Mate choice in the dung beetle *Onthophagus sagittarius*: are female horns ornaments?

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Sexual selection typically operates via male contest competition and female choice, favoring the evolution of secondary sexual traits in males. However, there are numerous taxa in which females possess secondary sexual traits and the selective pressures operating on female ornamentation are not well understood. We examined sexual selection operating via mate choice in the dung beetle *Onthophagus sagittarius*, a species exhibiting sex-specific ornamentation. Precopulatory mate preferences and their subsequent effects on breeding success were examined. Female preferences favored large males and when paired with small males, males with high courtship rates. Large females were choosier than small females. No overt male preferences for female size or ornamentation were found; thus, we conclude that female horns in this species are unlikely to have evolved as ornaments via precopulatory male mate choice. Relative horn length in females determined brood ball productivity, whereas female body size and an interaction between male courtship rate and male body size determined brood ball weight. Our results provide evidence for female but not male mate choice. They suggest that attractive males increase reproductive performance, but it is unclear whether the effect of male phenotype is mediated via differences in paternal investment or female differential allocation toward attractive mates. *Key words:* female horns, mutual mate choice, parental provisioning.

Since Darwin (1871) first proposed his theory of sexual selection, understanding how and why mate preferences evolve has been the subject of considerable research effort. Mate choice functions to screen prospective mates on the basis of quality and is favored when there are high breeding costs, high encounter rates, and high variation in mate quality (Kokko and Johnstone 2002). These conditions are usually most relevant to females in conventional mating systems in which the bulk of parental investment is maternally provided, limiting female availability and intensifying competition between males for mating opportunities (Bateman 1948; Trivers 1972; Emlen and Oring 1977; Clutton-Brock and Vincent 1991). Such systems favor the evolution of secondary sexual characteristics in males and choosiness in females (Andersson 1994). Males are predicted to be less discriminating than females in their choice of mates due to the comparatively low costs of reproduction they face (Trivers 1972). However, even in conventional mating systems, males may face significant reproductive costs resulting from courtship displays, ejaculate production, nuptial gifts, and paternal care. Where reproduction is costly for males and there exists high variation in female quality, male mate choice is likely to be favored by selection (Owens and Thompson 1994; Servedio and Lande 2006; Servedio 2007). Male mate preferences are expected to favor cues correlated with fecundity, such as body size or traits that reliably signal fecundity (Bonduriansky 2001).

Secondary sexual traits in females have traditionally been regarded as a rare phenomenon confined to role-reversed systems or occurring as a result of genetic correlations with male secondary sexual traits (Lande 1980). The rarity of female secondary sexual traits has typically been attributed to the high costs associated with maternal investment constraining trait development in females more than in males (Fitzpatrick et al. 1995). However, several phylogenetic analyses across a range of taxa have revealed numerous evolutionary gains of female-specific ornaments, suggesting that the evolution of secondary sexual traits may not be as constrained in females as previously assumed (e.g., Irwin 1994; Burns 1998; Wiens 1999). Recent studies on extant species have revealed direct selection operating via male mate choice and female competition on female ornamentation (e.g., Amundsen and Forsgren 2001; Heinsohn et al. 2005; Cornwallis and Birkhead 2007; Kraaijeveld et al. 2007).

Female ornaments have been shown to correlate with a range of parameters relating to female quality, acting as reliable cues for fecundity (Rosenqvist 1990; Berglund et al. 1997; Pizzari et al. 2003; Griggio et al. 2005; Servedio and Lande 2006; Inger and Hill 2005; Morales et al. 2007), condition (Velando et al. 2001; Jawor et al. 2004), immunocompetence (Roulin et al. 2000; Hanssen et al. 2006), parental quality (Linville et al. 1998; Massaro et al. 2003), dominance (Johnson 1998; Trail 1990; Bleiweiss 1992; Hill 1993; Jones and Hunter 1999), status (Murphy et al. 2009), and offspring quality (Weiss et al. 2009), thus providing considerable scope for adaptive male preferences to evolve. However, empirical evidence for the evolution of female ornaments via male mate choice is mixed. Studies show that in some species, males prefer to mate with more ornamented females (e.g., Johnson 1988; Amundsen et al. 1997; LeBas and Marshall 2000; Amundsen and Forsgren 2001; LeBas et al. 2003; Torres and Velando 2005; Griggio et al. 2009), whereas in others males prefer low (e.g., Nordeide 2002), and even intermediate levels of ornament expression (Chenoweth et al. 2007). The ambiguity of these findings may reflect variation in the ornament–fecundity relationship, which will dictate the adaptive value of male preferences. Directional male mate preferences are not expected where ornaments and fecundity are targets of a shared resource pool, rather convex male preferences can function to optimize the trade-off between signal exaggeration and female
Sex-specific ornamentation in *Onthophagus sagittarius*. Male (left) with cephalic horns and female (right) with cephalic horn and pronotal horn.
Thus, we took the residuals of the relationship between horn length and body size and used this as a measure of relative female horn size in our analyses. We adopted a general linear modeling approach, entering all potential independent variables and their interactions in the first step and reducing the models via stepwise deletion of nonsignificant terms as recommended by Crawley (1993), presenting the minimum adequate model to explain the variation in our dependent variable.

RESULTS

Courtship behavior

There was significant covariation between our different measures of male courtship behavior and between male courtship behavior and male body size. Males that began courtship sooner had higher rates of courtship ($r = -0.654$, degrees of freedom [df] = 119, $P < 0.001$), and males with higher rates of courtship had longer copula durations ($r = 0.422$, df = 59, $P < 0.001$). Male pronotum and courtship rate were positively correlated ($r = 0.593$, df = 119, $P < 0.001$). There were no significant relationships between female body size and her partner’s courtship or mating behavior (correlations between female body size and log latency to court, $r = -0.122$, df = 119, $P = 0.184$; log courtship rate, $r = 0.109$, df = 119, $P = 0.237$, copula duration, $r = -0.002$, df = 59, $P = 0.989$). Neither were there any significant relationships between the size of a female’s horns and her partner’s courtship and mating behavior (partial correlations controlling for female pronotum width, between female horn length and log latency to court, $r = 0.119$, df = 117, $P = 0.199$; copula duration, $r = -0.095$, df = 61, $P = 0.475$; log courtship rate, $r = -0.106$, df = 117, $P = 0.250$).

Mating success

To investigate how variation in male and female phenotypes and courtship parameters influenced mating success, we fitted a nominal logistic regression with courtship rate and body size entered as independent variables, and mating success as the response ($0 =$ unsuccessful, $1 =$ successful). The model explained 25% of the observed variation ($\chi^2 = 41.69$, df = 4, $P < 0.001$). Stepwise deletion of nonsignificant terms left significant relationships between body size and mating success (Table 1). Large females were less likely to mate than small females (Figure 2). Male body size and courtship rate both had significant effects on mating success, and there was a significant interaction effect such that the effect of courtship rate was dependent on male body size.

To examine the nature of the interaction, we reanalyzed the data after splitting the brood balls produced ($F_{3,45} = 5.918$, $P < 0.001$). Female body size and the interaction between male size and courtship rate were significant positive predictors of brood ball weight (Table 2). To examine the nature of the interaction effect, the data were again split on the basis of male size. Separate analyses entering male and female body size and courtship rate as variables affecting brood ball weight revealed that there was no effect of courtship rate on productivity when females were paired with small males ($F_{3,45} = 1.747$, $P = 0.171$). However, when females were breeding with large males, the weight of the brood balls produced increased with males that had delivered high courtship rates ($F_{3,32} = 4.607$, $P = 0.009$, see Figure 3).

Breeding success

To investigate how traits that convey success in courtship influenced breeding success, we entered body size and courtship rate as independent variables into least squares models: one with brood ball weight as the dependent variable and one with brood ball number.

Brood ball weight

The model explained 23% of the variance in the weight of brood balls produced ($F_{3,80} = 5.918$, $P < 0.001$). Female body size and the interaction between male size and courtship rate were significant positive predictors of brood ball weight (Table 2). To examine the nature of the interaction effect, the data were again split on the basis of male size. Separate analyses entering male and female body size and courtship rate as variables affecting brood ball weight revealed that there was no effect of courtship rate on productivity when females were paired with small males ($F_{3,45} = 1.747$, $P = 0.171$). However, when females were breeding with large males, the weight of the brood balls produced increased with males that had delivered high courtship rates ($F_{3,32} = 4.607$, $P = 0.009$, see Figure 4).

Brood ball number

After stepwise deletion of nonsignificant terms, only female residual horn was a significant predictor of fecundity. The reduced model explained just 5% of the variance in the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of body size and courtship rate on mating success</th>
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<tbody>
<tr>
<td>Source</td>
<td>$\chi^2$ (df = 1)</td>
</tr>
<tr>
<td>Male pronotum</td>
<td>20.05</td>
</tr>
<tr>
<td>Female pronotum</td>
<td>6.15</td>
</tr>
<tr>
<td>Log courtship rate</td>
<td>6.55</td>
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<tr>
<td>Log courtship rate $\times$ male pronotum</td>
<td>5.48</td>
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Figure 2

Relationship between the probability of mating and female pronotum width (mm). Points represent predicted values based on real data. The cubic spline is the best-fit curve for predicted probability of mating for a given female body size and was fitted by minimizing the generalized cross-validation score using GLMS 4.0 of Schluter (1988). The standard error of the spline was calculated from 50 bootstrap replications.
number of brood balls produced ($F_{1,84} = 1.692, P = 0.034$); females with relatively longer horns for their body size produced more broods (effect estimate = $12.72 \pm 5.89$).

**DISCUSSION**

We found little evidence to suggest that males exerted selection on females through mate choice. Males did not initiate courtship sooner, court more vigorously, or copulate for longer with females of a larger body size or with females bearing longer horns. A previous study of *O. sagittarius* revealed that absolute horn length and body size accurately indicate the number and quality of offspring females are capable of producing (Simmons and Emlen 2008), and the current study found that females with relatively longer horns produced more broods. The fact that female phenotype signals fecundity and that increasing courtship rate increases a male’s chances of mating, male mate choice might be expected (Servedio and Lande 2006; Servedio 2007). However, our study suggests that direct male choice does not act on female body size or ornament expression in this beetle. Therefore our data suggest that male mate preferences are unlikely to be responsible for the evolution of female horns in *O. sagittarius*. However, because mate preferences can operate across all stages of reproduction (Cunningham and Birkhead 1998), it is possible that males in this species attempt matings with all females but exert cryptic preferences, via a bias in their investment in sperm and/or seminal fluid products (e.g., Cornwallis and Birkhead 2007) or via biased investment in offspring (e.g., Roulin 1999; Pilastro et al. 2003).

The present study found residual horn length to be the only significant influence on brood ball numbers, indicating that it is the relative amount of investment in horn exaggeration that correlates with variation in brood ball productivity. Female *O. sagittarius* with relatively larger horns for their body size had higher fecundity than individuals of the same body size with smaller horns. We note that this effect was very weak explaining only 5% of the variation in brood ball productivity. The effect is also in contrast to the finding by Simmons and Emlen (2008) on the same species, where relative horn length did not predict fecundity. Although Simmons and Emlen (2008) calculated lifetime brood production, they eliminated the effects of male assistance by forcing females to breed alone. As such our results are not directly comparable. It may be that in our study males differentially allocated parental investment to females with relatively longer horns, elevating their productivity. It is even possible that females with longer horns are better
able to coerce or manipulate males into providing greater assistance. Further work will be required to determine if the effect of female horns on productivity we observed here is robust.

We found that larger females were less likely to mate. This may reflect a difference in female prudence in mating decisions between large and small females, such that larger females are choosier. Variation in female sexual responsiveness has previously been shown to be influenced by resource availability when mate choice has the potential to provide large direct benefits, with females in low condition being more sexually responsive than females in high condition (e.g., Brown 1997; Cratsley and Lewis 2003; Hunt et al. 2005). Onthophagine males can provide significant direct benefits by assisting females during brood ball provisioning; increasing the weight and number of brood balls produced which gives rise to larger and more numerous offspring. Because offspring body size determines female fecundity and male competitive ability (Lee and Peng 1981; Cook 1998; Emlen 1994, 1997; Hunt and Simmons 1997, 1998, 2000, 2002a; Sowig 1996), male care will enhance offspring fitness. Smaller females may therefore be more willing to accept matings in order to maximize fitness benefits. An alternative or additional explanation might be that female horns are used to repel unwanted mating attempts, such that larger females are better able to reject males. Male–female head to head encounters were occasionally observed during courtship trials, and similar interactions have been mentioned in other species with female horns (Otronen 1988).

We found a significant preference among female O. sagittarius for large males and for males with high courtship rates. Our findings are consistent with Kotiaho’s (2002) study of 3 congeners of O. sagittarius that also demonstrated significant female preferences for high courtship rates. In O. taurus, O. binodus, and O. australis courtship rate was shown to be a condition-dependent trait, with its expression being dependent on food availability (Kotiaho 2002). Moreover, in O. taurus a significant proportion of phenotypic variation in male courtship rate was due to additive genetic variation and was genetically correlated with male condition (Kotiaho et al. 2001). Females may thereby receive indirect fitness benefits by choosing to mate with males with high courtship rates via the increased condition, and thus attractiveness of their own male offspring (Kotiaho et al. 2001; Kotiaho 2002; Kotiaho and Simmons 2003).

In contrast with previous studies of Onthophagini, we found that the effect of courtship rate on male mating success in O. sagittarius was dependent on male body size. Among small males, those with high courtship rates had higher mating success. Yet for large males, body size was the significant determinant of mating success. One explanation for this may be that courtship rate and body size offer cues to different aspects of mate quality. As noted above, in O. taurus, courtship rate is heritable and genetically correlated with male condition, offering cues to indirect genetic benefits for offspring. In contrast, body size in Onthophagini has been linked to levels of paternal provisioning, with large males typically offering assistance in brood provisioning (Cook 1998; Sowig 1996; Hunt and Simmons 2000, 2002a) that can ameliorate the costs of reproduction for females (Hunt et al. 2002). Thus, one interpretation of the interaction effect between male size and courtship rate on male mating success could be that female preferences in O. sagittarius target both direct and indirect benefits. The importance of indirect benefits may have been overemphasized in many studies of sexual selection (Kotiaho and Puurtinen 2007) because in general, the importance of direct benefits in mate choice are often greater than indirect benefits (Møller and Jennions 2001). In this system, adult body size is largely environmentally determined, rendering the importance of direct benefits likely to be greater than indirect benefits. But because courtship rate is a correlate of body size, any female preferences targeting courtship rate would also be favoring larger males, and thus targeting both direct and indirect benefits that these males provide. Because female Onthophagini cannot be forced to mate, the higher mating success of males with high courtship rates is unlikely to be attributed to their better ability to force copulations. However, there is some evidence of fitness costs to females from exposure to males (Hunt and Simmons 1998, but see Hunt and Simmons 2000), such that female “preferences” observed here could represent an avoidance of fitness costs, rather than the seeking of fitness benefits. Nevertheless, if courtship rate is heritable (as in O. taurus, Kotiaho et al. 2001), the benefit to females of accepting males with high courtship rates, whether it be via preference or resistance, is the same; their male offspring will also enjoy higher mating success (Cordero and Eberhard 2003).

Females produced heavier brood balls when paired with large males that also exhibited high courtship rates. These male phenotypes were also those that were most successful in gaining matings. Therefore, reproductive performance was higher for females that mated with attractive males. The mechanism through which this effect is manifest is unclear. Theory predicts 2 contrasting relationships between male attractiveness and parental care. The “good parent” hypothesis predicts that male advertisement signals honestly indicate the quality of paternal care (Hoelzer 1989), whereas the “differential allocation” hypothesis predicts that females may accept and have to compensate for low levels of paternal investment in exchange for mating with and retaining a male of good quality (Burley 1988), thus trading off indirect (“good genes”) and direct benefits (paternal care). Females that allocate more resources to investment in offspring sired by the most “attractive” males can maximize the fitness benefits gained from each reproductive opportunity and should be favored by selection (De Lope and Møller 1993; Sheldon 2000). In this study, male size and courtship rate may act as honest signals of a male’s intention and/or ability to provide assistance, following the prediction of the good parent hypothesis. Alternatively, male attractiveness may correlate negatively with paternal assistance, yet females may be facilitating the observed effect of male phenotype on brood ball weight via facultative adjustments in maternal investment. Evidence from O. taurus indicates that Onthophagine females do differentially allocate increasing resource allocation to offspring when mated with large males, even when those males are unavailable to assist in brood production (Kotiaho et al. 2003). Furthermore, because large males are those that provide assistance during offspring provisioning, differential allocation may act to strengthen the intensity of sexual selection, amplifying any indirect good genes effects (Kotiaho et al. 2003; Head et al. 2006). The likelihood of either mechanism (male assistance or female differential allocation) occurring in this system may depend on whether direct or indirect benefits exert the greatest influence on offspring fitness (Kokko 1998).

In conclusion, we found significant sexual selection operating on male body size and courtship rate via female choice. Females paired with attractive males produced heavier broods, which may be due to differential allocation by females and/or through the paternal provisioning that is typical of Onthophagini. Although this species exhibits female-specific ornamentation, we found no evidence to suggest that female horns were subject to precopulatory male mate choice. We therefore reject the notion that female horns of O. sagittarius are ornaments based on precopulatory male choice. It has been suggested that secondary sexual traits in females are more likely to be...
under selection through resource competition than for competition for mates because the direct benefit of gaining resource access has a potentially greater selective strength than any indirect benefits associated with attracting a high-quality male as a mate (LeBas 2006; Clutton-Brock 2009). Onthophagine females rely on dung for reproduction because it is necessary for brood ball construction, without it they cannot breed. Dung is an ephemeral resource that is transportable and defendable, rendering an individuals ability to acquire dung a strong determinant of fitness. Female Onthophagine have been found to parasitize broods of other females, with rates of parasitism increasing when dung availability is reduced (Moczek and Cochrane 2006). Moreover, female Onthophagine can be the victims of parasitic species of dung beetles (González Megías and Sánchez-Piñeiro 2004). These studies suggest that reproductive competition may represent a significant selective pressure acting on female dung beetles. Future work should focus on the role of female competition in the evolution of female horns in this species, to address how female phenotype influences competitive ability, and whether horns offer a competitive advantage in inter- and intra-specific interactions. We hope that the role of female competition in the evolution of female-specific secondary sexual traits will receive further research attention across taxa.

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REFERENCES


