

Original Article

The cost of mating and mutual mate choice in 2 role-reversed honey locust beetles

Yassaman Salehialavi, Karoline Fritzsche, and Göran Arnqvist

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

Situations where both males and females simultaneously exercise mate choice may be much more common than previously believed. Yet, experimental studies of mutual mate choice are rare as is information on the types of female traits that are favored by male mate choice. We first assessed the cost of mating to males under different feeding regimes in 2 honey locust beetles (Bruchidae, *Megabruchidius* spp.) where females actively search for and court males. Further, in a series of mate choice trials, we manipulated female mating status and male food provisioning to assess how male and female characteristics affected the outcome of male–female interactions. Mating carried substantial costs to males, but these costs were independent of food availability. Males generally showed a preference for large females but also for females that delivered a more vigorous courtship display. Moreover, males preferred virgin females in one species but nonvirgin females in the other species, and we provide data suggesting that this choice is adaptive. Female choice was restricted to a lower rate of female mate rejection of larger males in one of the species. Our results reveal a striking interspecific variation in mutual mate choice, even between these closely related species, and show that sexual selection in females can act on much the same types of traits that are commonly considered sexually selected in males, such as size-related traits and courtship vigor. This suggests that a preference for condition-dependent traits may be a commonality that is shared between mate choice by both sexes. *Key words:* allometry, Bruchidae, mate choice, nuptial gifts, sex-role reversal, sexual selection. [*Behav Ecol* 22:1104–1113 (2011)]

INTRODUCTION

The last decade has seen a gradual conceptual reappraisal of the sex roles in evolutionary biology (e.g., Johnstone et al. 1996; Bonduriansky 2001; Forsgren et al. 2004; Clutton-Brock 2007, 2009). The classic foundation of behavioral ecology laid by Darwin (1871), Bateman (1948), and Trivers (1972), stating that rigid sexual asymmetries in investment in gametes and parental care generate monotypic sex roles, has been gradually modified. Although sexual selection is stronger in males in many, or even most, animals (Eens and Pinxten 2000), an increasing number of studies have documented cases where the classic sex roles of competitive, indiscriminating, and eager males that interact with discriminating and coy females are a poor fit (Kraaijeveld et al. 2007).

The relative strength of intrasexual competition is determined by the operational sex ratio (OSR), that is, the ratio of sexually active individuals of each sex in a population at any given time (Trivers 1972; Emlen and Oring 1977). The OSR of a given population is largely determined by relative parental investment by males and females, and from this derives the classic prediction that the sex with fewest sexually active individuals should be the choosy one (Emlen and Oring 1977; Clutton-Brock and Parker 1992; Kokko and Jennions 2008). Under this general framework, we would expect to see mutual mate choice primarily in situations where the OSR is near unity. Interestingly, however, new theory suggests that the

conditions for the evolution of male mate choice may be much broader than previously realized. First, there are reasons to believe that factors that are not traditionally thought of as affecting parental investment, such as the cost of mating, sex-specific mortality, and mate encounter rates can affect the evolution of sex roles (Dewsbury 1982; Kvarnemo and Ahnesjö 1996; Okuda 1999; Kokko and Monaghan 2001; Kokko and Johnstone 2002). Second, if high enough, variation in female quality can favor the evolution of male mate choice (Owens and Thompson 1994; Johnstone et al. 1996; Reinhold et al. 2002; Servedio and Lande 2006; Rowell and Servedio 2009). Therefore, male mate choice can be favored even under conventional sex roles and male-biased OSR. In combination with the fact that an increasing number of empirical studies have revealed sizeable costs of mating to males (e.g., Brown and Weatherhead 2004; Paukku and Kotiaho 2005; Oliver and Cordero 2009; South et al. 2009; Wedell 2010), indicating that these costs have often been underestimated in the past, this body of theory suggests that male mate choice may be widespread and consequential.

A general view is thus that male should evolve to prefer female traits that signal direct benefits (i.e., high fecundity of fertility) to males (Gwynne 1981). In accordance, there is much documentation of male preferences for large female body size and for unmated females over mated in insects (Bonduriansky 2001; Thomas and Simmons 2010). Because of trade-offs between investment in signals and fecundity, theory suggests that the evolution of costly female secondary sexual traits should be constrained (Fitzpatrick et al. 1995; Servedio and Lande 2006), suggesting that secondary sexual traits such as signals and ornaments should be rare (Bonduriansky 2001; LeBas 2006). Yet, the evolution of secondary sexual signals in females does not seem as rare as one

Address correspondence to G. Arnqvist. E-mail: goran.arnqvist@ebc.uu.se.

Received 9 December 2010; revised 18 May 2011; accepted 18 May 2011.

might expect (Amundsen 2000; Clutton-Brock 2007, 2009; Kraaijeveld et al. 2007) in light of theory suggesting that costly traits that are honest indicators of female fecundity can only evolve under rather restrictive conditions (Fitzpatrick et al. 1995; Chenoweth et al. 2006; Servedio and Lande 2006; Hooper and Miller 2008; Nakahashi 2008). In some cases, direct resource competition between females may have led to the evolution of costly and condition-dependent female signals (Gwynne 1991; Gwynne and Bussiere 2002; LeBas et al. 2003; Heinsohn et al. 2005; Clutton-Brock 2007; Gwynne et al. 2007) which have then been exploited by males during male mate choice (LeBas 2006).

Our understanding of the evolutionary dynamics of male mate choice is limited. Although it is clear that female preferences for male signals evolve both rapidly and divergently (e.g., Andersson 1994), we know much less about divergence in male preferences. Here, one may take 1 of 2 contrasting stands. If males are selected to simply favor large females (or other general indicators of high fecundity), then male preferences may be evolutionarily conserved. We would then expect to see little evolutionary divergence in female sexual signals. On the other hand, if male preferences for traits, which are honest indicators of female quality (Amundsen 2000; Clutton-Brock 2007), are widespread, male preferences and female signals may evolve as divergently as female preference for secondary sexual traits in males. We note that the best-studied model system in this regard is nocturnal moths, where females of most species emit pheromone signals to males. Here, both female signals and male preferences for such signals evolve rapidly and are generally highly species specific (Löfstedt 1993; Phelan 1997).

In seed beetles (Coleoptera, Bruchidae), males of many species provide females with a large and nutritious ejaculate, which places significant constraints on male ejaculate production and which provides females with direct benefits (Moya-Larano and Fox 2006; Rönn et al. 2008). Yet, seed beetles generally show conventional sex roles. The only known exception is the honey locust beetle *Megabruchidius dorsalis*. This oriental species is known to show 5 hallmarks of many sex-role reversed taxa, defined loosely as systems with predominantly female–female competition for access to mates (Vincent et al. 1994; Berglund and Rosenqvist 2003). First, as in for example some pipefish (Berglund and Rosenqvist 1993), females show active and extended courtship of males (Takakura 1999). In *M. dorsalis*, females initiate mating by antennating the male's head and thorax in a face-to-face position. The female then turns 180° and presents her abdominal plate (i.e., the pygidium) in front of the male. The male antennates and palpates the pygidium and may accept or reject the female. If not accepted, the female generally turns back 180° and resumes antennation. Typical courtships last from less than a minute to several minutes and consist of multiple repetitions of this dual-stage behavioral sequence (on average 10 times; Takakura 1999). Second, females are equipped with a sex-limited secondary sexual character which is employed during courtship, something that is rare even in role-reversed taxa (Funk and Tallamy 2000; Clutton-Brock 2009): The enlarged female pygidium has 2 patches (oval depressions; Tuda and Morimoto 2004) that are presented to males during courtship and which carry numerous pores that may emit pheromones. Third, as in many role-reversed insects (Vahed 1998), females derive direct benefits from mating. Female *M. dorsalis* mated 10 times lay as much as 8 times as many eggs as those mated only once (Takakura 1999). The fact that females mate more often when kept on a low-quality diet (Takakura 2004a, 2004b) even suggests that females in effect forage for matings (Kaitala and Wiklund 1994). Fourth, as in role-reversed pipefish (Berglund and Rosenqvist 2003),

male reproductive investment is considerable and constrained. Male *M. dorsalis* transfer a large and nutritious ejaculate to females (7% of male body weight), and the weight of the ejaculate decreases in successive matings (Takakura 1999), although the cost of mating to males is unknown. Fifth, as has been found in a few other insects (Gwynne 1990; Simmons 1992) and pipefish (Vincent et al. 1994; Berglund and Rosenqvist 2003), the estimated male reproductive investment exceeds that of females in *M. dorsalis* (Takakura 2006), suggesting that the OSR may effectively be female biased.

A previous study failed to find evidence of female mate choice in *M. dorsalis* (Takakura 2001), despite the fact that females should benefit directly from preferring to mate with large (Takakura 2004b) and well-fed (Takakura 2004a) males. The current study examines mutual mate choice in 2 species of honey locust beetles and has 3 main goals. First, we estimate the cost of mating to males and assess whether such costs are contingent on male resource availability. We predict a sizeable cost of mating that is inversely related to resource abundance. Second, we seek to identify those phenotypic traits in both sexes, which are preferred by members of the other sex. We predict that females should prefer large and well-fed males, because these provide females with more direct benefits, and that males should discriminate among females based on female size and mating status, as these should reflect female fecundity and the level of sperm competition. Third, we test for differences in the pattern of mutual mate choice between 2 closely related species, using an identical and standardized experimental protocol. We achieved these goals by careful observations of staged encounters between males and females with experimentally manipulated phenotypes.

MATERIALS AND METHODS

Study taxa and rearing

The genus *Megabruchidius* Borowiec is an oriental genus of seed beetles (Coleoptera, Bruchidae) with 3 known species (Tuda and Morimoto 2004). The 2 sister species *M. tonkineus* and *M. dorsalis* (Kergoat et al. 2007) show an originally oriental distribution, but both species have spread throughout much of Europe as a result of the cultivation of their host plants as an ornamental tree (honey locusts; *Gleditsia* spp.). Although the biology of *M. dorsalis* has been studied in some detail (e.g., Kurota and Shimada 2002; Takakura 2004a, 2006) much less is known about *M. tonkineus* (György 2007). We established large (>500 individuals) laboratory stock populations of both species (26 °C, 16:8 light:dark, 70% relative humidity) reared on seeds of *Gleditsia triacanthos*, from original field samples of *M. dorsalis* (Inogashira Park, Tokyo, Japan; ≈3000 adults, June 2009) and *M. tonkineus* (Orczy-kert, Budapest, Hungary; ≈300 adults, May 2009) at Uppsala University. Stock populations were reared in multiple 1-l glass containers and were fed 20% sucrose solution, pollen, and water. Virgin beetles were obtained by isolating single seeds, collecting individual beetles as they hatched, and keeping them isolated until the onset of the experiment. The experiments described below were performed in a manner identical for both species.

Male mating cost experiment

In order to assess the cost of mating to males, defined as those costs that derive from courtship and copulation, we studied the effects of mating rate and food provisioning on male life span (Paukku and Kotiaho 2005; Pomiankowski et al. 2005; Hall et al. 2009; South et al. 2009; Wedell 2010). All males were kept individually in 90 mm ø petri dishes throughout this experiment. We randomly assigned 96 virgin 1- to 2-day-old males of

each species to a treatment level in a crossed 4×2 factorial and balanced design. First, we varied male exposure to females. Our mating rate treatments consisted of (I) virgin males, (II) males mated once only, (III) males exposed to a female once a week, and (IV) males exposed to a female 5 times a week. At each exposure to females, a single virgin female was introduced to each male, and the female was removed once copulation was observed or after 120 min if copulation was not observed. Secondly, we varied male food availability. Although all males were provided with a continuous supply of deionized water, (I) one group of males were fed 20% sucrose solution and pollen during the weekends (48 h), whereas (II) another group received no food. Male life span was determined by daily inspections. We note that honey locust beetles, like other seed beetles, do not require food as adults in order to reproduce successfully. Variation in male life span was subsequently analyzed by means of parametric survival analysis for uncensored data (JMP 9.0). We based this on a log-logistic probability distribution of survival time because this distribution yielded the lowest Akaike information criterion.

In order to assess difference between the species in realized copulation rates, we also performed a more detailed copulation rate assay at one specific exposure to females during the second week of our experiment (mating rate treatment groups III and IV) where all replicates were monitored closely and continuously for copulation.

Mutual mate choice experiment

We employed a no-choice experimental design (Coyne et al. 2005), which was deemed most realistic because males and females do not tend to aggregate in the field (Takakura 2004a). Our basic approach was to carefully record the reproductive behavior of male–female pairs, the phenotypes of which had been experimentally manipulated (a 2×2 design for each species; see below). Following the experimental mating trials, the morphology of all beetles was measured.

Males were either (I) fed or (II) not fed. We first set up mating pools of 10 virgin males and 10 virgin females in petri dishes (all individuals were 1 day post emergence) to provide males with experience of male–female interactions prior to our mating trials. After 24 h, when direct observations ensured us that all males had experienced mating, males were removed from these mating pools and were individually housed in petri dishes. Half of the males were provided with water, 20% sucrose solution, and pollen, whereas the other half were provided only with water, for 48 h prior to the mating trials. Thus, all experimental males had experienced female courtship and mating and were 4 days old at the time of the mating trial, whereas half of the males were fed and half were not fed prior to the focal mating trials.

Females were either (I) virgins or (II) mated. Virgin females were individually housed in petri dishes provided with water 1 day after emergence. Half of these females were allowed to cohabit with a virgin male for 24 h (at least one copulation per pair was verified) and half were not. Females were then isolated individually for 48 h and were thus all 4 days old at the time of the focal mating trials.

Focal mating trials were performed by placing pairs of males and females in circular glass arenas (33 mm ϕ), in a fully factorial and balanced design with regards to food and mating treatment. Total sample size per species was 60 pairs. The behavior of each pair was recorded with a Panasonic macro-enabled digital video camera (NV-Mx300EG) for 60 min. Following these trials, all individuals were preserved in 96% ethanol for subsequent morphometric analyses. The following behavioral parameters were then extracted from the digital recordings: 1) which sex initiated the first^t courtship, 2) duration of the first courtship, 3)

number of 180° turns performed by the female during the first courtship, 4) whether or not the first courtship resulted in copulation, 5) the total number of courtship interactions that occurred, 6) whether or not copulation occurred during the 60 min, 7) copulation duration, and 8) for all courtships, we recorded which sex rejected their mate in cases where the courtship did not result in copulation. Here, movement directed away from the interacting partner was deemed a rejection. Two measures of the outcome were used as main response variables in subsequent analyses: 1) whether the first courtship resulted in copulation or not and 2) mating speed. Mating speed was constructed as an ordinal variable (copulation during first encounter = 2, copulation during a later encounter = 1, and no copulation = 0).

We measured the morphology of all individuals by using a digitizing tablet (Summasketch III) placed under a side-mounted camera lucida attached to a Leica MZ8 dissecting microscope. In both sexes, we measured the length of both antennae and both elytra. Elytra length was used as a measure of body size. In females, we also measured the length of the pygidium and the length and width of both pygidial patches. Patch area (calculated as the area of an ellipse) was then used as measure of pygidial patch size. The repeatability of all bilaterally symmetrical traits was high (males: elytra = 0.99, antenna = 0.73; females: elytra = 0.99, antenna = 0.75, and pygidial patch = 0.74), and we used the average value of the left and right side for all subsequent analyses.

Female age and fecundity

Whether mating with virgin females is beneficial to males depends on how soon after the first copulation female egg production commences (Alonzo and Pizzari 2010). In order to characterize this, we set up replicate male–female groups, each consisting of 5 virgin males and 5 virgin females ($N = 20$ groups per species; all individuals were aged 3–5 days post emergence) that were housed together in glass petri dishes (120 mm ϕ) provided with 20 *Gleditsia* seeds. Initial mating activity in these groups was high, and all females were deemed to have mated at least once during day zero. The average number of eggs produced per female per day in each group was then recorded for 6 consecutive days, following the first mating of females.

Allometry

Because traits under sexual selection often show positive static allometric scaling (Kodric-Brown et al. 2006), we assessed the pattern of allometry for a few key traits using reduced major axis regression to estimate the allometric slopes (Sawada 1999) (\log_{10} transformed trait values). First, we assessed the sex- and species-specific pattern of static allometric scaling between antennal length and body length because antennae are employed by females during active courtship (Takakura 1999), following an inferential global analysis of covariance ($N = 238$). Second, because the sexually dimorphic pygidium and pygidial patches presumably result from sexual selection in females, we also estimated the allometric slopes for the length of these traits (in females only).

All linear modeling were performed in Systat 13.0 and Genstat 10.2. All morphological variables were standardized to a mean of zero and unit variance, for each species separately, prior to inclusion as covariates in the linear models.

RESULTS

The pre-mating female courtship behavior of *M. dorsalis* and *M. tonkineus*, as well as their general mating behavior, were very similar indeed. In fact, we observed no qualitative differences,

Table 1
Analysis of the effects of species, feeding regime, and mating regime on male survival

Source	df	χ^2	P
Species	1	0.94	0.333
Feeding	1	619.65	<0.001
Mating	3	80.54	<0.001
Species \times feeding	1	3.56	0.059
Species \times mating	3	4.41	0.221
Feeding \times mating	3	1.79	0.615
Species \times feeding \times mating	3	1.75	0.624
Test of entire model	15	624.05	<0.001

df, degrees of freedom.

and the detailed account given by Takakura (1999) for *M. dorsalis* applies in full for both species. In our experiments, female courtships that led to copulation lasted on average 134.8 s (standard error [SE] = 29.5) and included 3.2 (SE = 0.6) female turns in *M. tonkineus*, whereas the corresponding numbers for *M. dorsalis* were 60.5 s (SE = 6.9) and 3.4 (SE = 0.4).

Cost of mating

Our analysis of male survival in the cost of mating experiment (Table 1) showed that both feeding and mating had large effects on male survival (Figure 1). However, mating rate

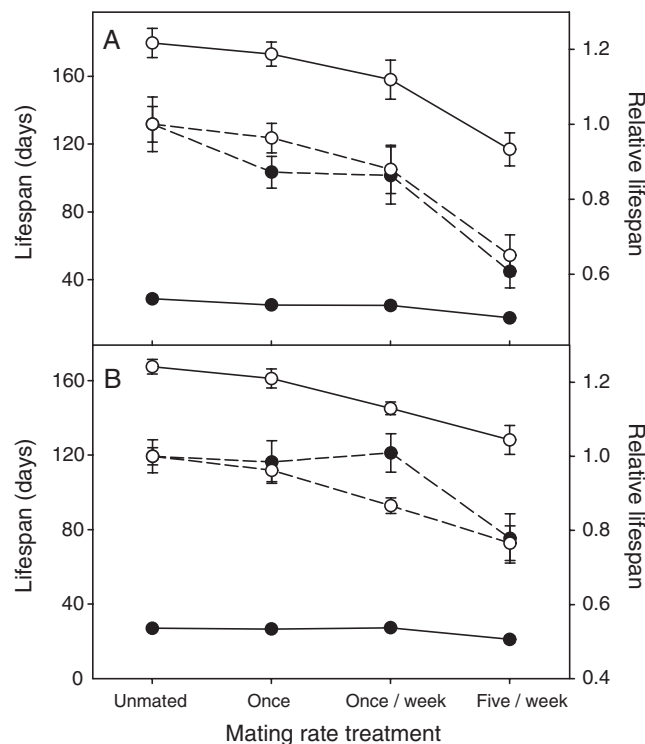


Figure 1
Average (\pm SE) life span of males that were either fed (open circles) or unfed (filled circles) and experienced either of 4 mating rate regimes: unmated, mated once only, exposed to females once per week, and exposed to females 5 times per week throughout their life (A *Megabruchidius tonkineus*, B: *M. dorsalis*). Shown are both absolute life span (left ordinate; solid lines) and life span relative to unmated males of each food treatment and species (right ordinate; dashed lines).

did not interact significantly with feeding regime. Thus, although mating was costly to males, the reduction in survival that resulted from increased mating was not larger in unfed than in fed males (Figure 1). Overall, the estimated 50% survival time of males exposed to females 5 times per week was 0.69 of that of unmated males. Finally, the effects of our treatments on male survival did not differ significantly in *M. dorsalis* and *M. tonkineus*. We note that, in our mating rate assay, 58% of the *M. dorsalis* males and 83% of the *M. tonkineus* males mated ($\chi^2_1 = 13.34$, $P < 0.001$). Thus, the realized copulation rate was somewhat higher in *M. tonkineus*, suggesting that the cost per copulation is somewhat lower.

Mate choice trials

Our 2 main measures of the mating outcome of trials, that is, mating speed and whether the first courtship resulted in copulation or not, yielded quantitatively very similar and qualitatively identical results. To restrict our presentation, we report only the results of the latter measure here. Throughout, we employed generalized linear models to analyze variation across pairs in the probability of copulation, using a binomial error distribution, a logit link function, and empirically derived scaling factors. In our analyses of the probability of copulation at the first encounter, we first fitted the main effects of our 3 treatment factors, retaining only those factorial interactions that were significant. We then tested the effects of courtship behavior and morphology by adding sets of covariates accounting for variation in behavior, female morphology, and male morphology to the model. Our final inferential model is presented in Table 2. Male food treatment had no effect on the probability of copulation, and the 2 species did not differ. However, female mating status showed a rather strong effect, but the direction of this effect was opposite in the 2 species: although virgin *M. dorsalis* females were more likely to mate at their first encounter with the male, virgin *M. tonkineus* females were less likely to do so (Figure 2). Models fitted for the 2 species separately showed the effect of female mating status was significant in both *M. dorsalis* ($F_{1,53} = 10.51$, $P = 0.002$) and *M. tonkineus* ($F_{1,52} = 6.41$, $P = 0.014$). Further, female size (Table 2), but not male size ($F_{1,106} = 0.61$, $P = 0.435$), their interaction ($F_{1,106} = 0.35$, $P = 0.555$), or any other morphological variable ($P > 0.3$ in all cases), affected the probability of copulation. Again, the effect of female size was significant in both species but differed in sign across species. Although small *M. tonkineus* females were more likely to mate at the first encounter with the male ($t_{52} = 2.35$, $P = 0.023$), large *M. dorsalis* females were more likely to do so ($t_{53} = 3.91$, $P < 0.001$) (Figure 3). However, female courtship behavior

Table 2
Analysis of deviance of the probability of mating during the first courtship in staged mating trials

Source	df	Deviance	F^a	P
Species	1	0.41	0.71	0.401
Female mating status	1	0.07	0.12	0.732
Male food treatment	1	0.41	0.72	0.399
Species \times female mating status	1	7.67	13.27	<0.001
Courtship duration	1	33.52	58.00	<0.001
No. of female turns	1	47.99	83.02	<0.001
Female elytra size	1	0.01	0.01	0.935
Species \times female elytra size	1	6.60	11.42	<0.001
Residual	108	62.42		

Omnibus test of the entire model: $F_{8,108} = 20.91$, $P < 0.001$. df, degrees of freedom.

^a Deviance ratio

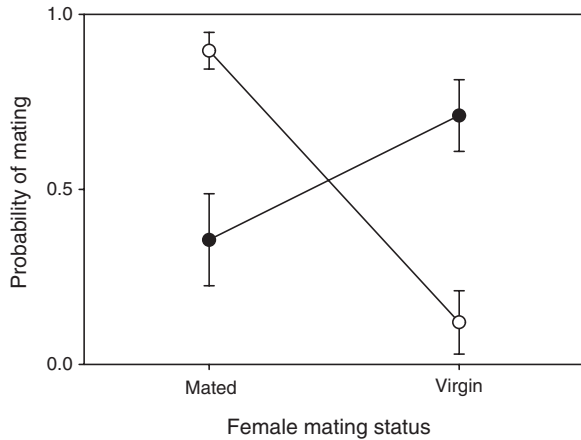


Figure 2

Predicted probability (\pm SE) that focal males mate with females that are virgins or already mated at their first encounter, for *Megabruichidius tonkineus* (open circles) and *M. dorsalis* (filled circles).

had by far the strongest independent effect on the probability of copulation at the first encounter. A multiple logistic regression including both the duration of courtship ($\beta = 0.017$, $t_{108} = 2.64$, $P = 0.009$) and the number of female turns ($\beta = 1.608$, $t_{108} = 6.44$, $P < 0.001$) showed that both had independent and positive effects (see also Table 2). Males were thus more likely to mate in cases where females delivered longer courtships (Figure 4) with a higher rate of turns (i.e., a higher number of turns when the duration of courtship is kept constant). We note that virgin and mated females did not differ in courtship duration (*Mt*: $t_{56} = 0.30$, $P = 0.76$; *Md*: $t_{58} = 0.39$, $P = 0.69$) or in the number of female turns (*Mt*: $t_{56} = 0.20$, $P = 0.84$; *Md*: $t_{58} = 1.45$, $P = 0.15$) in either of the 2 species.

In order to gain further insights into the relative roles of males and females in determining the outcome of the trials in the 2 species, we analyzed the data in some more detail. In *M. tonkineus*, 54% of the first male–female interactions were initiated by males, whereas 34% of the initial male–female interactions were initiated by males in *M. dorsalis* (Mann–Whitney

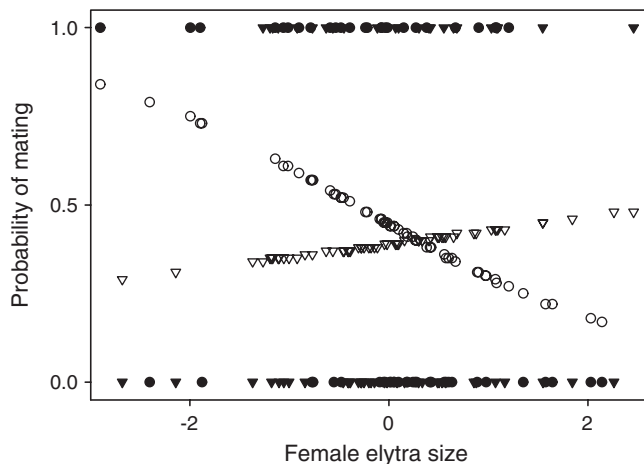


Figure 3

Observed (filled) and predicted (open) probability of mating at their first encounter with the focal male for *Megabruichidius tonkineus* (circles) and *M. dorsalis* (triangles) females as a function of their elytra size. Predicted values shown were generated in a generalized linear model including species, female elytra size, and their interaction.

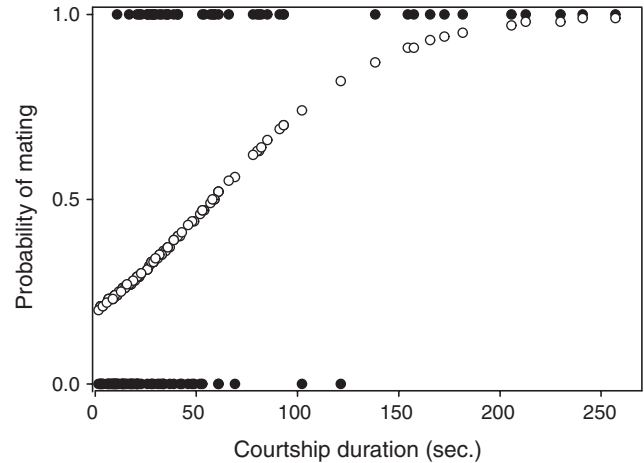


Figure 4

Observed (filled) and predicted (open) probability of mating at the first encounter as a function of the duration of pre-mating courtship. Predicted values shown were generated in a generalized linear model including only courtship duration.

U test, $P = 0.032$). In total, 45% of the *M. tonkineus* pairs and 40% of the *M. dorsalis* pairs mated at their first encounter. Out of the first courtships that did not result in copulation, males were the rejecting sex in 19% of the cases in *M. tonkineus* and in 47% of the cases in *M. dorsalis* ($\chi^2_1 = 6.13$, $P = 0.013$).

In total, 60% of the pairs mated in *M. tonkineus*, and 50% mated in *M. dorsalis*. Out of the pairs that did mate, copulation occurred on average after 2.2 interactions in *M. tonkineus* and 2.1 in *M. dorsalis*. In all but 2 pairs, the pairs that did not mate interacted at least once. The average number of courtship interactions in pairs that did not mate was 12.8 in *M. tonkineus* and 10.4 in *M. dorsalis*. Thus, the number of interactions seems high enough to allow mate assessment. The average proportion of male rejects among pairs that did not mate was 20% in *M. tonkineus* and 39% in *M. dorsalis* ($\chi^2_1 = 5.40$, $P = 0.024$). Thus, although the 2 species were similar in their general mating behavior, male *M. dorsalis* rejected more female courtships than did *M. tonkineus* males.

We also investigated the effects of courtship behavior and morphology on male and female rejection behavior. We first divided the number of male and female mate rejections in each replicate with the total number of male–female interactions in each replicate. These 2 proportions (male and female rejection rates; correlation across replicates: $r = -0.58$) were then treated collectively as response variables in a MANCOVA, where observations were weighted by the square root of the total number of interactions. This analysis yielded several insights (see Table 3). Species differed significantly in their rejection behavior, as *M. dorsalis* males rejected more than *M. tonkineus* males. Further, courtship behavior had a strong effect on mate rejection behavior. This was primarily due to an effect of the rate of female turning on female mate rejection ($F_{1,105} = 39.62$, $P < 0.001$), such that elevated female turning rate during courtship was associated with a lower degree of female resistance to mating. This analysis also indicated that female antennal length, rather than female body size, was associated with a high rate of male mate acceptance, and this effect did not differ between species. Finally, the model suggested that male elytra size affects female rejection behavior, but that this effect differs in the 2 species (Table 3). This was confirmed in weighted linear regressions in the 2 species, showing that male elytra size was negatively related to the proportion of female mate rejections in *M. tonkineus* ($\beta' = -0.271$, $t_{58} = 2.11$, $P = 0.040$) but not in *M. dorsalis* ($\beta' = 0.076$, $t_{59} = 0.44$, $P = 0.661$).

Table 3
Multivariate analysis of covariance of male and female rejection behavior

Source	Wilks's λ	$F_{2,105}$	P
Species	0.933	3.79	0.026
Courtship duration	0.941	3.31	0.040
No. of female turns	0.561	41.07	<0.001
Female elytra size	0.962	2.05	0.134
Female pygidium size	0.980	1.09	0.340
Female pygidial patch size	0.969	1.68	0.192
Female antennal length	0.925	4.28	0.016
Male elytra size	0.991	0.46	0.634
Male antennal length	1.000	0.02	0.984
Species \times male elytra size ^a	0.943	3.15	0.047

^a All other interactions between species and covariates were insignificant (Wilks's $\lambda > 0.95$, $F_{2,105} < 2.6$, $P > 0.08$; in all cases) and were therefore excluded from the inferential model.

In order to assess whether any phenotypic characteristics of females determined whether males rejected or accepted females during the first encounter, we used only the subset of the data where males either rejected females in the first encounter or where the first encounter led to copulation. Similarly, we assessed determinants of whether females rejected or accepted males during the first encounter, using only the subset of the data where females either rejected males or where the first encounter led to copulation. These more focused analyses should thus incorporate only those first interactions where a focal partner (male or female) decided to accept or reject their partner. Males were more likely to accept those females that had a large pygidium and that showed a courtship with a high turning rate (Table 4A). In addition, the probability that a male would accept rather than reject a female was, again, influenced by an interaction between species and female mating status (see also Figure 2). In contrast, we failed to find any male characteristics that significantly affected the probability that females would accept rather than reject males in the first encounter (Table 4B).

Finally, we tested for the effects of our factorial treatments on copulation duration. Both female mating status ($F_{1,56} = 6.45$, $P = 0.014$), species ($F_{1,56} = 15.99$, $P < 0.001$), and male

Table 4
Analysis of deviance of the probability that males (A) and females (B) accept rather than reject their mates during the first courtship (see text)

Source	df	Deviance	F^a	P
A				
Species	1	4.77	6.33	0.014
Female mating status	1	0.01	0.02	0.897
Species \times female mating status	1	3.36	4.45	0.039
Female elytra size	1	0.34	0.45	0.505
Female antennal length	1	0.18	0.24	0.627
Female pygidial patch size	1	0.41	0.54	0.465
Female pygidium size	1	3.03	4.02	0.049
Courtship duration	1	22.34	29.62	<0.001
No. of female turns	1	9.02	11.96	<0.001
Residual	62	46.76		
B				
Species	1	0.21	0.15	0.703
Male food treatment	1	0.75	0.52	0.471
Species \times male food treatment	1	1.97	1.37	0.245
Male elytra size	1	0.35	0.24	0.624
Male antennal length	1	0.01	0.00	0.961
Residual	88	126.85		

df, degrees of freedom.

^a Deviance ratio

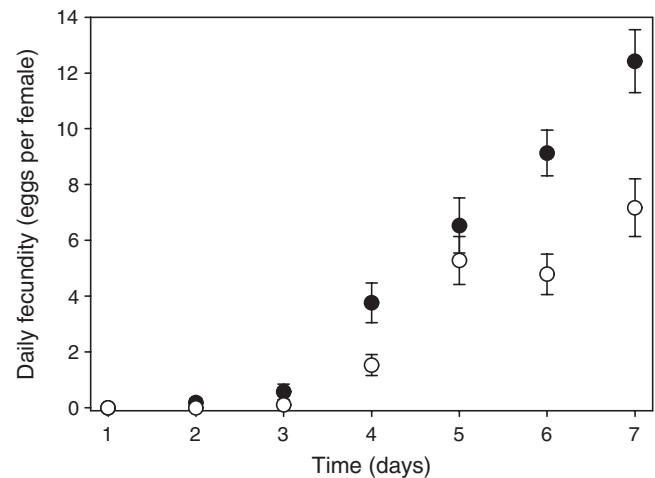


Figure 5
Average daily fecundity (\pm SE) over time (days after first mating) for *Megabruchidius dorsalis* (filled) and *M. tonkineus* (open).

food treatment ($F_{1,56} = 6.69$, $P = 0.012$) had significant effects on copulation duration, but none of the interactions between factorial variables ($F_{1,50} < 2.66$, $P > 0.109$ in all cases) nor the addition of male and female elytra size (partial F test; $F_{2,52} = 0.718$, $P = 0.493$) had any significant effects. Mean copulation durations in seconds (SE) were 384.9 (10.8) for mated females, 420.5 (10.7) for virgin females, 428.9 (11.4) for *M. dorsalis*, 376.5 (9.9) for *M. tonkineus*, 381.2 (10.4) for fed males, and 424.2 (11.1) for unfed males. Thus, although *M. dorsalis* exhibited a longer copulation duration, virgin females copulated for longer than nonvirgins, and unfed males copulated for longer than did fed males in both species.

Female age and fecundity

Daily fecundity increased with days because first copulation in both species (Figure 5) but did so more rapidly in *M. dorsalis* compared with *M. tonkineus* (repeated measures analysis of variance of log-transformed fecundities; effect of species $F_{1,38} = 10.33$, $P = 0.003$; effect of species \times time $F_{5,190} = 2.81$, Huynh-Feldt adjusted $P = 0.029$).

Allometry

In both species, the allometric relationship between antennal length and body length differed between the sexes (Table 5). The allometric slopes were steeper in females than males in both *M. dorsalis* (females: $\beta = 1.47$, $SE_{\beta} = 0.14$; males: $\beta =$

Table 5
Analysis of covariance of the length of antennae in males and females of *M. dorsalis* and *M. tonkineus*

Source	SS ($\times 10^{-3}$)	df	F	P
Sex	0.66	1	7.17	0.008
Species	0.10	1	1.13	0.288
Elytra length	20.07	1	219.75	<0.001
Sex \times species	0.07	1	0.76	0.384
Sex \times elytra length	0.69	1	7.55	0.006
Species \times elytra length	0.13	1	1.45	0.230
Sex \times elytra length \times species	0.09	1	0.99	0.319
Residual	21.01	230		

df, degrees of freedom; SS, sums of squares.

0.91, $SE_{\beta} = 0.09$) and *M. tonkineus* (females: $\beta = 1.01$, $SE_{\beta} = 0.08$; males: $\beta = 0.85$, $SE_{\beta} = 0.08$). However, the slope differed significantly from isometry only in *M. dorsalis* females ($t_{60} = 3.31$, $P = 0.002$). Females also tended to show positive allometry for both pygidium length (*M. dorsalis*: $\beta = 1.10$, $SE_{\beta} = 0.09$; *M. tonkineus*: $\beta = 1.07$, $SE_{\beta} = 0.07$) and pygidial patch length (*M. dorsalis*: $\beta = 1.31$, $SE_{\beta} = 0.14$; *M. tonkineus*: $\beta = 1.13$, $SE_{\beta} = 0.13$), but this was significantly different from isometry only for pygidial patch length in *M. dorsalis* ($t_{60} = 2.26$, $P = 0.033$).

DISCUSSION

Experimental tests of mutual mate choice are essential but rare (e.g., Jones and Hunter 1993; Hunt et al. 1999; Sandvik et al. 2000; Romero-Pujante et al. 2002; Aquiloni and Gherardi 2008; Kemp 2008). This is no doubt in part due to the fact that many taxa in which male mate choice is evident, such as role-reversed species, are nonmodel organisms that are not amenable for experimental work. Our experimental dissection of mutual mate choice in honey locust beetles yielded several novel insights. Below, we will restrict our discussion to 4 facets of the patterns that emerged. First, the cost of mating to males was substantial in these beetles and was independent of food availability. Second, we found a consistent pattern of male mate choice for females that delivered vigorous courtship displays and for indices of female size. Third, although male mate choice was manifest in our data, evidence for female mate choice was less obvious. Fourth, the pattern of mate choice showed some striking differences between these 2 closely related species, in particular with regards to female mating status.

Reproductive investment manifested as a cost of mating to males is a key parameter in some mutual mate choice theory (Wedell et al. 2002; Härdling et al. 2008; South et al. 2009), simply because this form of reproductive investment by males has major direct and indirect effects on the OSR (Okuda 1999; Kokko and Monaghan 2001; Kokko and Johnstone 2002). Mating is clearly costly for males in both *M. dorsalis* and *M. tonkineus*: Frequent mating reduced survival and life span by about 30% compared with unmated males. Moreover, the costs of mating were additive such that more frequent mating led to a larger reduction in survival. Although the existence of male mating costs is crucial for our understanding of sex roles in these beetles, theory makes no quantitative predictions regarding the absolute size of costs required to favor male mate choice. Although the cost of mating to males documented here seems sizeable, it is not clear whether these costs alone are large enough to favor male mate choice when placed beside the benefits that may derive from male mate choice. In fact, the cost of mating to males may seem rather modest considering the fact that males transfer an average of 7% of their body weight at each mating, which constitutes a very valuable nutritional resource for females (Takakura 1999, 2001). This is substantiated by the fact that male mating costs are significant also in seed beetles with conventional sex roles and a much smaller male investment in ejaculates: Paukku and Kotiaho (2005) found that mating only 3 times reduced male life span with as much as 15% in *Callosobruchus maculatus*. We note that encounter rates between the sexes and realized mating rates in the wild are largely unknown for both *M. dorsalis* and *M. tonkineus*.

Male and female honey locust beetles show partly distinct ecologies. Although adult females reside on host plants for mating and ovipositing, males are much more mobile and frequently visit flowers of nonhost plants to forage on pollen and nectar (Takakura 2004a). The food provided to males in our experiment (water/sucrose/pollen) is a very close match to their natural food supply, and we predicted that the cost of mating to males would be alleviated when provided with food. Yet, although absolute life span was much increased by feeding,

the proportional cost of mating was unaffected by resource availability. This suggests that a large component of the cost of mating to adult males derives from producing components of the ejaculate from resources that males recruit during their juvenile stage in the host seed and that they do not refuel as adults (Conner et al. 2000). This may, for example, be various phospholipids or free fatty acids, which are common in the seminal fluid of other insects (Poiani 2006). In any case, honey locust beetle males seem unable to evade the cost of mating by foraging as adults and that, therefore, food conditions may have little effect on the OSR. This is consistent with the fact that Takakura (2006) failed to find any difference between ejaculates of well fed and poorly fed males in terms of the nutritional value to females. This contrasts with role-reversed bush crickets where the cost of mating to males is diet dependent (Simmons 1992, 1993) as are the sex roles (Gwynne and Simmons 1990). As in bush crickets, however, it is likely that poor food conditions aggravate competition among females for males (Gwynne 1990; Takakura 2004a).

Male honey locust beetles consistently showed a mating preference for females that delivered a more intense courtship display and for females that were large, although the latter effect was evident in different indices of female size in different analyses and was less consistent in *M. tonkineus*. Male mating preferences for large females are common (Bonduriansky 2001) and preferences for female sexual signals that are expressed by both sexes (i.e., sexually homologous signals, sensu Arnold 1985) have been documented in a range of taxa (e.g., Jones and Hunter 1993; Hunt et al. 1999; Sandvik et al. 2000; Romero-Pujante et al. 2002; Aquiloni and Gherardi 2008; Kemp 2008), but our study is a rare example of a male preference for a secondary sexual signal present only in females (i.e., courtship behavior) (Funk and Tallamy 2000; Amundsen and Forsgren 2001). All of these traits are likely to be, or even known to be (Potti and Merino 1996; Amundsen 2000; Domb and Pagel 2001; LeBas et al. 2003; Weiss et al. 2009; Kekalainen et al. 2010), condition dependent (Rowe and Houle 1996) in females. This suggests that traits favored by males in mate choice may generally be of the same type as those favored by females in mate choice: traits that reveal the phenotypic condition of potential mates (Rowe and Houle 1996; Tomkins et al. 2004). Because the vigorous and extended courtship behavior exhibited by honey locust beetle females must carry costs, certainly in terms of time and energetic expenditure and possibly in terms of increased predation risk, these insects illustrate that male mate choice can lead to the evolution of costly sexual signals in females (Servadio and Lande 2006; Nakahashi 2008). Finally, we note that our results are consistent with male mate choice resulting primarily from direct benefits to male honey locust beetles because large females and females in good phenotypic condition are known to produce more and larger eggs in other seed beetles (Yanagi and Miyatake 2002; Czesak and Fox 2003; Gonzalez-Teuber et al. 2008).

Female courtship behavior suggests that males may, at least in part, use female antennation and the pygidium to assess female size. This is supported both by our analyses (Tables 3 and 4) and by the positive allometry of antennal length and pygidial patch length in females of one species. Positive allometry is a hallmark of many (Kodric-Brown et al. 2006), but not all (Bonduriansky 2007), traits under directional sexual selection and has previously been documented for female sexual signals in another insect (South and Arnqvist 2009).

Female mate choice was much less obvious in our experiments and was restricted to a negative relationship between male size and female mate rejection behavior in *M. tonkineus*, although this did not translate into a significantly higher probability of mating for large males. In sex-role reversed species, such as honey locust beetles, one might argue that we would predict

female choice to be weak or absent for the same reasons that we by convention expect little male mate choice in species showing conventional sex roles. Yet, several factors may lead to the evolution of mate choice also in the sex experiencing the most intense intrasexual competition (Owens and Thompson 1994; Johnstone et al. 1996; Reinhold et al. 2002; Servedio and Lande 2006; Rowell and Servedio 2009).

There are at least 3 implications of the fact that the pattern of male mate choice differed in the 2 closely related honey locust beetle species studied here under a “common garden” protocol. First, several facts suggests that role reversal is more pronounced in *M. dorsalis* compared with *M. tonkineus*: More male–female interactions were initiated by females in the former species, it showed a lower male mating rate, a higher rate of male mate rejections, no evidence for female mate choice, a more pronounced male preference for large females, and a positive allometry for antennal length and pygidial patch length in females. These differences in mating system and mating pattern may result from, for example, deterministic differences in ecology or more arbitrary differences in male–female coevolutionary trajectories (Arnqvist and Rowe 2005). Secondly, our results show that male mate choice is labile and that important aspects of male mating preferences can apparently evolve quite rapidly.

Third, the fact that male *M. dorsalis* prefer virgin females, whereas *M. tonkineus* males prefer already mated females is interesting when seen in light of the time-specific fecundities of females. Male preference for virgin females is a very common empirical observation in arthropods and no doubt generally reflects the fact that virginity in females signifies high residual reproductive value and/or reduced sperm competition (Bonduriansky 2001; Thomas and Simmons 2010). However, this need not be the case (Engqvist and Reinhold 2006). In polyandrous taxa, the relative reproductive value of virgin females can be reduced by an interaction between the pattern of sperm precedence, female mating rate, and female egg production. If there is last male sperm priority, females remate frequently, and females do not start producing eggs directly following their first mating, then the first male to mate with a female may fertilize very few or even no eggs. In cases where males make a sizeable nutritional investment in the ejaculate, such as honey locust beetles, the first male may in essence have his reproductive investment exploited by subsequent males (Alonzo and Pizzari 2010). The fact that male *M. tonkineus* preferred mated over virgin females is at least consistent with such a scenario: Honey locust beetles show last male sperm priority and females remate at least every 24 h (Takakura 2001), but *M. tonkineus* females mate more often and require a longer period following their first mating until egg production plateaus (Figure 5). Given the higher mating rate and longer fecundity time lag of *M. tonkineus*, it is possible that virgin females have a relative lower reproductive value to males in this species. A similar scenario seems to be responsible for the evolution of male preference for mated females in the Asian citrus psyllid (Wenninger et al. 2008).

In conclusion, matings are costly for honey locust beetle males, males reject many mating opportunities, and male mate choice is more pronounced than female mate choice. Further, the types of female traits preferred by males are all likely to reflect the direct reproductive value of their mates. This includes both female size and mating status but also the vigor of the female courtship display. The fact that males preferred virgin females in one species but mated female in the other can be understood in light of the species-specific mating rates and female fecundity functions. Our study adds to a growing number of reports of male mate choice for traits in females that indicate high phenotypic condition or reproductive value to males.

FUNDING

The Swedish Research Council (621-2007-4835); the University of Uppsala; and the Zoologiska Stiftelsen.

We are very grateful indeed to Masakazu Shimada and Zoltan György for help with collecting the beetles. Ingrid Ahnesjö, Anders Berglund, and Sandra South provided constructive comments on a previous version of our manuscript.

REFERENCES

- Alonzo SH, Pizzari T. 2010. Male fecundity stimulation: conflict and cooperation within and between the sexes: model analyses and co-evolutionary dynamics. *Am Nat.* 175:174–185.
- Amundsen T. 2000. Why are female birds ornamented? *Trends Ecol Evol.* 15:149–155.
- Amundsen T, Forsgren E. 2001. Male mate choice selects for female coloration in a fish. *Proc Natl Acad Sci U S A.* 98:13155–13160.
- Andersson M. 1994. *Sexual selection*. Princeton (NJ): Princeton University Press.
- Aquiloni L, Gherardi F. 2008. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. *J Zool.* 274:171–179.
- Arnold SJ. 1985. Quantitative genetic models of sexual selection. *Experientia.* 41:1296–1310.
- Arnqvist G, Rowe L. 2005. *Sexual conflict*. Princeton (NJ): Princeton University Press.
- Bateman AJ. 1948. Intra-sexual selection in *Drosophila*. *Heredity.* 2:349–368.
- Berglund A, Rosenqvist G. 1993. Selective males and ardent females in pipefishes. *Behav Ecol Sociobiol.* 32:331–336.
- Berglund A, Rosenqvist G. 2003. Sex role reversal in pipefish. *Adv Study Behav.* 32:131–167.
- Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol Rev.* 76:305–339.
- Bonduriansky R. 2007. Sexual selection and allometry: a critical reappraisal of the evidence and ideas. *Evolution.* 61:838–849.
- Brown GP, Weatherhead PJ. 2004. Sexual abstinence and the cost of reproduction in adult male water snakes, *Nerodia sipedon*. *Oikos.* 104:269–276.
- Chenoweth SF, Doughty P, Kokko H. 2006. Can non-directional male mating preferences facilitate honest female ornamentation? *Ecol Lett.* 9:179–184.
- Clutton-Brock T. 2007. Sexual selection in males and females. *Science.* 318:1882–1885.
- Clutton-Brock T. 2009. Sexual selection in females. *Anim Behav.* 77:3–11.
- Clutton-Brock TH, Parker GA. 1992. Potential reproductive rates and the operation of sexual selection. *Q Rev Biol.* 67:437–456.
- Conner WE, Boada R, Schroeder FC, Gonzalez A, Meinwald J, Eisner T. 2000. Chemical defense: bestowal of a nuptial alkaloidal garment by a male moth on its mate. *Proc Natl Acad Sci U S A.* 97:14406–14411.
- Coyne JA, Elwyn S, Rolan-Alvarez EL. 2005. Impact of experimental design on *Drosophila* sexual isolation studies: direct effects and comparison to field hybridization data. *Evolution.* 59:2588–2601.
- Czesak ME, Fox CW. 2003. Evolutionary ecology of egg size and number in a seed beetle: genetic trade-off differs between environments. *Evolution.* 57:1121–1132.
- Darwin C. 1871. *The descent of man and selection in relation to sex*. London: John Murray.
- Dewsbury DA. 1982. Ejaculate cost and male choice. *Am Nat.* 119:601–610.
- Domb LG, Pagel M. 2001. Sexual swellings advertise female quality in wild baboons. *Nature.* 410:204–206.
- Eens M, Pinxten R. 2000. Sex-role reversal in vertebrates: behavioural and endocrinological accounts. *Behav Processes.* 51:135–147.
- Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science.* 197:215–223.
- Engqvist L, Reinhold K. 2006. Theoretical influence of female mating status and remating propensity on male sperm allocation patterns. *J Evol Biol.* 19:1448–1458.
- Fitzpatrick S, Berglund A, Rosenqvist G. 1995. Ornaments or offspring—costs to reproductive success restrict sexual selection processes. *Biol J Linn Soc.* 55:251–260.
- Forsgren E, Amundsen T, Borg AA, Bjelvenmark J. 2004. Unusually dynamic sex roles in a fish. *Nature.* 429:551–554.

- Funk DH, Tallamy DW. 2000. Courtship role reversal and deceptive signals in the long-tailed dance fly, *Rhaphomyia longicauda*. *Anim Behav*. 59:411–421.
- Gonzalez-Teuber M, Segovia R, Gianoli E. 2008. Effects of maternal diet and host quality on oviposition patterns and offspring performance in a seed beetle (Coleoptera: Bruchidae). *Naturwissenschaften*. 95:609–615.
- Gwynne DT. 1981. Sexual difference theory—mormon crickets show role reversal in mate choice. *Science*. 213:779–780.
- Gwynne DT. 1990. Testing parental investment and the control of sexual selection in Katydid—the operational sex-ratio. *Am Nat*. 136:474–484.
- Gwynne DT. 1991. Sexual competition among females—what causes courtship-role reversal. *Trends Ecol Evol*. 6:118–121.
- Gwynne DT, Bussiere LF. 2002. Female mating swarms increase predation risk in a 'role-reversed' dance fly (Diptera: Empididae: *Rhaphomyia longicauda* Loew). *Behaviour*. 139:1425–1430.
- Gwynne DT, Bussiere LF, Ivy TM. 2007. Female ornaments hinder escape from spider webs in a role-reversed swarming dance fly. *Anim Behav*. 73:1077–1082.
- Gwynne DT, Simmons LW. 1990. Experimental reversal of courtship roles in an insect. *Nature*. 346:172–174.
- György Z. 2007. To the biology of the honey locust seed beetle, *Megabruichidius tonkineus* (Pic, 1904) (Coleoptera: Chrysomelidae: Bruchinae). *Folia Entomol Hung*. 68:89–96.
- Hall MD, Bussiere LF, Brooks R. 2009. Diet-dependent female evolution influences male lifespan in a nuptial feeding insect. *J Evol Biol*. 22:873–881.
- Hårdling R, Gosden T, Aguilera R. 2008. Male mating constraints affect mutual mate choice: prudent male courting and sperm-limited females. *Am Nat*. 172:259–271.
- Heinsohn R, Legge S, Endler JA. 2005. Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science*. 309:617–619.
- Hooper PL, Miller GF. 2008. Mutual mate choice can drive costly signaling even under perfect monogamy. *Adapt Behav*. 16:53–70.
- Hunt S, Cuthill IC, Bennett ATD, Griffiths R. 1999. Preferences for ultraviolet partners in the blue tit. *Anim Behav*. 58:809–815.
- Johnstone RA, Reynolds JD, Deutsch JC. 1996. Mutual mate choice and sex differences in choosiness. *Evolution*. 50:1382–1391.
- Jones IL, Hunter FM. 1993. Mutual sexual selection in a monogamous seabird. *Nature*. 362:238–239.
- Kaitala A, Wiklund C. 1994. Polyandrous female butterflies forage for matings. *Behav Ecol Sociobiol*. 35:385–388.
- Kekalainen J, Huuskonen H, Tuomaala M, Kortet R. 2010. Both male and female sexual ornaments reflect offspring performance in a fish. *Evolution*. 64:3149–3157.
- Kemp DJ. 2008. Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). *Behav Ecol*. 19:1–8.
- Kergoat GJ, Silvain JF, Delobel A, Tuda M, Anton KW. 2007. Defining the limits of taxonomic conservatism in host-plant use for phytophagous insects: molecular systematics and evolution of host-plant associations in the seed-beetle genus *Bruchus* Linnaeus (Coleoptera: Chrysomelidae: Bruchinae). *Mol Phylogenet Evol*. 43:251–269.
- Kodric-Brown A, Sibly RM, Brown JH. 2006. The allometry of ornaments and weapons. *Proc Natl Acad Sci U S A*. 103:8733–8738.
- Kokko H, Jennions MD. 2008. Parental investment, sexual selection and sex ratios. *J Evol Biol*. 21:919–948.
- Kokko H, Johnstone RA. 2002. Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philos Trans R Soc Lond Ser B Biol Sci*. 357:319–330.
- Kokko H, Monaghan P. 2001. Predicting the direction of sexual selection. *Ecol Lett*. 4:159–165.
- Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J. 2007. The evolution of mutual ornamentation. *Anim Behav*. 74:657–677.
- Kurota H, Shimada M. 2002. Geographical variation in the seasonal population dynamics of *Bruchidius dorsalis* (Coleoptera: Bruchidae): constraints of temperature and host plant phenology. *Environ Entomol*. 31:469–475.
- Kvarnemo C, Ahnesjö I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol Evol*. 11:404–408.
- LeBas NR. 2006. Female finery is not for males. *Trends Ecol Evol*. 21:170–173.
- LeBas NR, Hockham LR, Ritchie MG. 2003. Nonlinear and correlational sexual selection on 'honest' female ornamentation. *Proc R Soc Lond B Biol Sci*. 270:2159–2165.
- Löfstedt C. 1993. Moth pheromone genetics and evolution. *Philos Trans R Soc Lond Ser B Biol Sci*. 340:167–177.
- Moya-Larano J, Fox CW. 2006. Ejaculate size, second male size, and moderate polyandry increase female fecundity in a seed beetle. *Behav Ecol*. 17:940–946.
- Nakahashi W. 2008. Quantitative genetic models of sexual selection by male choice. *Theor Popul Biol*. 74:167–181.
- Okuda N. 1999. Sex roles are not always reversed when the potential reproductive rate is higher in females. *Am Nat*. 153:540–548.
- Oliver C, Cordero C. 2009. Multiple mating reduces male survivorship but not ejaculate size in the polygamous insect *Stenomacra marginella* (Heteroptera: Largidae). *Evol Ecol*. 23:417–424.
- Owens IPF, Thompson DBA. 1994. Sex-differences, sex-ratios and sex-roles. *Proc R Soc Lond Ser B Biol Sci*. 258:93–99.
- Paukku S, Kotiaho JS. 2005. Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. *J Insect Physiol*. 51:1220–1226.
- Phelan PL. 1997. Genetics and phylogenetics in the evolution of sex pheromones. New York: Chapman & Hall Inc.
- Poiani A. 2006. Complexity of seminal fluid: a review. *Behav Ecol Sociobiol*. 60:289–310.
- Pomiankowski A, Denniff M, Fowler K, Chapman T. 2005. The costs and benefits of high early mating rates in male stalk-eyed flies, *Cyrtodiopsis dalmanni*. *J Insect Physiol*. 51:1165–1171.
- Potti J, Merino S. 1996. Decreased levels of blood trypanosome infection correlate with female expression of a male secondary sexual trait: implications for sexual selection. *Proc R Soc Lond B Biol Sci*. 263:1199–1204.
- Reinhold K, Kurtz J, Engqvist L. 2002. Cryptic male choice: sperm allocation strategies when female quality varies. *J Evol Biol*. 15:201–209.
- Romero-Pujante M, Hoi H, Blomqvist D, Valera F. 2002. Tail length and mutual mate choice in bearded tits (*Panurus biarmicus*). *Ethology*. 108:885–895.
- Rönn JL, Katvala M, Arnqvist G. 2008. Interspecific variation in ejaculate allocation and associated effects on female fitness in seed beetles. *J Evol Biol*. 21:461–470.
- Rowe L, Houle D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc R Soc Lond B Biol Sci*. 263:1415–1421.
- Rowell JT, Servedio MR. 2009. Gentlemen prefer blondes: the evolution of mate preference among strategically allocated males. *Am Nat*. 173:12–25.
- Sandvik M, Rosenqvist G, Berglund A. 2000. Male and female mate choice affects offspring quality in a sex-role-reversed pipefish. *Proc R Soc Lond B Biol Sci*. 267:2151–2155.
- Sawada M. 1999. Model II: geometric mean regression add-in for Excel '97. Freeware by M. Sawada. Ottawa (Canada): University of Ottawa.
- Servedio MR, Lande R. 2006. Population genetic models of male and mutual mate choice. *Evolution*. 60:674–685.
- Simmons LW. 1992. Quantification of role reversal in relative parental investment in a bush cricket. *Nature*. 358:61–63.
- Simmons LW. 1993. Some constraints on reproduction for male bush-crickets. *Requena verticalis* (Orthoptera, Tettigoniidae)—diet, size and parasite load. *Behav Ecol Sociobiol*. 32:135–139.
- South SH, Arnqvist G. 2009. Morphological variation of an ornament expressed in both sexes of the mosquito *Sabethes cyaneus*. *Evol Ecol Res*. 11:1–21.
- South SH, Steiner D, Arnqvist G. 2009. Male mating costs in a polygynous mosquito with ornaments expressed in both sexes. *Proc R Soc Lond Ser B Biol Sci*. 276:3671–3678.
- 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.
- Takakura K. 2001. Courtship-role-reversal in the bean weevil, *Bruchidius dorsalis* (Coleoptera: Bruchidae): interplay between male-male competition and cryptic female choice. *Appl Entomol Zool*. 36:311–316.
- Takakura KI. 2004a. The nutritional contribution of males affects the feeding behavior and spatial distribution of females in a bruchid beetle, *Bruchidius dorsalis*. *J Ethol*. 22:37–42.
- Takakura KI. 2004b. Variation in egg size within and among generations of the bean weevil, *Bruchidius dorsalis* (Coleoptera, Bruchidae): effects of host plant quality and paternal nutritional investment. *Ann Entomol Soc Am*. 97:346–352.
- Takakura KI. 2006. Estimation of relative reproductive expenditure in the courtship-role-reversed bean weevil, *Bruchidius dorsalis* (Fahraeus). *J Ethol*. 24:33–36.

- Thomas ML, Simmons LW. 2010. Cuticular hydrocarbons influence female attractiveness to males in the Australian field cricket, *Teleogryllus oceanicus*. *J Evol Biol*. 23:707–714.
- Tomkins JL, Radwan J, Kotiaho JS, Tregenza T. 2004. Genic capture and resolving the lek paradox. *Trends Ecol Evol*. 19:323–328.
- Trivers RL. 1972. Mother-offspring conflict. *Am Zool*. 12:648.
- Tuda M, Morimoto K. 2004. A new species *Megabruchidius sophorae* (Coleoptera, Bruchidae), feeding on seeds of *Styphnolobium* (Fabaceae) new to Bruchidae. *Zool Sci*. 21:105–110.
- Vahed K. 1998. The function of nuptial feeding in insects: review of empirical studies. *Biol Rev*. 73:43–78.
- Vincent A, Ahnesjö I, Berglund A. 1994. Operational sex-ratios and behavioral sex-differences in a pipefish population. *Behav Ecol Sociobiol*. 34:435–442.
- Wedell N. 2010. Variation in male courtship costs in butterflies. *Behav Ecol Sociobiol*. 64:1385–1391.
- Wedell N, Gage MJG, Parker GA. 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol Evol*. 17:313–320.
- Weiss SL, Kennedy EA, Bernhard JA. 2009. Female-specific ornamentation predicts offspring quality in the striped plateau lizard, *Sceloporus virgatus*. *Behav Ecol*. 20:1063–1071.
- Wenninger EJ, Stelinski LL, Hall DG. 2008. Behavioral evidence for a female-produced sex attractant in *Diaphorina citri*. *Entomol Exp Appl*. 128:450–459.
- Yanagi SI, Miyatake T. 2002. Effects of maternal age on reproductive traits and fitness components of the offspring in the bruchid beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Physiol Entomol*. 27:261–266.