

The form of sexual selection arising from male–male competition depends on the presence of females in the social environment

D. S. PROCTER*, A. J. MOORE*† & C. W. MILLER‡

*Centre for Ecology & Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn, UK

†Department of Genetics, University of Georgia, Athens, GA, USA

‡Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA

Keywords:

male–male competition;
selection analysis;
selection gradients;
selection surface;
sexual selection.

Abstract

Sexual selection arises from social interactions, and if social environments vary so too should sexual selection. For example, male–male competition often occurs either in the presence or in the absence of females, and such changes in the social environment could affect the form and strength of sexual selection. Here we examine how the presence of a female influences selection arising from male–male competition in a leaf-footed cactus bug, *Narnia femorata*, which has a resource defence mating system. Males compete for territories on cacti because females lay eggs on the cactus plants. Females are not always present when this competition first occurs; however, the presence or absence of the female matters. We found that both the form and strength of selection on male traits, those traits that influenced success in intrasexual competition, depended on the social context. When a female was not present, male size and the area of the sexually dimorphic hind legs was only marginally important to winning a contest. However, males with larger overall size and leg area were more likely to win in the presence of a female. There was also positive quadratic selection on these traits when a female was present with both the largest and the smallest males winning. The implication is unexpected alternative strategies when females are present. Our results support the notion that sexual selection should be studied under all relevant social contexts.

Introduction

There is an increasing awareness that sexual selection is dynamic. The form and strength of sexual selection can vary both temporally (Mateos, 1998; Ferguson & Fairbairn, 2000; Jann *et al.*, 2000; Preziosi & Fairbairn, 2000; Gosden & Svensson, 2008; Kasumovic & Andrade, 2008; Punzalan *et al.*, 2008, 2010; Sullivan-Beckers & Crocroft, 2010) and spatially (Baird *et al.*, 1997; Ferguson & Fairbairn, 2000; Kraushaar & Blankenhorn, 2002; Kwiatkowski & Sullivan, 2002; Gosden & Svensson, 2008). This variation is not unexpected as changeable

abiotic environments can alter sexual selection (Maan & Seehausen, 2011). Higher rainfall increases the opportunity for sexual selection in grey seals (Twiss *et al.*, 2007). In sand gobies, the strength of male–male competition and female choice depends nest resource availability, with opposite effects on each (Forsgren *et al.*, 1996; Lindström, 2001). Food resource quality influences which sex is choosy, independent of population density, in Mormon crickets (Gwynne, 1993). The temperature environment individuals experience during development influences both male–male competition and mate choice in *Nauphoeta cinerea* (Clark *et al.*, 1997). Sexual selection is clearly environmentally sensitive.

Social environments are also important in sexual selection. Social conditions, particularly population density and opportunities for interactions, have long been considered to provide important contexts for sexual

Correspondence: Christine W. Miller, Department of Entomology and Nematology, University of Florida, PO Box 110620, Steinmetz Hall Natural Area Drive, Gainesville, FL 32611-0620, USA.
Tel.: +1 352 273 3917; fax: +1 352 392 0190; e-mail: cwmiller@ufl.edu

selection (Emlen & Oring, 1977). Group size can fluctuate rapidly in natural populations and influence social information that informs aggressive interactions, mating decisions and offspring production (Dall *et al.*, 2005; Fletcher & Miller, 2008). However, the composition of the social environment independent of the density is also very important. Variation along a social axis is of particular interest because it is potentially much more dynamic than other environmental variables studied (Moore *et al.*, 1997; Wolf *et al.*, 1999; McGlothlin *et al.*, 2010). Yet the effects of variable social environments on male–male competition are poorly explored outside of studies that examine the demographic effects on sexual selection (Moore, 1987, 1989; Michener & McLean, 1996; Jirotkul, 1999; Head *et al.*, 2008; Kasumovic *et al.*, 2008).

One way in which social environments may vary is in the presence or absence of the opposite sex. Female presence is known to strongly influence male–male competition (Cox & Le Boeuf, 1977; Hand, 1986). Yet many studies of male–male competition have estimated selection with limited female contact (Ligon *et al.*, 1990; Petersson *et al.*, 1999) or none (Bakker & Sevenster, 1983; Clark & Moore, 1995; Candolin, 2000; Bonduriansky & Rowe, 2003; Benson & Basolo, 2006), which may not realistically reflect the natural environment in which selection occurs and so may not accurately estimate the selection reported.

In this study, we examined male–male competition under different social conditions. The species we studied, the leaf-footed cactus bug *Narnia femorata* (Fig. 1), exhibits resource defence polygyny in the wild, with males establishing territories on *Opuntia* and *Cylindropuntia* sp. cacti with fruit and attracting females. Male *N. femorata* compete for territories on this cactus, especially around fruits, and females use cacti pads and (preferentially) fruit for oviposition. Male–male competition can occur before a female arrives and so can be independent of any female effect. Thus, we are measuring one component of sexual selection; it is likely that total sexual selection will involve a combination of first male–male competition and then female choice (Hunt *et al.*, 2009). Competition for a cactus

can be described as competition over a mating resource, as territories are held on these cacti and reproduction requires cacti for egg laying and subsequent juvenile development. However, males that lose this initial competition do not necessarily disperse, or competition may not necessarily occur. Male–male competition may also (or instead) take place once a female arrives. Competition under these conditions likely differs, reflecting direct competition for mates when females are present as well as potential breeding sites. Sexual selection arising from male–male competition therefore must incorporate both situations.

We measured the effect of variable social environment in *N. femorata* by measuring selection gradients (Lande & Arnold, 1983) and visualizing selection (Schluter, 1988) resulting from male–male competition under different social conditions. Estimating selection gradients and visualizing the selection surface is a standard method to examine the selection acting on traits that influence ‘winning’ a competition, one potential component of fitness (e.g. Moore, 1990; Brodie *et al.*, 1995; Brodie & Janzen, 1996; Hunt *et al.*, 2009). Our goal was to determine whether selection arising from male–male competition differs under the two common social conditions that are experienced by *N. femorata* males. Thus, our approach is analogous to a partitioned selection approach (Arnold & Wade, 1984; Wade & Kalisz, 1989; Hunt *et al.*, 2009). We predicted that selection on male traits that are related to success in male–male competition would be similar in form (directional) under both resource and mating competitions. However, we also predicted stronger (steeper) selection gradients in the presence of a female because of the ‘added value’ of winning in that context; that is, because the probability of immediately mating was greater.

Materials and methods

Research organism

Narnia femorata is a member of the leaf-footed bugs (family Coreidae) and is between 10 and 18 mm in length. This genus of insects is native to the south-western United States, Mexico and parts of Central America, where it lives on *Opuntia* and *Cylindropuntia* sp. cacti, and was only recently introduced to Florida (Baranowski & Slater, 1986). In our study area in north-central Florida, USA, it lives on *O. humifusa*. Males establish territories and defend areas on cacti by physical competition. The form of this competition is not ritualized. Competitions can end by one male retreating after just antennal contact, after physical contact or after fully escalated competition. During escalated competition, males face rear to rear and grab each other using their hindmost legs (Fig. 1), as is common throughout the Coreidae (Mitchell, 1980; Miyatake, 1997; Eberhard, 1998; Miller & Emlen, 2010a,b).

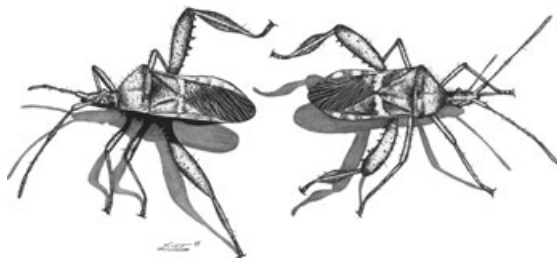


Fig. 1 Diagram of male–male competition in *Narnia femorata*. During fights, males grab and squeeze each other with their hind legs. Drawing by David Tuss.

We collected *N. femorata* from the wild at the Ordway-Swisher Biological Station, University of Florida (29°41'N, 82°W), and brought them to the laboratory to form populations. The majority (73%) of the males were first-generation laboratory insects, whereas the remainder were second generation. Adults were paired so as to maximize genetic diversity in the population. The *O. humifusa* for the study was collected from both the Ordway-Swisher Biological Station and also the Camp Blanding Joint Training Centre (29.95°N, 81.98°W).

Both male and female *N. femorata* complete five nymphal instars before eclosion. Until 4th instar, nymphs were kept in communal 'deli' containers with soil, cladode and ripe fruit in heated incubators at 26 (\pm 1)°C and on a light cycle of 14 h : 10 h light/dark. At 4th instar, we separated nymphs into their own containers, in which there was soil, cladode and ripe fruit. We then moved them to a greenhouse on a 15 h : 9 h light/dark cycle with mean temperature of 26 °C.

Experiment 1: male competition in the absence of a female (MM)

The arenas in which we measured selection were containers containing soil, a cactus pad (cladode) and a fruit. Where possible, we used fruit naturally attached to the pad; when this was not possible, we artificially attached fruit to the top of the cladode with toothpicks. We randomly selected two males, marked one with water-soluble paint and placed both into an arena together. We allowed them to acclimatize and establish dominance for 2 h prior to observations. Males were unrelated and matched as closely as possible in age post-eclosion (median = 0 days, mean = 1.86 days) and always within 7 days in age. All males were over 14 days of age to ensure sexual maturity, with a median age of 22 days post eclosion (maximum 45 days). Observations commenced after the males had been in the arena for the 2-h acclimatization period and lasted for 2 h in a room maintained at 24 °C. A single observer conducted the 85 behavioural trials. Not all pairings resulted in competitions (see Results). We chilled bugs after observations, placed them into labelled tubes to be frozen and later measured morphological traits.

Experiment 2: male competition in the presence of a female (MMF)

The experimental set-up was the same as Experiment 1 except that after the males had been together for 2 hours, a marked, sexually mature female was added and then observations were begun. Females were selected randomly from the available population and were unrelated to either male. Female age varied more widely than in selected males, with female median age 35 days post

eclosion (minimum = 14 days; maximum = 97 days). A single observer again conducted 85 behavioural trials, and again, not all pairings resulted in competitions. Different males were used for experiment 1 and experiment 2. We froze bugs after observations and later measured morphological traits.

Outcome of interactions as a component of fitness

We used two measures to score fighting success. We scored 'absolute male competitive success', with the bug that withdrew most per interaction scored the loser (fitness = 0) and the bug that withdrew the least per interaction scored the winner (fitness = 1). We also used a continuous measure of fitness, 'relative male competitive success', calculated as $[1 - (\text{proportion of withdrawals})]$. In the end, both measures provided identical qualitative results, including patterns and statistical significance, so we only present the results from absolute male competitive success. A zero-one (win-lose) measure is a commonly used fitness surrogate for male-male competition, so adopting this measure facilitates comparisons (Hunt *et al.*, 2009). Fitness was transformed to relative fitness for selection analyses (Brodie *et al.*, 1995), although for zero-one scoring this does not matter (Brodie & Janzen, 1996).

This measure of fitness we use is indirect; that is, it assumes that males compete for territories that later influence mating success. Although we did not then measure male mating success or female mate choice of males that competed, this is a parsimonious assumption giving the resource defence polyandry system of mating for these bugs. Male-male competition in the absence of a female may commonly represent the first component of sexual selection, followed by sexual selection in the presence of a female. Our measures of selection therefore reflect a partition of sexual selection (e.g. Moore, 1990).

Morphological measurements

We dissected frozen bugs into their component parts (legs and body) to facilitate measurement of characteristics. Each component was then photographed using a Canon EOS 50D digital camera attached to a dissecting microscope along with a reference measurement. We used IMAGEJ 1.42q (<http://rsb.info.nih.gov/ij/>, 1997–2009) to measure straight line and area measurements from the photographs. We measured the pronotum width, head length, body length (thorax + abdomen), front femur length and front tibia length using a single linear measure. We also used IMAGEJ to trace the outline of the rear (elaborated) leg segments and obtain measures of the rear femur area and rear tibia area (Fig. 1). For leg characters where there were right and left measurements, we measured both and used the mean in our analyses.

Statistical analysis

We determined the form and strength of selection by using parametric regressions to estimate linear (β) and nonlinear (γ) coefficients (Lande & Arnold, 1983; Arnold & Wade, 1984). Linear coefficients were determined using a multiple linear regression of all the single terms and nonlinear coefficients using a multiple linear regression of single terms, squared terms and interaction terms (Brodie *et al.*, 1995). Regression coefficients for nonlinear coefficients were doubled to give the correct γ value (Stinchcombe *et al.*, 2008). All morphological variables were transformed to mean = 0, standard deviation = 1 before analysis. Although we chose morphological measurements that can be independently derived during development, extreme correlation of variables is problematic for selection analyses (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987) and so we used Pearson's product-moment correlations to examine the extent of correlation between variables. Highly correlated variables were converted to a single composite measure with principal component analysis. Because fitness was measured as two categories, we used logistic regression to determine the significance of the selection coefficients (Janzen & Stern, 1998).

Using multiple regression to estimate the form of selection has limitations, as it fits a specific model (Schluter, 1988; Brodie *et al.*, 1995). Therefore, we also used a nonparametric method to determine whether more complex selection, especially nonlinear such as disruptive or stabilizing, was present. Nonparametric estimations of the fitness function (f) were made using cubic splines (Schluter, 1988; Brodie *et al.*, 1995), with λ set to 1, using JMP 8.0; SAS Institute Inc. Cary, NC, USA.

Results

We found no statistically significant differences between males used in the MM or MMF experiments in any of the morphological measurements (Table 1) (pronotum width: $t_{402} = 1.468$, $P = 0.143$; head length: $t_{402} = 1.855$, $P = 0.064$; body length: $t_{402} = 1.313$, $P = 0.190$; front

tibia length: $t_{402} = 1.641$, $P = 0.102$; front femur length: $t_{402} = 1.629$, $P = 0.104$; hind tibia area (HTA): $t_{402} = 1.375$, $P = 0.170$; hind femur area (HFA): $t_{402} = 1.612$, $P = 0.110$). We also found no difference in any of the variances between males in the two treatments (Table 1) (Levene's test; pronotum width: $F_{1,402} = 0.077$, $P = 0.781$; head length: $F_{1,402} = 0.844$, $P = 0.359$; body length: $F_{1,402} = 0.348$, $P = 0.555$; front tibia length: $F_{1,402} = 0.210$, $P = 0.647$; front femur length: $F_{1,402} = 0.572$, $P = 0.450$; HTA: $F_{1,402} = 0.004$, $P = 0.984$; HFA: $F_{1,402} = 0.005$, $P = 0.985$). Competition between males was no more likely in MM (68.2% of pairs expressed dominant/subordinate behaviour) than in MMF (57.6%; $\chi^2_1 = 1.614$, $P = 0.204$).

We found strong correlations between all measured variables (Table 2). Given the strong correlations, we used PCA to reduce our measures to a single composite measure of size (Table 3). PC1 had strong positive loadings on all traits, explained 87% of the variance and provides a robust measure of general body size. PC2 had a single strong loading on head length, with all other traits having negative but weaker loadings, but this component had an eigenvalue less than one and explained only 9% of the variance. No other PC explained more than 2% of the variance. We therefore only used PC1 in further analyses.

Table 2 Pearson's product-moment correlation coefficients for all measured traits.

	PW	HL	BL	FTL	FFL	HTA	HFA
PW	1						
HL	0.669	1					
BL	0.947	0.640	1				
FTL	0.914	0.685	0.912	1			
FFL	0.936	0.701	0.941	0.957	1		
HTA	0.906	0.690	0.901	0.946	0.924	1	
HFA	0.951	0.698	0.943	0.930	0.951	0.928	1

PW, pronotum width; HL, head length; BL, body length; FTL, front tibia length; FFL, front femur length; HTA, hind tibia area; HFA, hind femur area. All pairwise correlations are highly statistically significant. Measurements from $N = 404$ individuals.

Table 1 Summary statistics of untransformed phenotypic characters. All means (SD in parentheses) are given in mm except for areas, which are in mm².

Trait	MM males			MMF males		
	Population	Win	Lose	Population	Win	Lose
Pronotum width	3.71 (0.44)	3.81 (0.47)	3.67 (0.45)	3.77 (0.43)	3.91 (0.44)	3.67 (0.35)
Head length	2.97 (0.18)	3.01 (0.22)	2.98 (0.18)	3.01 (0.17)	3.05 (0.16)	2.98 (0.14)
Body length	10.52 (1.04)	10.76 (1.05)	10.38 (1.08)	10.66 (1.01)	11.02 (1.01)	10.41 (0.91)
Front tibia length	3.54 (0.30)	3.62 (0.31)	3.51 (0.32)	3.59 (0.30)	3.70 (0.31)	3.52 (0.27)
Front femur length	3.93 (0.34)	4.02 (0.34)	3.90 (0.36)	3.98 (0.31)	4.10 (0.32)	3.90 (0.27)
Hind tibia area	4.12 (0.95)	4.37 (1.00)	4.03 (1.01)	4.25 (0.96)	4.64 (0.98)	4.02 (0.81)
Hind femur area	6.14 (1.71)	6.57 (1.87)	5.96 (1.82)	6.42 (1.72)	7.16 (1.74)	5.86 (1.37)
<i>N</i>	218	62	62	186	52	52

Table 3 Principal component analysis of linear morphological measurements, explaining 96.2% of the variance.

Trait	PC1	PC2
Pronotum width	0.463	-0.198
Head length	0.374	0.922
Body length	0.460	-0.266
Front femur length	0.469	-0.137
Front tibia length	0.463	-0.143
Eigenvalue	4.346	0.462
% Variance explained	86.9	9.2

Table 4 Linear (β) and nonlinear (γ) selection coefficients (Lande & Arnold, 1983) on size (PC1) and elaborated traits during male–male competition when there was no female present. Fitness was measured as lose/win (0, 1). Significance was determined by logistic regression (Janzen & Stern, 1998).

	Linear selection		Nonlinear selection	
	β (SE)	P	γ (SE)	P
Size (PC1)	0.038 (0.021)	0.071	0.004 (0.015)	0.758
Hind tibia area	0.085 (0.045)	0.059	-0.019 (0.074)	0.794
Hind femur area	0.081 (0.045)	0.071	-0.015 (0.073)	0.850
PC1 \times hind tibia area			-0.803 (0.592)	0.160
PC1 \times hind femur area			-0.565 (1.153)	0.622
Tibia area \times femur area			-0.124 (1.183)	0.921

Experiment 1: male competition in the absence of a female

When competitive outcomes were scored as absolute competitive success, we found no statistically significant linear or nonlinear selection coefficients (Table 4). We did find that positive linear selection approached statistical significance for overall size (0.071) and nearly reached conventional significance for tibia area ($P = 0.059$). Thus, if any selection arises from male–male competition when no female is present, it is weak and for larger size. This interpretation is supported by the visualizations of selection provided by the univariate cubic splines (Fig. 2). The selection surface for all traits, although often rugged, was primarily directional.

Experiment 2: male competition in the presence of a female

In contrast to the results found when no female was present, when a female was present we found statistically significant positive linear and nonlinear selection on overall size (Table 5). We also found significant positive linear selection on HTA and significant disruptive nonlinear selection on HFA (Table 5). These suggest a combination of positive linear selection and divergent selection (positive selection of trait variance), a pattern that was supported by the visualizations of selection

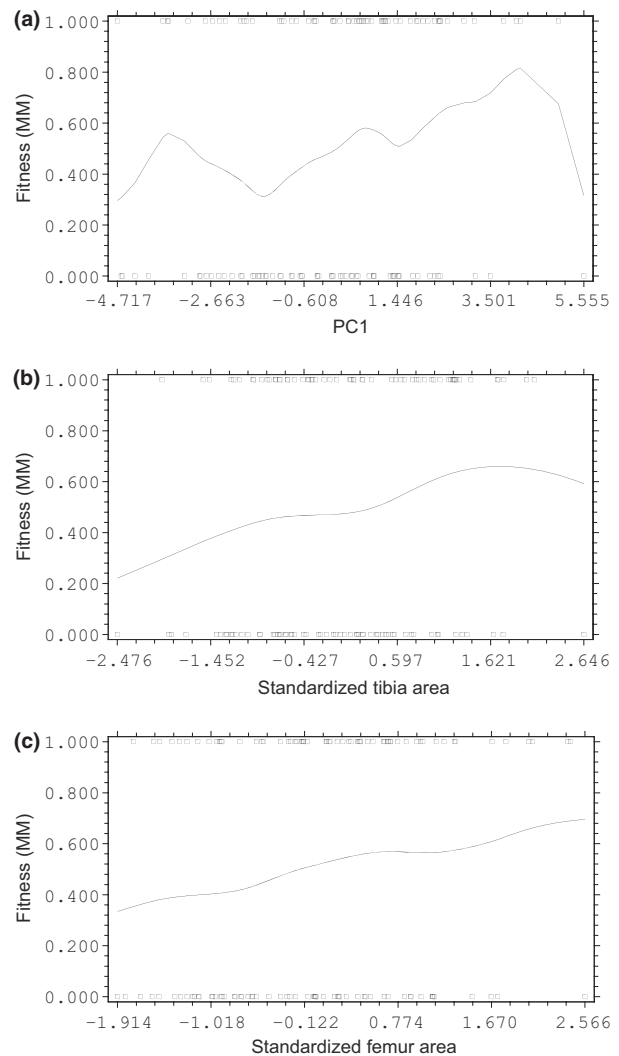


Fig. 2 Univariate cubic splines for (a) overall size (PC1), (b) the elaborated tibia area and (c) the elaborated femur area when there was not a female present (MM social condition). These splines provide a nonparametric visualization of selection on the male traits, with the component of fitness scored as absolute competitive success (0, 1). For the splines, all lambda values set to 1.

using splines (Fig. 3). For all traits, there were multiple peaks on the selection surface.

Comparison of selection in two environments

Determining the extent of the differences in selection in the two environments is complicated. We can test for differences in the selection gradients, but this is a very conservative test as it reduces selection to just the linear or nonlinear components described by regression coefficients. In our experiments, the linear selection gradients on the HTA were statistically significantly different

Table 5 Linear (β) and nonlinear (γ) selection coefficients (Lande & Arnold, 1983) on size (PC1) and elaborated traits during male–male competition with a female present. Fitness was measured as lose/win (0, 1). Significance determined by logistic regression (Janzen & Stern, 1998).

	Linear selection		Nonlinear selection	
	β (SE)	P	γ (SE)	P
Size (PC1)	0.077 (0.023)	0.021	0.032 (0.018)	0.045
Hind tibia area	0.166 (0.047)	0.0005	0.106 (0.084)	0.150
Hind femur area	0.192 (0.046)	<0.0001	0.123 (0.080)	0.053
PC1 \times hind tibia area			-0.776 (0.556)	0.158
PC1 \times hind femur area			0.842 (1.220)	0.469
Tibia area \times femur area			-0.924 (0.591)	0.182

(two-tailed t -test, d.f. = 400, $P = 0.035$), as was linear selection on HFA in the two environments (d.f. = 400, $P = 0.029$). Linear selection gradients on PC1 were not statistically significantly different (d.f. = 400, $P = 0.321$). None of the nonlinear selection gradients were statistically significantly different from each other based on a t -test (all d.f. = 400; PC1, $P = 0.174$; HTA, $P = 0.879$; HFA, $P = 0.874$; PC1 \times HTA, $P = 0.878$; PC1 \times HFA, $P = 0.287$; HTA \times HFA, $P = 0.566$). However, visual inspection of the selection surfaces supports the less conservative interpretation based on which gradients were statistically significantly different from zero, and which were not. Directional selection is stronger in the presence of a female, and there also tends to be more disruptive selection when a female is present.

Discussion

The shape and strength of selection reflected the social context. Qualitatively, we found that linear selection on overall size (PC1) was weaker when no female was present than when a female was in the environment (Tables 4 and 5). In addition, when a female was present there were significant nonlinear components, suggesting disruptive selection, that were not seen when no female was present. We also examined selection on the rear leg area, the part of the males that is most elaborated and where we expected the strongest sexual selection arising from male–male competition. An even stronger pattern was seen for selection on the elaborated traits: both qualitatively and quantitatively, there was stronger linear selection in the presence of a female. We also found more evidence for a nonlinear component indicating disruptive selection on rear leg area. Although the elaborated traits were highly correlated with our overall size measure, there was no indication of correlational selection integrating size and elaboration, or integrating the two elaborated leg areas. These findings are more nuanced than our original predictions.

We did not predict selection would change form, only that the strength might differ. Both the cactus and the

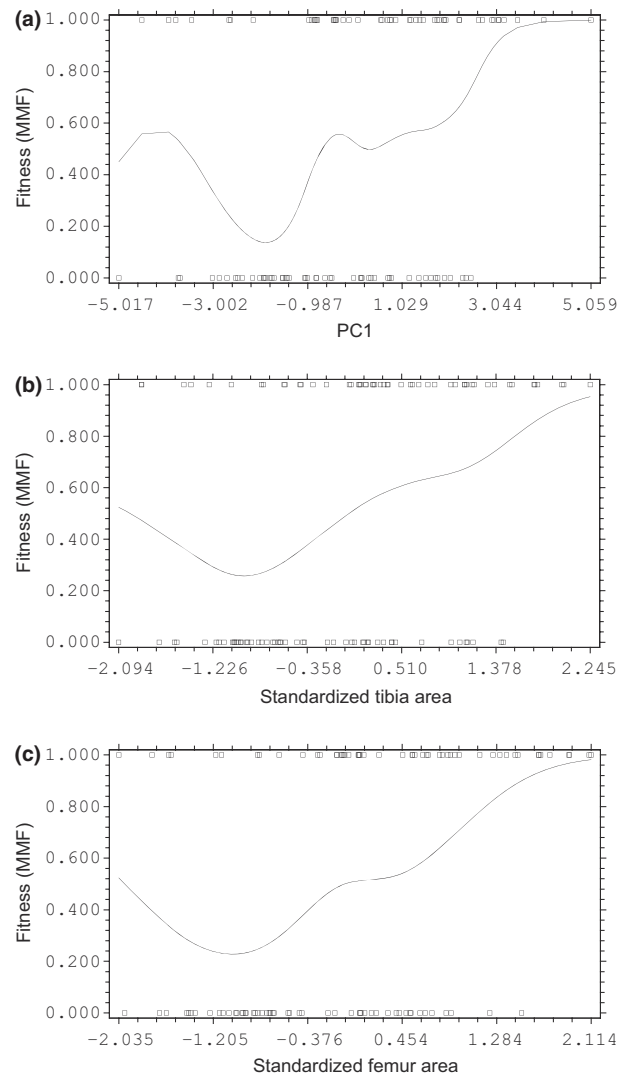


Fig. 3 Univariate cubic splines for (a) overall size (PC1), (b) the elaborated tibia area and (c) the elaborated femur area when there was a female present (MMF social condition). These splines provide a nonparametric visualization of selection on the male traits, with the component of fitness scored as absolute competitive success (0, 1). For the splines, all lambda values set to 1.

female are mating resources for a male; therefore, we predicted that the two resources together should represent a higher value resource. Females are expected to incite male–male competition when possible to ensure they mate with more dominant males (Cox & Le Boeuf, 1977; Pizzari, 2001); however, we would expect incitation of competition to result in stronger linear gradients in the presence of a female. There is no a priori reason to predict that selection should be nonlinear. We suggest the difference between our observations and initial predictions reflects a failure to consider that directly achieving access to females is essential to reproduction,

while successfully defending a host plant in the absence of a female is one additional step removed. The evidence for quadratic selection in the presence of a female suggests that males in this situation may have alternative strategies for reproduction. Our findings might also help explain why females are bigger than males. It is very likely that there is fecundity selection for larger size on females, as this is true for most insects (Reeve & Fairbairn, 1999), but selection on males is not simply for larger size. For males, we found that bigger is not always best.

The difference in selection surfaces under different social conditions suggests variation in mating tactics with body size in *N. femorata*. There are many examples of alternative-mating strategies associated with sexual selection (Ra'anan & Sagi, 1985; Shuster & Wade, 1991; Akagawa & Okiyama, 1993; Sinervo & Lively, 1996; Calsbeek *et al.*, 2002; Hankison & Ptacek, 2007); some of which vary with body size (Ra'anan & Sagi, 1985; Shuster & Wade, 1991). Male–male competition is costly to males (Andersson & Iwasa, 1996), and so males might be expected to change their tactics depending on the necessity and importance of the resource over which they compete. Fights in *N. femorata* are not without costs. During our observations, competition resulted in the severing of body parts on several occasions. Given that the smallest males have very little chance of establishing and maintaining a territory for very long, it may be to their benefit to risk injury only in the presence of a female, where there is likely to be an immediate gain of fitness after the fight. Larger males could expect to maintain a territory in the future and so have a significantly higher future reproductive success than small males, therefore reducing the benefit of potentially costly competition immediately. This would result in increased aggressiveness of small males in the presence of a female, which may explain their observed increase in competitive success. It is still of greater benefit to be large rather than small in the presence of a female (Fig. 3).

These findings add further support to the increasing body of evidence suggesting that the environment in which selection occurs has a large bearing on the form of selection itself. Social environments can be much more dynamic than other forms of environmental variation that affect sexual selection. Most existing studies of temporal variation in sexual selection have shown change over breeding seasons (Jann *et al.*, 2000; Punzalan *et al.*, 2008, 2010) or between years (Ferguson & Fairbairn, 2000; Preziosi & Fairbairn, 2000; Gosden & Svensson, 2008), but our study system could vary in social environment and therefore selection on male–male competition in a matter of hours. Social information can provide powerful cues to adaptive behaviour (Dall *et al.*, 2005). Where studied, rapidly varying social environments appear to be important influences on sexual selection arising from male behaviour. For example, the

social composition within a group influences male pheromonal communication and sexual selection in *Drosophila melanogaster* (Kent *et al.*, 2008; Krupp *et al.*, 2008). 'Audience effects' influence male *Poecilia mexicana* courtship (Plath *et al.*, 2008a,b). This suggests that social contexts should be measured and varied whenever sexual selection is studied. For field studies, estimates of the proportion of competitions with or without a female present can add an important layer of information, suggesting how selection may lead to further evolution of male traits. Incorporating these factors into sexual selection can be accomplished by multiple partitioning of selection (Arnold & Wade, 1984; Wade & Kalisz, 1989). This then can be combined with selection arising from mate choice. Ultimately, to determine total sexual selection (Hunt *et al.*, 2009), we will also have to examine how females influence mating success subsequent to the outcome of male–male competition. It is clear that a complete picture of sexual selection can only be gained when sexual selection is measured under all socially and ecologically relevant conditions.

Finally, we found that we could not predict a target of contemporary selection. Enlarged hind legs are important in male–male competition throughout the Coreidae (Miyatke, 1997; Eberhard, 1998; Miller & Emlen, 2010a), so selection on size of the elaborated hind leg areas could be expected in *N. femorata*. We found strong correlations between all measurements of hind leg dimensions and other morphological variables (Table 2), so there is no signal of these being isolated as separate targets of selection. This does not preclude a role for leg elaboration in determining the outcome of male–male competition at some point in the past; it is just that current associations between body size and leg size are complete.

These results suggest many follow-up experiments and manipulations that could provide a more refined view of what is occurring with sexual selection on males. Here we present one experiment that we ran as a quick follow-up to the results we found and present here. Males in the study described above were randomly paired, lessening our power to detect the independent role of leg size in contest outcome. Therefore, we later size matched males (difference of 5% or less) based on pronotum size (a reliable indicator of overall size for many insects, including *N. femorata*, that is easily measured) but not femur size. Males with larger legs won 31 contests but males with smaller legs won 27, regardless of the presence or absence of a female. However, the larger the difference in weapon size, the more likely the male with the larger femur won the contest (Fig. 4). Thus, although there may be little or no contemporary selection on weapon size, it appears as if the femurs can and therefore perhaps did, in the past, influence the outcome of male–male competition. Although this is an attractive and parsimonious explanation, the experiment we present here is just a 'quick and dirty' summary.

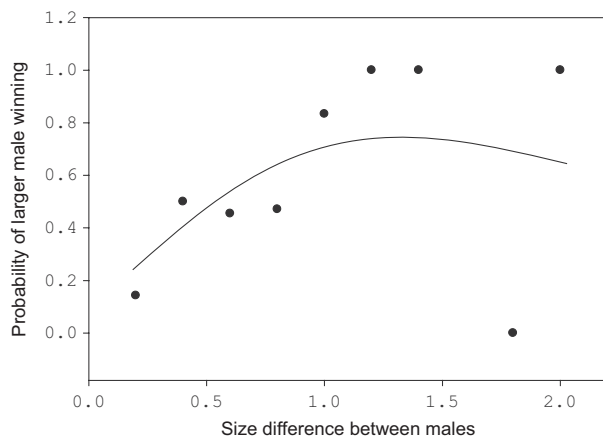


Fig. 4 The importance of larger femurs depends on extent of difference between males ($N = 58$). The difference is defined as size of larger male femur – size of smaller male femur. For all contests, males were within 5% of each other in size, as assessed by their pronotum width. Thus, controlling for size experimentally, the bigger the difference between the males, the more likely the larger male wins in male–male competition. However, in 27 pairings males did not interact and so there was no winner or loser. Cubic spline visualizing likelihood of males with larger femur winning depending on extent of size difference between males.

Manipulative experiments under all social conditions are required to disentangle the contributions of size and elaboration to male success.

Acknowledgments

We thank the members of the Miller Lab, especially Allison Bechard and Stephanie Gillespie, for help throughout the investigation. Stephanie ran the follow-up experiment. Tom Pizzari and an anonymous referee provided very helpful comments that refined our arguments and presentation. NSF grant IOS-0926855 (to CWM), along with grants from the European Social Fund and NERC (to AJM), supported this research.

References

Akagawa, I. & Okiyama, M. 1993. Alternative male mating tactics in *Hypopytychus dybowskii* (Gasterosteiformes): territoriality, body size and nuptial colouration. *Jpn. J. Ichthyol.* **40**: 343–350.

Andersson, M. & Iwasa, Y. 1996. Sexual selection. *Trends Ecol. Evol.* **11**: 53–58.

Arnold, S.J. & Wade, M.J. 1984. On the measurement of natural and sexual selection: theory. *Evolution* **38**: 709–719.

Baird, T.A., Fox, S.F. & McCoy, J.K. 1997. Population differences in the roles of size and coloration in intra- and intersexual selection in the collared lizard, *Crotaphytus collaris*: influence of habitat and social organisation. *Behav. Ecol.* **8**: 506–517.

Bakker, T.C.M. & Sevenster, P. 1983. Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus*). *Behaviour* **86**: 55–71.

Baranowski, R.M. & Slater, J.A. 1986. *Coreidae of Florida (Hemiptera: Heteroptera)*. Florida Department of Agriculture and Consumer Services, Gainesville, FL.

Benson, K.E. & Basolo, A.L. 2006. Male–male competition and the sword in male swordtails, *Xiphophorus helleri*. *Anim. Behav.* **71**: 129–134.

Bonduriansky, R. & Rowe, L. 2003. Interactions among mechanisms of sexual selection on male body size and head shape in a sexually dimorphic fly. *Evolution* **57**: 2046–2053.

Brodie, E.D. III & Janzen, F.J. 1996. On the assignment of fitness values in statistical analyses of selection. *Evolution* **50**: 437–442.

Brodie, E.D. III, Moore, A.J. & Janzen, F.J. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.* **10**: 313–318.

Calsbeek, R., Alonzo, S.H., Zamudio, K. & Sinervo, B. 2002. Sexual selection and alternative mating behaviours generate demographic stochasticity in small populations. *Proc. Biol. Sci.* **269**: 157–164.

Candolin, U. 2000. Male–male competition ensures honest signalling of male parental ability in the three spined stickleback (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.* **49**: 57–61.

Clark, D.C. & Moore, A.J. 1995. Variation and repeatability of male agonistic hiss characteristics and their relationship to social rank in *Grophadorhina portentosa*. *Anim. Behav.* **50**: 719–729.

Clark, D.C., DeBano, S.J. & Moore, A.J. 1997. The influence of environmental quality on sexual selection in *Nauphoeta cinerea* (Dictyoptera: Blaberidae). *Behav. Ecol.* **8**: 46–53.

Cox, C.R. & Le Boeuf, B.J. 1977. Female incitation of male competition: a mechanism in sexual selection. *Am. Nat.* **111**: 317–335.

Dall, S.R.X., Giradeau, L.-A., Olsson, O., McNamara, J.M. & Stephens, D.W. 2005. Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.* **20**: 187–193.

Eberhard, W.G. 1998. Sexual behaviour of *Acanthocephala declivis guatealana* (Hemiptera: Coreidae) and the allometric scaling relationship of their modified hind legs. *Ann. Entomol. Soc. Am.* **91**: 863–871.

Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science* **197**: 215–223.

Ferguson, I.M. & Fairbairn, D.J. 2000. Sex-specific selection and sexual size dimorphism in the waterstrider, *Aquarius remigis*. *J. Evol. Biol.* **13**: 160–170.

Fletcher, R.J. Jr & Miller, C.W. 2008. The type and timing of social information alters offspring production. *Biol. Lett.* **4**: 482–485.

Forsgren, E., Kvarnemo, C. & Lindström, K. 1996. Mode of sexual selection determined by resource abundance in two sand goby populations. *Evolution* **50**: 646–654.

Gosden, T.P. & Svensson, E.I. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution* **62**: 845–856.

Gwynne, D.T. 1993. Food quality controls sexual selection in Mormon crickets by altering male mating investment. *Ecology* **74**: 1406–1413.

Hand, J.L. 1986. Resolution of social conflicts: dominance, egalitarianism, spheres of dominance, and game theory. *Q. Rev. Biol.* **61**: 201–220.

Hankison, S.J. & Ptacek, M.B. 2007. Within and between species variation in male mating behaviours in the maxican sailfin mollies *Poecilia velifera* and *P. petenensis*. *Ethology* **113**: 802–812.

Head, M.L., Lindholm, A.K. & Brooks, R. 2008. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. *Evolution* **62**: 135–144.

- Hunt, J., Breuker, C.J., Sadowski, J.A. & Moore, A.J. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. *J. Evol. Biol.* **22**: 13–26.
- Jann, P., Blanckenhorn, W.U. & Ward, P.I. 2000. Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dung fly *Scathophaga stercoraria*. *J. Evol. Biol.* **13**: 927–938.
- Janzen, F.J. & Stern, H.S. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* **52**: 1564–1571.
- Jirotkul, M. 1999. Operational sex ratios influences female preference and male-male competition in guppies. *Anim. Behav.* **58**: 287–294.
- Kasumovic, M.M. & Andrade, M.C.B. 2008. A change in competitive context reverses sexual selection on male size. *J. Evol. Biol.* **22**: 324–333.
- Kasumovic, M.M., Bruce, M.J., Andrade, M.C.B. & Herberstein, M.E. 2008. Spatial and temporal demographic variation drives within season fluctuations in sexual selection. *Evolution* **62**: 2316–2325.
- Kent, C., Azanchi, R., Smith, B., Fomosa, A. & Levine, J.D. 2008. Social context influences chemical communication in *D. melanogaster* males. *Curr. Biol.* **18**: 1384–1389.
- Kraushaar, U. & Blankenhorn, W.U. 2002. Variation in sexual selection and its effect on size allometry in two dung fly species with contrasting sexual size dimorphism. *Evolution* **56**: 307–321.
- Krupp, J.J., Kent, C., Billeter, J.-C., Azanchi, R., So, A.K.-C., Shonfeld, S.A. *et al.* 2008. Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. *Curr. Biol.* **18**: 1373–1383.
- Kwiatkowski, M.A. & Sullivan, B.K. 2002. Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus* (=ater). *Evolution* **56**: 2039–2051.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. 1990. Male-male competition, ornamentation and the role of testosterone in sexual selection in red jungle fowl. *Anim. Behav.* **40**: 367–373.
- Lindström, K. 2001. Effects of resource distribution on sexual selection and the cost of reproduction in sandgobies. *Am. Nat.* **158**: 64–74.
- Maan, M.E. & Seehausen, O. 2011. Ecology, sexual selection and speciation. *Ecol. Lett.* **14**: 591–602.
- Mateos, C. 1998. Sexual selection in the ring-necked pheasant: a review. *Ethol. Ecol. Evol.* **10**: 313–332.
- McGlothlin, J.W., Moore, A.J., Wolf, J.B. & Brodie, E.D. III 2010. Interacting phenotypes and the evolutionary process. III. Social evolution. *Evolution* **64**: 2558–2574.
- Michener, G.R. & McLean, I.G. 1996. Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. *Anim. Behav.* **52**: 743–758.
- Miller, C.W. & Emlen, D.J. 2010a. Across- and within-population differences in the size and scaling relationship of a sexually selected trait in *Leptoscelis tricolor* (Hemiptera: Coreidae). *Ann. Entomol. Soc. Am.* **103**: 209–215.
- Miller, C.W. & Emlen, D.J. 2010b. Dynamic effects of oviposition on offspring sexually-selected traits and scaling relationships. *Evol. Ecol.* **24**: 375–390.
- Mitchell, P.L. 1980. Combat and territorial defense of *Acanthocephala femorata* (Hemiptera, Coreidae). *Ann. Entomol. Soc. Am.* **73**: 404–408.
- Mitchell-Olds, T. & Shaw, R.G. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.
- Miyatake, T. 1997. Functional morphology of the hind legs as weapons for male contests in *Leptoglossus australis* (Heteroptera: Coreidae). *J. Insect Behav.* **10**: 727–735.
- Moore, A.J. 1987. The behavioral ecology of *Libellula luctuosa* (Burmeister) (Anisoptera: Libellulidae): I. Temporal changes in the population density and the effects on male territorial behavior. *Ethology* **75**: 246–254.
- Moore, A.J. 1989. The behavioral ecology of *Libellula luctuosa* (Burmeister) (Odonata:Libellulidae): III. Male density, OSR, and male and female mating behavior. *Ethology* **80**: 120–136.
- Moore, A.J. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. *Evolution* **44**: 315–331.
- Moore, A.J., Brodie, E.D. III & Wolf, J.B. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* **51**: 1352–1362.
- Petersson, E., Järvi, T., Olsén, H., Mayer, I. & Hedenskog, M. 1999. Male-male competition and female choice in Brown Trout. *Anim. Behav.* **57**: 777–783.
- Pizzari, T. 2001. Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus gallus domesticus*. *Proc. R. Soc. Lond. B* **268**: 181–186.
- Plath, M., Blum, D., Schlupp, I. & Tiedemann, R. 2008a. Audience effect alters mating preferences in Atlantic molly (*Poecilia mexicana*) males. *Anim. Behav.* **75**: 21–29.
- Plath, M., Kromszczyński, K. & Tiedemann, R. 2008b. Audience effect alters male but not female mating preferences. *Behav. Ecol. Sociobiol.* **63**: 381–390.
- Preziosi, R.F. & Fairbairn, D.J. 2000. Lifetime selection on adult body size and components of body size in a waterstrider: opposing selection and the maintenance of sexual size dimorphism. *Evolution* **54**: 558–566.
- Punzalan, D., Rodd, F.H. & Rowe, L. 2008. Contemporary sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*. *Behav. Ecol.* **19**: 860–870.
- Punzalan, D., Rodd, F.H. & Rowe, L. 2010. Temporally variable multivariate sexual selection on sexually dimorphic traits in a wild insect population. *Am. Nat.* **175**: 401–414.
- Ra'anan, Z. & Sagi, A. 1985. Alternative mating strategies in male morphotypes of the freshwater prawn *Macrobrachium rosenbergii* (De Man). *Biol. Bull.* **169**: 592–601.
- Reeve, J.P. & Fairbairn, D.J. 1999. Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* **83**: 697–706.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* **42**: 849–861.
- Shuster, S.M. & Wade, M.J. 1991. Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**: 608–610.
- Sinervo, B. & Lively, C.M. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**: 240–243.
- Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. 2008. Estimating nonlinear selection gradients using quadratic regression: double or nothing? *Evolution* **62**: 2435–2440.
- Sullivan-Beckers, L. & Crocroft, R.B. 2010. The importance of female choice, male-male competition, and signal

- transmission as causes of selection on male mating signals. *Evolution* **64**: 3158–3171.
- Twiss, S.D., Thomas, C., Poland, V., Graves, J.A. & Pomeroy, P. 2007. The impact of climatic variation on the opportunity for sexual selection. *Biol. Lett.* **3**: 12–15.
- Wade, M.J. & Kalisz, S. 1989. The additive partitioning of selection gradients. *Evolution* **43**: 1567–1569.
- Wolf, J.B., Brodie, E.D. III & Moore, A.J. 1999. Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. *Am. Nat.* **153**: 254–266.

Received 26 October 2011; revised 27 December 2011; accepted 25 January 2012