals other than the intended receiver, including neighbouring competitors, sometimes use the information transmitted by a signaller when making decisions about their own signalling strategy [3]. For instance, on detecting another male's courtship signal, competing males may alter specific properties of their own signals, such as the rate of production, so as to increase their relative attractiveness and thereby their chance of obtaining a mate [4]. For example, in the gray treefrog (*Hyla versicolor*) males increased their call rate with an increase in chorus size [5].

Several studies in acoustic signalling systems have shown that males alter call rate, duration and/or complexity in response to the presence and/or calling of other males [4-10]. Surprisingly few studies have, however, examined how competing sexual signals influence male behaviour in a visual signalling context [11-13]. Nevertheless, plasticity in visual signalling behaviour during mate attraction is likely to be widespread. Courtship signals are often costly because of the increased expenditure of energy and time, and a greater risk of predation. Selection should therefore favour males that minimize these costs by adjusting their signalling behaviour according to the level of competition and/or likelihood of attracting a mate [13,14].

In fiddler crabs (genus *Uca*), males attract females by waving their one greatly enlarged claw in a conspicuous courtship display [15]. In *Uca annulipes*, females preferentially visit males that wave at a faster rate (waves/min) and produce more leading waves [16,17] implying that wave rate and wave leadership are targets of female choice. In the related species, *U. mjoebergi*, experiments show that leadership and wave rate are direct targets of female choice [18]. Waving is, however, extremely costly, in terms of energy, time and increased conspicuousness to predators. For example, wave rate in *U. annulipes* decreases with lower food availability [19], and predation rate by Black-bellied plovers (*Pluvialis squatarola*) and Ruddy Turnstones (*Arenaria interpres*) on *U. uruguayensis* is greater for courting males [20]. Consequently, males should facultatively adjust their wave rate according to the level of male competition to

increase their chance of attracting a mate while minimizing the costs of producing courtship signals. This was, however, not the case in a study of *U. tangeri* where large males reduced their wave rate when competitors were present. It was argued that this decline in wave rate increased signal effectiveness by producing more lagging waves. It should be noted, however, that *U. tangeri* does not wave synchronously and that the sample size was only six males [21].

Here we investigate how the presence of competing males influences wave display rate in the synchronously displaying fiddler crab *Uca annulipes*.

METHODS

We studied *Uca annulipes* on the Chukwani mudflats (S6°13'19.37" E39°12'13.56"), Zanzibar, Tanzania from August to October 2010. *Uca annulipes* occur in dense mixed sex colonies and both sexes defend territories that are centered on a burrow [22]. During the mating period, receptive females leave their territory and wander through the population in search of a mate [16]. Courting males cluster around the female and wave their one greatly enlarged claw. Males tend to produce waves that are in close synchrony with each other [23]. Female *U. annulipes* preferentially approach males with faster wave rates, greater wave leadership (slightly earlier waves during a synchronous bout) and larger claws [16,17,23]. In combination with studies on the closely related *U. mjoebergi* [18] we assume here that females show a direct preference for higher wave rates. Final mate choice is based on burrow quality (mating and incubation occurs within the male's burrow). Courting males occur at an average density of 19.6 males/m² and females sequentially sample as many as 24 males and travel as far as 28 meters in search of a mate [17].

To determine whether the presence of a competing male affects the waving display of a courting male, we conducted an experiment that manipulated the social environment of a focal male. We used a repeated-measures design with two treatments (high competition and low competition) We located naturally occurring groups of males and removed any wandering females within 50cm of the group. A group of males was classified as 2-5 neighbouring males that were all waving at the same female at the time of initial sighting. For each trial, we tethered a female (superglued a 1cm piece of cotton

thread to the crab's carapace and attached it to a nail pushed into the sediment) and placed her 10cm in front of a randomly designated focal male that could either see (high competition) or not see (low competition) his neighbours.

For the low competition treatment, we startled all crabs into their burrows by standing up and approaching them. We then placed bottle caps over all burrows within 50 cm of the focal male to prevent the neighbouring crabs from emerging during the trial. Caps were removed immediately following the trial. We then waited approximately 3 min (up to 2 minutes to allow the male to surface and then 1 min to locate and start waving at the female) and measured the wave rate of the focal male by counting the number of waves produced over a 30 s period.

For the high competition treatment, we scared all crabs into their burrows and randomly placed 10 or more bottle caps on the sediment surface within 50 cm of the focal male's burrow, without covering any neighbouring burrows. As described above we then waited approximately 3 minutes (up to 2 minutes to allow all males to surface and then 1 min for the males to locate and start waving at the female) before recording the focal male's wave rate. The number of waving neighbours, as well as the size (measured to the nearest 0.1mm using dial calipers) and distance of the nearest male neighbour was also recorded.

The same male was designated the focal male for both treatments and the same group of males was used for both treatments. To avoid any bias due to an order effect, the treatment order was random. We tested the wave rate of 50 males in 50 different groups of males.

We used Wilcoxon signed-rank tests to compare wave rate between the two treatments. All tests were two-tailed. Data are presented as medians and upper and lower quartiles.

RESULTS

Wave rate differed significantly between the high and low competition treatments ($p < 0.001$, $Z = -5.167$). The median wave rate of the focal male during the high competition treatment was 16.5 waves per minute (upper and lower quartile: 12.75-

20.25). In the low competition treatment, where all neighbouring males were enclosed in their burrow, the focal male's wave rate was 11.5 waves per minute (7.75-15).

There was a positive relationship between the number of waving neighbours and the number of waves produced by the focal male during the high competition treatment ($r_s = 0.43$, $p = 0.002$). Males with more neighbours produced a greater number of waves (Fig. 1). There was no relationship between the distance to the nearest waving male neighbour and the number of waves produced by the focal male ($r_s = 0.112$, $p = 0.486$). There was also no relationship between the size of nearest waving male neighbour and the number of waves produced by the focal male ($r_s = 0.21$, $p = 0.190$).

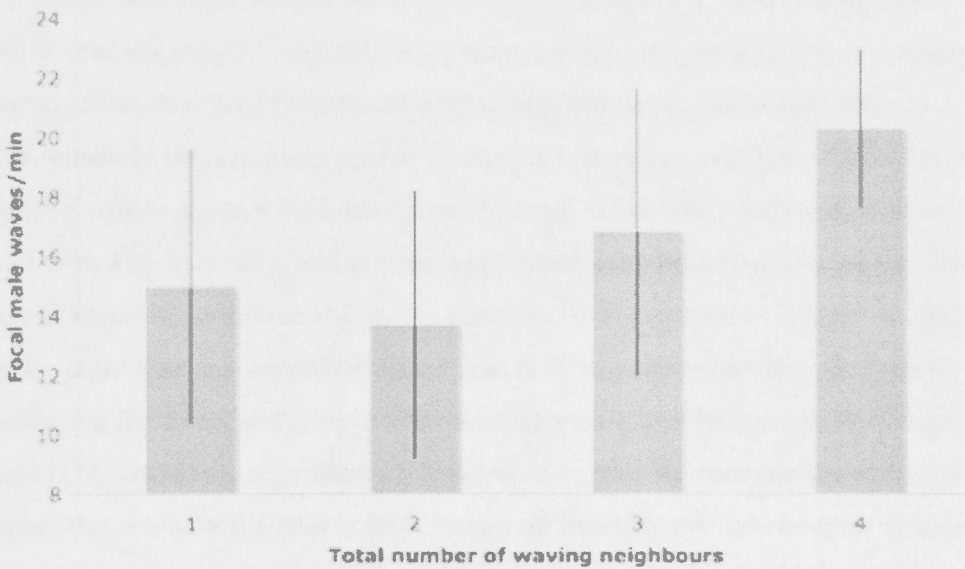


Figure 1. Focal male wave rate with 1, 2, 3, and 4 waving neighbours. Error bars indicate ± 1 standard deviation.

DISCUSSION

We have shown that male display rate in the fiddler crab *Uca annulipes* is strongly influenced by the presence and number of nearby displaying males. Males whose male neighbours were not on the surface produced 30% fewer waves than males whose male neighbours were present. Furthermore, males with many male neighbours displayed

more often than those with fewer male neighbours. While a number of studies have shown similar alterations to signal rate in response to competition in acoustic signalling species [e.g. 4-6,8,9], surprisingly few studies have empirically shown an increase in courtship rate in response to elevated mating competition in a visual signal context [review: 13]. One example comes from guppies (*Poecilia reticulata*), where Farr (1974) showed that males increased courtship rate in the presence of other males. Contrasting results were, however, reported by Jirotkul (1999), who showed that with greater competition male courtship rate actually decreased.

While we expected males to adjust their wave rate according to the current level of competition, there are several reasons why it might be beneficial for males to maintain high wave rates regardless of the level of competition. First, in fiddler crabs the operational sex ratio is extremely male-biased with males infrequently encountering receptive females [24,25]. In the closely related species *U. mjoebergi*, there is approximately one sampling female to every 45 courting males [25]. Opportunities to attract a female are therefore infrequent. Second, wave rate is believed to be an indicator of male quality, and females appear to have a preference for wave rates that exceed a minimum threshold [18,19]. Third, in fiddler crabs mate choice is often based on the sequential assessment of male traits. In *U. annulipes* females often travel substantial distances and sample multiple males from multiple groups before selecting a mate [17]. Therefore, regardless of the level of immediate competition, one might expect that males will signal at their maximum intensity not only because females are rare, but also to increase the probability of exceeding the female's mating preference threshold. The latter will decrease the likelihood of the female rejecting the male and moving on to sample another male.

The above factors are likely to generate selection on males to court all receptive females at their maximum intensity regardless of the level of competition. Our results suggest, however, that the costs of excessive waving might over-ride these potential benefits. We suggest that our results are best explained by the fact that waving is extremely costly and that males can reduce these costs by adjusting their wave rate according to the level of competition and/or the likelihood of attracting a female. By doing so, males effectively reduce the overall cost of courtship.

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CHAPTER 5: THE BATTLE OF THE SEXES? TERRITORY ACQUISITION AND DEFENCE IN MALE AND FEMALE FIDDLER CRABS

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ABSTRACT

In many territorial species males invest substantially in weapons, which they use in fights to acquire and maintain territories that ultimately give them access to females. Females often live within male territories or in female-only areas that are segregated from males. Under these circumstances, females do not have to compete directly with males for space, and in the context of territory acquisition and defence there is therefore no cost to being weaponless. In fiddler crabs, however, weaponless females hold territories among well-armed males. How do they do this? Observations and replacement experiments were carried out on *Uca annulipes* to determine how males and females acquire and defend territories. Weaponless females were as capable as well-armed males at both acquiring and defending a burrow. Females acquired burrows by seeking out empty burrows, while males acquired burrows by evicting male and female burrow owners. After acquiring a burrow, males mainly fought with male neighbours and females with female neighbours. Both sexes were equally likely to be evicted from their burrow and the likelihood that they remained on their territory for 24 hours or more was the same. Males and females employ different strategies of burrow acquisition and defence, and the male strategy appears to be more costly. We discuss possible explanations for these differences.

Keywords: fiddler crabs, sexual dimorphism, territoriality, territory defence, *Uca annulipes*

INTRODUCTION

In many sexually dimorphic species, males have large imposing weapons while females are weaponless (Andersson 1994). Males fight to acquire and maintain territories that ultimately give them access to females, or they directly engage in contests for the females themselves (Emlen 2008). In such circumstances females often live within male territories (Lopez & Martin 2002) or in female-only areas that are segregated from areas occupied by reproductively active males (Ruckstuhl 2007). In these cases, females do not compete directly with males for space or resources, and in the context of territory defence and acquisition there is therefore no cost to females of being weaponless (see Marra 1999). In fiddler crabs, however, the sexes are interdispersed, and weaponless females must maintain territories adjacent to those of well-armed males. Females are able to obtain and defend territories even when space is limited and therefore extremely valuable (Crane 1975).

Fiddler crabs live in dense mixed-sex colonies. Both males and females defend territories containing a central burrow. Burrows are a vital resource as they provide shelter from tidal inundation, predation and desiccation (Koga et al. 2001; Smith & Miller 1973). They are also used during reproduction as a site of mating and incubation (Backwell & Passmore 1996). The territory surrounding the burrow consists of a small area of surface sediment that is used both as a source of food (organic matter deposited on the sediment each high tide) and as a platform for mating displays (Crane 1975). Territories are therefore essential for survival and reproduction in both sexes.

Fiddler crabs display pronounced sexual dimorphisms. Females are small, usually dull-coloured with two small feeding claws. Males are large, often brightly coloured, with one feeding claw and one greatly enlarged claw (up to 50% of total body weight, making it the largest weapon relative to size in any animal) (Crane 1975; Emlen 2008). The enlarged claw is used both for mate attraction and as a weapon during fights with other males (Allen & Levinton 2007).

Fights occur over territory ownership, either with burrowless, wandering intruders or resident neighbours (Morrell et al. 2005). Males that lose their burrow, either through being evicted by another male or by forfeiting it to a female for incubation after mating with her, must quickly find a new burrow. To do so, they must fight and evict a resident

male or female crab, find an unoccupied burrow, or dig a new burrow (Jennions & Backwell 1996). Fights between males begin with opponents lining up and pushing their major claws against each other, and can escalate to a grapple where the claws are interlocked. Fights often last over a minute and can be extremely costly (Jennions & Backwell 1996). As females are weaponless and therefore disadvantaged in physical combat, the least costly method for a male to gain a new territory would presumably be to evict a resident female. How then do females obtain and defend territories within a population of heavily armed males?

This study aims to compare how well-armed males and weaponless females obtain and defend territories in the fiddler crab *Uca annulipes*.

METHODS

The study was carried out on Inhaca island, Mozambique (25°59'10"S, 32°56'35"E), during September and October 2008.

In *Uca annulipes*, mating occurs over 5-6 days each semilunar spring tide cycle, throughout the year (Backwell & Passmore 1996). Mating can occur underground in the male's burrow, or on the surface outside the female's burrow. Burrow mating is the most common type of mating. It occurs when a female leaves her burrow and wanders through the population in search of a mate. Once an appropriate male has been chosen, the female follows him back into his burrow. The male then seals himself and the female into the burrow by blocking the burrow entrance with a ball of mud and the pair mate (Backwell & Passmore 1996). After mating, the male vacates the burrow, leaving the female to incubate the clutch. Unlike burrow mating, surface mating mainly occurs between neighbours (Milner et al. *in press*) and can occur at any time during the tidal cycle (R. Slatyer & P.R.Y. Backwell 2009, unpublished data). Following a surface mating, the female will return to her own burrow to incubate her clutch or go on to burrow mate with a different male (R. Slatyer & P.R.Y. Backwell 2009, unpublished data).

Burrow acquisition and defence

To determine how female and male *U. annulipes* obtain territories, size-matched (within 1mm for carapace width) male-female pairs were captured, measured and individually marked (N=40 males, 40 females). One at a time, each crab was released and followed until a territory was gained. The sex of the crab released was alternated between trials. The following was documented: 1. distance travelled before acquiring a territory (straight line measurement from release point to new burrow); 2. time spent searching for a territory; 3. number of burrows visited prior to acquiring a new territory; 4. whether the focal crab fought with burrow owning males or females; and 5. method of territory acquisition (i.e. fought and evicted another crab or found an empty burrow). For male-male encounters, interactions were classified as fights if there was direct contact between the males' major chelae. For encounters involving females (female-female or male-female), inter-locking of the ambulatory legs was classified as a fight. Behaviour classified as a fight was unambiguously associated with initiation of a contest between the two crabs.

Burrow ownership and defence

Following the acquisition of a territory, the focal crab was observed for one hour to determine how female and male burrow owners defend new territories against both intruding and neighbouring crabs. We documented: 1. whether or not the crab was aggressively approached by a male or female neighbour (aggressive approaches included rapid movement towards the focal crab or antagonistic waves, if male, in the direction of the focal crab); 2. whether or not the focal crab fought with male or female neighbours or intruders (aggressive approaches that resulted in a fight were scored as a fight and not an aggressive approach); 4. whether or not the focal crab abandoned its burrow, sealed itself into the burrow by closing the entrance, or was evicted; and 5. whether the crab was still resident approximately 3 and 24hrs after burrow acquisition (i.e shortly before tidal inundation on the same and the following day).

Replacement experiments

Replacement experiments were performed to compare the responses of established burrow owning (henceforth 'resident') males and females to new female and male

neighbours while controlling for factors such as the distance to the nearest neighbour, and neighbour size and sex that could not be controlled for during the one-hour observations. Two similar sized males that had similar sized male neighbours (size matched by eye) at approximately the same distance away were removed from their burrows and replaced with a new male and female occupant ('replacement crabs') that were size matched to within 1mm of each other using dial callipers. The pairs were then observed for one hour and all interactions with neighbours (resident male and female neighbours) were documented: whether the neighbours aggressively approached the replacement crabs; whether the replacement crabs aggressively approached the neighbours; and whether the replacement crabs fought with their male or female neighbours.

Statistical Analysis

We tested for a difference between the sexes in the proportion of each assigned to one of two classes (e.g. did or did not fight a female neighbour) using log-likelihood ratio tests (LLR; $df = 1$). For continuous variables we tested for a difference between the sexes using Mann-Whitney U-tests. All tests were conducted using SPSS 15.0; alpha was set at 0.05 and tests were two-tailed. We used Bonferroni corrections to take into account multiple testing in each data set ($P_{\text{critical}} = 0.05/17 = 0.0029$ for burrow acquisition experiment; $P_{\text{critical}} = 0.05/6 = 0.0083$ for replacement experiment). It has, however, been suggested that presentation of effect sizes is a better approach than using Bonferroni correction when conducting multiple testing (see Nakagawa 2004). We therefore calculated the common effect size of Pearson's r and the 95% confidence interval for all tests using standard formulae (Cooper et al. 2009). If the male response was greater than the female response r was assigned a positive value.

RESULTS

Burrow acquisition and defence

Females did not differ significantly from males in the distance travelled, search duration, or number of burrows they approached before obtaining a burrow (Table 1). Both sexes were equally likely to fight with burrow owning females (19 out of the 40

males tracked compared to 22 out of 40 females tracked fought at least one female burrow owner). Males were, however, significantly more likely than females to fight with burrow owning males (38 males versus 9 females fought a burrow owning male (Table 2a).

Table 1. Burrow search period for male and females

	Median (lower, upper quartiles)		Mann-Whitney U	Effect size (r)	P value
	Male (N = 40)	Female (N = 40)			
Distance travelled (cm)	209 (114, 390)	210 (104, 690)	668.00	-0.103 (-0.32, 0.12)	0.355
Search time (sec)	1006 (329, 1710.3)	951 (264.3, 1843)	780.50	0.021 (-0.20, 0.24)	0.851
Burrows visited	10 (3.3, 21.5)	9.5 (3.3, 16.8)	756.50	0.047 (-0.17, 0.26)	0.675

Males and females differed significantly in their method of burrow acquisition: males were significantly more likely to evict a resident crab (evicted 13 females, 14 males, and 1 heterospecific crab), while 33 of 40 females found an empty burrow (Table 2a). There was no relationship between male size and the method of burrow acquisition (ANOVA, $F_{2,36}=0.211$, $p=0.811$, $r=0.11$). Females that evicted a resident crab either fought and evicted a female ($n=4$) or stole a male's burrow by beating him back to it ($n=3$). Such burrow 'sneaking' was only observed in females and involved no observable fighting (i.e. no fighting on the surface). Furthermore, there was no significant association between mating period (i.e. mating/spring tide and non-mating/neap tide) and whether males evicted a male or female to acquire a burrow (LLR=1.93, $df=1$, $p=0.165$) or whether males evicted a male or found an empty burrow to acquire a burrow (LLR=0.622, $df=1$, $p=0.430$).

Following the acquisition of a burrow, males were significantly more likely than females to fight with their male neighbours and with intruding males (Table 2b). Females were significantly more likely than males to fight with female neighbours (95% CI for effect size r : 0.27-0.62; although the difference is not significant if one controls for multiple testing using Bonferroni's correction). There was no significant difference between the sexes in the number of fights with intruding females, or the rate at which they were aggressively approached by male or female neighbours (Table 2b).

Furthermore, males and females were equally likely to abandon or seal their burrow, or to be evicted within the first hour, as well as being equally likely to retain their burrow 3 and 24 h after burrow acquisition.

Table 2. Burrow search and defence period for male and females

	Observed/Total		Likelihood	Effect size (r)	P value
	Males	Females	ratio		
A.					
Search period					
Evicted resident to acquire burrow	28/40	7/40	23.68	0.545 (0.37, 0.68)	<0.001 *
Fought burrow owning male(s)	38/40	9/40	49.91	0.790 (0.69, 0.86)	<0.001 *
Fought burrow owning female(s)	19/40	22/40	0.45	-0.075 (-0.29, 0.15)	0.502
B.					
Resident period					
Fought male neighbour(s)	16/40	3/40	12.56	0.460 (0.27, 0.62)	<0.001 *
Fought female neighbour(s)	3/40	11/40	5.83	-0.269 (-0.46, -0.05)	0.016
Fought male intruder	16/40	3/40	12.56	0.460 (0.27, 0.62)	<0.001 *
Fought female intruder	1/40	2/40	0.35	-0.066 (-0.28, 0.16)	0.553
Aggressively approached by male neighbour	13/40	7/40	2.43	0.174 (-0.05, 0.38)	0.119
Aggressively approached by female neighbour	2/40	5/40	1.45	-0.135 (-0.34, 0.09)	0.228
Abandoned burrow	5/40	4/40	0.13	0.040 (-0.18, 0.26)	0.723
Sealed burrow entrance	9/40	15/40	2.16	0.164 (-0.06, 0.37)	0.142
Evicted from burrow	5/40	3/40	0.56	0.084 (-0.14, 0.30)	0.454
Present 3hrs after burrow acquisition	13/24	16/24	0.79	0.128 (-0.16, 0.40)	0.375
Present 1 day after burrow acquisition	9/23	12/23	0.79	0.128 (-0.17, 0.40)	0.374

* $P < 0.05$ with Bonferroni correction

Replacement experiments

Replacement males were significantly more likely than replacement females to fight resident males, while replacement females were significantly more likely than replacement males to fight resident females (Table 3). There was, however, no significant difference between the sexes in the likelihood of a replacement crab being aggressively approached by resident males or females (Table 3). Replacement males were significantly more likely than replacement females to aggressively approach resident male neighbours (Table 3), but there was no significant difference in the likelihood they aggressively approached resident females (Table 3).

Table 3. Burrow defence period for replacement males and females

	Observed/Total		Likelihood ratio	Effect size (r)	P value
	Males	Females			
Fought resident male	17/20	0/20	37.64	0.970 (0.94, 0.98)	<0.001 *
Fought resident female	0/20	8/20	13.11	-0.573 (-0.75, -0.32)	<0.001 *
Aggressively approached by resident male	17/20	16/20	0.17	0.066 (-0.25, 0.37)	0.677
Aggressively approached by resident female	0/20	0/20	0	0 (-0.31, 0.31)	1.00
Replacement crab aggressively approached resident male	8/20	0/20	13.11	0.573 (0.32, 0.75)	<0.001 *
Replacement crab aggressively approached resident female	2/20	2/20	0	0 (-0.31, 0.31)	1.00

* $P < 0.05$ with Bonferroni correction

DISCUSSION

Burrow acquisition

In *Uca annulipes* well-armed males and weaponless females were equally successful at obtaining burrows, however, their methods of doing so differed significantly. While

there was no significant difference in the distance travelled, time taken, or number of burrows visited before obtaining a burrow, wandering males were significantly more likely than wandering females to fight with resident males and obtain burrows by evicting resident males and females. In contrast, most wandering females obtained an empty burrow and largely avoided aggressive encounters with males. The nine cases where females were aggressive towards males most likely involved small males. Since both methods are equally successful, and obtaining an empty burrow is probably less costly than fighting for an occupied burrow, why do males not simply seek out empty burrows? Burrow quality is of particular importance to male *U. annulipes* as it is an important criterion assessed by females during mate choice (Backwell & Passmore 1996). Empty burrows are often of poor quality (i.e. are damaged, unstable due to composition of substrate, or situated in low quality habitat) (Lim & Heng 2007). The costs incurred in obtaining a higher quality burrow by evicting another male would therefore likely be less than the benefits gained through an increased chance of mating (Backwell and Passmore 1996).

Given the difference in weaponry, and an approximately 1:1 adult sex ratio in this species (Litulo 2005), why do males not preferentially evict females? First, although not tested, female burrows are more likely to be of poorer quality than male burrows as females largely acquire empty burrows that have been abandoned. Due to the importance of burrow quality during mate selection (Backwell & Passmore 1996), males might avoid these burrows to some extent and seek out higher quality burrows owned by males. Second, male neighbours defend their female neighbours. Protecting female neighbours from wandering males increases a resident male's potential surface-mating opportunities (Milner et al. 2010). As a result, regardless of the burrow owner's sex, a wandering male might still have to fight a male. Evidence of territorial coalitions has been found in the fiddler crab *Uca mjoebergi*, where males assisted familiar male neighbours against intruders as it was often less costly than establishing new territory boundaries with a new, possibly stronger, male neighbour (Backwell & Jennions 2004). Similar defence coalitions have recently been shown to occur in *U. annulipes* (Detto et al. 2010).

Burrow ownership and defence

Well-armed males and weaponless females were equally successful at holding a territory over a 24 hour period, and equally likely to be evicted from their burrow. These results are consistent with those recorded during the search period, which showed wandering males to evict equal numbers of males and females. Also consistent with the results obtained during the search period, resident males were more likely than females to fight with wandering males. We suggest that due to the asymmetry in fighting ability of males and females, females are less likely to retaliate to male intrusions or initiate defensive attacks towards passing males. Instead, females generally retreat into their burrow at the first sign of an approaching male. Furthermore, because females receive protection from their male neighbours as outlined above (Milner et al. 2010), it might prove less costly in certain circumstances (i.e. when a female has numerous male neighbours) for wandering males to attempt to evict male rather than female burrow owners.

Male residents were more likely than female residents to fight a new male neighbour, while female residents were more likely to fight a new female neighbour (with the caveat that the trend for females was not significant if Bonferroni correction was applied; but see Nakagawa 2004). Fights between neighbours due to boundary disputes (including the establishment of new territory boundaries) and these fights appear to be sex assortative. We suggest that due to the asymmetry in fighting ability between sexes, females are less likely to encroach onto a male's territory, and if they do they will simply retreat when threatened (i.e. no fight would be recorded and the interaction would be recorded as an aggressive approach). Furthermore, given female neighbours appear to present males with potential mating benefits, agonistic behaviour towards neighbouring females could reduce male surface-mating success so that males might be more tolerant of female than male neighbour intrusions (Milner et al. 2010).

In summary we have shown that although female *Uca annulipes* are weaponless they are as capable as well-armed males of acquiring and holding burrows and territories. Females mainly acquire empty burrows, while males acquire burrows by fighting and evicting a resident. Males and females are equally likely to be evicted from their burrow, and both are equally likely to retain their territory for at least the first 24 hours after burrow acquisition. The fact that females are weaponless and still compete

successfully for the same general resource (although there might be a difference in the quality of the burrows they obtain) as well-armed males is an extremely interesting result that, to the best of our knowledge, has not been shown in any other species with such extreme sexual dimorphism in weaponry.

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CHAPTER 6: SAFE SEX: MALE-FEMALE COALITIONS AND PRE-COPULATORY MATE GUARDING IN A FIDDLER CRAB

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ABSTRACT

In fiddler crabs both males and females defend territories that are essential for survival. Given pronounced sexual dimorphism in weaponry, how do weaponless females defend their territory from well-armed males? Using observational data and two simple experiments, we test whether male *Uca annulipes* protect their female neighbours from conspecific intruders. We show that males defend their female neighbours against male but not female intruders. We also show that females sometimes mate with their immediate neighbours. Thus male defence of female neighbours might represent both pre-copulatory mate guarding and a territorial coalition. Males who ensure that their neighbour remains female could benefit through increased opportunity for future reproductive success and lower boundary maintenance costs.

Keywords: coalitions; female protection; Fiddler crabs; mate guarding; mating benefits; surface mating; *Uca annulipes*

INTRODUCTION

In many animal species males bear weapons such as horns, tusks or claws that are used in territorial defence or fighting for access to females (Emlen 2008). In contrast, females are often weaponless, even in species where both sexes hold territories (Keltie 1985). Males that have lost their territories should benefit from challenging weaponless female residents rather than fighting well-armed males. How, then, do females maintain their territories? One explanation is that females gain protection from their male neighbours through neighbour coalitions and/or mate guarding (where females exchange sex for protection).

Neighbour coalitions can occur in territorial species if it is less costly to help a neighbour defend its territory against intruders than renegotiate boundaries with a new, potentially stronger individual (Getty 1987; Mesterton-Gibbons & Sherratt 2009). Such by-product mutualisms have only been convincingly reported in three species: rock pipits (*Arthus petrosus*) (Elfstrom 1997), and fiddler crabs (*Uca mjoebergi* and *U. annulipes*) (Backwell & Jennions 2004; Detto *et al.* in prep). In each case, males left their territories to help familiar male neighbours fight off intruders. The authors argued that the costs of helping were outweighed by the benefit of not having to re-establish territory boundaries with new, stronger neighbours (e.g. in *U. annulipes* and *U. mjoebergi* males more often help when the intruder is larger than the neighbour). As males are often physically dominant over females, boundary maintenance should be less costly for males with a female neighbour. Nevertheless, helping can also be extremely costly. In fiddler crabs, fights can result in injury, claw loss and death (Milner *et al.* 2009), and males that leave their territory to help a neighbour run the risk having their own unattended burrow usurped.

Males might also protect females in exchange for mating opportunities. Trading sex for material benefits is known from several taxa. For example, female red-winged blackbirds (*Agelaius phoeniceus*) that mate with an extra-pair male are subsequently allowed to forage on his territory (Gray 1997). In the Adelie penguin (*Pygoscelis adeliae*) females exchange copulations for highly sought-after stones used for nest building (Hunter & Davis 1998). Furthermore, in pigeons, females trade copulations for protection. Females keep their male partner close by initiating frequent copulations, thereby avoiding harassment from other males (Lovell-Mansbridge & Birkhead 1998).

Fiddler crabs

Fiddler crabs display pronounced sexual dimorphism. Females have two small feeding claws while males have one feeding claw and one greatly enlarged major claw (up to 50% of total body weight) that is used during courtship and as a weapon during antagonistic interactions (Crane 1975). They live in dense, mixed sex colonies and both sexes defend territories that contain a burrow. Burrows are vital as shelter from tidal inundation, predation and desiccation (Koga *et al.* 2001). They are also used during reproduction as the site of mating and incubation, with females in some species choosing mates based on male burrow quality (e.g. *U. annulipes* Backwell & Passmore 1996).

Recent work on territory defence and acquisition in *U. annulipes* has shown that males and females are equally successful at gaining and holding a territory, despite the strong competition for burrows between weaponless females and well-armed males (Milner *et al.* 2010). Females rarely evict resident crabs and instead tend to search for empty burrows, while males usually fight and evict a resident male or female. This study raised an important question: why don't burrow-searching males always evict female residents? Fights between males can be extremely costly, and it would seem that a well-armed burrow-searching male should target female-owned territories. We proposed three non-mutually exclusive explanations for these results. 1. Female burrows could be of poorer quality than male burrows as females generally acquire abandoned, empty burrows. Males might therefore avoid evicting females because burrow quality is a criterion for female mate choice. 2. Males might assist in the defence of neighbouring females' territories (as they do for males) to escape renegotiation costs with a new resident. 3. Neighbouring males might defend female residents to increase their potential future mating opportunities (Milner *et al.* in prep).

In fiddler crabs, mating can occur underground in the male's burrow, or on the surface outside the female's burrow. In some species only surface or burrow mating occurs (Crane 1975). However, in several species, both mating behaviours occur (Koga *et al.* 1998). Burrow matings require females to leave their burrow and wander through the population in search of a mate. Wandering females are highly vulnerable to predation, as they do not have a burrow to retreat into (Koga *et al.* 1998). When surface mating, a

male approaches a resident female and the pair copulate at the entrance of her burrow (Yamaguchi 2001). Although copulating on the surface is probably more risky than inside a burrow, the overall risk is likely to be lower than that arising from mate searching prior to burrow mating (Koga *et al.* 1998).

Uca annulipes is known to both surface and burrow mate, and previous work has shown males to form defence coalitions with male neighbours (PRY Backwell, unpublished data; Detto *et al.* 2010). Our study was designed to determine whether: 1. males form similar defence coalitions with female neighbours; 2. there is evidence of females exchanging sex, with their nearest male neighbour, for protection. We predicted that males would protect their female neighbours, both to avoid renegotiating territory boundaries with a new male neighbour and to increase their reproductive success through future surface mating opportunities.

METHODS

Does surface mating occur only between females and their nearest neighbouring males?

We observed surface matings ($n=52$) in a population of *U. annulipes* in Durban Harbour, South Africa from November to December. We noted whether or not mating crabs were immediate neighbours that shared a territory boundary.

Do male residents help defend their female neighbours' territories from intruding males?

To determine whether male residents protect female neighbours from intruding males, we tethered (superglued a 1 cm length of cotton thread to the crab's carapace and attached it to a nail pushed into the sediment) and placed a male at the entrance of a female's burrow to simulate an intrusion. To avoid confusing male defence of his own territory with female protection, the intruder male was always placed on the side of the female's burrow furthest from the focal male's burrow. The intruder male (major claw length: 20.7 ± 4.2 mm, $n=20$) was always smaller than the male neighbour (25.1 ± 5.3 mm, $n=20$). This increased the likelihood of a male neighbour response, as he was more

likely to beat the intruder (Backwell & Jennions 2004). The focal male neighbour was always the closest neighbour to the focal female. Female protection was scored if the male neighbour approached and fought with the intruder male within 5 minutes of re-emerging from his burrow.

Do male residents help in territorial defence if the intruder is a female?

To determine whether male residents protect female neighbours from intruding females, we repeated the previous experiment but replaced the tethered male with a tethered female (female intruder). Half of the female intruders (carapace width: 14 ± 0.9 mm, $n=10$) used were larger than the focal female (11.7 ± 1.5 mm, $n=10$) and half were smaller (12.1 ± 1.1 mm, $n=10$) than the focal female (13.1 ± 0.8 mm, $n=10$). This allowed us to test whether there was any relative female size effect present. All female intruders were smaller than the focal male.

Both protection experiments were carried out on tidal mudflats on Inhaca Island, Mozambique from October to November.

RESULTS

Does surface mating occur only between females and their nearest neighbouring males?

In 44 of the 52 surface matings we observed (85%), the mated male was a neighbour that shared a territory border with the resident female. In the remaining 15%, the mated male was a stranger: either a burrowless male or a non-neighbouring resident.

Do male residents help their female neighbours defend their territories from intruding males and female?

In 20 of 21 experimental trials (95%), the male neighbour approached and fought off the tethered intruding male. This required that the male left his own territory unprotected while he fought the intruder on the female's territory. In only 3 of 20 trials (15%) did the neighbouring male approach and fight off the tethered intruding female.

There was a significant relationship between the sex of the intruder and whether or not a male protected his female neighbour (Fisher's Exact test: $N=41$, $P<0.001$). Unlike the case in male-male coalitions (see Backwell & Jennions 2004), whether the intruding female was smaller or larger than the neighbouring female did not significantly affect the likelihood that the male helped (0/10 vs 3/10; Fisher's Exact test: $N=20$, $P=0.211$).

DISCUSSION

We have shown that male *U. annulipes* protect their female neighbours from intruding males but not intruding females; and that females that surface mate mainly do so with their immediate neighbours. We suggest that males: 1. form defensive coalitions with their female neighbours against intruding males; and 2. protect their female neighbours in exchange for future mating benefits. These results support those of Backwell & Jennions (2004), who showed that males protect their male neighbours from intruders, smaller than themselves but larger than their neighbour, to avoid renegotiating territory boundaries with a new, stronger individual. We argue that males are protecting their female neighbours for a similar reason, but, in addition, do so to increase their opportunity for surface matings.

Males protected their female neighbours in 95% of instances where the intruder was male and only 15% of instances where the intruder was female. This suggests that males either do not care who their neighbour is, as long as they are female, or that they are unable to individually distinguish females. While we did not test whether *U. annulipes* recognise their neighbours, individual recognition based on carapace colouration occurs in other fiddler crabs (Detto *et al.* 2006). Regardless, by ensuring that their neighbour is female, males will benefit through increased opportunity for future reproductive success as well as reduced boundary maintenance costs (i.e. it is less costly to maintain a territory boundary with a female than a male).

While it is possible guarding is post- rather than pre-copulatory, we believe this is highly unlikely. Our results imply that males do not care whether one female replaces another as a neighbour. If guarding occurs post-copulation one might expect a male to

guard a female regardless of the intruder's sex. As this does not occur, we suggest that the available evidence is more consistent with pre-copulatory guarding.

Our results can partly explain why burrow-seeking males do not always target burrow-owning females. Although females are apparently defenceless, Milner *et al.* (2010) showed that they are as capable as males at holding territories. Here we show that females might hold their territory by gaining protection from their male neighbour. Therefore burrow-searching males might have to contend with fighting a male regardless of whether they attempt to usurp a female's or a male's burrow.

To the best of our knowledge we have provided the first experimental evidence for pre-copulatory mate guarding in fiddler crabs and for defence coalitions between territorial males and female neighbours. This might explain why weaponless females and well-armed males are similarly capable of retaining a territory (Milner *et al.* 2010). More generally, we have exposed some important links between coalition formation, the evolution of surface mating and pre-copulatory mate guarding in fiddler crabs that can now be explored more thoroughly.

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CHAPTER 7: KNOW THINE ENEMY'S NEIGHBOR: NEIGHBOR SIZE AFFECTS FLOATERS' CHOICE OF WHOM TO FIGHT

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ABSTRACT

It can be less costly to help a neighbor repel an intruder than to renegotiate boundaries with a new and potentially stronger individual. Male fiddler crabs will help a smaller neighbor fight off an intruder when the intruder is intermediate in size relative to the two neighbors. Fights involving neighbor coalitions are costly for an intruder because he rarely wins when a larger neighbor intervenes. It might therefore be expected that territory-seeking males will avoid fighting residents that have large neighbors. We found a strong effect of the neighbor's size on whether or not a territory-seeking male initiated a fight with a resident male. Although territorial coalitions in the fiddler crab *Uca mjoebergi* are relatively uncommon, the potential for them to occur appears to impose strong selection on the fighting decisions of territory-seeking males.

Keywords: coalitions; fiddler crabs; fight choice; territoriality; *Uca mjoebergi*

INTRODUCTION

Fight outcome is largely determined by differences in the resource holding potential (RHP) of opponents (Parker 1974). As fights are often costly due to greater energetic demands, lost time and the risk of injury, those individuals that can accurately assess the RHP of an opponent before participating in a contest should be at a selective advantage (Maynard Smith and Parker 1976). However, there are factors other than RHP that can also contribute to contest outcome, such as the relative value of the contested resource to each opponent (Krebs 1982), body condition (Fitzstephens and Getty 2000), age (Kemp 2003), energy reserves (Marden and Waage 1990), fighting experience (Hsu and Wolf 2001) and territorial coalitions (Detto et al. 2010).

In some territorial species, residents are less aggressive towards neighbors than towards strangers who are non-territorial ‘floaters’ (‘dear enemy’ effect). In most cases this occurs because neighbors are familiar and/or pose less of a threat (i.e. they are only temporarily intruding onto the focal individual’s territory) compared to unknown, territorial intruders who could potentially challenge the resident for his territory (Temeles 1994). This creates a stable community due to the benefit of reduced conflict that accrues to all residents. This can, in some cases, lead to residents helping their neighbors defend their territory against intruders (Booksmythe et al. 2010). By retaining an existing neighbor, a resident removes the cost of having to renegotiate boundaries with a new neighbor. Furthermore, because a new neighbor has evicted the former neighbor it is, on average, likely to be a relatively stronger individual. This increases the likelihood that the new neighbor will reduce the size of the resident’s territory (Getty 1987; Mesterton-Gibbons and Sherratt 2009). Coalitions that arise due to this type of by-product mutualism have been reported in four species: rock pipits (*Arthus petrosus*), and three fiddler crabs (*Uca mjoebergi*, *Uca annulipes* and *Uca elegans*) (Elfstrom 1997; Backwell and Jennions 2004; Booksmythe et al. 2010; Detto et al. 2010; Milner et al. 2010a). In each case, males have been seen to leave their own territory to help familiar male or female neighbors repel intruders (i.e. territory-less ‘floaters’). In fiddler crab species, male-male coalitions mainly occurred when the ally was larger than the intruder who, in turn, was larger than the assisted male (Backwell and Jennions 2004; Detto et al. 2010). Males appear to make judicious size-based decisions as to when they should help, which suggests that they can determine the size of an intruder relative to themselves and their neighbor. We now have a relatively good understanding of the

circumstances under which coalitions are formed in fiddler crabs (Detto et al. 2010), but we know nothing of the broader selective pressures imposed by their potential formation. For example, does the formation of defensive coalitions influence which individuals a territory-seeking male challenges?

In fiddler crabs, territory-seeking males frequently bypass and avoid many resident males prior to initiating a fight (Milner et al. 2010b). Fight outcome in fiddler crabs is largely determined by claw size (Morrell et al. 2005). In order to counteract an ownership advantage (Fayed et al. 2008), territory-seeking males tend to choose rivals that are slightly smaller than themselves (Jennions and Backwell 1996). This can account for males avoiding larger residents when searching for a territory, but it does not explain why they pass and avoid numerous males of a seemingly ‘contestable’ size. Why are these males avoided? Fighting in fiddler crabs is costly and can result in wasted time, claw loss, injury, and even death (Milner et al. 2010c). The costs imposed on an intruding male are likely to be exaggerated during coalition fights. This suggests that selection should favor males that reduce the risk of these potentially costly interactions. More importantly, if a resident assists his neighbor the intruder has a much lower likelihood of winning a fight (Milner et al. 2010c).

Here we investigate whether the occurrence of defensive coalitions in the fiddler crab *Uca mjoebergi* influences a territory-seeking male’s choice of opponent. Specifically, do territory-seeking males take into account the size of neighboring males before they initiate a fight with a resident?

METHODS

The study was conducted at East point reserve, Darwin, Australia from September to December 2009.

Study system

Uca mjoebergi is a small fiddler crab (carapace width <20mm) that inhabits intertidal mudflats in northern Australia. They occur in dense mixed sex colonies (37 ± 17 crabs m^{-2} ; R. Slatyer, L. T. Reaney and P. R. Y. Backwell unpublished data; inter-burrow

distance between neighboring males: 11.84 ± 3.49 cm; *see results*) and both sexes defend a territory centered on a burrow (Reaney and Backwell 2007). Territories are uniformly distributed. Burrows are a vital resource as they are used as a site of reproduction, as well as shelter from tidal inundation, predation and desiccation (Smith and Miller 1973; Backwell and Passmore 1996; Koga et al. 2001). The small area of sediment surrounding the burrow is the source of food (organic matter deposited on the sediment each high tide; Crane 1975). The species shows pronounced sexual dimorphisms. Males have one small feeding claw and one greatly enlarged ('major') claw (up to 50% of total body weight; 14.97 ± 4.29 mm; *see results*). Females lack a major claw and instead have two small feeding claws (Crane 1975). The enlarged claw is used both for mate attraction and fighting. Fights occur over territories and either involve boundary disputes between two resident neighbors or a territory-seeking male (hereafter, "floater") trying to evict a resident.

Do floaters take into account the size of their opponent's nearest neighbor?

To determine whether a floater takes into account the size of the neighbor when deciding whether or not to challenge a resident for his territory, we monitored the fighting decisions of males in the field. We captured males and then measured their major claw length (± 0.1 mm), after which they were individually released (≥ 2 m from initial capture point so that they no longer had a burrow). We then followed them until they had fought with two resident males. We did not record a focal male's first fight to ensure that he had recovered from any short-term effects associated with his capture (e.g. scared responses). Focal males that won a burrow on their first fight were excluded from the analysis. A fight was classified as any interaction where two males touched major claws. After the second fight, the challenged resident male and his nearest neighbor were captured and measured ($N = 50$ pairs of males). To provide a 'control' we then located the nearest male of equivalent size (< 1 mm) to the fought resident. The control male and his nearest neighbor were then captured and measured. Neither the control male nor his nearest neighbor were ever the challenged resident or the challenged resident's neighbor. The distance between the burrows of the challenged resident and his nearest neighbor, and between the control resident and his nearest neighbor were also measured. We used paired *t*-tests to compare the claw size of the different sets of males.

An experimental test: do floaters avoid fighting males with large neighbors?

To determine whether floaters avoid fighting males with large neighbors, we conducted a choice experiment *in situ*. A focal male was given a choice between two pairs of males ($N=30$). Pair 1 consisted of two size-matched males (<1 mm) that were 1-2 mm smaller than the focal male. Pair 2 consisted of a male that was also 1-2 mm smaller than the focal male, and a male that was 2-6 mm larger than the focal male. Males in each pair were tethered (2 cm piece of cotton glued to the carapace and attached to a nail inserted into the sediment) and placed 8 cm apart and each 3 cm in front of their own artificial burrow. The two pairs were on opposite sides of a test arena equidistant from the focal male release point (20 cm away) (Figure 1). A barrier was placed around the arena to prevent other males entering and/or the focal male leaving the arena. A positive choice was scored if the focal male moved in a direct line towards and then fought with one of the four tethered males ($N = 20$). A trial was discarded if the male ran immediately after being released or did not initiate a fight within 3 min ($N=10$). A new set of males was used in each trial. To determine whether the focal male avoided fighting a male with a neighbor larger than himself, we conducted a Chi-square goodness-of-fit test. We were only interested in cases where a male chose a similar sized opponent. In such cases the null hypothesis is that the ratio of approaches to Pair 1 to Pair 2 should be 2:1.

Summary statistics are presented as mean $\pm$ SD. All tests are two tailed with $\alpha = 0.05$.

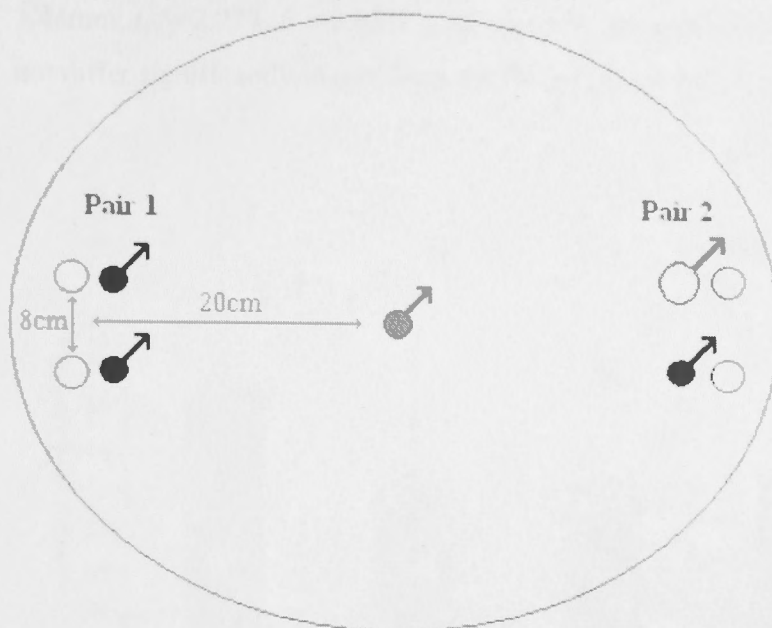


Figure 1. Fight choice arena for experiment testing whether territory seeking males avoid contesting males with large neighbours.  : Focal/floater male;  : large male resident;  : size matched male residents;  : resident male burrow.

RESULTS

Do floaters take into account the size of their opponent's nearest neighbor?

The mean size of the floater males was 15.55 ± 3.16 mm ($n=50$). These males were of average size and did not differ significantly from the population mean of 14.97 ± 4.29 mm ($n = 222$) (Independent sample *t*-test, unequal variances: $t_{94.55} = 1.098$, $P = 0.275$). In agreement with earlier studies we found that floaters generally fought males that were slightly smaller than themselves (resident: 13.26 ± 3.44 mm) (Paired *t*-test: $t_{49} = 4.721$, $P < 0.001$) (Figure 2).

The opponent's nearest neighbor was, on average, significantly smaller than the floater (neighbor: 13.49 ± 3.59 mm $t_{49} = 4.721$, $P < 0.001$). The size difference between the floater and his opponent's nearest neighbor was not simply a byproduct of the floater's choice of a smaller opponent combined with smaller males clumping together. Although control and opponent males were closely size-matched, the nearest neighbors of control males were significantly larger than those of opponent males (control neighbor: $14.96 \pm$

3.44mm; $t_{49} = 2.227$, $P = 0.031$). Consequently, the control male's nearest neighbor did not differ significantly in size from the floater ($t_{49} = 0.982$, $P = 0.331$) (Figure 2).



Figure 2. Major claw size (mm) of the intruding floater male, resident male fought, control male resident, fought male resident's neighbor, and control male resident neighbor.

The inter-burrow distance between the opponent and his nearest neighbor (11.39 ± 3.68 cm) did not significantly differ from that of the control resident and his nearest neighbor (11.84 ± 3.49 cm; $t_{48} = 0.601$, $P = 0.551$).

Do floaters avoid fighting males with large neighbors?

In 18 of 20 fights, the focal 'floater' male fought a size-matched male slightly smaller than himself. In 17 of these 18 fights the floater avoided fighting a male who had a larger neighbor. This is significantly more often than predicted by the null hypothesis ($\chi^2_1 = 6.25$, $P = 0.012$).

DISCUSSION

We have provided strong evidence that territory-seeking male *Uca mjoebergi* choose whom to fight based partly on the size of the potential opponent's nearest neighbor. Floaters preferentially fought slightly smaller males but both the observational study and experiment test show that they tend to avoid such males if their nearest neighbor was larger than themselves. Previous studies have shown that floaters usually target slightly smaller males, presumably to counter a well-documented ownership advantage (Jennions and Backwell 1996; Fayed et al. 2008). However, no study has shown that the size of neighbors influences which resident a floater will attack. Our results might help to explain why territorial coalitions are rarely seen in fiddler crabs: only 6.3% of fights between an intruder and floater resulted in a territorial coalition in *U. mjoebergi* (Backwell and Jennions 2004). Specifically, if floaters avoid males with larger neighbors this decreases the likelihood that neighbors will form a territorial coalition to repel the floater. Our result might also account for the observation that floaters frequently avoid males of a seemingly suitable size (i.e. slightly smaller than the floater) when searching for a new territory.

It is clear floaters can reduce the costs associated with coalition fights by paying attention to the size of potential opponents' neighbors. In addition, however, floaters that target males with smaller neighbors are effectively determining their social environment and thereby altering the selection pressures that both they and residents experience (Oh and Badyaev 2010). In general, much emphasis has been placed on the benefits of associating with weaker and/or less attractive males. For example, it is beneficial for a male to have a small neighbor when establishing and maintaining territory boundaries because smaller males are less costly to fight (i.e. fights are shorter as smaller neighbors retreat sooner) (Pratt and McLain 2006). In addition, by preferentially associating with smaller or less attractive conspecifics, males can increase their perceived attractiveness to mate-searching females (Bateson and Healy 2005). Callander et al. (2011) recently showed in *U. mjoebergi* that there was a mating cost to being surrounded by more attractive males and argued that by retaining (i.e. helping) smaller neighbors, males could potentially increase their relative attractiveness. Similar results have been reported in the house finch *Carpodacus mexicanus*, whereby some less attractive males increase their attractiveness by actively seeking out social groups composed of even less attractive males (Oh and Badyaev 2010).

Our study is a reminder that there can also be costs to associating with weaker males. In *U. mjoebergi* floaters are less likely to attack residents with large neighbors (this study), and when they do the neighbor will often help the resident retain his territory (Backwell and Jennions 2004; Detto et al. 2010). Given the costs and benefits of larger neighbors, it is unclear what size neighbor will maximize a given male's fitness. Answering this question is beyond the scope of the current study. Nonetheless, our results provide strong evidence to suggest that even though the formation of territorial coalitions in *U. mjoebergi* is relatively uncommon, the potential for such coalitions imposes strong selective pressures on the fighting decisions of territory seeking males, as well as the helping decisions of residents. Ultimately these selective pressures should affect the social structure of the population.

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**CHAPTER 8: EVERYBODY NEEDS GOOD NEIGHBOURS:
COALITION FORMATION INFLUENCES FLOATER FIGHT CHOICE**

Richard N. C. Milner

In press: Ethology

ABSTRACT

In territorial species it is sometimes less costly to help a neighbour fight off an intruder than to reestablish territory boundaries with a new, potentially stronger neighbour. In fiddler crabs a male resident will only help his neighbour if he is larger than the intruder who, in turn, is larger than the challenged neighbour. Does this influence with whom a territory-seeking male decides to fight? I show that territory seeking males appear to choose opponents based partly on the size of the resident's nearest neighbour. By avoiding challenging resident males with larger neighbours, territory-seeking males can reduce the likelihood of initiating a fight with a resident who might gain help from his neighbour that decreases the likelihood that the intruder will win the fight.

Keywords: coalitions, fiddler crabs, fight choice, territoriality, *Uca annulipes*

INTRODUCTION

Differences in the resource holding potential (RHP) of opponents largely determines fight outcome (Parker 1974). However, there are factors other than RHP that also influence fight outcome such as resource value (Krebs 1982), body condition (Fitzstephens and Getty 2000), age (Kemp 2003), energy reserves (Marden and Waage 1990) and territorial coalitions (Detto et al. 2010).

In territorial species it can be beneficial to help a neighbour defend its territory against intruders. By doing so, a resident removes the cost of having to re-establish territory boundaries with a new male neighbour that is on average likely to be larger and stronger than the former neighbour. Such defensive coalitions have now been reported in a bird species (*Arthus petrosus*, Elfstrom 1997) and three fiddler crabs (*Uca mjoebergi*, *U. annulipes*, and *U. elegans*; Backwell and Jennions 2004; Booksmythe et al. 2010; Detto et al. 2010; Milner et al. 2010a). In each case, in fiddler crabs, a resident male left his own territory to help a neighbouring male (or female) fight off an intruding, territory seeking male. However, the male only helped if he was larger than the intruding male who, in turn, was larger than the assisted, neighbouring male (Backwell & Jennions 2004; Detto et al. 2010). This suggests that males make judicious size-based decisions about when to help. Furthermore it suggests they can determine the size of an intruding male relative to themselves and their neighbour.

In fiddler crabs, territory seeking-males frequently bypass and avoid fighting resident males of contestable size (i.e. slightly smaller than themselves; Morrell et al. 2005). Fight outcome in fiddler crabs is largely determined by major claw size and in order to overcome an ownership advantage territory seeking-males often approach and fight resident males that are slightly smaller than themselves (Morrell et al. 2005; Jennions & Backwell 1996; Fayed et al. 2008). Although this can explain why territory seeking-males avoid fighting resident males slightly larger than themselves, it does not explain why territory seeking-males avoid and bypass fighting resident males of an apparently contestable size.

While we now have a relatively good understanding of when and how territorial coalition arise in fiddler crabs (Detto et al. 2010), it is only recently that we have begun to explore the subsequent selective pressures imposed by the formation of territorial

coalitions. Recently Milner et al. (2011) showed that the formation of defensive coalitions in the fiddler crab *U. mjoebergi* could strongly influence whom territory seeking-male choose to fight. Neighbour size appears to strongly influence whether or not a territory seeking-male initiated a fight with a resident male because burrowless males tend to avoid otherwise suitable opponents if they have large neighbours. This is a fine scale level of adaptation so it is important to test the generality of this finding, both to confirm the validity of the earlier study and to test whether the same behavioural adaptation occurs in other species. Due to publication bias there is currently a growing concern over the lack of replicate studies testing the generality of findings both within and across taxa (Jennions & Møller 2002; Kelly 2006). By ignoring replicate studies and tests of biological generalizations, we greatly hinder our ability to fully understand evolutionary phenomena (Kelly 2006).

Here I investigated whether the formation of defensive coalitions in another fiddler crab species, *U. annulipes*, leads to similar tactics as those used by *U. mjoebergi* (Milner et al. 2011) when choosing an opponent. Specifically, I asked whether territory-seeking males avoid challenging resident males that have neighbours larger than themselves? According to the results obtained from a previous study on the influence of neighbour size on fight choice in *U. mjoebergi* (Milner et al. 2011), I predict that intruding males will avoid challenging resident males that have neighbours larger than themselves.

METHODS

The study was conducted on the Chukwani mudflats (S6°13'19.37" E39°12'13.56"), Zanzibar, Tanzania from August to October 2010.

Study system

Uca annulipes occur in dense, mixed sex colonies. Both sexes defend a territory centered on a burrow (Milner et al. 2010b). Territories are uniformly distributed. Males have one greatly enlarged claw, which is used during mate attraction and as a weapon during fights with other males. Fights occur over territories, either between residents over the use of contested space at the edge of their home ranges, or between a resident and a territory-seeking male (hereafter, "floater") for territory ownership. Burrows are a

vital resource. They are the site of reproduction and provide protection from predators, desiccation and the incoming tide (Smith & Miller 1973; Koga et al. 2001). Furthermore, the small area of sediment surrounding the burrow is used as a source of food (Crane 1975). Males that lose their burrow, either to an intruding male or through forfeiting it to a female following mating, must quickly find a new burrow.

To determine whether a floater takes into account the size of a potential opponent's neighbour when deciding whom to fight, I monitored the fighting decisions of males in the field. To do this I captured males, measured their major claw length (to the nearest 0.1mm using dial calipers) and individually released them at least 2m from their initial capture point (so that they no longer had a burrow). I then followed them until they had fought with at least two resident males. Fights were classified as any interaction where the floater male and a resident male touched major claws. Details of the first fight were not recorded to make sure that the male had recovered from any short-term effects associated with their capture. Floater males that won their first fight with a resident male were excluded from the analysis. Following the second fight, I measured the major claw length of the challenged resident male and his nearest male neighbour ($n=50$), as well as the distance between their burrows. To provide a control, I then captured the closest male of equivalent size to the challenged resident male. I then measured the control male and his nearest male neighbour for major claw length and inter-burrow distance. Neither the control resident male nor his neighbour was ever the challenged resident male or his nearest neighbour. I used a one-way ANOVA to compare the mean claw size of the different sets of males and the population mean claw size (unpublished data, Hayes, C., Jennions, MD, Backwell, PRY). I ran pairwise multiple comparisons using a post-hoc Scheffe test.

Summary statistics are presented as mean $\pm$ S.D. All tests are two tailed with $\alpha = 0.05$.

RESULTS

Mean claw size differed significantly between the different groups of males (floaters, residents, neighbours, control neighbours and population mean; $F_{5,384} = 8.368$, $P < 0.001$). Floater male size (19.38 ± 0.55 mm, $n=50$) did not significantly differ from the population mean for surface-active males (20.33 ± 0.39 mm, $n=144$; $P = 0.881$).

Fighting was size-assortative ($r = 0.429$, $P = 0.002$, $n = 50$). However, floater males did not fight resident males that were significantly smaller than themselves (resident: 19.53 ± 0.61 mm, $n=50$; $P = 1$). The fought resident's neighbour (16.5 ± 0.605 mm, $n=50$) was significantly smaller than the floater male ($P = 0.018$). The control and fought resident did not differ significantly in size (control: 19.13 ± 0.63 mm, $n=48$; $P = 0.999$), and the control resident did not differ in size from the population average ($P = 0.742$). The control neighbour (21.11 ± 0.55 mm, $n=48$) was, however, significantly larger than the fought resident's neighbour ($P < 0.001$), but not significantly different in size from the population average ($P = 0.948$) (Fig. 1).

The distance between the burrows of the opponent and his nearest neighbour (11.6 ± 0.4 cm) did not significantly differ from the distance between those of the control resident and his nearest neighbour's burrow (12.0 ± 0.5 cm; $t_{45} = 0.692$, $P = 0.492$).



Fig. 1 Mean major claw size (mm) of population, territory-seeking floater male, fought resident male, control resident male, fought male resident's neighbour, and control male resident neighbour. Error bars indicate $\pm$ S.E. of the mean.

DISCUSSION

Fighting was extremely size assortative, with resident and floater size only differing, on average, by 0.15mm (<1%). Unlike previous findings on fight choice in fiddler crabs (Morrell et al. 2005; Jennions & Backwell 1996; Fayed et al. 2008; Milner et al. 2011) floater males tended to challenge resident males slightly larger than themselves. This was unexpected as floater males in the closely related fiddler crab *U. mjoebergi* challenge residents slightly smaller than themselves, which is suggested to occur in order to counteract a fighting advantage that results from burrow ownership (Fayed et al. 2008). Intriguingly, however, floater males in my study appear to choose whom to fight with based on the size of the resident's nearest neighbour. Floaters chose males with neighbours substantially smaller than themselves: on average the nearest neighbour was ~15% smaller than the floater. Further, neighbours of targeted residents were ~19% smaller than the population average. Therefore, floater males appear to preferentially fight resident males of approximately the same size, but only when the resident's nearest neighbour is significantly smaller.

My results are generally in agreement with an earlier study by Milner et al. (2011), showing similar fight choice decision are made by another fiddler crab *Uca mjoebergi*. Like *U. annulipes*, *U. mjoebergi* also form territorial coalitions and it appears that the formation of such territorial coalitions places significant selective pressure on the decision making of territory-seeking males in both species. That is, by avoiding resident males with larger neighbours, territory-seeking males can reduce the likelihood of initiating a fight with a resident who might then gain help from his neighbour. By paying attention to the size of a resident's nearest neighbour, a floater can reduce the overall costs associated with coalition fights (i.e. a greater likelihood of losing the fight when a larger helper takes over from the challenged resident). Furthermore, the fact that floating males do not target residents slightly smaller than themselves, but instead choose to fight residents with neighbours smaller than themselves, suggests that the formation of territorial coalitions (i.e. three-way fights) in *U. annulipes* is more influential than an ownership advantage in decisions regarding fight choice.

Fighting a resident with a small neighbour can have immediate benefits by increasing the likelihood of attaining a territory in the first place (Milner et al. 2011; *this study*). It might also have longer-term benefits. For instance, by obtaining a territory with a

smaller neighbour, a male is effectively determining his social environment (Oh & Badyaev 2010). This is potentially beneficial when establishing and maintaining territory boundaries because smaller male neighbours are less costly to fight (Pratt & McLain 2006). Furthermore, a number of studies have now shown that associating with smaller, less attractive males is advantageous when attempting to attract mate-searching females (Bateson & Healy 2005). That is, a male can increase his relative attractiveness by associating with less attractive competitors. Callander et al. (2011) have recently showed in *U. mjobergi* that there is a mating disadvantage to being surrounded by larger males. They suggested that by keeping and protecting smaller neighbours, a male could potentially increase his relative attractiveness to mate-searching females. The same could apply in *U. annulipes*. As demonstrated within this study, however, there are also disadvantages to associating with weaker males. By obtaining a territory with small neighbours a male effectively removes the possibility of receiving help during an intrusion by a territory-seeking male. The fact that males still choose to fight opponents with small neighbours suggests that, given the trade-off between these two benefits, increasing the likelihood of mating is a more important determinant of fitness than improving territory retention by increasing the likelihood of receiving help from a neighbour.

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CHAPTER 9: HUNTING AND PREDATION IN A FIDDLER CRAB

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ABSTRACT

Fiddler crabs are known primarily to be deposit feeders. They eat detritus, bacteria, and other small particles of organic material found in the sandy or muddy substrate on which they live. They have highly specialized mouthparts used to separate edible matter from non-digestible material. Here we provide evidence of cannibalism and predation in a fiddler crab, *Uca annulipes*. We additionally provide the first evidence of a fiddler crab hunting shrimp and insects. This study is an exemplary reminder that, even though an animal may have evolved highly specialized feeding traits, this need not preclude it from opportunistically acting as a generalist feeder.

Keywords: cannibalism; feeding; fiddler crab; predation; *Uca annulipes*

INTRODUCTION

Fiddler crabs are accurately described as surface deposit feeders. They eat detritus, bacteria, algae and other small particles of organic material found in the sandy or muddy substrates on which they live (Dye & Lasiak 1986). To feed, a crab scoops up sediment onto its small feeding claw and then transfer the sediment to its mouth. Specialized setae located on the first and second maxillipeds separate edible matter from non-digestible material (Miller 1961). The buccal cavity is then flooded with water from the branchial chamber. Lighter, edible particles float to the top of the cavity and are washed into the gut (Maitland 1990; Miller 1961). Indigestible coarser material, such as sand, sinks to the bottom where it is expelled from the mouth as a small feeding pellet that is deposited onto the substrate (Maitland 1990; Takeda et al 2004). An indication of the degree of specialization this mode of feeding promotes is seen in the way in which species are adapted to the type of habitat in which they live. For example, those living on muddy sediments have ‘woolly’ hairs on their mouthparts since they ingest much of the mud along with the fine-grained rich organic matter it contains (see Maitland 1990; Crane 1975). Species living on sandier sediments have stiff, spoon-tipped hairs on their mouthparts that allow for greater sorting ability (see Maitland 1990). The feeding mechanisms of fiddler crabs are therefore highly specialized adaptations for extracting food from inedible surface materials.

Here, we report observations of the fiddler crab, *Uca annulipes*, hunting and eating live prey. Over a two month period of opportunistic observations we witnessed eight attempted and seven successful predation events on shrimp (probably juvenile *Penaeus indicus*), an attempt to catch an insect (an unknown bee species), two predation attempts on sand bubbler crabs (*Dotilla fenestrata*) and seven cannibalistic acts. These observations were all made on the tidal mudflats of Inhaca Island, Mozambique from September to October 2008.

RESULTS

Predation on shrimp

Juvenile penaeid shrimp live on the fringe mangroves and adjacent mudflats of Inhaca Island (Ronnback et al 1981). *Uca annulipes* were witnessed on eight occasions

attacking shrimp that were flicking themselves over the mud, presumably because they had become isolated in a pool of water that was receding and were trying to reach another pool of water. Individual crabs of both sexes and all sizes would pounce on the shrimp and attempt to pin it down by standing on top of it. They attempted to grab the shrimp with their walking legs, as well as the major claw if they were male. Usually only one crab at a time was seen attacking a shrimp but, as the shrimp moved across the mudflat, crabs that had failed to immobilize it would relinquish their pursuit, and another crab would then initiate an attack. Predatory crabs would often move far away from their territories to pursue a shrimp, thereby risking losing their territory and access to a burrow. On a few occasions, we saw several crabs attack a shrimp simultaneously. We also observed at least seven fights between crabs competing for ownership of a captured shrimp (see S1). These fights lasted up to 25 minutes, which is an unusually long fight for a fiddler crab (see Morrell 2005). Once a crab had successfully immobilized a shrimp (7 of the 8 occasions), it immediately started to eat it (see figure 1). This involved the crab using its feeding claw/s to rip minute pieces of tissue from the still alive shrimp. Males would occasionally crush the shrimp with their major claw, making access to softer tissue easier. In seven cases the crab eventually attempted to drag the shrimp down its burrow (see S1). All seven successful captures were by males. On three occasions however, a female was found feeding on and in possession of the shrimp (see figure 2). Only on one of the eight occasions did we not see the shrimp successfully captured and consumed.

Predation on Sand Bubbler crabs

In both cases where we observed *Uca annulipes* eating a sand bubbler crab, the prey was already dead when we first observed it. In both cases we saw a territory-less male fiddler crab carrying its prey in its major claw through the population, and stopping occasionally to feed on the sand bubbler. It is therefore unknown whether *U. annulipes* scavenged or actively hunted sand bubblebers.

Predation on an insect

A male crab pounced on a bee that had landed on the mudflat. It crushed the bee with its major claw before being disturbed and retreating down its burrow. After reemerging, the male returned to the bee and struggled to subdue it. The bee eventually escaped by flying away.



Fig. 1. Male *U. annulipes* feeding on a shrimp (*P. indicus*)



Fig. 2. Female *U. annulipes* feeding on a shrimp (*P. indicus*)

Cannibalism

In four of seven cases, we did not see the prey being killed. We simply observed a large male feeding on the carapace of a dead, medium-sized male conspecific (see figure 3 and S2). In the remaining three cases we observed prey capture. In one case, a large

male (carapace > 14 mm) chased after a small female. He crushed and killed her and then dragged her down his burrow (see S3). In the second case, a large male was seen dragging an injured medium-sized male across the mudflat. The victim appeared to have recently molted and its carapace was crushed and its eyestalks were missing. We observed the crab tearing off and eating the flesh of its prey. In the third case, a large male was witnessed capturing, crushing and eventually dragging a tethered male (for the purpose of another experiment) down his burrow.



Fig. 3. Male *U. annulipes* feeding on and attempting to drag a male conspecific down his burrow.

DISCUSSION

Cannibalism and predation on congeners has been documented in the wild in only three of the 100+ species within the genus *Uca*. Cannibalism has been reported in *Uca tetragonon* where males prey upon females and smaller males (Koga *et al.* 1995). Predation upon smaller-bodied congeners has also been reported in: *U. tetragonon*

(Koga et al. 1995); *U. minax*, which prey upon *U. pugnax* and *U. pugilator* (Pratt et al., 2002; McLain et al., 2003); and *U. rapax*, which also prey upon *U. pugilator* (McLain et al., 2003). To our knowledge, however, we have provided the first documentation of fiddler crabs actively hunting and feeding upon shrimp and bees. Furthermore, we have provided the first evidence of female fiddler crabs hunting. Given the level of feeding specialization needed to extract food from the substrate by fiddler crabs, it is rather surprising that they are also able to hunt and capture shrimp and other crabs. It is an exemplary reminder that, even though an animal may have evolved highly specialized feeding traits, this need not preclude it from opportunistically acting as a generalist feeder.

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APPENDIX 1: HOW DO WEAPONLESS MALE FIDDLER CRABS AVOID AGGRESSION?

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ABSTRACT

Mimicry of females enables weaker males in many species to avoid intrasexual aggression. In fiddler crabs (*Uca annulipes*) males use their major claw in aggressive interactions to acquire and defend a territory. Males that have autotomised their major claw will be disadvantaged in fighting, but might use their temporary resemblance to females to avoid costly aggressive encounters with other males. We investigated whether: (i) courting males mistake clawless male fiddler crabs for females; (ii) clawless males are able to acquire, defend and retain territories as successfully as intact males; and (iii) clawless males are more cautious than intact males. Clawless and intact males differed in burrow acquisition methods and fighting behaviour, but were equally successful at acquiring and retaining burrows. While courting males treated clawless males as female, we found no evidence that clawless males mimic the behaviour of females, or that they exploit the advantage of their mistaken identity. Clawless males further appear to avoid male aggression by altering their territorial strategies to minimise the potential for conflict.

Keywords: Autotomy; Female mimicry; Fiddler crab; Territory acquisition; Territory defence

INTRODUCTION

In sexually dimorphic species, it can sometimes be advantageous for an individual to resemble a member of the opposite sex. Such mimicry can reduce intrasexual aggression, and in some cases improve mating success. For example, the plumage colouration of some male pied flycatchers (*Ficedula hypoleuca*) is very similar to that of females, enabling them to avoid aggressive encounters with other males and to obtain better-quality breeding territories (Slagsvold and Saetre 1991). In the blennioid fish *Salaria pavo*, small males display the colour pattern and courtship behaviour of spawning females, allowing them to fertilise eggs laid by females in the nests of large males (Gonçalves et al. 1996).

Fiddler crabs (*Uca* spp.) provide a striking example of the morphological differences that can occur between males and females of the same species. Females are small, often drab, and have two small feeding claws, while males are larger, brightly coloured and have one feeding claw and one greatly enlarged ('major') claw (Crane 1975). The major claw is extremely important, as it is used both in a waving display for mate attraction and courtship, and as a weapon in aggressive interactions with other males. Claw length is a predictor of mating success (Backwell and Passmore 1996), and also acts as a signal of fighting ability (Jennions and Backwell 1996). Fighting plays an important role in the frequent territorial interactions experienced by male fiddler crabs. Each individual occupies a small territory containing a burrow, which is a vital resource for every crab, providing shelter from predation and tidal inundation (Crane 1975). The burrow must be defended against wandering individuals that have lost their own territories and will fight to evict residents. Additionally, residents fight their neighbours to expand their territory boundaries and increase their feeding area. Territorial fights are usually initiated by the intruding individual, and fighting is strongly size-assortative (Jennions & Backwell 1996). There is a close relationship between the size of residents and their burrows (Reaney & Backwell 2007). So, while it may be less costly for a large intruder to evict a smaller resident, there is then the added cost of enlarging the burrow.

Male fiddler crabs are able to autotomise their major claw, and do so to escape a predator (Backwell et al. 2000) or to emerge from a difficult moult (Maginnis 2006). While autotomy is preferable to death or serious injury, there are large costs associated with the loss of an appendage. Limb regeneration is a slow and energetically expensive

process that diverts resources away from other functions such as reproductive activity and general growth (Juanes and Smith 1995). Furthermore, in several species of fiddler crab a regenerated claw is a lighter, less robust structure than an original claw (Crane 1975) and a less effective weapon (eg. *Uca annulipes*, Backwell et al. 2000). Although males with regenerated claws are able to deceive conspecifics in some mating and aggressive contexts, there is still a net cost to having a regenerated claw as territory defence is usually compromised (Backwell et al. 2000; Reaney et al. 2007).

One large cost of claw loss must occur immediately following autotomy. Until the next moult, a newly autotomised male has no claw at all, and the regenerating claw will not reach the size of the lost original for several moult cycles (Skinner 1985). In *U. annulipes*, for example, complete claw regeneration takes about six months (Backwell et al. 2000). Clawlessness presents a serious impediment to many aspects of life for a male fiddler crab. In many cases, a male that has lost his claw is also likely to have lost his territory (for example, through being removed by a predator or large rival). His first concern is therefore likely to be acquiring a new territory, which must be achieved without the use of weaponry. If successful, the autotomised male then has to retain ownership of the territory. Despite these difficulties, the fact that many males survive claw loss is demonstrated by the presence of males with regenerated claws. In *U. annulipes* the proportion of individuals with regenerated claws was as high as 44% in one population (Backwell et al. 2000). This suggests that behaviours that ameliorate the costs of claw loss have arisen in fiddler crabs.

It is possible that clawless male fiddler crabs are mistaken for females, and as a result experience reduced aggression from other males. As the major claw is the main distinguishing feature, it is superficially difficult, for human observers at least, to tell clawless males from females except at close range. As the fiddler crab visual system does not involve high resolving power or visual acuity, relying instead on movement, colour, reflectance and patterning for species, sex and individual recognition (Detto et al. 2006, Zeil and Hemmi 2006), it is likely that the major claw is an important cue for sex recognition in the crabs themselves. Female fiddler crabs can acquire and hold territories without the use of an enlarged claw. When searching for a burrow, female fiddler crabs use different tactics to males. While most males fight to evict residents of either sex, females are more likely to seek out unoccupied burrows (R.N.C. Milner, I. Booksmythe, M.D. Jennions & P.R.Y. Backwell, unpublished data). Females also

obtain territories after mating as the male gives up his burrow for the incubation of eggs (Backwell and Passmore 1996). Despite having no apparent defence against them, burrow-owning females do not appear to suffer much harassment by burrow-seeking males (Jennions and Backwell 1996), who often bypass them to approach male residents. If clawless males use similar territorial strategies to females, or can deceive clawed males by mimicking female behaviour, they might also be successful in avoiding excessive harassment, and survive this vulnerable period.

Another potential cost of claw loss is increased susceptibility to predation. Limb autotomy increased susceptibility to predation in house crickets (*Acheta domestica*), due to a reduction in escape speed following limb loss (Bateman and Fleming 2006). Loss of a claw should not impede running in fiddler crabs. In fact, endurance capacity was greater for clawless than intact *U. pugilator* males, and sprint speed was unaffected by claw presence or absence (Allen and Levinton 2007). However, other aspects of defence could be impaired in clawless individuals, as they have lost both a weapon and the opportunity to escape another predation attempt via autotomy. The behavioural decisions of individuals can be strongly influenced by their perceived risk of danger (Lima and Dill 1990). For example, in striped plateau lizards (*Sceloporus virgatus*) the distance from a predator at which individuals took flight was greater for individuals that had previously been captured than for control individuals. Of the previously captured individuals, those that autotomised their tail showed greater refuge use than intact individuals (Cooper 2007). A fiddler crab that has recently lost a claw to a predation attempt is likely to have a higher perceived risk of threat than one that has not. Consequently a clawless male might be expected to exhibit greater caution than an intact male. A trade-off between predator avoidance and necessary activities such as foraging is found in most animals (Lima and Dill 1990). An increase in cautiousness could be costly if it results in reduced foraging time for a crab with the increased energetic requirements of claw regeneration. However, being more cautious might help clawless males avoid threats posed by conspecifics, as well as predators.

This study aims to examine whether clawless male *U. annulipes*

1. are recognised as male by resident males, or are courted like females
2. are able to acquire and defend burrows as effectively as intact males
3. can retain tenancy of a burrow for as long as intact males
4. are more cautious than intact males after exposure to a threat.

Together these data will indicate whether clawless males are at a disadvantage in terms of male-male competition, and whether they are mistaken for females in a courtship context.

METHODS

We investigated the effects of claw loss on male fiddler crabs, *Uca annulipes*. This species occurs in dense mixed-sex populations on intertidal mudflats. Fieldwork was carried out on Inhaca island, Mozambique (25°59'10"S, 32°56'35"E), during September and October 2008.

All the crabs used were caught as resident (burrow-owning) individuals and had original (non-regenerated) major claws. To remove the major claw, it was gently twisted forwards until the crab autotomised the limb. Claw removal was done immediately on capture to minimise additional stress on de-clawed males. The carapace width and major claw length of all crabs were measured using dial callipers to the nearest 0.1 mm. When size-matching was required crabs were matched to within 1 mm using carapace width.

1. Can courting males recognise clawless males?

To determine whether courting male *U. annulipes* can distinguish between wandering females and clawless males, we released clawless male, intact male, and female crabs onto the mudflat and documented the waving response of resident courting males. Size-matched trios ($N = 15$) of one female and two male crabs, were caught, measured and marked with quick-drying white paint on the carapace. The major claw of one of the males was then removed. The crabs were individually released on the mudflat and tracked for five minutes as they moved through the population. The number of resident males within 20 cm of the focal crab and the number of these that waved at the focal crab were recorded. For a crab to be counted as waving it had to directly face the focal crab and perform a full courtship wave. Data were analysed using linear mixed models in SPSS 17.0, with crab type (female/intact male/clawless male), number of males passed and the interaction between these terms as fixed factors, and trio identity as a random factor.

2. Can clawless males acquire and defend burrows as effectively as intact males?

To determine how intact and clawless males acquire and defend burrows, we released males of both types onto the mudflat and tracked them as they searched for and established a territory. Eighty males were caught and their carapace width and claw length measured. The major claw of 40 males was removed. Each crab was individually marked and released back into the population. The release point was marked with a flag, and the crab was tracked until it acquired a burrow. The duration of the search, number of burrows the crab approached, whether it fought with male or female residents, method used to obtain the final burrow, and the straight-line distance between the release point and the final burrow were recorded. Once the focal crab had secured a burrow, it was observed for one hour to record: whether it fought male and female neighbours and wandering individuals, whether it was aggressively approached by neighbours, and whether it sealed the burrow entrance, abandoned the burrow, or was evicted, within the first hour of occupation. We used Mann-Whitney U tests to compare continuous variables, and likelihood ratio tests to compare binomial response variables between clawless and intact males.

3. Can clawless males occupy a territory for as long a tenancy as intact males?

To determine whether clawless males retain burrows as successfully as intact males, we compared the length of tenancy for intact and clawless males experiencing similar pressures from neighbours. Size-matched pairs of males ($N = 37$) were caught, measured and marked, and one male in each pair was made to autotomise his claw. The marked males were placed in a pair of flagged empty burrows (the burrow entrance marked by a small, numbered flag) on the mudflat, whose male occupants we had previously evicted. The nearest neighbour to each flagged burrow was male, and the size of the nearest neighbour and distance to the nearest neighbour's burrow were judged by eye to be similar for each flagged burrow in a pair. After 30 minutes the burrows were checked to see whether the new occupant had sealed the entrance. Flagged burrows were checked twice daily for the presence of the marked occupants until the male with the longer tenancy could be determined. We used a binomial test to analyse the effect of clawlessness on which male in a pair had the longer tenancy. Binomial response data were analysed using likelihood ratio tests ($df = 1$).

4. Are clawless males more cautious than intact males following a threat?

To determine whether clawless males were more cautious than intact males after being exposed to a threat, we scared a pair of intact and clawless males ($N = 20$ pairs) and recorded the time they took to re-emerge after retreating into their burrows. A resident clawless male was located by scanning the mudflat, and a nearby resident male with an original claw was identified. An observer sat approximately 1 m from both crabs and waited until all crabs in the area had resumed normal activity. When both focal crabs were on the surface the observer stood suddenly to scare them into their burrows. The fiddler crab visual system is highly sensitive to movement and particularly to objects moving above the visual horizon (Zeil & Hemmi 2006). Thus quickly standing elicits a threat response indistinguishable from that provoked by a predator (Jennions et al. 2003). The time from the scare to the re-emergence of each crab was recorded. When all crabs in the area had resumed normal feeding activity, the trial was repeated for the same focal pair. Both crabs were then caught and measured. Data were analysed using linear mixed models in S-Plus 7.0, with male type included as a fixed factor and male identity nested within pair identity as random factors. The significance of random factors was tested using a log-likelihood ratio test ($df=1$) of the model with and without the terms of interest. For fixed factor, we simply present the F-ratio for the parameter estimate.

Unless otherwise stated all tests are two-tailed and alpha was set at 0.05. We used standard formulae to calculate the common effect size of Pearson's r for all tests (Cooper et al. 2009). If the intact male response was greater than the clawless male response r was assigned a positive value.

RESULTS

1. Can courting males recognise clawless males?

Released females and clawless males provoked the same level of courtship waving from resident males while released intact males provoked a much lower rate of waving. In the initial model, when all crab type categories were included, there was a significant

interaction between crab type and the number of males passed ($F_{2,27} = 25.9$, $P < 0.001$). The number of resident males waving at the focal crab increased strongly with the number of resident males passed for both females and clawless males, but barely increased for intact males (Fig. 1). When only females and clawless males were included in the model, the interaction between crab type and the number of males passed was not significant, and was removed from the model. In the final model there was no difference in the slope of the line for females and clawless males ($F_{1,14} = 2.85$, $P = 0.11$), indicating that resident courting males responded in the same way to clawless males and females.

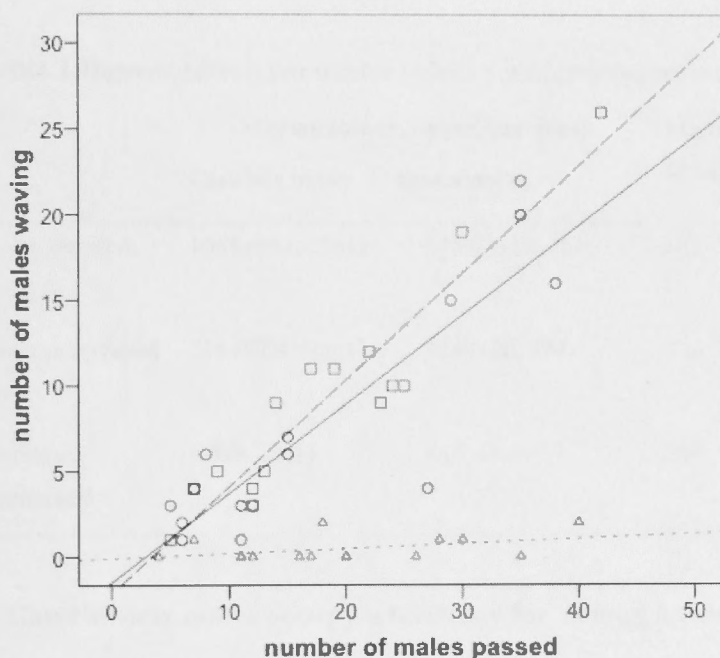


Figure 1 Waving response of resident males to females (circles and solid line; $Y = 0.522X - 1.52$), clawless males (squares and dashed line; $Y = 0.626X - 2.165$) and intact males (triangles and dotted line; $Y = 0.029X - 0.1$).

2. Can clawless males acquire and defend burrows as effectively as intact males?

Clawless males did not differ significantly from intact males in search duration, number of burrows approached, or the distance travelled during the search (Table 1). Compared to intact males, significantly fewer clawless males fought resident males, but significantly more fought resident females. Clawless and intact males also differed significantly in the method used to finally acquire a burrow, with clawless males significantly less likely than intact males to evict a resident, instead searching out empty

burrows (Table 2). Although clawless and intact males differed in the behaviours they engaged in while searching, over all these results do not indicate a difference in the ability of each type of male to acquire a burrow. After obtaining a burrow, fewer clawless males than intact males fought neighbouring males. Conversely, more clawless than intact males fought neighbouring females, although this difference was not significant ($P = 0.07$). Clawless and intact males did not differ significantly in the likelihood they would: fight wandering male or female crabs; be aggressively approached by male or female neighbours; seal the burrow entrance; or abandon the burrow. However, significantly more clawless than intact males were evicted from their burrows within the first hour of occupation (Table 2).

Table 1 Burrow search parameter values for clawless and intact males

	Median (lower, upper quartiles)		Mann-Whitney U	Effect size (<i>r</i>)	P value
	Clawless males	Intact males			
Search duration (sec)	1068 (382, 2381)	846.5 (329, 1629.8)	682	-0.13	0.26
Distance travelled (cm)	215 (87.4, 504.1)	228 (120, 390)	772.5	-0.01	0.94
Burrows approached	9.5 (4, 21.8)	8 (4, 19.5)	754	-0.05	0.66

3. Can clawless males occupy a territory for as long a tenancy as intact males?

In replaced pairs, intact males were no more likely to have a longer burrow tenancy than clawless males (clawless 54%; intact 46%; $P = 0.85$). There was no difference in the likelihood that they seal their burrow entrance within the first 30 minutes of occupancy (clawless 13/24; intact 9/24; $G_1 = 1.35$, $P = 0.25$). Crabs that sealed their burrow entrance were slightly more likely to be the longer tenant than crabs that did not seal, but this trend was not significant (sealed 14/22; didn't seal 10/26; $G_1 = 3.05$, $P = 0.08$).

4. Are clawless males more cautious than intact males following a threat?

Clawless and intact males did not differ in the time they took to re-emerge following retreat from a threat ($F_{1,36} = 0.84$, $P = 0.36$). This result is not due to inaccuracy in measurement as there was significant repeatability in re-emergence time among

individual males (log likelihood ratio = 3.87, $df=1$, $P = 0.05$), even after controlling for the effect of pair identity (LLR = 5.55, $P = 0.02$).

Table 2 Incidence of aggressive, defensive and other territorial behaviours in tracking and subsequent one-hour observations of clawless and intact males

	Observed/Total		Likelihood ratio	Effect size (<i>r</i>)	P value
	Clawless males	Intact males			
Burrow-searching					
Fought males	14/40	38/40	15.58	0.670	<0.001
Fought females	29/40	19/40	5.28	-0.260	0.02
Acquired burrow by evicting resident	6/39	27/39	24.65	0.562	<0.001
1st hour of occupation					
Fought male neighbours	2/36	16/39	14.41	0.440	<0.001
Fought female neighbours	8/36	3/39	3.24	-0.209	0.07
Fought male intruders	13/36	18/39	0.78	0.101	0.38
Fought female intruders	3/36	1/39	1.28	-0.130	0.26
Aggressively approached by male neighbours	7/34	13/35	2.33	0.182	0.13
Aggressively approached by female neighbours	6/34	2/35	2.49	-0.187	0.12
Sealed burrow entrance	17/36	11/39	2.9	-0.196	0.09
Abandoned burrow	1/36	6/39	3.9	0.217	0.06
Evicted from burrow by intruder	11/36	4/39	4.95	-0.257	0.026

DISCUSSION

Burrow acquisition

While clawless male *U. annulipes* were not observed to actively mimic female behaviour, resident males courted wandering clawless males and wandering females equally. This was clearly different to their response to intact males, who were rarely courted. This suggests that clawless males are misidentified as females by male crabs. Whether males always perceive clawless males as female is unclear, although it is likely that at short range, cues besides the presence or absence of a major claw would indicate the sex of an individual. It is common in systems of mimicry for only one of multiple signals to be deceptive. For example, in Augrabies flat lizards (*Platysaurus broadleyi*), young males mimic females visually, but can still be identified as male by their scent (Whiting et al. 2009). While courting males were deceived, we found no evidence that clawless males exploited the advantage of their mistaken identity with behavioural mimicry of females.

Compared to intact males, burrow-searching clawless males fought significantly less often with resident males. Our observations suggest this was less a function of resident males' inability to recognise clawless males than of the tendency of clawless males to avoid approaching burrows owned by males (personal observation). Clawless males did not directly obtain burrows by deceiving male residents. On the single observed occasion when a resident male allowed a clawless male to enter his burrow, the resident followed the clawless male into the burrow as would happen in a successful mate-attraction event. At this point the two crabs appeared to reach a stalemate in the burrow entrance where they both remained stationary for at least two hours, when the tide covered the burrow and the observation had to be terminated.

Clawless males exhibited very different burrow acquisition methods to intact males, but they did not differ significantly in search duration, travel distance, or the number of burrows approached. This suggests that the strategy adopted by males that have lost their claw (that is, locating empty burrows rather than fighting to evict residents) is as successful as the normal male burrow-searching strategy. This raises a question: why do intact males fight and evict residents to obtain burrows, when they could instead search out abandoned burrows at no apparent extra cost? One reason might be that wandering

intact males, unlike clawless males, present an obvious threat to residents, and are therefore less able to avoid fights initiated by defensive resident males. Such an unsolicited increase in fights could simply result in more opportunities to win burrows through fighting. However, this seems insufficient to explain the far greater likelihood that intact males evict a resident. Furthermore, intact males evicted both male and female residents in equal numbers, and resident females are unlikely to initiate fights with wandering males. Finally, previous studies and our observations suggest that territorial fights are mainly initiated by wandering males (eg. Jennions and Backwell 1996).

Another explanation for the differences observed is that the burrow-searching strategy employed by clawless males could impose costs not measured in our study. For example, the quality of a male's burrow is an important criterion assessed by females searching for mates in *U. annulipes* (Backwell and Passmore 1996), and burrow structural qualities can be affected by factors such as substrate composition (Lim 2006). Abandoned burrows may be damaged or of poor structural quality, or situated in resource-depleted or otherwise lower quality habitat. Consequently, for an intact male it might be worth the cost of fighting to acquire a high-quality, well-situated burrow.

Burrow ownership

In the first hour after obtaining a burrow, clawless and intact males did not differ in the number of aggressive approaches from neighbours of either sex, but clawless males had significantly fewer fights with male neighbours, suggesting there are some key behavioural differences between clawless and intact males. Clawless males might initiate fewer fights with male neighbours, and/or exhibit greater caution than intact males, retreating as soon as a neighbouring male displays aggression to avoid any escalation of the encounter. While our experimental results showed that clawless males were not more cautious than intact males after exposure to a threat, the threatening stimulus presented was like that of a potential predator rather than a conspecific competitor. Although studies of other fiddler species have found higher predation on both males (*U. beebei*, Koga et al. 2001) and females (*U. pugilator*, Bildstein et al. 1989), sex biased predation has not been shown in *U. annulipes* (Walker 1972), suggesting the predator-avoidance benefits of hiding for longer would not differ for *U. annulipes* with or without a major claw.

Once resident, clawless and intact males did not differ in the number of fights they had with wandering males or females. However, clawless males were more frequently evicted by wandering crabs, indicating they were more likely to lose these fights when they occurred. Despite this vulnerability clawless males do not appear to be specifically targeted by burrow-searching individuals. Differences in the burrow quality of clawless and intact males might contribute to the pattern observed. If clawless males tend to occupy poorer quality burrows the benefit from evicting a clawless male is reduced as well as the cost. Depending on their own condition, intruders could therefore be choosing between two roughly equal strategies: reduced costs for reduced benefits or increased costs for greater gain.

Our tracking observations showed that newly resident clawless males had a greater likelihood of eviction within the first hour of burrow occupancy. The replacement experiment, however, showed that clawlessness did not predict shorter burrow tenancy. These apparently contradictory results could be explained by the greater (though not significant) likelihood for intact males to abandon a burrow. The method we used to assess burrow tenancy did not allow for a detailed examination of the reasons for departure from a burrow, and was only intended to provide a broad measure of the general ability of each male type to retain a burrow.

In summary, clawless male *U. annulipes* experience reduced aggression from males, which might be a result of their temporary resemblance to females. However, we found no evidence that clawless males mimic the behaviour of females, or that they exploit the advantage of their mistaken identity. Even so, an incidental resemblance to females decreases aggression directed at burrow-searching clawless males, increasing the probability that they will survive to regenerate a claw. Clawless males further appear to avoid male aggression by altering their territorial strategies to minimise the potential for conflict.

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**APPENDIX 2: INBREEDING AND ADVERTISMENT CALLING IN
THE CRICKET *TELEOGRYLLUS COMMODUS*: LABORATORY AND
FIELD EXPERIMENTS**

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ABSTRACT

If sexually selected traits reveal a male's heterozygosity or condition to females, then such traits should exhibit declines with inbreeding. We tested this by examining the effect of inbreeding on advertisement calling in male crickets *Teleogryllus commodus*. We investigated the effect of one generation of full-sibling mating on calling effort and fine-scale call structure. Inbreeding reduced calling effort but had no effect on call structure. We then compared the attractiveness of inbred and outbred calls in the field by monitoring how many wild females were attracted to each call type. From the field data we conducted a selection analysis to identify the major axes of linear and non-linear multivariate sexual selection on call structure. A comparison of multivariate attractiveness of inbred and outbred calls along each major axis of selection revealed no difference in attractiveness. Our results suggest that inbred male calls have a fine-scale structure that is no less attractive to females than that of outbred calls. However, because inbred males call less often, and female *T. commodus* prefer males with a higher calling effort, inbred males will suffer reductions in mating success. Females who base mate choice on call rate are therefore using a signal correlated with male heterozygosity and/or condition.

Keywords: male attractiveness, selection analysis, heterozygosity, calling effort, call structure, sexual selection

INTRODUCTION

For many years, theoretical models have been based on the assumption that female mate choice in non-resource based mating systems is driven by males signaling additive genetic benefits that increase offspring fitness through the inheritance of ‘good genes’ (Hunt et al. 2004a; Radwan 2008). More recently, however, attention has focused on non-additive genetic benefits. Specifically, it has been asked whether females show directional mating preferences for males with higher than average levels of genome wide heterozygosity (e.g. Mays and Hill 2004; Kempenaers 2007; Fromhage et al. 2009). This is because, under certain conditions, more heterozygous males are more likely to carry locally rare or dissimilar alleles to those of a female, so that mating with such males increases offspring heterozygosity (i.e. heterozygosity is heritable in the sense that the heterozygosity of offspring will resemble that of their parents; Mitton et al. 1993, but see Puurtinen et al. 2009). For example, if an individual migrates into a new population, it might bring with it locally rare alleles. If this individual then mates with a local individual, the resulting offspring will not only be relatively heterozygous, but they will also carry the rare alleles brought in by their immigrant parent. Other females then choosing these heterozygous sons as mates will, in effect, be choosing carriers of recent immigrant genes that are probably dissimilar to their own, thereby producing relatively heterozygous offspring themselves (Reid et al. 2006). This should elevate fitness because inbred organisms and/or those with lower heterozygosity tend to perform less well than outbred ones (although there is still debate about the strength of the correlation between heterozygosity and fitness; reviewed by Kempenaers 2007). Consequently, females mating with more heterozygous males should produce fitter offspring. Females can detect such males using male secondary sexual traits if the expression of these traits is correlated with heterozygosity. For example, if male condition is positively correlated with heterozygosity, then increased heterozygosity will correlate with the expression of condition-dependent sexual traits. Females can then express a directional preference for highly ornamented and therefore relatively heterozygous males (Brown 1997; Fromhage et al. 2009; Ilmonen et al. 2009).

It is possible to test for a correlation between heterozygosity and the expression of sexually selected ornamental traits using inbreeding. Inbreeding (the mating of relatives) increases homozygosity across the genome and tends to reduce fitness (inbreeding depression). Inbreeding depression has been reported in a wide range of

traits across numerous taxa (Lynch and Walsh 1998; Crnokrak and Roff 1999; DeRose and Roff 1999; Keller and Waller 2002; Armbruster and Reed 2005). Inbreeding depression is thought to arise from either or both the unmasking of recessive deleterious alleles and the loss of advantageous heterozygosity (Charlesworth and Charlesworth 1987; 1999). In addition, epistatic interactions between loci might play a role in some species (van Oosterhout et al. 2003). Consequently, inbreeding depression is largely due to the effects of dominance interactions, specifically directional dominance, and does not occur in traits with a purely additive genetic basis (Charlesworth and Charlesworth 1987; Lynch and Walsh 1998). Traits that are closely related to fitness (i.e. life history traits, including sexual signals) are predicted to show reduced additive genetic variance because they are more closely associated with fitness and are therefore under stronger selection which depletes variation (Mousseau and Roff 1987; DeRose and Roff 1999; Blows and Hoffmann 2005; Hunt et al. 2007). Such traits are also expected to show higher levels of directional dominance, and therefore inbreeding depression, because mutations affecting these traits are typically deleterious and recessive. Traits that are less closely related to fitness (i.e. morphological traits) show substantial levels of additive genetic variance and less directional dominance, and therefore tend to exhibit lower levels of inbreeding depression (Lynch and Walsh 1998; Roff 1998; DeRose and Roff 1999).

Inbreeding depression in sexually selected traits might be due to effects of changes in heterozygosity at loci that directly code for sexual traits. Many sexually selected traits are condition dependent (reviewed by Cotton et al. 2004), however, so if inbreeding reduces the general ability of males to acquire and assimilate resources (i.e. lowers condition), then sexually selected traits are predicted to show strong inbreeding depression. Hence, sexually selected traits will signal the negative effects of reduced heterozygosity at the many loci across the genome that affect condition (Rowe and Houle 1996; Tomkins et al. 2004). These two mechanisms of inbreeding depression on sexual traits (direct effects or via inbreeding depression of condition) are not mutually exclusive.

More generally, if sexually selected traits are condition dependent, and inbreeding reduces male condition, then sexual traits should show declines with inbreeding. In this case, it may not be a male's heterozygosity *per se* that sexual traits will signal but rather his underlying condition. In such systems, females choosing males based on sexually

selected traits will normally gain genetic benefits for their offspring if some of the variation in male signaling is due to additive genetic variation in condition (Tomkins et al. 2004). In this case, inbreeding can be viewed as way of experimentally reducing a male's condition if condition is subject to inbreeding depression (see Drayton et al. 2007).

Several studies report substantial declines in sexually selected traits with inbreeding. For example, inbreeding in guppies *Poecilia reticulata* impairs male reproductive and courtship behaviour (van Oosterhout et al. 2003; Mariette et al. 2006), reduces sexual colouration (Sheridan and Pomiankowski 1997; van Oosterhout et al. 2003) and decreases actual mating success (Mariette et al. 2006). Likewise, inbreeding in the least killifish, *Heterandria formosa*, reduces the frequency of male mating attempts (gonopodial thrusting) (Ala-Honkola et al. 2009). Similarly, female mice *Mus musculus* find the courtship scent marks of inbred males less attractive than those of outbred males (Ilmonen et al. 2009), and inbred male *Drosophila* have delayed and abnormal courtship (Miller et al. 1993), altered courtship songs (Aspi 2000) and reduced competitive mating success (Sharp 1984; Miller et al. 1993). Finally, inbred male butterflies *Bicyclus anynana* suffer a reduction in mating success (Joron and Brakefield 2003). Traits that are involved in post-copulatory sexual selection (e.g. sperm quality and quantity) can also show substantial inbreeding depression (e.g. Roldan et al. 1998; Margulis and Walsh 2002; Konior et al. 2005; Gage et al. 2006; Zajitschek et al. 2009). Importantly, several of the studies that have investigated multiple traits and/or different populations have shown that the traits that females explicitly use as cues during mate choice are those that tend to show more substantial inbreeding depression (e.g. Sheridan and Pomiankowski 1997; Aspi 2000). Although these data are limited, they suggest that such traits might be good indicators of genome wide heterozygosity and/or male condition.

Here, we test the prediction that traits that are important mate choice cues will be sensitive to the effects of inbreeding in the Australian black field cricket, *Teleogryllus commodus*, using a population from Smith's Lakes, Australia. Specifically we predict that if such mate choice cues reveal condition and/or relative heterozygosity they should be reduced by inbreeding. Males produce a long-distance advertisement call to attract sexually receptive females (Campbell and Shipp 1979; Evans 1988). We have already shown that female choice exerts strong positive directional sexual selection for greater

calling effort and moderate levels of stabilising and directional multivariate sexual selection on finer-scale features of advertisement calls (for details of which traits or trait combinations are favored see Brooks et al. 2005; Bentsen et al. 2006). Furthermore, through diet manipulations, calling effort has been shown to be affected by nutrition (Hunt et al. 2004b; Maklakov et al. 2008). We have previously found substantial inbreeding depression in advertisement calling in another Australian population of *T. commodus* from Canberra (Drayton et al. 2007). In the current study, we quantify the effect of one generation of brother-sister mating (inbreeding coefficient $F = 0.25$) on nightly calling effort and finer-scale advertisement call structure. Studies have shown that the effect of inbreeding on male reproductive success can be masked in the laboratory relative to natural conditions (e.g. Joron and Brakefield 2003). We therefore examined the attractiveness of inbred and outbred advertisement calls in the field by monitoring how many wild females were attracted to each call type. This did not allow us to test whether natural conditions increase the relative difference in the expression of calls by inbred and outbred males compared to those in the laboratory (because calls were recorded in the laboratory, see below), but it did allow us to ensure that female choice was measured in a more natural context (i.e. over greater distances and with confounding natural effects on signal propagation such as wind and vegetation). The field experiment allowed us to conduct a formal selection analysis (e.g. Brooks et al. 2005; Bentsen et al. 2006; Hall et al. 2008) to identify the major axes of linear and non-linear multivariate sexual selection on the finer scale call structure. We could then compare the multivariate attractiveness of inbred and outbred males along each major axis of selection under field conditions.

METHODS

Generation and rearing of inbred and outbred crickets

Inbred individuals were created by one generation of full sibling matings ($F = 0.25$) and their fitness compared with that of outbred crickets ($F = 0$). Full sibling families were derived from ~ 70 wild caught gravid females collected in May 2006, at Smith's Lakes (at the Bungwahl cemetery), New South Wales, Australia. Male and female offspring from wild caught females were reared to adulthood (males and females were separated before sexual maturity to ensure virginity) and then paired at random (such that pair

members did not share a wild caught mother) to create full sibling families. Each full sibling family was reared communally in separate large plastic tubs with cat food (KiteKat Krunch) and water *ad libitum*. Females were separated from their brothers before sexual maturity to ensure virginity.

The full sibling families were grouped in sets of two to generate mating blocks ($N = 33$ blocks). We ensured that the two families in a block did not share a grandmother. In each block, brothers and sisters from both full sibling families were mated to create two inbred genotypes. Outbred genotypes were created by reciprocal matings of a male and a female from each family in the block. For example, in a block consisting of full sibling families A and B, inbred progeny were generated by mating brothers and sisters from family A, and brothers and sisters from family B, while outbred progeny were generated by mating a male from family A with a female from family B, and a male from family B with a female from family A. This design generated four offspring genotypes (two inbred and two outbred) per block such that maternally and paternally derived genes from each family are, on average, equally represented in inbred and outbred males. After mating females were provided with moist cotton wool ("egg pads") in which to lay their eggs. Egg pads were checked every three days for hatching nymphs. Upon hatching, nymphs from a given pairing were reared communally for 20 days in 9x9x5cm plastic tubs with food and a piece of moist cotton wool. They were then transferred to individual containers (9x9x5cm) with a water tube, cat food and a cardboard refuge. Nymphs were not transferred to individual containers immediately after hatching because they are small and easily crushed by a water tube. On average, 37.5 ± 3.3 ($\pm$ SE) nymphs of each inbred genotype and 39.4 ± 3.3 nymphs of each outbred genotype were set up individually for each block. Food and water was replaced every 10 days. Nymphs nearing maturity were checked daily to record development time (days from hatching to adult eclosion). The males used to test the effect of inbreeding on calling were >10 days post maturation to ensure sexually maturity. All crickets were kept at 26-28°C on a 12:12 photoperiod.

Calling effort

Male nightly calling effort was measured using a custom-built electronic monitoring device similar to that of Hunt et al. (2004b) and Drayton et al. (2007). In brief, the device consists of 128 recording chambers (9x9x5cm). Each chamber has one

condenser microphone mounted in the lid and housed one male. The device samples the chambers throughout the night. During sampling, the microphone of one chamber is activated and, if the sound level produced by the male in that chamber is 10 dB or more above the background noise, a 1 representing that the male is calling is recorded. Otherwise, a 0 representing no calling is recorded. The microphone of that chamber is then de-activated and the next microphone in the next chamber is activated, so that only one microphone (and therefore only one chamber) is sampled at a given time. Each recording chamber (and therefore each male) was sampled 10 times per second. The nightly calling effort of a male was calculated in two ways. First, if a male was recorded as calling for any of the 10 sampling events/second, he was recorded as calling for that second, and nightly calling effort was defined as the total number of seconds called per night. Second, calling effort was calculated as the total number of sampling events (i.e. the total number of tenths of a second) that a male called per night. The two measures of calling effort are highly correlated ($r_s = 0.993$, $P < 0.001$, $N = 1409$), so we only present the results of analyses using seconds called/night. Calling effort was measured for 10 hours per night (19h00- 05h00) at 26-28°C. Males were 12-23 days post maturation (14.4 ± 0.02 days), and their calling effort was recorded over 2-5 nights (3.2 ± 0.01 nights). Males were weighed before they were put into a chamber.

The structure of the advertisement call

The advertisement call

Male crickets call using a stridulatory apparatus consisting of a file and scraper on both elytra. The most basic call unit is a pulse. Each pulse is produced by closing the wings once (Kavanagh 1987). In the advertisement call of *T. commodus* there are two types of sound pulses. The first are longer, more intense and grouped into chirps. The second are shorter, softer and grouped into trills. Chirps and trills are arranged into the main repeated unit of calling, a phrase, which consists of a single chirp followed by one to several trills (Bentley and Hoy 1972; Hill et al. 1972) (see Fig. 1).

Recording and analysis of the advertisement call

Males were set up in individual, acoustically isolated 9x9x5cm recording chambers with a condenser microphone mounted in the lid. We recorded calls using a digital recorder

(MicroTrack 24/96, M-Audio). A power unit, containing a 9 volt battery powered the microphone. Chambers were checked though out the evening for calling males. If a male was heard calling, we recorded 1-2 minutes of his call. He was then weighed. Calls were recorded 15-36 days post maturation (23.0 ± 0.46 days). Because advertisement calls were recorded opportunistically and males do not call on demand, despite intensive effort on our part, sample sizes for advertisement call structure are lower than those for calling effort.

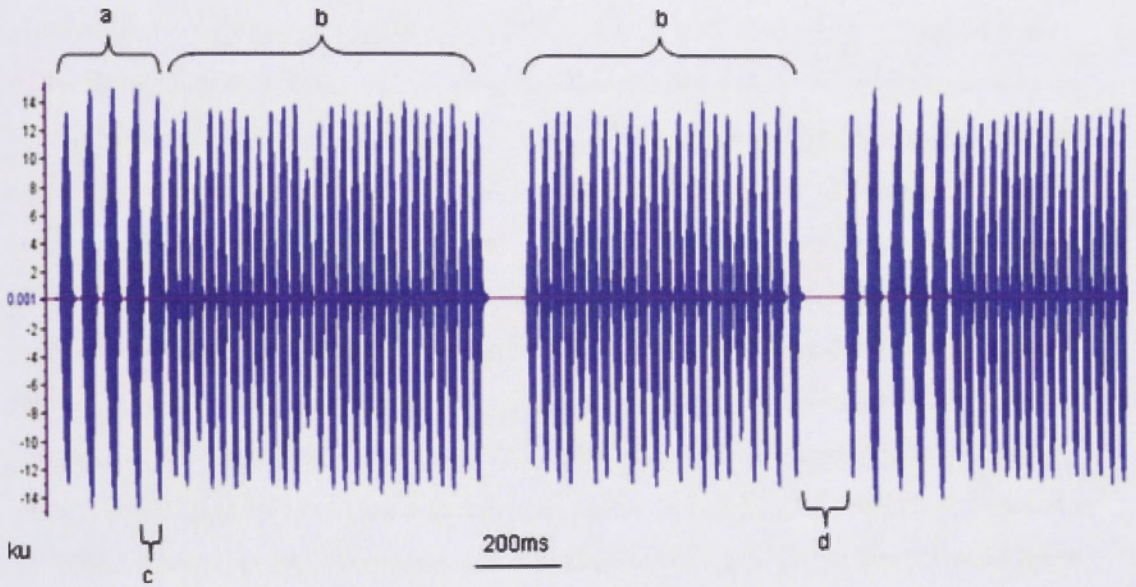


Figure 1. The advertisement call of male *T. commodus*. Louder pulses repeated at a lower rate are group into chirps (a) and softer pulses repeated at a higher rate are grouped into trills (b). Each phrase consists of a single chirp followed by a variable number of trills (the phrase in the figure has two trills). We measured the dominant frequency (DF), the number of pulses per chirp (CPN), the chirp inter-pulse duration (CIPD) (c), the inter-call duration (ICD) (d), and the number of trills in a phrase (TN). The horizontal axis is time (ms), while the vertical is amplitude (kU).

Calls were analyzed using Raven Pro 1.3 sound analysis software (Cornell Laboratory of Ornithology, Ithaca, NY, USA www.birds.cornell.edu/raven). We measured the following five parameters of five randomly chosen phrases from each male: dominant frequency (DF), the interval between the last trill pulse of the selected phrase and the first chirp pulse of the next [inter-call duration (ICD)], the number of pulses per chirp [chirp pulse number (CPN)], the duration of the interval between the last two pulses in the chirp [chirp inter-pulse duration (CIPD)] and the number of trills in a phrase [trill number (TN)] (Fig. 1). These five call parameters were measured because they have

been shown to capture variation that predicts male attractiveness to females (e.g. Brooks et al. 2005; Bentsen et al. 2006; Hunt et al. 2007).

Male attractiveness in the field

Playback experiments were conducted at Smith's Lake, Australia (32°22'S, 152°30'E) in February-March, 2009, on a grassy area surrounded by Eucalypt forest. Playbacks were conducted at night. Eight playback units were evenly spaced around the circumference of a circular arena (20 m diameter). A playback unit consisted of a pair of speakers (Logitech LS11 stereo speakers, one speaker faced into, the other out of the arena), a digital audio player (SanDisk Sansa c200) and a small tent (for rain protection) covering the unit. The speakers were placed on a 60x60cm piece of black Coreflute that was coated with sticky insect trap coating (TangleFoot) to capture approaching females (Fig. 2). Each playback unit continuously played the call of one male each night. Speakers were powered from the mains by cables that ran around the circumference of the arena. The calls of inbred and outbred males were played from alternating units around the circumference of the arena (Fig. 2). Call playback intensity was calibrated nightly to 60dB at 80 cm using a sound level meter (Digitech QM-1589) (see Bentsen et al. 2006). To ensure that there were females present during trials, at least 50 wild adult females were collected from a nearby site (<5km, Bungwahl cemetery) and released in the centre of the arena, under cardboard egg cartons, immediately before each trial. The collected females were released to boost an unknown number of wild females that were already at the field site. Consequently, the females attracted to the calls could have been released females and/or resident females. Because females were from the wild, we had no prior knowledge of their previous mating or rearing experience and were therefore unable to control for factors such as females mating status, age or condition. In the wild, however, males will encounter females that vary in such factors, and so we were recreating similar conditions under which mate choice occurs in nature. Playbacks were started between 21h00-22h00 and ended at 05h30. The number of trapped females at each unit was counted when the trial ended. The number of females caught at a unit provides a measure of attractiveness of the call played by that unit.

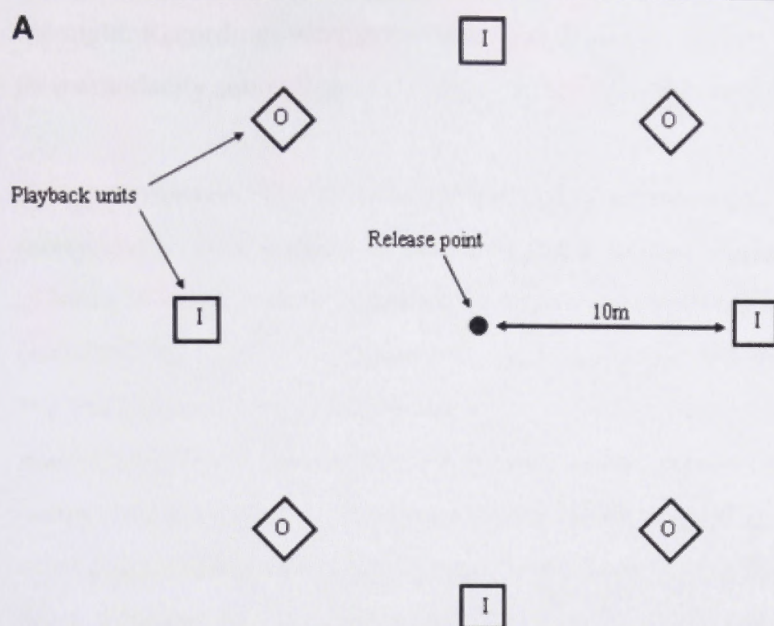


Figure 2. Set up of the field experiment (a) Schematic diagram showing the placement of the playback units around the arena. The calls of inbred (I) and outbred (O) males were played in an alternating pattern around the circumference of the arena (b) one playback unit, showing the tent, speakers and coreflute covered in Tangle-Trap. The digital audio player was housed in the plastic box for rain protection.

To create the playback recordings for each test male we selected 15 consecutive phrases from his original laboratory recording, selecting from the beginning of the first chirp pulse of the first phrase to the end of the intercall duration (ICD) of the 15th phrase. We then repeated this selection to create a continuous call loop that was played throughout

the night. Recordings were generated using Audacity 1.2.6 Free Digital Audio Editor (www.audacity.sourceforge.net) and saved as uncompressed WAV audio files.

The attractiveness of inbred and outbred calls was compared in two experiments. First, inbred and outbred males were paired by block. Second, males were tested irrespective of block of origin. Of the males whose advertisement calls we recorded, 42 could be paired by block (i.e. $N = 21$ pairs of an inbred and outbred male from the same block). For the first experiment, male pairs were randomly assigned into groups of four (i.e. 8 males per group). Each night, the eight calls of one group were played. The calls of males from the same pair were played from adjacent units. To test the 21st pair, three other pairs that had already been tested were grouped with this pair to create the same level of background calling experienced by other pairs tested.

The second experiment tested the attractiveness of males that could not be paired by block ($N = 16$ inbred and 16 outbred males). Males were randomly assigned into groups of four inbred and four outbred males. Each night, the calls of one group were tested.

We also included a silent control (a playback unit was set up and powered, but no call was played) in the second experiment. The control was placed between two units that were playing calls. Its position was randomly changed each night.

Statistics

Calling effort

Calling effort (seconds/night) was not normally distributed, so we used non-parametric tests. The calling effort of each male was measured over several nights, so we could test repeatability among males using a Kruskal-Wallis test. Calling effort was repeatable (see Results), so we calculated a mean value per male. We tested for any relationship between calling effort, and weight, development time and adult age using Spearman rank correlations (r_s).

To examine inbreeding depression in calling effort we took two approaches. First, to control for block/family effects, we conducted a meta-analysis with each block contributing one effect size. For each block we calculated the mean and standard

deviation for the number of seconds of calling/night by inbred and outbred males respectively. We then calculated Hedge's g as the difference between the inbred and outbred means standardised by the pooled standard deviation. We corrected for small sample size effects using J to generate the final effect size Hedge's d . All the equations used are in Rosenberg et al. (2000) (note: some authors refer to Hedge's d as g , e.g. Cooper et al. 2009). We then conducted a standard random effects model meta-analysis in Metawin 2.0 to calculate the grand mean effect size after weighting effect sizes by the inverse of their variance. In practice this means that we calculated the mean effect size across blocks giving greater weighting to blocks where more males were monitored. Inspection of the 95% confidence interval of the mean effect size reveals whether there is a significant difference in call rate between inbred and outbred males. We also tested whether there was greater variation in effect sizes among blocks than expected by chance using the test statistic Q_T . Statistical methodology and equations are in Rosenberg et al. (2000). Second, we ran a Wilcoxon matched-pairs test to compare the mean calling effort per block between inbred and outbred males. This test need not give the same result as the meta-analysis as it makes a different assumption (i.e. equal weighting for each block).

Structure of the advertisement call

The repeatability of each call parameter among males was assessed using a one-way ANOVA or Kruskal-Wallis test. All parameters were highly repeatable (see Results), so we calculated a mean value for CIPD, ICD, CPN, DF and TN for each male. To test for an effect of inbreeding on CIPD, ICD and CPN we ran separate linear mixed models in S+ 7.0. Where necessary, variables were transformed to ensure that residuals were normally distributed and homoscedastic. We used a model simplification approach, initially fitting a full model, including all two-way interactions, and then removed fixed terms until the final model only contained significant terms (random terms were always retained as part of the experimental design) (Crawley 2002). For all models, block and the interaction 'block by inbreeding' were included as random factors, allowing us to control for any differences in call parameters across blocks (i.e. random intercepts) and differences in the effect of inbreeding among blocks. The initial models contained inbreeding as a fixed factor and development time, adult age (days post maturation) and male mass as fixed effect covariates. To allow the reader to assess the influence of

terms excluded from the final model, we present the P - value associated with the parameter estimate for each term if it is included in the final model.

DF and TN were not normally distributed and could not be transformed. Because of lower sample sizes, we used a different non-parametric approach than the one taken for calling effort. To test for inbreeding depression, we calculated the mean value of inbred and outbred males for each block and compared these using Mann-Whitney U tests (we do not present Wilcoxon tests, as pairing by block reduced the sample size due to missing values in blocks; Wilcoxon tests did, however, yield the same conclusions). We assessed relationships between the two call parameters and development time, age and mass using Spearman Rank correlation (r_s).

To compare traits, we calculated the standardized coefficient of inbreeding δ (Lande and Schamske 1985) which is the percentage change with inbreeding, calculated as (outbred trait value- inbred trait value)/outbred trait value. A negative value indicates that inbred individuals had a larger value for the trait, interpretation of which depends on the direction of selection on the trait. We also present δ for calling effort and the five call parameters after one generation of full sibling inbreeding in a Canberra population of *T. commodus* (Drayton et al. 2007). Furthermore for both studies we calculated Hedge's d (see above) to compare the effect of inbreeding between the current study and Drayton et al. (2007) (to allow the comparison we treat males as independent data points). We then calculated the difference in effect sizes between the two studies for each trait and tested whether they differed significantly using a Z-test.

Male attractiveness in the field

For the first experiment we tested for a difference in the number of females attracted to the calls of inbred and outbred males with a paired t -test (i.e. paired by block, note that a highly conservative approach of pairing by night yielded the same conclusion). For the second experiment we used an independent samples t -test. Finally, to maximize the sample size, we pooled the data from both experiments and ran an independent samples t -test (i.e. one data point per mating type per block).

Summary statistics are presented as mean $\pm$ standard error. When two sample size values are presented the first is for inbred, and the second for outbred males. All tests are two-tailed and $\alpha = 0.05$.

Estimating linear and non-linear selection, and comparing multivariate attractiveness

To estimate linear, quadratic and correlational selection on the five call parameters we used the multiple regression approach of Lande and Arnold (1983). We standardized call traits using a Z- transformation (i.e. mean = 0, variance = 1) and converted absolute fitness (i.e. the number of females attracted to a call) to relative fitness by dividing the absolute fitness of each call by the mean across all calls (i.e. population mean). Linear and non-linear selection gradients were estimated in separate regression models. We doubled quadratic regression coefficients to obtain the correct quadratic selection gradients (Stinchcombe et al. 2008). To start, we ran separate multiple-regression models for inbred and outbred calls and then used a sequential model building approach to compare linear and non-linear selection between the call types (Draper and John 1988; Chenoweth and Blows 2005). To determine if sexual selection differed between breeding types we evaluated if the addition of linear by breeding type, quadratic by breeding type or correlational by breeding type interaction terms improved the fit of the linear and non-linear regression models, respectively (see Appendix A in Chenoweth and Blows 2005; Hall et al. 2008 for application of this approach).

There were no detectable differences between breeding types (see Results) so we re-ran the analysis on the full dataset to generate linear selection gradients (β) and the γ matrix of quadratic and correlational selection coefficients. Relative fitness was not normally distributed, so we used randomization testing (see Mitchell-Olds and Shaw 1987) to assess the significance of each β and γ . Relative fitness values were randomized across the calls and selection gradients re-calculated using the Monte Carlo option in PopTools (ver. 2.6.9, CSIRO). This process was repeated 9999 times to obtain the expected distribution for each gradient under random female choice. The P -value is the proportion of randomizations in which the gradient estimate was equal to or farther away from zero than the original estimate (Bisgaard and Ankenman 1996; Blows and Brooks 2003).

To detect the major axes of nonlinear sexual selection we conducted a canonical rotation of the γ matrix (Phillips and Arnold 1989). This produced a $\mathbf{M}$ matrix containing 5 eigenvectors $\mathbf{m}_{1-5}$ (linear combinations of weighted values of the original five call parameters). The strength of linear and nonlinear sexual selection on each eigenvector is given by θ and λ_i (i.e. the eigenvalue), respectively. The values of θ and λ_i are the regression coefficients when $\mathbf{m}$ scores are regressed against relative fitness in separate linear and quadratic regression models (Bisgaard and Ankenman 1996). As with terms in the γ matrix, we tested the significance of θ and λ_i using randomization tests in PopTools.

Finally, to compare the multivariate attractiveness of the calls of inbred and outbred males we compared the $\mathbf{m}$ scores between inbred and outbred males using randomization tests (with 10,000 permutations) implemented in Poptools. To compare each $\mathbf{m}$ score, we first ran an ANOVA comparing the $\mathbf{m}$ score across inbred and outbred males. The $\mathbf{m}$ scores were then randomly shuffled between treatments (i.e. inbred versus outbred) and a second ANOVA on this shuffled data set performed. A Monte-Carlo simulation with 10,000 permutations was then performed and the number of times the F value of the ANOVA based on the real data exceeded the F value based on the shuffled data was calculated and converted to a two-tailed P -value following the protocol outlined in Manly (1997).

It might be argued that although we have treated them as such in the statistical analyses, the attractiveness scores of each male call are not independent of those of other male calls that were played on a given night (i.e. for every female that was attracted to one male call, there is one fewer choosing another male). However, the entire basis of selection analyses of mating success in large, natural populations [i.e. using the standard multiple regression methods of Lande and Arnold (1983) and subsequent elaborations], is that this non-independence is ignored. In contrast, say, two-choice mating experiments in the laboratory often explicitly take into account non-independence in the statistical analysis (e.g. use of the difference in association time between two males). Our study is an intermediate between these two extremes. We therefore also performed an extremely conservative analysis in which we simply compared the total number of females that were attracted to each call type (inbred or outbred) in a night using a paired t -test.

Visualization of the fitness surface

We used thin-plate splines (Green and Silverman 1994) to visualize the major axes of the fitness surface extracted from the canonical rotation. This non-parametric approach provides a less constrained view of the surface than the best quadratic approximation (Blows et al. 2003). We used the *Tsp* function in the *fields* package in R (<http://www.r-project.org>) to fit a spline surface using the value of the smoothing parameter, λ , that minimized the generalized cross-validation (GCV) score. We then plotted the surface in R using both the perspective and contour map views.

RESULTS

Calling effort

We monitored the calling effort of 1409 males. Calling effort was repeatable ($\chi^2_{1408} = 3387.79$, $P < 0.001$, $r_1 = 0.550$). Both inbred and outbred males that took longer to mature had a lower calling effort (inbred males $r_s = -0.290$, $P < 0.001$, $N = 664$, outbred males $r_s = -0.235$, $P < 0.001$, $N = 745$). Both older ($r_s = 0.112$, $P = 0.002$, $N = 745$) and heavier outbred males ($r_s = 0.109$, $P = 0.003$, $N = 738$) had a higher calling effort. For inbred males, there was no significant relationship between age and calling effort ($r_s = 0.060$, $P = 0.120$, $N = 664$), or weight and calling effort ($r_s = 0.020$, $P = 0.612$, $N = 663$). The correlations are, however, not significantly different for inbred and outbred males (age: $Z = 0.97$, $P = 0.33$; weight: $Z = 1.66$, $P = 0.11$).

On average, outbred males called 0.13 standard deviation units more per night than inbred males (Hedge's $d = 0.135$; 95% CI: 0.01 - 0.240). There was therefore a significant negative effect of inbreeding on calling effort (~30% reduction, Table 1). There was no significant heterogeneity in effect size among blocks ($Q_{\text{Total}} = 29.73$, $df = 27$, $P = 0.327$). This suggests that inbreeding had a constant effect and did not differ among families. Any differences in the direction and/or magnitude of inbreeding depression among blocks is likely to be attributed to sampling error. In the second analysis, where each block was weighted equally, inbreeding still significantly reduced calling effort (Wilcoxon's test: $Z = 2.11$, $P = 0.035$, $N = 28$ blocks).

Structure of the advertisement call

In total, we measured call parameters for 98 males. All five call parameters were repeatable among males (Table 2). Inbreeding had no effect on any call parameter (CIPD, ICD and CPN: see Table 3, DF: Mann-Whitney test: $Z = 0.307$, $P = 0.759$, $N = 19, 24$, TN: Mann-Whitney test: $Z = 0.416$, $P = 0.677$, $N = 19, 24$, Table 1).

Older males had both shorter CIPDs and ICDs, but age had no effect on the CPN. In contrast, males who took longer to mature had longer ICDs and more pulses in their chirps, but there was no effect on CIPD. The only effect of mass on calling was that heavier males had a lower CPN (Table 3). There was no interaction between inbreeding and male age, development time or mass affecting CIPD, ICD or CPN [all $P > 0.05$, except for ICD: the interaction between inbreeding and development time ($P = 0.046$), and for CPN: the interaction between inbreeding and male age ($P = 0.042$). For these two interactions, inspection of the graphs of ICD on development time, and CPN on male age showed that the regression lines for inbred and outbred males intercepted outside the range of development times/ages that were included in the experiments. We therefore removed these interactions from the respective models].

Table 1. The means $\pm$ SE for inbred and outbred males, δ (% change with inbreeding) and Hedge's d for the effect of inbreeding on calling effort and the five call parameters from this study and from Drayton et al. 2007 for comparison. Z and P - values are for the test of a significant difference in effect sizes between the two studies for each trait.

Trait	Mean $\pm$ SE (N), this study		δ this study	δ Drayton et al. 2007	d , this study (95% confidence interval)	d , Drayton et al. 2007 (95% confidence interval)	Z	P
	Inbred	Outbred						
Calling effort (s/night)	234.06 $\pm$ 38.93 (664)	335.32 $\pm$ 39.56 (745)	30.2	-5.0	0.10 (-0.01, 0.20)	-0.02 (-0.50, 0.45)	0.49	0.63
CIPD (s)	0.015 $\pm$ 0.001 (37)	0.016 $\pm$ 0.001 (61)	6.3	-34.4	0.14 (-0.27, 0.55)	-2.34 (-3.06, -1.62)	5.86	<0.001
ICD (s)	0.179 $\pm$ 0.025 (37)	0.157 $\pm$ 0.007 (61)	-14.0	-350.8	-0.21 (-0.62, 0.20)	-1.92 (-2.59, -1.24)	4.24	<0.001
CPN	6.21 $\pm$ 0.16 (37)	5.74 $\pm$ 0.13 (61)	-8.0	-17.9	-0.45 (-0.86, -0.04)	-1.04 (-1.64, -0.44)	1.58	0.11
DF (Hz)	4031.93 $\pm$ 24.38 (37)	4018.85 $\pm$ 11.74 (61)	-0.3	0.0	-0.11 (-0.52, 0.30)	0 (-0.57, 0.57)	0.31	0.76
TN	2.13 $\pm$ 0.19 (37)	1.96 $\pm$ 0.13 (61)	-8.6	0.4	-0.16 (-0.57, 0.25)	0.01 (-0.56, 0.58)	0.46	0.64

CIPD: chirp inter-pulse duration, ICD: inter-call duration, CPN: chirp pulse number, DF: dominant frequency, TN: trill number.

Bold values of δ indicate a significant difference between inbred and outbred males.

Bold P -values indicate that the effect sizes for a trait differ significantly between the two studies.

Table 2. The repeatability of call parameters among males (five calls per male, $N = 98$ males).

Call Parameter	Test Statistic	P -Value	r_1
CIPD	$F_{97,392} = 16.917^*$	<0.001	0.888
ICD	$F_{97,392} = 5.832^*$	<0.001	0.707
CPN	$F_{97,392} = 10.384^*$	<0.001	0.824
DF	$\chi^2_{97} = 464.424^\dagger$	<0.001	0.979
TN	$\chi^2_{97} = 393.908^\dagger$	<0.001	0.908

CIPD: chirp inter-pulse duration, ICD: inter-call duration, CPN: chirp pulse number, DF: dominant frequency, TN: trill number. r_1 : intra-class correlation coefficient.

*One-way ANOVA, † Kruskall-Wallis test.

Table 3. The effect of inbreeding, development time, age and weight on CIPD, ICD and CPN. P -values are from the final model if the term was significant, or when it alone was added to the final model if non-significant (see text). P -values significant at the 0.05 level are in bold.

	CIPD				ICD				CPN		
	$d.f.$	F	P		$d.f.$	F	P		$d.f.$	F	P
Inbreeding	1,14	0.711	0.413		1,14	0.185	0.674		1,14	1.658	0.219
Development time	1,53	2.465	0.122		1,53	5.180	0.027		1,53	5.658	0.021
Age	1,54	9.857	0.003		1,53	4.193	0.046		1,52	0.223	0.639
Weight	1,53	1.719	0.196		1,52	1.761	0.190		1,53	4.613	0.036

DF was not correlated with development time, age or weight. TN was not correlated with development time or weight. None of these correlations differed significantly between inbred and outbred males (Table 4). Older inbred males had fewer trills/phrase in their calls but there was no such relationship for outbred males. The relationship between age and TN differed significantly between inbred and outbred males (Table 4).

Table 4. Correlations between DF (dominant frequency) and TN (trill number), and development time, age and weight. *Z* and *P* values are for the comparison of *r_s* between inbred and outbred males.

	DF					TN			
	<i>r_s</i> inbred (<i>P</i>)	<i>r_s</i> outbred (<i>P</i>)	<i>Z</i>	<i>P</i>		<i>r_s</i> inbred (<i>P</i>)	<i>r_s</i> outbred (<i>P</i>)	<i>Z</i>	<i>P</i>
Development time	-0.233 (0.165)	0.029 (0.823)	1.29	0.197		0.132 (0.436)	-0.166 (0.200)	1.45	0.146
Adult age	-0.241 (0.150)	0.111 (0.393)	1.73	0.084		-0.442 (0.006)	-0.020 (0.880)	2.28	0.023
Weight	-0.304 (0.067)	0.059 (0.651)	1.81	0.071		-0.036 (0.833)	-0.112 (0.389)	0.37	0.711

All *r_s* *N* = 37, 61. *P*-values significant at the 0.05 level are in bold.

Hedge’s *d* for the effect of inbreeding on calling effort and the five call parameters for both this study and Drayton et al. (2007) are presented in Table 1. The effect sizes for inbreeding differed significantly between the Canberra (Drayton et al. 2007) and Smith’s Lake populations (this study) for CIPD and ICD, with a significantly larger effect of inbreeding on both traits in the Canberra population.

Male attractiveness in the field

There was no difference in the number of females attracted to the calls of inbred and outbred males in either the first (*t*₂₀ = 1.083, *P* = 0.292, inbred males = 4.1 ± 0.75, outbred males = 3.2 ± 0.71 females/night) or second experiment (*t*₃₀ = 0.269, *P* = 0.790, inbred males = 2.0 ± 0.66, outbred males = 2.3 ± 0.66 females/night). The pooled data also showed no inbreeding effect (*t*₇₂ = 0.587, *P* = 0.559, inbred males = 3.1 ± 0.54, outbred males = 2.7 ± 0.50 females/night). In addition, when we simply compared the total number of females attracted to each call type (inbred or outbred) per night there was no effect of inbreeding (*t*₉ = 0.823, *P* = 0.432). Therefore when calling effort was standardized between inbred and outbred males (because calls were played continuously), there was no difference in attractiveness, suggesting that inbreeding did not alter any aspects of call structure that are important to females. No females were caught on the silent control. Therefore females caught at speakers are actively attracted to the call playbacks.

Estimating linear and non-linear selection, and comparing attractiveness

Inbred and outbred calls did not differ in the strength of linear ($F_{5,68} = 0.693, P = 0.630$), quadratic ($F_{5,58} = 1.123, P = 0.357$) or correlational sexual selection ($F_{10,38} = 0.582, P = 0.818$), so we estimated sexual selection gradients on the combined data set. There was significant linear selection favouring lower dominant frequency (DF), shorter intercall durations (ICD) and higher chirp pulse number (CPN). The only significant non-linear selection was negative quadratic selection (stabilizing) on CIPD (Table 5).

Table 5. The vector of standardized linear selection gradients (β) and the matrix of standardized quadratic and correlational selection gradients (γ).

		γ				
	β	CIPD	DF	ICD	TN	CPN
CIPD	0.228	-0.234				
DF	-0.275	-0.060	-0.009			
ICD	-0.258	-0.334	0.426	-0.045		
TN	-0.083	0.279	-0.241	-0.191	-0.023	
CPN	0.310	-0.349	0.009	0.493	-0.066	0.038

Gradients in bold are significant at $P < 0.05$. 10,000 permutations to test for significance.

Canonical rotation of the γ matrix of non-linear selection gradients revealed significant concave up (disruptive) selection along $\mathbf{m}_1$ and stabilizing selection along $\mathbf{m}_5$, indicating that sexual selection approximates a multivariate saddle (Fig. 3). There was also significant positive directional selection along $\mathbf{m}_2$ (favouring a combination of higher CPN, higher TN and a lower DF: Fig. 4a) and along $\mathbf{m}_4$ (favouring higher CPN, longer CIPD and shorter ICD: Fig. 4b) (Table 6). None of the axes differed between inbred and outbred calls: $\mathbf{m}_1$ ($P = 0.084$), $\mathbf{m}_2$ ($P = 0.107$), $\mathbf{m}_3$ ($P = 0.573$), $\mathbf{m}_4$ ($P = 0.702$) and $\mathbf{m}_5$ ($P = 0.695$), suggesting inbred and outbred males do not differ in multivariate attractiveness.

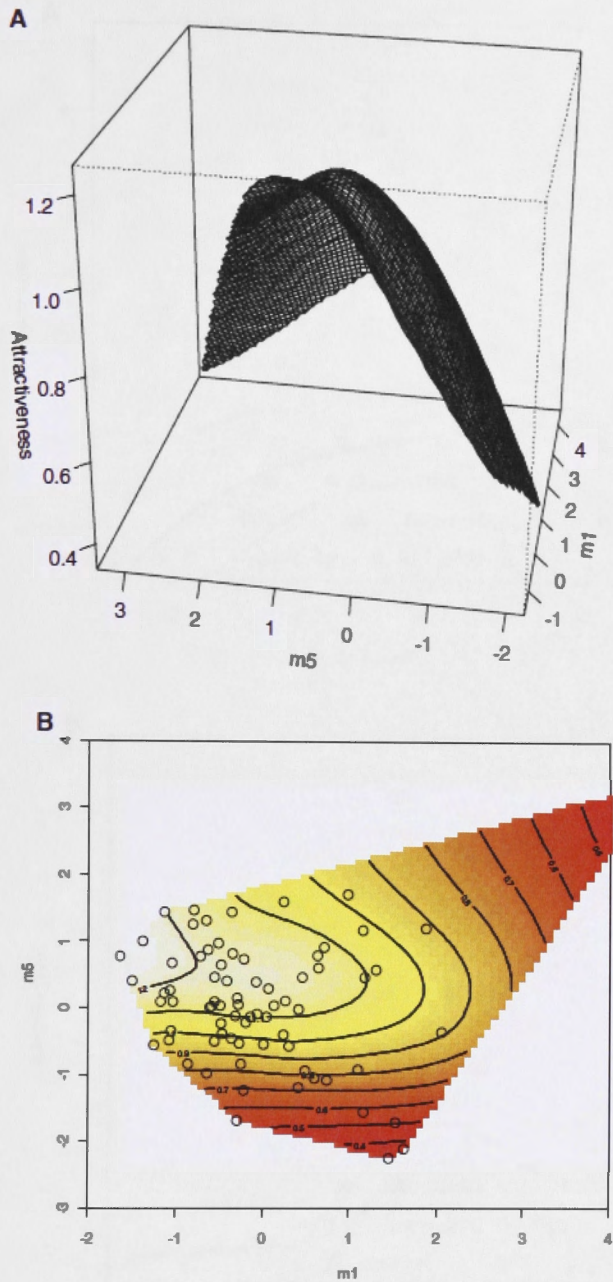


Figure 3. Thin-plate spline visualization of the fitness surface along the two major axes of non-linear selection, m_1 and m_5 . (a) Perspective view, (b) contour view with data points overlaid. Yellow regions indicate higher fitness.

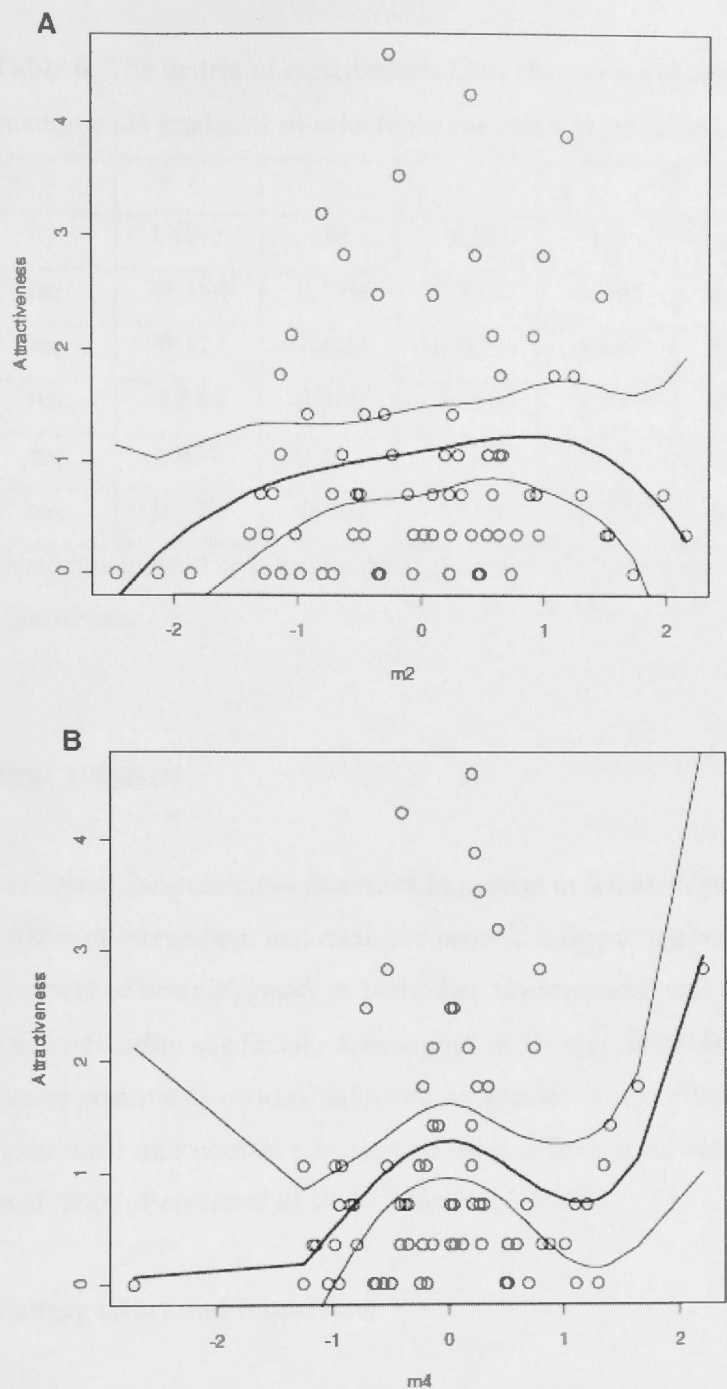


Figure 4. Univariate cubic spline visualization of sexual selection operating on (a) m_2 and (b) m_4 with data points overlaid. 95% confidence intervals are also provided.

Table 6. The matrix of eigenvectors from the canonical rotation of γ . The linear (θ) and quadratic (λ) gradients of selection along each eigenvectors are given.

						Selection	
	CIPD	DF	ICD	TN	CPN	θ	λ
m₁	-0.354	0.379	0.588	-0.345	0.515	-0.148	0.466
m₂	-0.124	-0.624	0.027	0.437	0.636	0.297	0.056
m₃	0.284	0.500	0.290	0.765	0.008	-0.209	-0.087
m₄	0.431	0.351	-0.574	-0.177	0.575	0.343	-0.326
m₅	0.770	-0.307	0.489	-0.271	0.015	0.161	-0.381

Gradients in bold are significant at $P < 0.05$. 10,000 permutations to test for significance.

DISCUSSION

We tested the prediction that traits important in female mate choice are sensitive to the effects of inbreeding, and therefore provide a signal of a male’s phenotypic condition, his level of heterozygosity or both. The advertisement call traits that we examined are known to influence female mate choice in *Teleogryllus commodus*: female choice exerts strong positive directional selection for greater calling effort and stabilising and directional multivariate selection on the five finer-scale aspects of call structure (Brooks et al. 2005; Bentsen et al. 2006; Hunt et al. 2007).

Calling effort and inbreeding

We found that one generation of full sibling mating substantially reduced male nightly calling effort. While this could be due to the effect of inbreeding at loci that directly code for calling behaviour (indicating directional dominance for the mechanical traits underlying calling), it seems more likely that inbreeding depression in calling effort is mediated by effects on condition. Calling in crickets, including *T. commodus*, has been shown to be condition dependent (Wagner and Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003; Hunt et al. 2004b; Hedrick 2005; Judge et al. 2008; Maklakov et al. 2008) and to incur substantial energetic costs (Prestwich and Walker 1981; Kavanagh 1987; Hack 1998). Given the detrimental effects of inbreeding on general

fitness in this species (Drayton et al. 2007), it is plausible that inbreeding reduces the ability of males to acquire and assimilate the nutritional and energetic resources needed for high calling rates. Studies with *T. commodus* (Bentsen et al. 2006), and other crickets (Hedrick 1986; French and Cade 1989; Crnokrak and Roff 1995; Holzer et al. 2003) have shown that females prefer males with higher calling effort. Female *T. commodus* may benefit from this preference because calling effort is condition-dependent and might therefore signal male genetic quality or direct benefits (Hunt et al. 2004b). It may also be easier to locate males that call for longer periods (Bentsen et al. 2006). Given the reduction in calling effort with inbreeding, females choosing males based on call rate are using a signal that is correlated with male condition and/or heterozygosity. The relative importance of male heterozygosity or condition in generating benefits of female mate choice in *T. commodus* is uncertain, although it is conceivable that calling effort signals both characters. Models have shown that the evolution of mate choice for heterozygosity requires a positive correlation between the heterozygosity of parents and offspring (Fromhage et al. 2009). The extent to which parents and offspring resemble each other in heterozygosity in the field population of *T. commodus* is unclear. Nevertheless, the decline in calling effort of inbred males indicates that calling effort does signal aspects of male quality.

Call structure, attractiveness and inbreeding

We found no effect of inbreeding on any of the finer-scale call parameters (ICD, CPN, CIPD, TN and DF), indicating low directional dominance variance for these traits (Roff 1998; Rantala and Roff 2007). These traits do not seem to be condition-dependent in *T. commodus* as reducing dietary protein had no effect on any of these call parameters (Hunt et al. 2004b). Nonetheless, these call parameters influence female choice (Brooks et al. 2005; Bentsen et al. 2006), even though they do not seem to reflect dietary condition or levels of inbreeding (in the $F = 0$ to 0.25 range). These finer scale call parameters might therefore have evolved to signal information other than male condition or heterozygosity to females. For example, finer scale call parameters could play a role in species recognition (Hill et al. 1972), which is supported by the significant stabilizing selection detected for finer scale call structure in this study and others (e.g. Brooks et al. 2005; Bentsen et al. 2006; Hunt et al. 2007), indicating that males who deviate in either direction from optimum trait value combinations might not be recognized by conspecific females.

Despite our study finding no effect of inbreeding on the five measured structural components of the advertisement call, it is possible that inbreeding altered unmeasured call characteristics that are still detected by females. Consequently, we tested the attractiveness of advertisement calls in the field by measuring the number of females that approached the calls of inbred and outbred males. We tested the attractiveness of advertisement calls in the field because studies have shown that estimates of inbreeding depression on male attractiveness can be masked in the laboratory. This could arise because the detrimental effect of inbreeding on trait development or production is magnified under harsher field conditions and/or because female choice differs between the laboratory and field. For example, Joron and Brakefield (2003) demonstrated that a small reduction in mating success of male butterflies in captivity was greatly accentuated in a greenhouse environment where unconstrained flight and a fuller expression of courtship behaviour were possible. Unlike the study by Joron and Brakefield, however, male sexual traits were not expressed under field conditions in our study as inbred and outbred calls were recorded from laboratory reared males. Nonetheless, we ensured that female choice was expressed in the field. By testing the attractiveness of the calls here we created conditions that were as close as possible to those under which females would naturally express a choice for inbred and outbred males while also controlling for calling effort by using a playback design.

In our field experiment call rate did not differ between inbred and outbred calls because each call was broadcast continuously throughout the night. This allowed us to isolate the effect of any structural changes in the advertisement call. There was no difference in the number of females that were attracted to each call type, suggesting that inbreeding had no effect on call structures important to females. This was further supported by the lack of any difference in the scores for the major axes of multivariate selection between inbred and outbred males. This, however, does not mean that inbreeding will not affect male reproductive success. Recall that inbred males called significantly less (30% reduction) than outbred males. They will therefore attract far fewer females as an earlier field study at the same site showed a very strong female preference for higher call rate (Bentsen et al. 2006). It is also noteworthy that we detected inbreeding depression in calling effort under favorable laboratory conditions of unrestricted food, no competition for calling sites and controlled temperatures. Under harsher field conditions, the reduction in calling would most likely exceed 30%. Furthermore, once a male has

attracted a female, he produces a different courtship song, to induce her to mate. Inbreeding depression in the courtship song is currently under investigation in our laboratory. There is evidence that courtship songs are condition dependent (Tregenza et al. 2006, but see Wagner and Reiser 2000; Gray and Eckhardt 2001). For example, male *T. oceanicus* produce different courtship songs after suffering an immune challenge (Tregenza et al. 2006). There is also a link between courtship call features and cryptic female choice (spermatophore retention by females) in *T. commodus* (Hall et al. 2008). Any changes in the courtship song with inbreeding might further reduce the reproductive success of inbred males if they fail to successfully court females.

A comparison of studies

We have previously investigated the effect of inbreeding on advertisement calling in a Canberra (Australia) population of *T. commodus*. In accordance with the results of the current study, we found no effect of inbreeding (one generation of brother sister mating) on dominant frequency and trill number in the Canberra population. In contrast, in the Canberra population inbreeding had no significant effect on nightly calling effort, but caused highly significant changes in CIPD, ICD and CPN (Drayton et al. 2007; Table 1). The effect sizes for inbreeding however, only differed significantly between studies for CIPD and ICD, with the effect of inbreeding being of a larger magnitude in the Canberra population for these two traits (Table 1). It would therefore seem that aspects of advertisement call structure vary in their susceptibility to inbreeding depression between the Canberra and Smith's Lake population (450 km apart). Several factors might account for these differences. One possibility is that the two populations differ in the level of directional dominance variance (and therefore susceptibility to inbreeding depression) underpinning different components of call structure because of differences in the strength of selection imposed by females (which depletes additive genetic variance) for different trait combinations between the populations. To test this prediction would require a population comparison of female choice criteria. In addition, natural selection on call traits might differ between the populations due to differences in the occurrence of acoustically orientating predators or parasitoids (e.g. Zuk et al. 1993; Rotenberry et al. 1996).

Multivariate sexual selection on call structure

While the main aim of the selection analysis was to compare the multivariate attractiveness of inbred and outbred males, the resulting measure of multivariate sexual selection deserves mention. The selection analysis revealed significant concave up (disruptive) selection along $\mathbf{m}_1$ (most heavily loaded with ICD and CPN) and stabilizing selection along $\mathbf{m}_5$ (heavily loaded with CIPD, and to a lesser degree, ICD). The resulting fitness surface is saddle shaped (Fig. 3), indicating two fitness peaks. The large loading of CIPD in $\mathbf{m}_5$ and the resulting involvement of this trait in significant stabilizing selection is in contrast to both Brooks et al. (2005) and Bentsen et al. (2006), who failed to find strong evidence for a role of CIPD in stabilizing selection in *T. commodus* from Smith's Lake. It is possible that there are temporal differences in multivariate sexual selection operating in the Smith's Lake population. We also found evidence of directional selection on call traits. Selection favoured a lower dominant frequency (DF), shorter intercall duration (ICD), a higher number of pulses in the chirp (CPN), a higher number of trills in a phrase (TN) and longer intervals between the pulses in the chirp (CIPD). Our results showing directional selection on DF, CPN and CIPD are consistent with Bentsen et al. (2006) who investigated multivariate sexual selection on call structure and calling effort in the same field population of *T. commodus*.

Interestingly, Bentsen et al. (2006) found significant directional selection for longer ICD, whereas Brooks et al. (2005) and our study found significant directional selection for shorter ICDs. In both our study and Brooks et al. (2005), calls were played to females in a continuous loop. However, Bentsen et al. (2006) varied the number of phrases repeated in a five minute loop, meaning that there was a silent period between the end of the last phrase in the loop and the first phrase in the next loop. Playbacks therefore consisted of discrete calling bouts and inter-bout intervals of silence. Females preferred males with longer call bouts, and these were the loops that contained both longer phrases and a greater number of phrases. A longer interval between phrases (i.e. longer ICD) increases the overall bout length by lengthening each phrase. Consequently, in this case, selection for shorter inter-bout intervals (less time 'off air') overrides selection for shorter intercall durations (ICD), which increases total time on air when calling is continuous (Bentsen et al. 2006).

In conclusion, our results show that the actual call of an inbred ($F = 0.25$) male *T. commodus* is no less attractive to a female than that of an outbred male in a natural field setting. This is either because inbreeding does not affect the structure of the advertisement call, or because any structural changes are inconsequential to females. It is, of course, possible that the effects of inbreeding on call structure will only become apparent in this population after more severe inbreeding (e.g. Zajitschek et al. 2009), or under harsher rearing conditions. We did find, however, that inbred males have a far lower call rate than outbred males. This should cause a marked reduction in the number of females mating with inbred males. This is because males who call less often are harder for females to locate and/or because female *T. commodus* actively prefer males who call more (Bentsen et al. 2006). In sum, advertisement calling (i.e. calling effort) signals a male's inbreeding status, and females can therefore discriminate against inbred males. Although the avoidance of inbred males may not be the reason why female mating preferences evolved, and the effect of mating with relatively outbred males on offspring fitness is unknown, the predicted reduction in the mating success of inbred males clearly has fitness consequences for individuals, and wider ramifications for population genetic structure.

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APPENDIX 3: INBREEDING AND COURTSHIP CALLING IN THE CRICKET *TELEOGRYLLUS COMMODUS*

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ABSTRACT

Male field crickets produce two acoustic signals for mating: advertisement calls and courtship calls. While the importance of advertisement calling in mate attraction is well understood, the function of courtship calling is less clear. Here we tested if the courtship call of male crickets *Teleogryllus commodus* signals aspects of male quality by comparing the calls of inbred and outbred males. We examined the effect of one generation of full sibling mating on fine-scale call structure, along with several life history traits. Inbreeding reduced nymph survival but had no significant effect on weight or development time. Inbreeding resulted in a small but significant change in two of the six call parameters measured. We then tested if inbreeding affects call trait combinations that are important to females by using the results of a previous selection analysis to compare the multivariate attractiveness of the calls of inbred and outbred males. There was no difference. We conclude that the courtship call of *T. commodus* is not a reliable signal of aspects of male quality that are affected by inbreeding (which generally reduces fitness-enhancing traits). It might, however, signal components of male fitness that are not affected by changes in heterozygosity.

Keywords: female choice, heterozygosity, condition dependence, selection analysis, courtship call, sexual selection

INTRODUCTION

Males of many species possess multiple sexually selected ornaments that females may use when evaluating prospective mates (Moller & Pomiankowski, 1993; Candolin, 2003). Field crickets are a useful model system to investigate multiple sexual signals during mate choice. Males produce two acoustic sexual signals prior to mating: an advertisement call and a courtship call. The advertisement call attracts sexually receptive females from a distance for mating (Loher & Rence, 1978; Campbell & Shipp, 1979; Evans, 1988). Female preferences for advertisement calls have been well studied (e.g. Hedrick, 1986; Stout & McGhee, 1988; Wagner, 1996; Wagner & Reiser, 2000; Holzer *et al.*, 2003; Brooks *et al.*, 2005; Bentsen *et al.*, 2006; Wagner & Basolo, 2007). Several studies have shown that preferences for specific aspects of advertisement calling are likely to exist because they provide information about male traits that confer direct benefits to females (Wagner & Basolo, 2007) as well as traits that are likely to confer indirect genetic benefits if they are heritable and positively correlated with net fitness, such as body condition (Scheuber *et al.*, 2003a, b; Judge *et al.*, 2008), dietary intake (Maklakov *et al.*, 2008) and immune function (Jacot *et al.*, 2004). At a higher level, it is also well established that advertisement calling signals species identity. As expected for a signal used in species recognition, there is evidence for strong stabilising selection on advertisement calls (e.g. Brooks *et al.*, 2005; Bentsen *et al.*, 2006). At a more proximate level, stabilising selection on advertisement calls can also be imposed by limitations of the female auditory and nervous system (Brooks *et al.*, 2005).

In contrast, the courtship call has received less attention and explaining its evolution and maintenance is more challenging (Gray & Eckhardt, 2001; Zuk *et al.*, 2008; Rebar *et al.*, 2009). Once a female approaches a male and the two are in physical contact, the male switches from advertisement calling to produce the distinctive courtship call. This raises an obvious question. If the advertisement call is sufficient to bring a male and a female together to mate, why produce a second signal (Tregenza *et al.*, 2006; Rebar *et al.*, 2009), especially when there are costs of acoustic signals for both males (review: Zuk & Kolluru, 1998; Kotiaho, 2001) and females (e.g. Martin & Wagner, 2010)? It appears, however, that the courtship call is important to induce female mounting behaviour (Crankshaw, 1979; Burk, 1983; Adamo & Hoy, 1994; Libersat *et al.*, 1994; Balakrishnan & Pollack, 1996; but see Bailey *et al.*, 2008), which is necessary for spermatophore transfer to the female. Several recent studies have demonstrated female

preferences for specific courtship call components that appear to signal aspects of male fitness. For example, female variable field crickets *Gryllus lineaticeps* prefer courtship calls with a higher chirp rate, which can generate a different pattern of mate choice than that initially predicted based on preferences for advertisement calls (Wagner & Reiser, 2000). Female Mediterranean field crickets *Gryllus bimaculatus* prefer courtship calls with a greater expression of components that are positively correlated with an immune response (encapsulation rate) (Rantala & Kortet, 2003). Likewise, male *Teleogryllus oceanicus* produce structurally different courtship calls after an immune challenge. Furthermore, females responded more rapidly to males in response to playback of courtship calls that were experimentally manipulated to resemble those of males with higher encapsulation ability (Tregenza *et al.*, 2006). Rebar *et al.*, (2009) showed that the courtship calls of attractive *T. oceanicus* males differ from those of less attractive males, and that a previously unattractive male could be made more attractive by muting him and playing the courtship call of a more attractive male.

One way to test whether male sexual traits signal aspects of male quality is to create classes of individuals that can be assigned high or low phenotypic quality and to compare the expression of sexual traits between these classes. An aspect of quality that can be easily manipulated in the laboratory is condition. This is usually achieved by changing external environmental factors that affect condition such as diet or food availability (e.g. Holzer *et al.*, 2003; Scheuber *et al.*, 2003a, b; Judge *et al.*, 2008). The few studies that have investigated the condition dependence of the courtship call by manipulating diet have found no effect (*G. texensis*: Gray & Eckhardt, 2001; *G. lineaticeps*: Wagner & Reiser, 2000). An alternative, yet underexploited method of creating classes of individuals that can be assigned high or low quality status is to manipulate condition via inbreeding (e.g. Bolund *et al.*, 2010; Drayton *et al.*, 2010; review: Cotton *et al.*, 2004). Inbreeding (the mating of relatives) increases homozygosity across the genome and usually results in a decline in fitness known as inbreeding depression (review: Keller & Waller, 2002; Armbruster & Reed, 2005). This reduction in fitness mainly arises due to the unmasking of partially or fully recessive deleterious alleles, as well as the loss of advantageous heterozygosity (Charlesworth & Charlesworth, 1999; Charlesworth & Willis, 2009). Inbreeding could affect sexual traits due to lower heterozygosity at loci that directly code for them. If, however, sexual traits are condition dependent (review: Cotton *et al.*, 2004), and inbreeding reduces the general ability of individuals to acquire and/or assimilate the energetic and nutrient

resources that are available to be allocated to sexual signalling (i.e. lowers condition *sensu* Rowe & Houle, 1996; review: Tomkins *et al.*, 2004), then we expect reductions in sexual traits with inbreeding.

Inbreeding depression in sexually selected traits may also indicate that these traits can reveal a male's genome wide heterozygosity to females. Recent models for the evolution of female mate choice have proposed that females can benefit from mating with males with above average heterozygosity (Mays & Hill, 2004; Kempenaers, 2007; Fromhage *et al.*, 2009). This is because, under certain conditions based largely on rates of immigration between meta-populations, males with greater heterozygosity are more likely to carry locally rare alleles dissimilar to those in the average female.

Consequently, mating with such males yields offspring with above average heterozygosity (i.e. mean heterozygosity is heritable; Mitton *et al.*, 1993; Brown, 1997 but see Puurtinen *et al.*, 2009). Female choice for heterozygous males will therefore elevate offspring fitness whenever heterozygosity increases offspring performance. A decline in sexual trait expression with inbreeding provides direct evidence that these traits can signal genome wide heterozygosity. More generally, inbreeding in normally outbreeding species almost always reduces fitness (Falconer & Mackay, 1996). We predict that inbreeding will reduce many components of male fitness such as immune function, performance capacity and condition. If a sexual trait is a signal of one or more of these measures of male quality, and/or genome wide heterozygosity, then inbreeding should lead to signals that are less attractive to females.

In this study, we investigate the extent to which courtship call parameters signal male fitness in the black field cricket *Teleogryllus commodus*. To do this, we quantified the effect of one generation of brother-sister matings ($F = 0.25$) on courtship calls. We have previously shown that inbreeding ($F = 0.25$) reduces egg hatching success, nymphal survival and adult lifespan in *T. commodus* (Drayton *et al.*, 2007). There is therefore good evidence for inbreeding depression in fitness. We have also shown that inbreeding reduces the advertisement call rate (Drayton *et al.*, 2010), and alters fine-scale advertisement call structure (Drayton *et al.*, 2007). In contrast, there is currently no data on whether or not the courtship call signals aspects of male quality. Hall *et al.* (2008) have shown that courtship calls of *T. commodus* are under post-copulatory sexual selection because call components affect the length of time that a female retains the externally attached spermatophore after mating. The longer the attachment time, the

greater the number of sperm transferred (Bussiere *et al.*, 2006; Hall *et al.*, 2010). Hall *et al.* (2008) characterized the major axes of multivariate sexual selection on courtship call structure using spermatophore attachment times as the measure of fitness. They found significant non-linear multivariate selection on call structure and male size (Hall *et al.*, 2008). We worked with the same study population and measured the same courtship call parameters as Hall *et al.* (2008). This allowed us to compare the multivariate attractiveness of inbred and outbred males along significant axes of multivariate selection. If the courtship call signals aspects of male quality that are reduced by inbreeding, such as male condition or heterozygosity, then we predict that the call will change significantly with inbreeding, and that this will lower the predicted multivariate attractiveness of inbred males. Finally, we also quantified the effect of inbreeding on three life history traits in both males and females: maturation weight, development time and nymph survival.

METHODS

Generation and rearing of inbred and outbred crickets

Inbred individuals were created by full sibling matings ($F = 0.25$) and their fitness compared with that of outbred crickets ($F = 0$). Full sibling families were derived from approximately 70 wild caught gravid females collected in May 2006, at Smiths Lakes, NSW. Offspring from wild caught females were reared to adulthood (when communally rearing all crickets in the study, males and females were always separated before sexual maturity to ensure virginity) and then paired at random (a male and female in a pair did not share the same wild caught mother) to create full sibling families. Each full sibling family was reared in a separate 43x30x13 cm plastic tub with dry cat food (KiteKat Krunch, Uncle Ben's, Raglan, Australia) and water provided *ad libitum*.

The full sibling families were grouped into pairs, hereafter known as blocks ($N = 33$ blocks). We ensured that the two families in a block were unrelated to each other. In each block, brothers and sisters from both full sibling families were mated to create two inbred genotypes. Outbred genotypes were created by reciprocal matings of a male and a female from each family in the block. For example, in a block consisting of full sibling families A and B, inbred progeny were generated by mating brothers and sisters

from family A, and brothers and sisters from family B, while outbred progeny were generated by mating a male from family A with a female from family B, and a male from family B with a female from family A (Fig. 1). This design generated four offspring genotypes (two inbred and two outbred) per block. After mating females were provided with moist cotton wool (“egg pads”) for egg laying. These were checked every three days for emerging nymphs. Upon emerging, nymphs from the same pairing were reared communally for 20 days in 9x9x5cm plastic tubs with food and a piece of moist cotton wool. After 20 days, each nymph was transferred to an individual container (9x9x5cm, i.e. one nymph/container) with a small tube of water plugged with cotton wool at the open end, a piece of cat food and a cardboard egg cup for shelter. Nymphs were not transferred to individual containers immediately after emerging because they were small enough to be crushed by the water tube. The density of nymphs during the communal stage was very similar because, on average, we set up 37.5 ± 3.3 ($\pm$ SE) nymphs of each inbred genotype and 39.4 ± 3.3 nymphs of each outbred genotype per block. It is unlikely that nymphs competed for resources during the communal rearing because they were very small and therefore spacing between individuals was large, and food and water was provided *ad libitum*. Once set up individually, food and water was replaced every 10 days. Dead nymphs were recorded to calculate nymph survival (the proportion of nymphs that reached the final adult moult). Nymphs nearing maturity were checked daily to record development time (days from hatching to the final adult moult). All crickets were weighed after their final adult moult (i.e. maturation weight). All the males used to test the effect of inbreeding on courtship calling were at least 10 days post maturation to ensure that they were sexually mature. Crickets were maintained at 26-28°C on a 12:12 photoperiod.

Block	Matings			
	Inbred 1	Outbred 1	Outbred 2	Inbred 2
1	A♂ A♀	A♂ B♀	B♂ A♀	B♂ B♀
2	C♂ C♀	C♂ D♀	D♂ C♀	D♂ D♀
3...	E♂ E♀	E♂ F♀	F♂ E♀	F♂ F♀
...33	Y♂ Y♀	Y♂ Z♀	Z♂ Y♀	Z♂ Z♀

Figure 1. The mating design showing Block 1 (comprising full sibling families A and B), Block 2 (comprising full sibling families C and D) and so on. There are two inbred and two outbred genotypes generated per block.

The structure of the courtship call

The courtship call

Male crickets call using a stridulatory apparatus consisting of a file and scraper on the forewings. The most basic unit of a call is the pulse. Each pulse is produced by closing the wings once (Kavanagh, 1987). Pulses of the courtship call are grouped together into chirps and trills. These are arranged into repeated phrases. Each phrase consists of a single, amplitude-modulated chirp, followed by a variable number of trills that are consistent in their intensity (Hall *et al.*, 2008) (Fig. 2).

Recording and analysis of the courtship call

To record courtship calls, we set up males in 9x9x5cm recording chambers (one male per chamber) with a condenser microphone mounted in the lid. To induce a male to call, a virgin stock female was introduced into the recording chamber. We recorded 1-2 minutes of the courtship call using a digital recorder (MicroTrack 24/96, M-Audio, Irwindale, CA, USA). A power unit, containing a 9 volt battery, was in series between the microphone and the recorder to power the microphone. If the female mounted the male during recording, we gently shook the chamber to interrupt the mating. After each recording, the male and female were removed from the chamber and weighed. We also noted the age of the male and the ambient temperature. Male crickets *T. oceanicus* can adjust the quality of their ejaculates according to female matings status (Thomas & Simmons, 2007). It is therefore possible that male *T. commodus* might adjust courtship depending on the phenotype of the female. Consequently, only virgin females were used to induce courtship and the allocation of females to inbred and outbred males was randomised.

Courtship calls were analyzed using Raven Pro 1.3 sound analysis software (Cornell Laboratory of Ornithology, Ithaca, NY, USA; www.birds.cornell.edu/raven). We measured the following six call parameters of five randomly chosen phrases from the

courtship call of each male: dominant frequency (DF), the number of pulses in the chirp [chirp pulse number (CPN)], the duration of the interval between the last two pulses in the chirp [the chirp inter-pulse duration (CIPD)], the duration of the interval between the chirp and the first trill [chirp-trill interval (CTI)], the number of trills in a phrase [trill number (TN)] and the length of the first trill [trill 1 length (T1L)] (Fig. 2). Calls were filtered before analysis to remove ambient noise below 3kHz and above 6kHz.

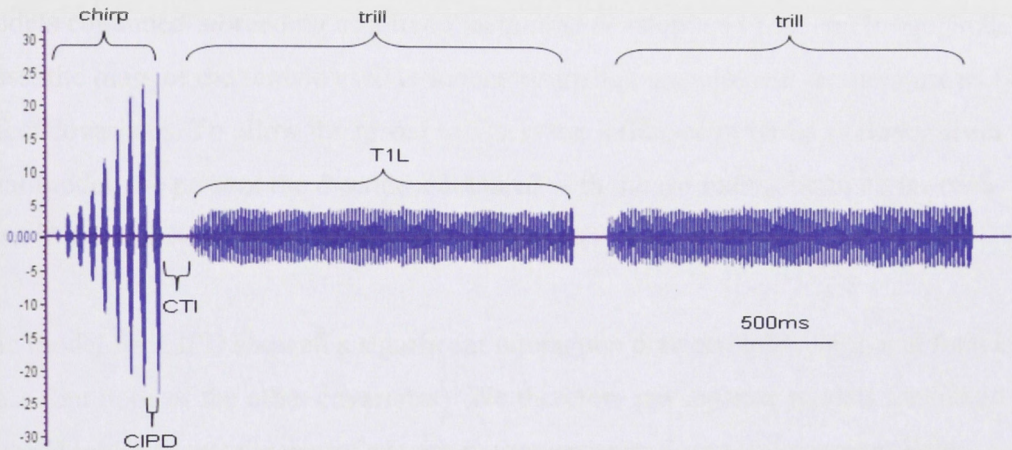


Figure 2. The courtship call of *T. commodus*. Each phrase consists of a single, amplitude-modulated chirp, followed by a variable number of trills. The phrase shown here has two trills. We measured: dominant frequency (DF), chirp pulse number (CPN), chirp inter-pulse duration (CIPD), chirp-trill interval (CTI), trill number (TN) and the length of the first trill (T1L). The vertical axis represents the amplitude of the sound produced.

Statistics

Structure of the courtship call

The repeatability of each call parameter among males was assessed using a one-way ANOVA or Kruskal-Wallis test. All call parameters were highly repeatable (see Results), so we calculated a mean value of CIPD, CTI, DF, T1L, TN and CPN for each male. To test for an effect of inbreeding on CIPD, CTI, T1L and CPN we ran separate linear mixed models in S-Plus 7.0. Where necessary, variables were transformed to ensure that residuals were normally distributed and homoscedastic. We used a model

simplification approach, initially fitting a full model that included all two-way interactions. We then removed fixed terms, starting with interactions, until the final model only contained significant terms. Random terms were always retained as part of the experimental design (Crawley, 2002). For all models, block and the interaction between inbreeding and block were included as random factors. This controlled for variation in the mean value of call parameters across blocks (i.e. random intercepts), and differences in the effect of inbreeding among blocks (i.e. random slopes). The initial models contained inbreeding as a fixed factor and development time, male age, male mass, the mass of the female used to induce courtship, and ambient temperature as fixed effect covariates. To allow the reader to assess the influence of terms excluded from the final model, we present the *P*-value associated with the parameter estimate for each term if it is individually included in the final model.

The model for CIPD showed a significant interaction between inbreeding and female mass (but none of the other covariates). We therefore ran separate models for inbred and outbred males to test for the effects of other covariates. We used Spearman Rank correlations (r_s) to assess the bivariate relationships between DF or TN and development time, male age, male and female mass and temperature, as neither call parameter could be transformed to fit parametric assumptions.

To test the effect of inbreeding on CIPD, TN and DF we ran separate meta-analyses. Each block contributed one effect size (Hedge's *d*) per call parameter based on the standardised difference in means between inbred and outbred males. We corrected for small sample size effects using *J* [see Rosenberg *et al.* (2000); note: some authors refer to Hedge's *d* as *g*, e.g. Cooper *et al.*, 2009)]. We then ran a random effects model in Metawin 2.0 to calculate the mean effect size. This weights each effect size by the inverse of its variance, which gives greater weighting to blocks where the estimate of the inbreeding effect was more precise. We then examined the non-parametric, bootstrapped 95% confidence interval to determine whether the mean effect of inbreeding differed significantly from zero. This meta-analysis is equivalent to a standard parametric two-way mixed model ANOVA (block by inbreeding), but does not require that the call parameters are normally distributed.

Maturation weight, development time and nymph survival

To test for an effect of inbreeding on maturation weight, we ran a linear mixed model in S-Plus 7.0 (see above). Block and the block by inbreeding interaction were random factors and the initial model contained inbreeding and sex as fixed factors. Development time could not be transformed to conform to parametric assumptions so we calculated separate means for each sex for inbred and outbred individuals in each block and compared the means using two Wilcoxon's tests. There was no inbreeding effect (see Results). To test for any difference between the sexes we therefore pooled inbred and outbred individuals, calculated the mean development time for males and females in each block, and compared the means with a Wilcoxon's test. For the effect of inbreeding on nymph survival, we calculated the proportion of inbred and outbred nymphs that survived to adult eclosion in each block and compared the values with a paired *t*-test.

To compare traits, we calculated the standardized coefficient of inbreeding, δ (Lande & Schamske, 1985) which is the percentage change with inbreeding, calculated as (outbred trait value - inbred trait value)/outbred trait value. A negative value indicates that inbred individuals had a larger value for the trait, interpretation of which depends on the direction of selection on the trait. We also calculated the effect size, *d* (see above), for inbreeding for each trait using the grand mean and standard deviation for all inbred and outbred crickets (i.e. ignoring block). A positive value of *d* indicates that the outbred trait value is larger than the inbred trait value (conversely a negative value indicates a larger inbred trait value).

Summary statistics are presented as mean $\pm$ standard error. When two sample size values are presented the first is for inbred, and the second for outbred crickets. All tests are two-tailed and $\alpha = 0.05$.

Selection analysis and comparing multivariate attractiveness

We measured the six courtship call parameters used by Hall *et al.* (2008) to characterise multivariate sexual selection on the courtship call, where male fitness was measured as spermatophore retention time for mating pairs of crickets collected from the same study site. The selection analysis of Hall *et al.* (2008) identified the major axes of multivariate

sexual selection on courtship call structure. Using the courtship call and weight data from our study, we could then calculate the value of each inbred or outbred male along each major axis of selection. This allowed us to compare the multivariate attractiveness of the courtship calls of inbred and outbred males. Full details of the selection analysis are given in Hall *et al.* (2008). In brief, to characterize sexual selection on the courtship call and body size, Hall *et al.* (2008) used the multiple regression approach of Lande & Arnold (1983). Before analysis, they converted absolute fitness (i.e. spermatophore attachment time) to relative fitness by dividing the attachment time of each male by the sample mean and standardized all other traits to a mean of zero and standard deviation of one. Separate multiple regressions (Lande & Arnold, 1983) were then used to estimate the vectors of linear selection gradients (β) and the matrix of quadratic and correlational selection gradients (γ), with the reported quadratic regression coefficients doubled to obtain quadratic selection gradients (Stinchcombe *et al.*, 2008). Finally, a canonical rotation of the γ matrix was used to detect the major axes of nonlinear sexual selection (Phillips & Arnold, 1989). This analysis produced an $\mathbf{M}$ matrix with 7 eigenvectors $\mathbf{m}_{1-7}$ (linear combinations of weighted values of the original traits) that describe the major axes of the nonlinear response surface (see Table 3 in Hall *et al.*, 2008), with the strength of nonlinear sexual selection on $\mathbf{m}_i$ given by the eigenvalue λ_i .

To compare the multivariate attractiveness of the courtship call between inbred and outbred males we calculated a score for the two eigenvectors with the strongest nonlinear selection ($\mathbf{m}_6$ and $\mathbf{m}_7$) for each male (Table 3: female only treatment in Hall *et al.*, 2008). Specifically, all call traits were first standardised, multiplied by the respective trait loadings of the eigenvectors and then summed in order to calculate an individual's multivariate attractiveness. We then compared $\mathbf{m}$ scores between inbred and outbred males using linear mixed models with inbreeding as a fixed factor, and block and the block by inbreeding interaction as random factors.

RESULTS

Courtship call parameters

In total we recorded and successfully analysed the calls of 451 males (199 inbred and 252 outbred). All six call parameters were repeatable (Table 1). Inbreeding had no

effect on CTI, T1L or CPN (Table 2 and 3). Inspection of the confidence intervals from the meta-analyses showed that inbreeding significantly increased courtship call DF (Hedge’s $d = -0.198$; 95% CI: -0.374 to -0.030). There was also a significant increase in the CIPD of the calls of inbred males (Hedge’s $d = -0.178$; 95% CI: -0.333 to -0.026), but there was no effect of inbreeding on TN (Hedge’s $d = -0.089$; 95% CI: -0.295 to 0.0740 , for all meta-analyses $N = 26$ blocks) (see Table 2).

Table 1. The repeatability of call parameters among males ($N = 451$ males).

Call parameter	Test Statistic	P -value	r_I
chirp inter-pulse duration (CIPD)	$F_{450, 1712} = 5.309^*$	<0.001	0.683
chirp-trill interval (CTI)	$F_{450, 1712} = 2.974^*$	<0.001	0.497
trill 1 length (T1L)	$F_{450, 1712} = 5.783^*$	<0.001	0.705
chirp pulse number (CPN)	$F_{450, 1712} = 7.329^*$	<0.001	0.760
dominant frequency (DF)	$\chi^2_{450} = 1823.283^\dagger$	<0.001	0.840
trill number (TN)	$\chi^2_{450} = 1376.254^\dagger$	<0.001	0.758

*One-way ANOVA, † Kruskall-Wallis test.

Table 2. Mean $\pm$ SE for inbred and outbred males, δ (% change with inbreeding) and Hedge’s d for the six courtship call parameters. Bold values of δ and d indicate a significant difference between inbred and outbred males.

Call parameter	Mean $\pm$ SE		δ	Hedges $d \pm$ SE
	Inbred ($N = 199$)	Outbred ($N = 252$)		
CIPD (ms)	29.3 ± 0.5	28.6 ± 0.4	-2.4	-0.097 $\pm$ 0.095
CTI (ms)	146 ± 5.0	143 ± 3.5	-2.1	-0.056 ± 0.095
T1L (s)	2.723 ± 0.092	2.668 ± 0.081	-2.1	-0.042 ± 0.095
CPN	9.302 ± 0.145	9.495 ± 0.115	2.0	0.100 ± 0.095
DF (Hz)	3978.55 ± 9.32	3947.48 ± 7.84	-0.8	-0.243 $\pm$ 0.095
TN	3.084 ± 0.122	3.078 ± 0.106	-0.2	-0.004 ± 0.095

Several of the covariates we examined affected call parameters (Tables 3 and 4). Males with a slower development time had a significantly higher CPN, older males had significantly shorter CTIs and, at higher temperatures, the CTIs were also shorter. Heavier males had a significantly longer TIL. None of these relationships differed between inbred and outbred males (all interactions $P > 0.05$). In contrast, the relationship between female mass and CIPD differed between inbred and outbred males ($F_{1,377} = 6.981$, $P = 0.009$). For inbred males courtship calls performed for heavier females had significantly longer CIPDs but there was no effect of ambient temperature. While for outbred males, courtship calls performed for heavier females had significantly shorter CIPDs, and at higher temperatures the CIPD was also significantly shorter (Table 3).

The calls of heavier inbred and outbred males had a significantly lower DF. For outbred, but not inbred, males those with a longer development time had a call with a significantly lower DF, and, at higher temperatures, the DF of the courtship call was also significantly higher. However, none of these correlations differ significantly between inbred and outbred males. Heavier inbred males had a significantly lower TN, while the TN of outbred males was significantly lower at higher temperatures. Finally, outbred male courtship calls directed at heavier females had a marginally non-significant increase in the number of trills ($P = 0.051$). The correlations between trill number and male mass, female mass and temperature were significantly different for inbred and outbred males (Table 4).

Table 3. The effect of inbreeding and five covariates on four courtship call parameters: CIPD, CTI, T1L and CPN. *P*-values are from the final model if the term was significant, or when it alone was added to the final model if non-significant. *P*-values significant at the 0.05 level are in bold.

	CIPD						CTI			T1L			CPN		
	Inbred			Outbred											
	<i>df.</i>	<i>F</i>	<i>P</i>	<i>df.</i>	<i>F</i>	<i>P</i>	<i>df.</i>	<i>F</i>	<i>P</i>	<i>df.</i>	<i>F</i>	<i>P</i>	<i>df.</i>	<i>F</i>	<i>P</i>
Inbreeding	-	-	-	-	-	-	1,27	0.01	0.907	1,27	0.07	0.797	1,27	2.64	0.116
Development time	1,164	1.78	0.184	1,207	1.67	0.198	1,378	3.19	0.075	1,353	1.88	0.172	1,390	6.61	0.011
Male age	1,164	0.36	0.551	1,207	0.55	0.460	1,379	6.81	0.009	1,353	0.35	0.555	1,389	0.11	0.745
Male mass	1,150	2.09	0.151	1,185	0.22	0.639	1,343	0.21	0.647	1,354	6.77	0.010	1,353	3.59	0.059
Female mass	1,165	4.36	0.038	1,208	4.51	0.035	1,367	0.34	0.563	1,341	0.12	0.725	1,377	0.02	0.894
Temperature	1,161	0.09	0.761	1,208	5.92	0.016	1,379	11.42	0.001	1,347	0.02	0.897	1,383	1.11	0.293

Table 4. Correlations between dominant frequency (DF) or trill number (TN), and development time, male age, male and female mass, and temperature. Z and P -values are for the comparison of r_s between inbred and outbred males. P -values significant at the 0.05 level are in bold.

	DF				TN			
	r_s inbred (P, N)	r_s outbred (P, N)	Z	P	r_s inbred (P, N)	r_s outbred (P, N)	Z	P
Development time	0.004 (0.951, 199)	-0.129 (0.041 , 252)	1.400	0.162	-0.031 (0.660, 199)	-0.030 (0.635, 252)	0.010	0.161
Male age	-0.110 (0.123, 199)	0.021 (0.739, 252)	1.377	0.169	-0.073 (0.303, 199)	-0.076 (0.231, 252)	0.032	0.976
Male mass	-0.319 (< 0.001 , 185)	-0.196 (0.003 , 230)	1.326	0.185	-0.276 (< 0.001 , 185)	-0.014 (0.830, 230)	2.707	0.007
Female mass	-0.121 (0.092, 194)	-0.061 (0.341, 244)	0.625	0.532	-0.065 (0.369, 194)	0.125 (0.051, 244)	1.969	0.049
Temperature	-0.004 (0.956, 196)	0.132 (0.037 , 249)	1.422	0.155	0.028 (0.697, 196)	-0.181 (0.004 , 249)	2.195	0.028

Maturation weight, development time and nymph survival

Inbreeding significantly reduced nymph survival ($t_{28} = 2.98$, $P = 0.006$; outbred survival: $71.9 \pm 2.0\%$, inbred survival: $64.5 \pm 2.1\%$, $\delta = 10.3\%$, Hedges $d = 0.642 \pm 0.269$). Despite the loss of over 30% of nymphs, we still recorded life history traits for 1584 females and 1620 males. There was a marginally non-significant trend for inbred crickets to have a lower maturation weight ($F_{1,28} = 3.773$, $P = 0.062$, inbred females: 564.7 ± 5.4 mg, $N = 732$, outbred females: 591.9 ± 5.2 mg, $N = 852$, $\delta = 4.6\%$, Hedges $d = 0.182 \pm 0.051$; inbred males: 611.9 ± 5.8 mg, $N = 764$, outbred males: 634.6 ± 5.4 mg, $N = 856$, $\delta = 3.6\%$, Hedges $d = 0.143 \pm 0.050$). Males were significantly heavier at maturation than females ($F_{1,3141} = 75.837$, $P < 0.001$).

Inbreeding had no effect on male or female development time (male: $Z = 1.37$, $P = 0.170$, $N = 29$ blocks, inbred males: 92.7 ± 0.7 days, $N = 767$, outbred males: 90.1 ± 0.7 days, $N = 867$, $\delta = -2.9\%$, Hedges $d = -0.130 \pm 0.050$; female: $Z = 0.44$, $P = 0.658$, $N = 29$ blocks, inbred females: 87.3 ± 0.8 days, $N = 736$, outbred females: 86.3 ± 0.7 days, $N = 868$, $\delta = -1.2\%$, Hedges $d = -0.051 \pm 0.050$). Males took significantly longer to mature than females ($Z = 3.51$, $P < 0.001$, $N = 33$ blocks) (all Wilcoxon's tests).

Selection analysis and comparing multivariate attractiveness

The position of inbred and outbred males along the two strongest vectors of selection did not differ significantly (**m**₆: $F_{1,27} = 0.105$, $P = 0.749$, Hedges $d = -0.061 \pm 0.095$, **m**₇: $F_{1,27} = 0.201$, $P = 0.658$, Hedges $d = 0.039 \pm 0.095$). There is therefore no evidence that inbred and outbred males differ in the multivariate attractiveness of their courtship calls and their ability to induce a female to retain their spermatophore for a longer period to increase the total amount of sperm transferred.

DISCUSSION*General evidence for inbreeding depression*

One generation of full sibling mating in the cricket *Teleogryllus commodus* significantly reduced nymph survival (δ : 10.3%). There was a marginally non-significant trend for

inbreeding to lower maturation weight (δ : 3.6 to 4.6%), but there was no detectable effect on development time (δ : -1.2 to -2.9%). Traits that are closely linked to fitness are expected to show higher levels of inbreeding depression than traits that are less closely related to fitness. Inbreeding depression is due to the effects of directional dominance, and does not occur in traits with a purely additive genetic basis (Charlesworth & Charlesworth, 1987; Lynch & Walsh, 1998). Traits that are more closely related to fitness are expected to be under stronger selection which depletes additive genetic variation (Mousseau & Roff, 1987; DeRose & Roff, 1999; Blows & Hoffmann, 2005; Hunt *et al.*, 2007) and because mutations affecting such traits are typically deleterious and recessive, traits closely tied with fitness are expected to show higher levels of directional dominance, and therefore inbreeding depression (see Tomkins *et al.*, 2010). In contrast, traits that are less closely related to fitness and therefore under weaker selection show substantial levels of additive genetic variance and less directional dominance, and therefore tend to exhibit lower levels of inbreeding depression (Lynch & Walsh, 1998; Roff, 1998; DeRose & Roff, 1999; Wright *et al.*, 2008). In accordance with these predictions, we found strong inbreeding depression in nymphal survival, a key fitness trait. Weight may also be closely linked to fitness because female fecundity generally increases with body size in insects (Honek, 1993), and male fighting success is strongly affected by size in *T. commodus* (Shackleton *et al.*, 2005). Inbreeding depression on weight was, however, marginally non-significant. We found no effect of inbreeding on development time, a result that is consistent with a previous study of *T. commodus* (Drayton *et al.*, 2007) and with the finding of low inbreeding depression on development time in the cricket *Gryllus firmus* (Roff, 1998). This indicates little directional dominance for development time. It is not known how strongly development time affects fitness. More generally, our results agree with those of two previous studies in *T. commodus* showing widespread inbreeding depression on major fitness traits. Drayton *et al.* (2007) found that inbreeding (full-sibling mating, $F = 0.25$) led to lower egg hatching success (δ : 11.9%), reduced nymph survival (δ : 33.2%) and a shorter adult lifespan (δ : 13.4 to 31.5%). Drayton *et al.* (2010) found that inbreeding ($F = 0.25$) significantly reduced male advertisement call rate (δ : 30.2%), which is a strong predictor of male mating success (Bentsen *et al.*, 2006). Taken together these results provide clear evidence that inbreeding reduces fitness in this species.

Courtship call structure and male attractiveness

A small but growing number of studies are now using inbreeding as an experimental method to manipulate male quality (e.g. Bolund *et al.*, 2010; Drayton *et al.*, 2010 and references therein). Given the well established effects of inbreeding on fitness (reviews: Keller & Waller, 2002; Armbruster & Reed, 2005), inbred individuals can be unambiguously assigned as low phenotypic quality relative to outbred individuals. If male sexually selected traits reflect aspects of quality that are reduced by inbreeding such as condition and/or heterozygosity then sexual traits should decline with inbreeding. Accordingly, several studies have documented strong inbreeding depression in sexually selected male traits: studies of guppies *Poecilia reticulata* (Sheridan & Pomiankowski, 1997; van Oosterhout *et al.*, 2003; Mariette *et al.*, 2006), least killfish, *Heterandria formosa* (Ala-Honkola *et al.*, 2009), mice *Mus musculus* (Ilmonen *et al.*, 2009), *Drosophila* (Sharp, 1984; Miller *et al.*, 1993; Aspi, 2000), butterflies *Bicyclus anynana* (Joron & Brakefield, 2003) and even of *T. commodus* itself (Drayton *et al.*, 2010) have all shown that inbreeding reduces traits known to attract and induce females to mate such as sexual colouration and displays (i.e. traits under pre-copulatory sexual selection), ultimately reducing male mating success. Traits subject to post-copulatory sexual selection (e.g. sperm quality and quantity) can also show substantial inbreeding depression (e.g. Roldan *et al.*, 1998; Margulis & Walsh, 2002; Konior *et al.*, 2005; Gage *et al.*, 2006; Fitzpatrick & Evans, 2009; Zajitschek *et al.*, 2009). In general, these studies suggest that a comparison of inbred and outbred males is a useful method to determine whether or not pre- or post-copulatory sexually selected traits are good indicators of male quality and/or genome wide heterozygosity.

It is plausible that the courtship call might signal male quality in *T. commodus*. Producing the courtship call in the cricket *Acheta domesticus* is twice as energetically expensive as producing advertisement calls (Hack, 1998). There is also evidence from the congeneric *T. oceanicus* that males produce structurally different courtship calls after suffering an immune challenge (Tregenza *et al.*, 2006), suggesting that the courtship call can signal male fitness. Furthermore, courtship call structure influences post-copulatory female choice in *T. commodus* (Hall *et al.*, 2008). We therefore predicted that if the courtship call signalled aspects of male quality that are reduced by inbreeding, such as condition, (and ultimately net fitness) there should be significant changes in the structure and attractiveness of the courtship call with inbreeding. We

found that that one generation of full sibling mating increased both the dominant frequency (DF, δ : -0.8%) and the chirp inter-pulse duration (CIPD, δ : -2.4%) of the courtship call. There was no effect on any other call parameter (δ : -2.1 to 2.0%). The effect of inbreeding on DF may have been mediated, in part, by the tendency for inbred males to be smaller, because both larger inbred and outbred males had calls with a lower DF. The increased CIPD of inbred males could indicate a slight reduction in male condition with inbreeding. Each pulse of the call and the silent interval that precedes it is produced by one cycle of wing closure (the pulse) and wing opening (the interval) (collectively termed one wing stroke). The duration of the CIPD therefore corresponds to the time taken for a male to reopen his wings after producing one pulse of sound (Prestwich & Walker, 1981; Kavanagh, 1987; Simmons *et al.*, 2005). The increase in the CIPD means that inbred males were taking slightly longer to reopen their wings after producing sound pulses than outbred males. Because the energetic cost of calling increases as the wing stroke rate increases (Prestwich & Walker, 1981), this suggests that inbred males may have slightly less energy reserves to allocate to calling. Despite finding significant, albeit small, changes in two structural components of the call, we found no evidence that inbreeding reduces the post-copulatory multivariate attractiveness of the courtship call.

There are several scenarios that could explain the lack of substantial inbreeding depression in the courtship call and resulting multivariate attractiveness. First, the courtship call is not a reliable signal of male quality or condition in *T. commodus*. The courtship call does not appear to reflect male condition in the cricket *Gryllus texensis* as it was unaffected by dietary treatment, and was unrelated to two measures of condition (residual mass and fat reserve) (Gray & Eckhardt, 2001). Likewise, female crickets *G. lineaticeps* prefer courtship calls with a higher chirp rate but this trait was not affected by nutrition (Wagner & Reiser, 2000). These studies and our data suggest that, at least for three cricket species, the courtship call, despite its potential energetic cost (Hack, 1998), is a poor indicator of male condition. Interestingly, work on the closely related species *T. oceanicus* suggests that the courtship call is not an obligatory requirement to induce females to accept a spermatophore. On the Hawaiian island of Kauai, a recent morphological mutation ('flatwing') has rendered males silent because they lack sound-producing wing structures. This mutation confers a selective advantage as it protects silent males against acoustically orientating parasitoid flies (Zuk *et al.*, 2006). Females must still mate with silent males or the mutation would have been eliminated by

selection. Comparison of courtship behaviour between the present population (where the flat wing mutation occurs) and the ancestral population (collected before the mutation arose; therefore, there are no flatwing males in the ancestral population) shows that in both populations approximately 55% of females mounted males in the absence of the courtship call. Consequently, females in the ancestral population were willing to mount silent males. However, the tendency for females to mount males was increased by broadcasting a courtship call when a silent male courted a female (Bailey *et al.*, 2008). Likewise, deafened female *T. commodus* will still mount males, but only after the courting male approaches and makes antennal contact with the female (Loher & Rence, 1978). These studies suggest that in both *T. commodus* and *T. oceanicus*, while the courtship call increases the tendency of females to mount males, it is not an absolute requirement for mating. Selection on the courtship call as an indicator of male quality might therefore be weak. In this case, the courtship call may be a sexual signal that provides unreliable information to females but is maintained because it is cheap to produce (although potentially energetically costly it is only produced for a few minutes) and there is still a weak vestigial female preference for it (Moller & Pomiankowski, 1993).

Second, it is possible that the courtship call might show stronger inbreeding depression in response to more severe inbreeding (i.e. $F > 0.25$, see Zajitschek *et al.*, 2009). The available evidence suggests, however, that the courtship call of *T. commodus* does not signal male condition or genome-wide heterozygosity. Even if more severe inbreeding does change courtship call structure or attractiveness, the fact that inbreeding at the same level as the current study had a strong effect on advertisement call rate (δ : 30.2%; Drayton *et al.*, 2010) implies that the courtship call is a far less accurate indicator of traits that are strongly correlated with fitness and are affected by inbreeding. This is further supported by the fact that the males used by Drayton *et al.* (2010) (where a 30.2% reduction in calling effort was found) are the same individuals used in this study to investigate inbreeding depression on courtship calling. Third, the courtship call might provide information to females about male traits that are not affected by inbreeding (i.e. traits with little directional dominance), in which case, we should not expect changes in the courtship call with inbreeding. If this is the case, then the advertisement call (which shows strong inbreeding depression, Drayton *et al.*, 2007; 2010) and the courtship call must signal different aspects of male quality to females (e.g. Moller & Pomiankowski, 1993). Consistent with this notion, females use the courtship call in post-copulatory

mate choice because it affects the timing of spermatophore removal by females (e.g. Hall *et al.*, 2008), and therefore the number of sperm transferred (Bussiere *et al.*, 2006; Hall *et al.*, 2010). Fourth, it is important to note that our measure of multivariate attractiveness was of post-copulatory attractiveness (i.e. spermatophore retention time, Hall *et al.*, 2008). It is likely that the courtship call of *T. commodus* is also under pre-copulatory sexual selection and will influence whether or not a female will mount a male (e.g. Wagner & Reiser, 2000; Tregenza *et al.*, 2006). It is possible that inbreeding will affect measures of pre-copulatory courtship call attractiveness (e.g. latency to mounting). This is an area for future study. Finally, by using the previous selection analysis to predict the multivariate attractiveness of males, we have assumed that the fitness surface described by Hall *et al.* (2008) is stable across time. Although we worked on the same population of *T. commodus*, under almost identical laboratory conditions, our study was done approximately two years later than Hall *et al.* (2008). We therefore can not rule out the possibility that we found no difference in multivariate attractiveness because the fitness surface has shifted over time.

On a more general note, it is worth noting that a female may not necessarily produce lower quality offspring after mating with an inbred male. An inbred male can not pass on his inbred status to his offspring, because diploid sexual organisms inherit genes, not genotypes. Genes inherited from each parent combine to produce new offspring genotypes (Falconer & Mackay, 1996; Szulkin & Sheldon, 2006), and if an inbred male mates with an unrelated, genetically dissimilar female, then the resulting offspring should be heterozygous and have higher fitness relative to their father. In this sense, the negative effects of inbreeding will be wiped clean, at least genetically, in the offspring of an inbred male (Szulkin & Sheldon, 2006). Consequently, an inbred male will not necessarily be a lower quality sire relative to an outbred male because the offspring of an inbred male will not necessarily have reduced fitness (provided that the female is unrelated to the inbred male). In this case, there may be no reason to expect that a sexual trait will reveal the poor genetic quality, in relation to inbreeding, of an inbred male. If however, due to population structuring, a female is more likely to be related to an inbred male compared to an outbred male, she will benefit by mating with the outbred male, thereby producing outbred, heterozygous offspring (e.g. Reid *et al.*, 2006). Females can detect such outbred males if male sexual traits are correlated to levels of genome wide heterozygosity (Kempnaers, 2007; Fromhage *et al.*, 2009). In any case, if sexually selected traits reveal information about male characteristics that

could confer direct or indirect benefits to females (e.g. sperm quality, nuptial feeding, condition or heterozygosity) and if inbreeding adversely affects such characteristics, then sexual traits should show declines with inbreeding. In this sense, inbreeding can be viewed as a way of experimentally reducing fitness to then determine if such reductions in fitness are mirrored in sexual traits (see Bolund *et al.*, 2010; Drayton *et al.*, 2010). Whether or not the offspring of inbred males have reduced fitness is an area for future study.

In conclusion, inbreeding had only a small effect on the structure of the courtship call and did not change post-copulatory multivariate attractiveness. It is therefore unlikely that the courtship call signals aspects of male quality that are reduced by inbreeding such as condition or heterozygosity. However, the courtship call influences female post-copulatory choice in this species (Hall *et al.*, 2008) and consequently, it is possible that the courtship call signals information to females about other male properties that are not affected by inbreeding.

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