



HOMAGE TO BATEMAN: SEX ROLES PREDICT SEX DIFFERENCES IN SEXUAL SELECTION

Karoline Fritzsche^{1,2} and Göran Arnqvist¹

¹*Animal Ecology, Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36, Uppsala, Sweden*

²*E-mail: karoline.fritzsche@ebc.uu.se*

Received June 12, 2012

Accepted January 8, 2013

Data Archived: Dryad doi:10.5061/dryad.650gr

Classic sex role theory predicts that sexual selection should be stronger in males in taxa showing conventional sex roles and stronger in females in role reversed mating systems. To test this very central prediction and to assess the utility of different measures of sexual selection, we estimated sexual selection in both sexes in four seed beetle species with divergent sex roles using a novel experimental design. We found that sexual selection was sizeable in females and the strength of sexual selection was similar in females and males in role-reversed species. Sexual selection was overall significantly stronger in males than in females and residual selection formed a substantial component of net selection in both sexes. Furthermore, sexual selection in females was stronger in role-reversed species compared to species with conventional sex roles. Variance-based measures of sexual selection (the Bateman gradient and selection opportunities) were better predictors of sexual dimorphism in reproductive behavior and morphology across species compared to trait-based measures (selection differentials). Our results highlight the importance of using assays that incorporate components of fitness manifested after mating. We suggest that the Bateman gradient is generally the most informative measure of the strength of sexual selection in comparisons across sexes and/or species.

KEY WORDS: Bateman gradient, mating system, sexual dimorphism, sperm competition.

Sexual selection is a major generator of diversity, both in terms of trait diversity (Darwin 1871; Andersson 1994) and speciation (Ritchie 2007). Although it is clear that sexual selection regimes are related to variation in mating systems in a very general sense (Trivers 1972; Shuster and Wade 2003), the causal evolutionary relationship between mating system parameters and the strength of sexual selection is much less obvious (Emlen and Oring 1977; Arnqvist and Rowe 2002). Furthermore, there is little consensus on how sexual selection is best defined (Bonduriansky 2001, 2009; Carranza 2009; Shuker 2010; Rosvall 2011) and quantified (Arnold and Duvall 1994; Jones 2009; Klug et al. 2010a; Krakauer et al. 2011). Although Darwin's (1871) original definition of sexual selection was broad and did not exclude sexual selection in females, subsequent research became focused almost entirely on males. However, this view has recently been reevaluated and it is now widely recognized that sexual selection can occur in fe-

males (Bonduriansky 2009; Clutton-Brock 2009; Rosvall 2011). Yet, the relative strength of sexual selection in females is largely unknown at this stage, as very few empirical studies have directly compared the strength of sexual selection in males and females (see Lorch et al. 2008 for review; Fitze and Le Galliard 2011) and no single study has provided comparable estimates of sexual selection in males and females across species with divergent mating systems and sex roles. This is an unfortunate gap in the empirical foundation of the Bateman—Trivers paradigm (Bateman 1948; Trivers 1972).

Comparisons of sexual selection across the sexes and species are made problematic by the fact that there is little consensus over the relative utility of different measures of sexual selection (Table 1; Shuster and Wade 2003; Jones 2009; Klug et al. 2010a,b; Fitze and Le Galliard 2011; Krakauer et al. 2011). Three points, in particular, are discussed. First, is there a single sufficient

Table 1. Empirical measures of sexual selection (see Jones 2009).

Abbreviation	Name	Calculation	Description
β_{ss}	Bateman gradient (sexual selection gradient)	Slope of the least squares regression of relative reproductive success on relative mating success	The strength of selection on mating rate
I	Opportunity for selection	Variance in relative reproductive success	The maximal strength of net sexual selection (i.e., pre- and postmating) ¹
I_s	Opportunity for sexual selection	Variance in relative mating success	The maximal strength of premating sexual selection ¹
s'	Standardized selection differential	Covariance between a trait (standardized) and relative reproductive success	The strength of net sexual selection on a trait (i.e., pre- and postmating) ¹
m'	Standardized mating differential	Covariance between a trait (standardized) and relative mating success	The strength of selection on a trait due to mating success ¹
$cov(z, \epsilon)$	Residual selection differential	Covariance between a trait (standardized) and the residual reproductive success from the Bateman gradient	The strength of selection on a trait due to factors other than mating success ¹

¹Assuming that the experimental design renders other sources of selection (e.g., mortality) negligible.

quantitative measure of sexual selection or do we need multiple measures to fully characterize sexual selection? Second, are measures based on variance across individuals (measures of sexual selection unrelated to phenotypic traits) preferable or do those that are based on phenotypic traits better reflect sexual selection? Finally, how important is it to measure sexual selection in both sexes? To date, the few studies that have compared different measures of selection within and across species (e.g., Bjork and Pitnick 2006; Mills et al. 2007; Paczolt and Jones 2010; Fitze and Le Galliard 2011; Munroe and Koprowski 2011) have done so within a single mating system and have provided somewhat contrasting answers to these questions. Empirical studies that systematically compare sexual selection in both sexes across species with distinct sexual selection regimes and/or mating system parameters, such as from sex role reversed to conventional mating systems, could help settle these issues (Jones et al. 2000).

The controversy over how to measure sexual selection is exacerbated by the fact that many studies estimating the strength of sexual selection suffer from two problematic limitations. First, there may not be direct observational data on all matings occurring. This will, unsurprisingly, render all sexual selection metrics that are based on mating success very difficult to interpret. This is common in field-based studies where mating success is measured as the number of genetic mates (i.e., the number of partners with which offspring is produced), using microsatellite parentage assignment. However, this will yield biased results both because matings that do not result in offspring will not be detected and/or because nonmating individuals will be missing from the

data (Shuster and Wade 2003). This is particularly problematic (i) when only offspring, and not adults, are genotyped (adults with zero reproductive success undetected); (ii) when litter sizes are small (more matings undetected); (iii) only a fraction of offspring are genotyped (some sires undetected); and/or (iv) postmating sexual selection occurs (more matings undetected). Second, laboratory-based studies sometimes estimate sexual selection in the absence of reproductive competition, which can be seen as estimating the upper limit of sexual selection (Lorch 2005). However, how such measures relate to factual selection is unclear as they exclude the two key processes of sexual selection: intrasexual reproductive competition and intersexual mate choice.

Generating a rich and maximally informative set of empirical measures of sexual selection is, thus, clearly very challenging. We suggest that studies should at least (i) gather data for both sexes; (ii) record all matings by direct observation; (iii) be conducted in a competitive setting where males and females can compete for and choose among mates; (iv) measure key phenotypic traits; (v) be conducted over a reasonably long period of time; and (vi) determine paternity/maternality of all offspring produced.

Our objective was to provide a quantitative comparison of the strength of sexual selection in both sexes in several species that vary in their mating system parameters. We conducted a standardized and controlled mating experiment, allowing for mate choice and reproductive competition, where mating success was determined by direct observations and reproductive success was recorded. Our main goals were (i) to test classic theory (Shuster and Wade 2003) by comparing the strength of sexual selection

in males and females in role reversed and conventional mating systems and (ii) to assess various measures (Table 1) of sexual selection in terms of how well they reflect the sex roles. We used four related seed beetle species of two genera (Coleoptera, Bruchidae) that differ strikingly in sexual dimorphism and sex roles (Fox 1993; Eady 1994; Fox et al. 1995; Takakura 1999, 2001; Sakurai and Kasuya 2008; Salehialavi et al. 2011). The mating system of the genus *Callosobruchus* is “conventional,” in the sense that males actively search for, court and compete for females (Rönn et al. 2006) whereas females are reluctant to mate and show female choice (Maklakov and Arnqvist 2009). In the genus *Megabruchidius*, the mating system is role “reversed” such that females actively search for and court males whereas males are choosy and often reject courting females (Takakura 1999; Salehialavi et al. 2011).

Comparisons of trait-specific measures of sexual selection across sexes and species require that selection is measured on a shared phenotypic trait that is under sexual selection in all sexes and species (Krakauer et al. 2011). We chose to measure sexual selection on body size for four related reasons. First, there is marked sexual dimorphism in body size in seed beetles, suggesting a history of sex specific selection on body size (Fox et al. 2007). Second, body size is a highly integrative trait that reflects both phenotypic (e.g., condition) and genetic variation in life-history traits (Fox et al. 2007). Third, the selection differential on body size does not only measure direct selection on body size but also indirect selection on all unmeasured phenotypic traits that correlate with overall size (Lande and Arnold 1983). Finally, several previous studies have indeed revealed pre- and postmating sexual selection on both male and female body size in seed beetles (e.g., Eady 1994; Fox et al. 1995; Savalli and Fox 1998, 1999; Takakura 2001; Salehialavi et al. 2011).

Materials and Methods

MODEL SYSTEM

We used four related (Tuda and Morimoto 2004) seed beetle species that share a common general ecology but differ in their mating system parameters; *Callosobruchus maculatus* (South India), *Callosobruchus chinensis* (Okayama), *Megabruchidius dorsalis* (Tokyo), and *Megabruchidius tonkineus* (Budapest). Beetles were reared on their natural host seeds in 1 L glass containers kept in climate chambers set to reproduce their natural climate and light conditions. *C. maculatus* and *C. chinensis* were reared on *Vigna radiata* (mung beans) and *Vigna angularis* (adzuki beans), respectively, at 29°C and 50% ($\pm 10\%$) relative humidity (RH) under a 12 h : 12 h light : dark cycle. *M. dorsalis* and *M. tonkineus* were maintained on seeds of *Gleditsia triacanthos* (honey locust) at 26°C and 70% ($\pm 10\%$) RH under a 16 h : 8 h light : dark cycle. All beetles were fed with 20% sucrose solution, pollen, and

water. Under these conditions, *Callosobruchus* spp. have a developmental time of approximately 3–4 weeks and *Megabruchidius* spp. of about 7 weeks. We generated virgin individuals for our experiments by isolating single beans (in 24-well culture plates) with larvae, collecting individual beetles as they hatched from the beans and keeping them isolated until the onset of the experiments.

In the genus *Callosobruchus*, one mating is sufficient to fertilize all of a female’s eggs (Eady 1994; Harano et al. 2008). Yet, females mate multiply during their life (Fox 1993; Savalli and Fox 1998; Arnqvist et al. 2005; Harano et al. 2006) and there are several distinct costs and benefits of mating. For example, although females are injured by the male genitalia during mating (Crudginton and Siva-Jothy 2000) they also benefit from the ejaculates transferred by males (Savalli and Fox 1998) which can be sizeable (about 5% of their body weight in *C. maculatus* and 1% in *C. chinensis*; Rönn et al. 2008). Previous work suggests, however, that females do not gain significantly more offspring with each further mating (Arnqvist et al. 2005). Within this genus, there is marked sexual dimorphism in size (females are larger) and antennal morphology (male antennae enlarged; e. g., Fox 1993; Fox et al. 1995; Savalli and Fox 1998; Colgoni and Vamosi 2006). Males use their antennae to detect female pheromones (Tanaka et al. 1981; Shimomura et al. 2008) during active mate search and males persistently court/harass females (Rönn et al. 2006). Females, in contrast, are generally reluctant to mate and resist male mating attempts (Maklakov and Arnqvist 2009).

In the genus *Megabruchidius*, females mate multiply and female offspring production increases with number of matings (Takakura 1999). Males transfer a large and nutritious ejaculate (about 7% of their body weight; Takakura 1999) that represents a costly reproductive investment by males (Takakura 2006; Salehialavi et al. 2011). Females actively search for and court males whereas males are choosy and often reject courting females (Takakura 1999; Salehialavi et al. 2011). Within this genus, sexual dimorphism is less pronounced and is opposite in direction compared to *Callosobruchus*, both in size (males are larger) and antennal morphology (female antennae enlarged; Takakura 1999; Salehialavi et al. 2011). Female *Megabruchidius* are also equipped with a secondary sexual character: the enlarged female abdominal plate has two red oval depressions (Tuda and Morimoto 2004) that are presented to males during courtship and which carry numerous pores that seem to emit pheromones (Salehialavi et al. 2011).

MATING SUCCESS ASSAYS

The experiments were conducted separately for all species using the same protocol. We first collected 150 virgin individuals of each sex and species, aged 1–2 days posteclosion in *Callosobruchus* spp. and less than 5 days in *Megabruchidius* spp. We then scored individual mating and reproductive success across many replicated “mating populations,” each consisting of five

males and five females introduced into a mating dish (6 cm Ø for *Callosobruchus*, 9 cm Ø *Megabruchidius*), using a design similar to that employed by Bjork and Pitnick (2006). We estimated sexual selection independently for males and females, using separate male ($N = 20$ mating populations per species) and female ($N = 10$ mating populations per species) assays. To enable recognition of individuals during the experiment, we anesthetized all beetles lightly with CO₂ (<1 min) prior to the experiment and color-marked them individually on their elytra (uni Paint marker PX-21; Mitsubishi®). Postexperimental tests showed that color marking had no effect on mating success and reproductive success ($P > 0.05$ in all cases). In the male assays, four randomly selected males in each mating population were made sterile for life by irradiation (100 Grey, cesium source) whereas a single focal randomly selected male was a normal fertile male. Sperm cells of irradiated males are fully motile and are able to successfully compete for fertilizations, but eggs fertilized by sperm of irradiated males do not hatch after laying (Harano et al. 2008; Maklakov and Arnqvist 2009). In the female assays, in contrast, all males were normal and fertile. Each replicate mating population yielded data on the mating and reproductive success of a single male in the male assays, whereas it yielded the same data for five females in the female assays.

In each mating population, we recorded the total number of copulations per individual by direct observation for 4 h each day, for five consecutive days, for all five females in the female assays and for the focal male in the male assays. These form our measures of mating success. Between these 4-h mating episodes, all beetles were isolated for 20 h in their individual petri dishes in climate chambers. They were provided with 20% sucrose solution, pollen (Bee Pollen Capsules, Manuak Health Ltd., New Zealand), and water and female dishes were also provided with an ad libitum supply of beans for oviposition (13 g of *Vigna* beans for *Callosobruchus* spp. and 100 g of *Gleditsia* for *Megabruchidius* spp.). Individual dishes of *Megabruchidius* spp. were 12 cm Ø glass petri dishes and those of *Callosobruchus* spp. were 9 cm Ø plastic petri dishes.

REPRODUCTIVE SUCCESS

Females were kept isolated in their individual petri dishes between the five mating episodes (above) and continuously after the last mating episode. They were allowed to oviposit until death, at most 33 days after the final mating episode. Beans were subsequently incubated in climate chambers (5 weeks for *Callosobruchus* and 9 weeks for *Megabruchidius*) after which all hatched offspring were counted. In the female assays, reproductive success of an individual female was measured as the total number of offspring she produced. In the male assays, reproductive success of the focal male was measured as the sum of the total number of offspring produced by all five females in his mating population.

BODY SIZE

We measured the mean length of the left and right elytra of each individual as an estimate of body size, as elytra length correlates very closely with body weight (Wilson and Hill 1989). Elytra length was measured using a digitizing tablet (Summsketch III; Summagraphics Corp., Austin, TX) placed under a dissecting microscope equipped with a side-mounted camera lucida (Leica® MZ8; Leica Microsystems GmbH, Wetzlar, Germany).

STATISTICS

We included all focal individuals, also those with zero mating success and/or zero reproductive success, in our analyses (Shuster and Wade 2003; Wade and Shuster 2004). We estimated all common measures of sexual selection (see Table 1), using the methods detailed in Jones (2009) (see also Wade 1979; Arnold and Duvall 1994). Standard errors for variances (I and I_s) were estimated by bootstrapping data (10,000 iterations). Data on mating rate and reproductive success was relativized, by dividing with the mean, and body size data were standardized to a mean of zero and unit variance prior to analyses (see Jones 2009). Data for males were relativized/standardized across all males (one per mating population) whereas data on females was relativized/standardized on a per mating population level (i.e., per dish), because relative fitness is best expressed as being relative to those individuals that a given individual directly competes with. We stress, however, that the alternative way of standardizing the data on females (i.e., over all females) yielded results that were very similar indeed to those reported here (see Supporting Information).

To compare the estimates of sexual selection across sexes and mating systems, we used F -ratio tests for variance-based measures and partially nested analyses of covariance (where species was nested within mating system) for all other measures of sexual selection. Statistical analyses were performed with Systat 13.0, JMP 9.0.0, and R 2.6.2 (R Development Core Team). Means are given \pm SE. We note that our manner of analyzing data on females could potentially be biased if selection differed markedly across mating populations. We assessed this and found no evidence for differences across mating populations (see Supporting Information).

Results

The average number of matings per individual differed across species ($F_{2,196} = 37.6$, $P < 0.001$), and was higher in species with reversed sex roles than those with conventional sex roles (focused post hoc contrast; $F_{1,196} = 103.2$, $P < 0.001$; *M. tonkineus*: 5.26 ± 0.40 ; *M. dorsalis*: 5.66 ± 0.27 ; *C. chinensis*: 2.2 ± 0.19 ; *C. maculatus*: 3.3 ± 0.15 ; data from female assays).

Table 2. Partially nested analyses of covariance of various measures of sexual selection. Inferential models also included mating system, sex, mating system \times sex and species (mating system) but these are not reproduced below as their marginal effects were all zero (marginal means of response variables were, by definition, identical across mating systems, sexes, and species).

Measure of sexual selection	Response variable	Source	Type I SS	F	df	P
β_{SS}	Relative reproductive success	Mating success	16.99	143.9	1	<0.001
		Sex \times mating success	0.54	4.5	1	0.034
		Mating system \times mating success	5.99	50.7	1	<0.001
		Mating system \times sex \times mating success	0.015	0.1	1	0.720
m'	Relative mating success	Body size	1.98	10.8	1	0.001
		Sex \times body size	0.04	0.2	1	0.642
		Mating system \times body size	0.81	4.4	1	0.037
		Mating system \times sex \times body size	0.27	1.4	1	0.231
s'	Relative reproductive success	Body size	2.30	12.1	1	<0.001
		Sex \times body size	0.12	0.7	1	0.422
		Mating system \times body size	0.20	1.1	1	0.307
		Mating system \times sex \times body size	0.83	4.4	1	0.038
$cov(z, \epsilon)$	Residuals	Body size	1.17	11.5	1	<0.001
		Sex \times body size	0.20	2.0	1	0.158
		Mating system \times body size	0.12	1.2	1	0.283
		Mating system \times sex \times body size	0.10	1.0	1	0.328

VARIANCE-BASED MEASURES OF SEXUAL SELECTION

Relative reproductive success increased overall with mating success (see Table 2, β_{SS}). Bateman gradients were steeper in males than in females, as evidenced by a significant interaction between sex and mating success. Mating system had an even stronger effect, such that the Bateman gradients were steeper in role reversed species (Fig. 1A).

A graphical inspection of the results suggests that the opportunity for premating sexual selection was higher in males than in females in species with conventional sex roles (see Fig. 1B). In contrast, in role-reversed species, the opportunity for premating sexual selection was similar across sexes or higher in females than in males. However, variance in mating success differs significantly between the sexes in only one species (*C. maculatus*: $F_{49,19} = 0.48$, $P = 0.042$).

The opportunity for selection seemed higher in males than in females in all species (see Fig. 1C). However, the difference between the sexes was only significant in species with conventional sex roles (*C. maculatus*: $F_{49,19} = 0.30$, $P = 0.001$; *C. chinensis*: $F_{49,18} = 0.12$, $P < 0.001$).

TRAIT-BASED MEASURES OF SEXUAL SELECTION

Body size was positively related to mating success in both sexes (Fig. 1E) and overall mating differentials were higher in species with conventional sex roles, as evidenced by a significant interaction between body size and mating system (Table 2, m'). The mating differential did, however, not differ significantly between the sexes.

Relative reproductive success was also positively related to body size (Fig. 1F) and the selection differentials were significantly higher in males than in females. This difference was, however, contingent upon mating system: the sex differences in selection differential were stronger in species with conventional sex roles (see Table 2, s' ; three-way interaction).

The residuals from the Bateman gradient were positively related to body size. A visual inspection of the residual selection differentials suggest that they were higher in males than in females (Fig. 1D), but the difference between the sexes was not statistically significant (Table 2, $cov(z, \epsilon)$).

COMPARISON BETWEEN VARIANCE- AND TRAIT-BASED MEASURES

There are theoretical reasons to expect I and s' to be related to one another and I_s and m' to be related to one another. An inspection of the correlation between measures of sexual selection (Table 3) and a comparison between Figure 1C and F and between Figure 1B and E verified these expectations. The residual selection, $cov(z, \epsilon)$, has been interpreted as a trait-based measure of postmating sexual selection and/or fecundity selection (Jones 2009). Our data are consistent with such an interpretation, as residual selection correlated with s' (which includes components of fitness manifested after mating) but not with m' (which does not include such components). We note, however, that residual selection will include both components of sexual (sperm competition in males, differential allocation in both sexes) and natural (fecundity selection in both sexes) selection. In an effort to assess the relative contribution of selection due to mating success and selection due to factors other

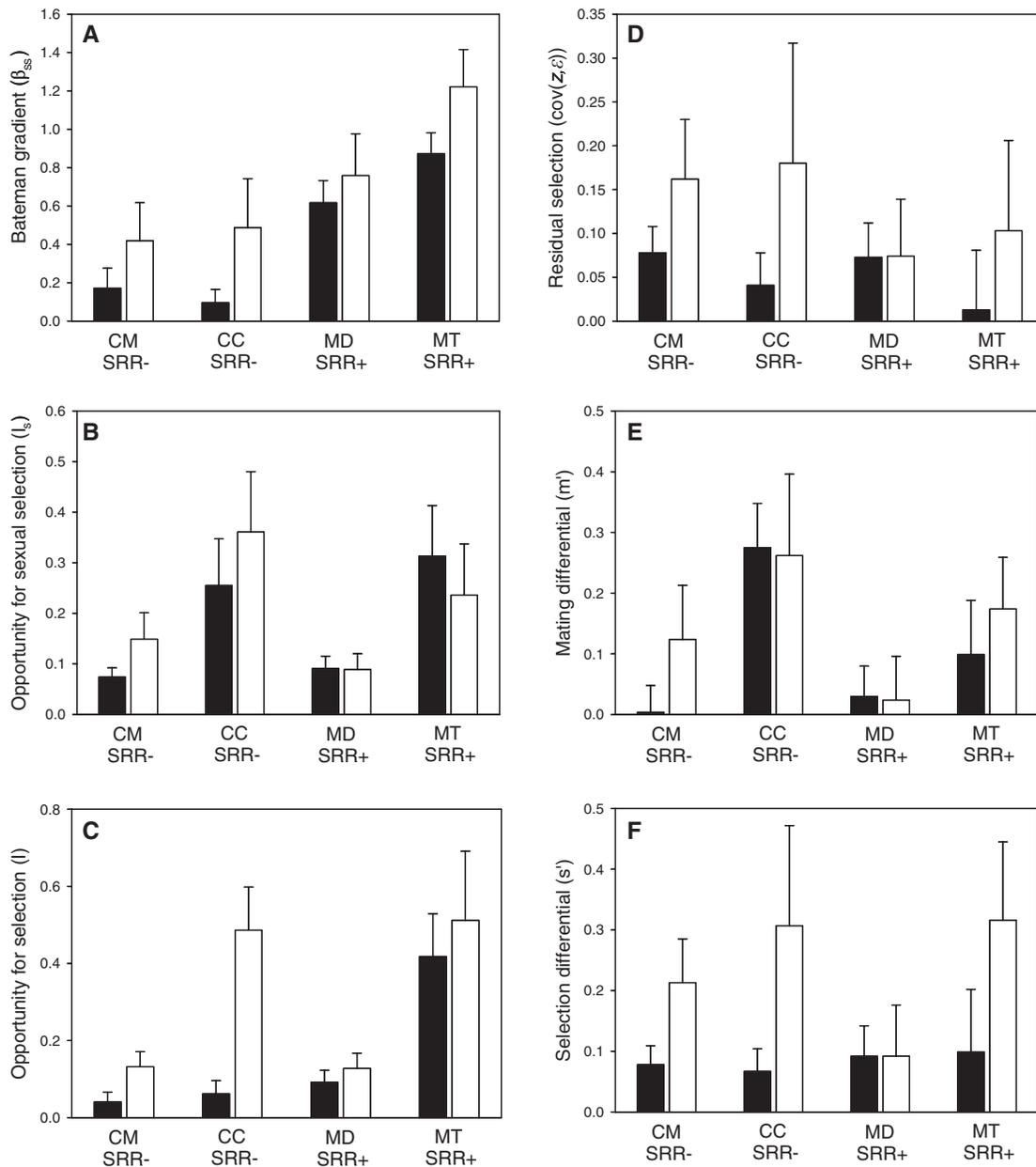


Figure 1. Six sex-specific measures of sexual selection (see Table 1) across four different seed beetle species (CM = *Callosobruchus maculatus*; CC = *Callosobruchus chinensis*; MD = *Megabruichidius dorsalis*; MT = *Megabruichidius tonkineus*; white bars = males; black bars = females). SRR+ denotes species that are sex-role-reversed and SRR- denotes species with conventional sex roles. Error bars represent SE.

than mating success to variation in s' across sexes and species, we regressed s' on m' and $cov(z, \varepsilon)$ simultaneously using multiple regression. The standardized partial regression coefficients suggested that the contribution of residual selection was about twice as large as that of m' (m' : $\beta' = 0.30$; $cov(z, \varepsilon)$: $\beta' = 0.67$). Finally, the lack of a positive correlation between the Bateman gradient and m' is noteworthy. This demonstrates a poor correspondence between selection on mating success (i.e., the Bateman gradient) and how important body size is in terms of determining mating success.

Discussion

Our work yields a series of novel insights into differences in the strength of sexual selection between the sexes and across mating systems. Below, we will discuss three distinct aspects of our work. We will first consider how well various measures of sexual selection reflect the underlying mating system parameters. We will then discuss differences in sexual selection in males and females across divergent mating systems. Finally, our work suggests that residual selection is an important and often omitted component of selection.

Table 3. Matrix of correlation coefficients (r_p) between the various measures of sexual selection across all species and both sexes ($n = 8$).

	β_{ss}	I	I_s	s'	m'
I	0.69				
I_s	0.18	0.76			
s'	0.45	0.76	0.49		
m'	-0.12	0.44	0.81	0.50	
$cov(z, \epsilon)$	-0.06	0.25	0.12	0.76	0.29

We first note that the experiments reported upon here were very labor intensive and, as a result, our taxon sampling was limited to four species from two genera. This prohibits the use of quantitative phylogenetic comparative methods and represents a limitation of our study. Although we thus cannot formally test for correlated evolution between, for example, mating system and sexual selection across species, we suggest that observations of the predicted association between mating system and sexual selection are informative and expand our understanding beyond single species studies.

MEASURES OF SEXUAL SELECTION

Previous comparisons of the correspondence between variance- and trait-based measures of sexual selection within-species have yielded somewhat mixed results (e.g., Jones et al. 2000, 2004; Bjork and Pitnick 2006; Mills et al. 2007; Paczolt and Jones 2010; Fitze and Le Galliard 2011; Munroe and Koprowski 2011). When comparing species with evolved differences in behavior and sexual dimorphism, we found a fairly close correspondence between variance-based measures of sexual selection (I and I_s) and trait-based measures (s' and m'). We suggest that this is due in part to the fact that the opportunity of sexual selection places an upper limit on trait-based measures of sexual selection (Shuster and Wade 2003) and in part to the fact that body size is an integrative key trait for both sexes in all four species.

The Bateman gradient measures the strength of selection on mating success and as such should predict the degree of competition for matings (Bateman 1948; Arnold and Duvall 1994; Shuster and Wade 2003; Wade and Shuster 2005; Shuker 2010). It is reasonable to assume that phenotypic variation should be more important for mating success (i.e., higher m') in situations where there is a high degree of competition for matings. Yet, we failed to find any correspondence between estimates of Bateman gradients and m' . This may be due to a fundamental difference between the Bateman gradient and trait-based measures of sexual selection. Bateman (1948) suggested that the Bateman gradient reflects inherent properties of male and female reproduction. As such, the Bateman gradient will primarily reveal basic aspects of

the economics of mating and relative parental investment and be less affected by variation in phenotypic traits and environmental conditions (Arnold and Duvall 1994; Wade and Shuster 2005). In contrast, trait-based variance-standardized estimates of sexual selection are affected by the absolute amount of phenotypic variation at any given point (Hereford et al. 2004) and often vary temporally and spatially with environmental conditions (Arnqvist 1992; Bussiere et al. 2008; Gosden and Svensson 2008). This problem is aggravated by the fact that strong directional selection may exhaust genetic variation in those traits that are under selection. For example, Reinhold (2011) recently found that traits under stronger sexual selection showed lower phenotypic variability. This is problematic when the aim is to compare the strength of sexual selection across sexes or species, because strong selection will reduce phenotypic variance which, in turn, will lower trait-based estimates of sexual selection.

Which measure of sexual selection is most appropriate will depend on the purpose of any given study (Krakauer et al. 2011). Our aim was to compare the overall strength of sexual selection across sexes and species. Sexual selection is, following the most general definition, generated by competition for matings or fertilizations (Darwin 1871; Andersson 1994). The strength of sexual selection should thus reflect the intensity of competition for matings or fertilizations. We suggest that, for our purposes, the Bateman gradient is the most relevant measure of the strength of sexual selection. This is due, in part, to the fact that it reflects latent properties of the mating system representing both male and female adaptations to mating. Furthermore, the explicit purpose of the Bateman gradient is to provide a measure of the strength of competition for matings (Arnold and Duvall 1994; Wade and Shuster 2005). Our suggestion is supported by two observations. First, sexual dimorphism in body size is more pronounced in *Callosobruchus* than in *Megabruchidius*. This is consistent with the fact that the relative difference between sexes in the Bateman gradient is smaller in *Megabruchidius* than *Callosobruchus* (Fig. 1A) and the observation that this is primarily due to steeper Bateman gradients in females in *Megabruchidius*. Furthermore, the difference in opportunity for selection between the sexes was larger in species with conventional sex roles. Second, and more importantly, the Bateman gradient, along with I and I_s , were better predictors of sexual dimorphism in reproductive behavior and secondary sexual traits across species. In fact, a survey (see Supporting Information) showed that the assignment of mating system to data was not significantly different from the random expectation for trait-based measures of sexual selection. The results of the survey show that the data for the Bateman gradient, I and I_s better mirror theoretical expectations, especially in terms of the relative strength of sexual selection in males and females (Fig. 1).

DIFFERENCES IN SEXUAL SELECTION IN FEMALES AND MALES

Overall, sexual selection was stronger in males than in females (Fig. 1A–F). In this very general sense, our comprehensive results are consistent with the view that sexual selection is typically stronger in males than in females (Bateman 1948; Trivers 1972; Andersson 1994; Arnold and Duvall 1994; Shuster and Wade 2003). Sexual selection was, however, sizeable in females and the relative strength of selection in males and females varied with mating system. In particular, we found that the strength of sexual selection in females was stronger in role-reversed mating systems compared to conventional ones (Fig. 1A–C). Because role reversal is typically rooted in male mate provisioning or male parental investment, our observation accords with theory (Trivers 1972). Male mate provisioning will elevate female Bateman gradients, as females may accumulate direct resources with each additional mating (Arnold and Duvall 1994; Lorch 2002), and females should thus compete for matings in role-reversed mating systems (Trivers 1972). Accordingly, strong sexual selection in females has been documented in a few role-reversed taxa, such as seahorses (Kvarnemo et al. 2007), jacanas (Emlen and Wrege 2004), and orthopterans (Simmons 1992; Gwynne 1993), although no previous study has been able to directly compare the relative intersexual strength of selection across related species with divergent mating systems. Male *Megabruchidius* donate a nutritional ejaculate to females and previous research has shown that female *M. dorsalis* gain approximately 10 eggs for each additional mating (Takakura 2006), which corresponds very well with the slope of the female Bateman gradient in this study (*M. dorsalis*: 9.8 ± 2.3 ; *M. tonkineus*: 14.9 ± 2.3). In contrast, there is no obvious increase in female fecundity with mating success in female *C. maculatus* (Arnqvist et al. 2005) and *C. chinensis* (Harano et al. 2006).

Male mate provisioning (e.g., “nuptial gifts”) should also affect male Bateman gradients. In the absence of a trade-off in males between mate provisioning and mating success, the evolution of increased mate provisioning should increase the Bateman gradient in males as much as in females (Lorch 2002, 2005) and Bateman gradients should be higher in males than in females even in the presence of male mate provisioning (Arnold and Duvall 1994). A higher Bateman gradient in females than males requires a strong trade-off in males between mating success and per-mating fertilization success, and is made more likely if the cost/value of the provision is high, resource levels are low, and male mating success is random with regards to male condition (Lorch 2002). Two facets of our results are consistent with these general predictions. First, the highest Bateman gradients were observed in males of the role-reversed *Megabruchidius* species, as expected if male provisioning elevates male Bateman gradients. Second, matings are clearly costly to male *Megabruchidius* (Takakura 2006) in

terms of decreased life span (Salehialavi et al. 2011), and a trade-off between mating success and per-mating fertilization success therefore seems likely. However, such a trade-off might not have had a strong effect in our experiments because we provided males with ad libitum food resources. This said, we note that the cost of mating to males is unaffected by food provisioning (Salehialavi et al. 2011). In addition, the fact that male mating success is positively related to male body size and phenotypic condition in *Megabruchidius* (Fig. 1D–F; Salehialavi et al. 2011) suggests that the males that mated most frequently were primarily those that could “afford” to mate at a high rate. Males may thus vary in terms of resources acquisition, which would further lessen the effect of a trade-off in terms of depressing the male Bateman gradient (Lorch 2002). These considerations suggest that the Bateman gradients need not necessarily be higher in females than in males in *Megabruchidius*, although the behavioral sex roles are reversed. We note that the larger opportunity for pre-mating sexual selection in females than in males is consistent with the reversal of the sex roles in *Megabruchidius* species (Fig. 1B).

POSTMATING SEXUAL SELECTION

Postmating sexual selection through sperm competition and/or cryptic female choice is a major component of sexual selection in general (e.g., Birkhead and Møller 1998), and in seed beetles in particular (Eady 1994; Wilson et al. 1997; Brown and Eady 2001; Fricke and Arnqvist 2004). Because sperm competition can affect both variance-based measures of net sexual selection (Simmons and Parker 1996) and the slope of the Bateman gradient (Lorch 2002; Wade and Shuster 2005), estimating its impact would be informative. Jones (2009) noted that residual selection will include both postmating sexual selection and fecundity selection. In males, residual selection measures both covariance between a trait z and mate fecundity and covariance between z and sperm competition success. The former could be caused by, for example, paternal investment or differential allocation by females. In females, residual selection measures covariance between a trait z and female fecundity. This could be caused by, for example, direct effects of the trait z on female fecundity or the effect that the trait z in females has on paternal investment (through differential allocation by males). Disentangling these possibilities requires specific experimental efforts (Jones 2009).

We suggest that both postmating sexual selection and fecundity selection contributes to the residual selection seen on z (body size). Both female body size and male body size are known to correlate with female fecundity in seed beetles, although the strength of these relationships are rather weak and variable across species (Savalli and Fox 1998; Fox et al. 2007). Similarly, large size in males is known to be favored by postmating sexual selection in seed beetles: large males tend to produce a larger ejaculate, which delays remating in females (Takakura 2001; Fox et al.

2007). Although our data does not allow us to assess the relative contribution of postmating sexual selection and fecundity selection to residual selection, it is clear that residual selection is a sizeable component of net selection. Moreover, the fact that variation in the Bateman gradient and I_s collectively predicted 90% of the variation in I across sexes and species (multiple regression; Bateman gradient: $\beta' = 0.58$, I_s : $\beta' = 0.66$, multiple $R^2 = 0.90$) suggests that the contribution of residual selection to the opportunity for selection was similar across sexes and species in our assays. We wish to highlight two points. First, the sign of the residual selection differential was significantly positive (Fig. 1D, Table 2), such that large individuals of both sexes gained more offspring per mating. We note that there were no significant differences across mating systems or sexes in residual selection (Table 2). Second, in the absence of residual selection, the product of m' and the Bateman gradient should equal s' (Jones 2009). In our assays, the average product was significantly lower than s' overall (0.07 vs. 0.16; paired t -test; $t_7 = 4.52$, $P = 0.003$), showing that residual selection was a significant contributor to variation in net selection on body size (mean $\text{cov}(z, \epsilon) = 0.09$). Similarly, the fact that the correlation between the residual selection differential and s' was higher than that between m' and s' (Table 3), shows that a sizeable proportion of variation in net selection on body size across sexes and species is due to residual selection.

Conclusion

Variation in the estimated strength of sexual selection across seed beetle species corresponded to evolved variation in sex roles and sexual dimorphism. Although we found a correspondence between variance- and trait-based measures of sexual selection, the variance-based measures were better predictors of sexual dimorphism in reproductive behavior and secondary sexual traits across species. We conclude that the Bateman gradient is the most informative measure of variation in the overall strength of sexual selection. Studies comparing the strength of sexual selection within and across species should ideally be conducted in a setting allowing for both pre- and postmating reproductive competition and should record all occurring matings.

ACKNOWLEDGMENTS

We thank N. Amvrosiadi, H. Chen, C. Hotzy, and B. Stenerlöw for technical assistance; I. Ahnesjö, A. Berglund, R. Bonduriansky, M. Pódevin, and two anonymous reviewers for discussions and comments. E. H. Duval, J. Heuschele, M. D. Jennions, A. G. Jones, H. Klug, H. Kokko, A. H. Krakauer, P. D. Lorch, S. M. Shuster, and M. S. Webster kindly participated in the blind survey. This work was supported by the European Research Council (AdG-294333, GA), the Swedish Research Council (621-2010-5266, GA), Zoologiska Stiftelsen (KF), and the EBC graduate school on genomes and phenotypes (KF).

LITERATURE CITED

- Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.
- Arnold, S. J., and D. Duvall. 1994. Animal mating systems—a synthesis based on selection theory. *Am. Nat.* 143:317–348.
- Arnqvist, G. 1992. Spatial variation in selective regimes—sexual selection in the water strider *Gerris odontogaster*. *Evolution* 46:914–929.
- Arnqvist, G., and L. Rowe. 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature* 415:787–789.
- Arnqvist, G., T. Nilsson, and M. Katvala. 2005. Mating rate and fitness in female bean weevils. *Behav. Ecol.* 16:123–127.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2:349–368.
- Birkhead, T. R., and A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press, London.
- Bjork, A., and S. Pitnick. 2006. Intensity of sexual selection along the anisogamy-isogamy continuum. *Nature* 441:742–745.
- Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* 76:305–339.
- . 2009. Reappraising sexual coevolution and the sex roles. *PLoS Biol.* 7: e1000255.
- Brown, D. V., and P. E. Eady. 2001. Functional incompatibility between the fertilization systems of two allopatric populations of *Callosobruchus maculatus* (Coleoptera : Bruchidae). *Evolution* 55:2257–2262.
- Bussiere, L. F., J. Hunt, K. N. Stolting, M. D. Jennions, and R. Brooks. 2008. Mate choice for genetic quality when environments vary: suggestions for empirical progress. *Genetica* 134:69–78.
- Carranza, J. 2009. Defining sexual selection as sex-dependent selection. *Anim. Behav.* 77:749–751.
- Clutton-Brock, T. 2009. Sexual selection in females. *Anim. Behav.* 77:3–11.
- Colgoni, A., and S. M. Vamosi. 2006. Sexual dimorphism and allometry in two seed beetles (Coleoptera : Bruchidae). *Entomol. Sci.* 9:171–179.
- Crudgington, H. S., and M. T. Siva-Jothy. 2000. Genital damage, kicking and early death—the battle of the sexes takes a sinister turn in the bean weevil. *Nature* 407:855–856.
- Darwin, C. 1871. The descent of man and selection in relation to sex. John Murray, London, UK.
- Eady, P. 1994. Intraspecific variation in sperm precedence in the Bruchid beetle *Callosobruchus-maculatus*. *Ecol. Entomol.* 19:11–16.
- Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and evolution of mating systems. *Science* 197:215–223.
- Emlen, S. T., and P. H. Wrege. 2004. Size dimorphism, intrasexual competition, and sexual selection in Wattled Jacana (*Jacana jacana*), a sex-role-reversed shorebird in Panama. *Auk* 121:391–403.
- Fitze, P. S., and J. F. Le Galliard. 2011. Inconsistency between different measures of sexual selection. *Am. Nat.* 178:256–268.
- Fox, C. W. 1993. Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera, Bruchidea). *Funct. Ecol.* 7:203–208.
- Fox, C. W., L. A. McLennan, and T. A. Mousseau. 1995. Male body-size affects female lifetime reproductive success in a seed beetle. *Anim. Behav.* 50:281–284.
- Fox, C. W., R. C. Stillwell, and J. Moya-Larano. 2007. Variation in selection, phenotypic plasticity, and the ecology of sexual size dimorphism in two seed-feeding beetles. Pp. 88–96 in D. J. Fairbairn, W. U. Blanckenhorn, and T. Székely, eds. Sex, size, and gender roles: evolutionary studies of sexual size dimorphism. Oxford Univ. Press, Oxford, UK.
- Fricke, C., and G. Arnqvist. 2004. Divergence in replicated phylogenies: the evolution of partial post-mating prezygotic isolation in bean weevils. *J. Evol. Biol.* 17:1345–1354.
- Gosden, T. P., and E. I. Svensson. 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.
- Harano, T., Y. Yasui, and T. Miyatake. 2006. Direct effects of polyandry on female fitness in *Callosobruchus chinensis*. *Anim. Behav.* 71:539–548.
- Harano, T., Y. Nakamoto, and T. Miyatake. 2008. Sperm precedence in *Callosobruchus chinensis* estimated using the sterile male technique. *J. Ethol.* 26:201–206.
- Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing strengths of directional selection: how strong is strong? *Evolution* 58:2133–2143.
- Jones, A. G. 2009. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution* 63:1673–1684.
- Jones, A. G., G. Rosenqvist, A. Berglund, S. J. Arnold, and J. C. Avise. 2000. The Bateman gradient and the cause of sexual selection in a sex-role-reversed pipefish. *Proc. R. Soc. B-Biol. Sci.* 267:677–680.
- Jones, A. G., J. R. Arguello, and S. J. Arnold. 2004. Molecular parentage analysis in experimental newt populations: the response of mating system measures to variation in the operational sex ratio. *Am. Nat.* 164:444–456.
- Klug, H., J. Heuschele, M. D. Jennions, and H. Kokko. 2010a. The mismeasurement of sexual selection. *J. Evol. Biol.* 23:447–462.
- Klug, H., K. Lindstrom, and H. Kokko. 2010b. Who to include in measures of sexual selection is no trivial matter. *Ecol. Lett.* 13:1094–1102.
- Krakauer, A. H., M. S. Webster, E. H. Duval, A. G. Jones, and S. M. Shuster. 2011. The opportunity for sexual selection: not mismeasured, just misunderstood. *J. Evol. Biol.* 24:2064–2071.
- Kvarnemo, C., G. I. Moore, and A. G. Jones. 2007. Sexually selected females in the monogamous Western Australian seahorse. *Proc. R. Soc. B-Biol. Sci.* 274:521–525.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Lorch, P. D. 2002. Understanding reversals in the relative strength of sexual selection on males and females: a role for sperm competition? *Am. Nat.* 159:645–657.
- . 2005. Using upper limits of “Bateman gradients” to estimate the opportunity for sexual selection. *Integr. Comp. Biol.* 45:924–930.
- Lorch, P. D., L. F. Bussiere, and D. T. Gwynne. 2008. Quantifying the potential for sexual dimorphism using upper limits on Bateman gradients. *Behaviour* 145:1–24.
- Maklakov, A. A., and G. Arnqvist. 2009. Testing for direct and indirect effects of mate choice by manipulating female choosiness. *Curr. Biol.* 19:1903–1906.
- Mills, S. C., A. Grapputo, E. Koskela, and T. Mappes. 2007. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 274:143–150.
- Munroe, K. E., and J. L. Koprowski. 2011. Sociality, Bateman’s gradients, and the polygynandrous genetic mating system of round-tailed ground squirrels (*Xerospermophilus tereticaudus*). *Behav. Ecol. Sociobiol.* 65:1811–1824.
- Paczolt, K. A., and A. G. Jones. 2010. Post-copulatory sexual selection and sexual conflict in the evolution of male pregnancy. *Nature* 464:U401–U494.
- Reinhold, K. 2011. Variation in acoustic signalling traits exhibits footprints of sexual selection. *Evolution* 65:738–745.
- Ritchie, M. G. 2007. Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* 38:79–102.
- Rönn, J., M. Katvala, and G. Arnqvist. 2006. The costs of mating and egg production in *Callosobruchus* seed beetles. *Anim. Behav.* 72:335–342.
- . 2008. Interspecific variation in ejaculate allocation and associated effects on female fitness in seed beetles. *J. Evol. Biol.* 21:461–470.
- Rosvall, K. A. 2011. Intrasexual competition in females: evidence for sexual selection? *Behav. Ecol.* 22:1131–1140.
- Sakurai, G., and E. Kasuya. 2008. The costs of harassment in the adzuki bean beetle. *Anim. Behav.* 75:1367–1373.
- Salehialavi, Y., K. Fritzsche, and G. Arnqvist. 2011. The cost of mating and mutual mate choice in 2 role-reversed honey locust beetles. *Behav. Ecol.* 22:1104–1113.
- Savalli, U. M., and C. W. Fox. 1998. Sexual selection and the fitness consequences of male body size in the seed beetle *Stator limbatus*. *Anim. Behav.* 55:473–483.
- . 1999. The effect of male size, age, and mating behavior on sexual selection in the seed beetle *Callosobruchus maculatus*. *Ethol. Ecol. Evol.* 11:49–60.
- Shimomura, K., S. Nojima, S. Yajima, and K. Ohsawa. 2008. Homofarnesals: female sex attractant pheromone components of the southern cowpea weevil, *Callosobruchus chinensis*. *J. Chem. Ecol.* 34:467–477.
- Shuker, D. M. 2010. Sexual selection: endless forms or tangled bank? *Anim. Behav.* 79:E11–E17.
- Shuster, S. M., and M. J. Wade. 2003. *Mating systems and strategies*. Princeton Univ. Press, Princeton, NJ.
- Simmons, L. W. 1992. Quantification of role reversal in relative parental investment in a bush cricket. *Nature* 358:61–63.
- Simmons, L. W., and G. A. Parker. 1996. Parental investment and the control of sexual selection: can sperm competition affect the direction of sexual competition? *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 263:515–519.
- Takakura, K. 1999. Active female courtship behavior and male nutritional contribution to female fecundity in *Bruchidius dorsalis* (Fahraeus) (Coleoptera : Bruchidae). *Res. Popul. Ecol.* 41:269–273.
- . 2001. Courtship-role-reversal in the bean weevil, *Bruchidius dorsalis* (Coleoptera : Bruchidae): Interplay between male-male competition and cryptic female choice. *Appl. Entomol. Zoolog.* 36:311–316.
- . 2006. Estimation of relative reproductive expenditure in the courtship-role-reversed bean weevil, *Bruchidius dorsalis* (Fahraeus). *J. Ethol.* 24:33–36.
- Tanaka, K., K. Ohsawa, H. Honda, and I. Yamamoto. 1981. Copulation release pheromone, erectin, from the azuki bean weevil (*Callosobruchus chinensis* L.). *J. Pestic. Sci.* 6:75–82.
- Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136–179 in B. Campbell, ed. *Sexual selection and the descent of man*. Heinemann, London.
- Tuda, M., and K. Morimoto. 2004. A new species *Megabruchidius sophorae* (Coleoptera, Bruchidae), feeding on seeds of *Styphnolobium* (Fabaceae) new to Bruchidae. *Zool. Sci.* 21:105–110.
- Wade, M. J. 1979. Sexual selection and variance in reproductive success. *Am. Nat.* 114:742–747.
- Wade, M. J., and S. M. Shuster. 2004. Sexual selection: Harem size and the variance in male reproductive success. *Am. Nat.* 164:E83–E89.
- . 2005. Don’t throw Bateman out with the bathwater! *Integr. Comp. Biol.* 45:945–951.
- Wilson, K., and L. Hill. 1989. Factors affecting egg maturation in the bean weevil *Callosobruchus maculatus*. *Physiol. Entomol.* 14:115–126.
- Wilson, N., S. C. Tubman, P. E. Eady, and G. W. Robertson. 1997. Female genotype affects male success in sperm competition. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 264:1491–1495.

Associate Editor: R. Bonduriansky

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Rater success in identifying mating system (conventional or role reversed) for various measures of sexual selection, when ratings were blind (i.e., raters were uninformed of which mating system was associated with a particular observation).

Table S1. Alternative ways of estimating sexual selection in females of the four species studied.