Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia

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In animals with internal fertilization and promiscuous mating, male genitalia show rapid and divergent evolution. Three hypotheses have been suggested to explain the evolutionary processes responsible for genital evolution: the lock-and-key hypothesis, the pleiotropy hypothesis and the sexual-selection hypothesis. Here, we determine whether variation in male genital morphology influences fertilization success in the dung beetle *Onthophagus taurus*, as predicted by the sexual-selection hypothesis. Variation in four out of five genital sclerites of the endophallus influenced a male's fertilization success, supporting the general hypothesis that male genitalia can evolve under sexual selection. Furthermore, different genital sclerites were found to enhance first versus second male paternity, indicating that different sclerites serve offensive and defensive roles. Genital-trait variability was comparable to that in other species but was less variable than a non-genital sexually selected trait (head horns). We suggest that directional selection for genital elaboration may be countered by natural selection, which should favour genitalia of a size and shape necessary for efficient coupling and sperm transfer.

Keywords: male genitalia; sexual selection; sperm competition; paternity; *Onthophagus*

1. INTRODUCTION

In animals with internal fertilization and promiscuous mating there is a remarkable diversity in the morphology of structures used in reproduction (Eberhard 1985; Andersson 1994; Arnqvist & Thornhill 1998). Male genitalia show rapid and divergent evolution (Eberhard 1985; Arnqvist 1997, 1998). Given the relatively simple function of sperm transfer it seems unlikely that such complicated genital morphology could have arisen purely for the mechanical transfer of sperm.

Three main hypotheses have been suggested to explain the evolutionary processes responsible for genital evolution: the lock-and-key hypothesis, the pleiotropy hypothesis and the sexual-selection hypothesis. The lock-and-key hypothesis states that genitalia evolve via selection for pre-insemination reproductive isolation (West-Eberhard 1983; Eberhard 1985; Arnqvist & Thornhill 1998). The hypothesis posits that female genitalia represent a 'lock' and male genitalia represent a 'key' whereby the female 'lock' allows access only to an appropriate male 'key', thus preventing cross-species mating (Eberhard 1985; Proctor *et al.* 1995). The findings of comparative studies (Eberhard 1985, 1992; Arnqvist 1998) and empirical data (Goulson 1993; Arnqvist & Thornhill 1998) conflict with the hypothesis although it is conceivable that genital traits contribute to the initial establishment of reproductive barriers during speciation events (Shapiro & Porter 1989; Civetta & Singh 1998).

Mayr (1963) proposed that genitalia evolve arbitrarily as a result of selection on genetically correlated traits via the pleiotropic effects of genes that code for sexual and non-sexual characters (Arnqvist & Thornhill 1998; Civetta & Singh 1998). Quantitative genetic approaches to the study of morphological variation are required to test the hypothesis. Indeed, there is evidence that pleiotropic genes can affect genital morphology in the water strider *Gerris incognitus* (Arnqvist & Thornhill 1998).

Finally, complexity in genital morphology may also evolve via sexual selection. Three main models of sexual selection have been proposed: sperm competition (Parker 1970), cryptic female choice (Eberhard 1985, 1996) and sexual conflict (Lloyd 1979; Alexander *et al.* 1997). First, when sperm from several males compete for the fertilization of a single ovum, sperm competition may select for genital traits that enable the male to pre-empt sperm stored by females from previous matings (Parker 1970; Simmons & Siva-Jothy 1998). Second, Eberhard (1985) proposed that cryptic female choice for genital stimulation, size or mechanical fit might account for the patterns of divergence of male genitalia. If females favoured males with certain heritable genital conformations, and their daughters inherited this preference, genitalia could evolve via Fisherian selection. Finally, sexual conflict between males and females over the control of reproduction could drive antagonistic coevolution of male and female sexual traits (Alexander *et al.* 1997; Arnqvist 1997; Watson *et al.* 1998; Arnqvist & Rowe 2002). Male genital traits that coerce the female to use a male's sperm, against her own best interests, could counter select for adaptations in female genitalia that circumvent male coercion. There is good comparative evidence that post-copulatory sexual selection has been important in the evolution of genital complexity (Arnqvist 1998). However, within-species studies that test the fundamental prediction that variance in fertilization success arises owing to variation in male genital morphology are limited (Otronen 1998;

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Arnqvist & Danielsson 1999; Danielsson & Askenmo 1999).

Each of the hypotheses makes different predictions about (i) the patterns of selection on, (ii) morphological variation in, and (iii) the inheritance of genital traits (Arnqvist 1997). A definitive difference between the hypotheses is the predicted relationship between genital morphology and fertilization success. Under the lock-and-key and pleiotropy hypotheses, genital morphology should not directly affect fertilization success, whereas this is a key prediction of the sexual-selection hypothesis. The objective of our research was to determine whether there is evidence for genital-trait evolution in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidea) via post-copulatory sexual selection.

Dung beetles in the genus *Onthophagus* exhibit dimorphic male morphology. Each phenotype adopts an alternative reproductive tactic. Large horned males (majors) guard the female against conspecifics and assist her in the provision of brood masses while small hornless males (minors) sneak copulations and do not invest in paternal care (Emlen 1997; Hunt & Simmons 1998*a*, 2000). Minor males outnumber majors in the dung pad so that both morphs face a high risk of sperm competition. Indeed, the two morphs invest equally in ejaculates (Simmons *et al.* 1999*b*), spend equivalent durations in copula and gain fertilizations in direct proportion to the relative number of copulations they perform with a female (Tomkins & Simmons 2000; Hunt & Simmons 2002). The average pattern of sperm use strongly suggests that sperm mix randomly in the female's genital tract. Nevertheless, there is a large amount of unexplained variation in P_2, the proportion of offspring sired by the second male to mate with a doubly mated female (Tomkins & Simmons 2000). Members of the genus *Onthophagus* show rapid and divergent variation in genital morphology (Howden & Gill 1993), a pattern characteristic of intense sexual selection (Eberhard 1985). In this study, we ask whether some of the variation in male fertilization success in *O. taurus* can be explained by variation in male genital morphology.

2. METHODS

Onthophagus taurus were collected from Serpentine, Western Australia. A mixed-sex population was kept in culture for two weeks to ensure that females were sexually mature and mated. Mated females were placed in breeding chambers (25 cm × 6 cm PVC piping, three-quarters filled with moist sand and topped with 250 ml of cow dung). Brood masses were sieved out and collected after 10 days. Each brood mass represents the resources available for the growth and development of a single beetle. Brood masses were incubated at 25 °C for three weeks. On emergence, adult females and males were separated and maintained in single-sex cultures with unlimited access to fresh dung for two weeks. Only minor males were used in the experiments described here. It should be noted, however, that male morph has no influence on fertilization success (Tomkins & Simmons 2000).

(a) *Paternity analysis*

We use the irradiated-male technique to evaluate the effect of male genital morphology on paternity in twice-mated females (Tomkins & Simmons 2000; Simmons 2001). A random sample of males was selected for sterilization. These males were exposed to 10 krad of gamma radiation from a ^{60}Co source, under nitrogen gas anaesthesia. Irradiated sperm are fertilization competent but lethal mutations cause early embryonic mortality so that eggs fertilized by irradiated sperm can be readily identified. The technique provides a reliable assignment of paternity in situations in which two males compete (Simmons 2001).

Matings occurred in plaster-of-Paris chambers (1 cm × 5 cm), in which a trace of dung had been smeared. A single virgin female was introduced into a chamber, followed by a single male. Beetles were observed under red light, and copula duration was recorded. After copulation the male was removed from the chamber and a second male was introduced. In many instances females would not mate twice on the same day. These females were housed in plastic boxes (5.5 cm × 4.5 cm) filled with moist sand and provided with fresh dung. Ultimately, all females mated twice, and the interval between the matings was recorded. After copulation males were placed in 1.5 ml Eppendorf vials, frozen and stored in 70% ethanol.

Females were mated with a normal (N) male and an irradiated (R) male. The irradiation sequence (RN or NR) was randomized across females ($n = 37$ and $n = 29$, respectively). Control double matings were also performed (NN and RR) to allow estimation of natural levels of infertility in females mated to normal males ($n = 10$) and residual fertility in females mated to irradiated males ($n = 10$).

Twice-mated females were placed in breeding chambers. After 12 days the female and her broods were collected. Females were placed in 1.5 ml Eppendorf vials, frozen and stored in 70% ethanol. Broods were opened and recorded as fertile if they contained larvae or sterile if they contained a rotting egg. Females that produced fewer than 10 broods ($n = 5$) were excluded from the analysis.

Estimates of fertilization success were calculated using the formulae given in Cook *et al.* (1997). The proportion of eggs sired by a normal male (P_N) was calculated as:

$$P_N = (x - z)/(p - z), \tag{2.1}$$

where x is the proportion of eggs that hatch from a double mating, p is the proportion hatching after a NN mating and z is the proportion hatching after an RR mating. In cases when the mating sequence was RN, $P_2 = P_N$, and when the sequence was reversed, $P_2 = 1 - P_N$.

Values of P_2 greater than one or less than zero can occur when estimates of x are higher than p or lower than z. P_2 values were therefore corrected using the formula given by Cook *et al.* (1997) so that the data lay within the range zero to one. P_2 data were normalized prior to statistical analysis by arcsine transformation.

(b) *Morphometrics*

Variation in genital morphology was examined by two dimensional measurement. The genitalia of *O. taurus* consist of an inflatable endophallus, which extends from the phallotheca into the bursa copulatrix during copula (Zunino 1979; Palestrini *et al.* 2000). The endophallus has several chitinous sclerites (figure 1). The phallotheca was removed, the connecting tissues macerated in 10% KOH for 2 h and the sclerites cleared in 50% aque-
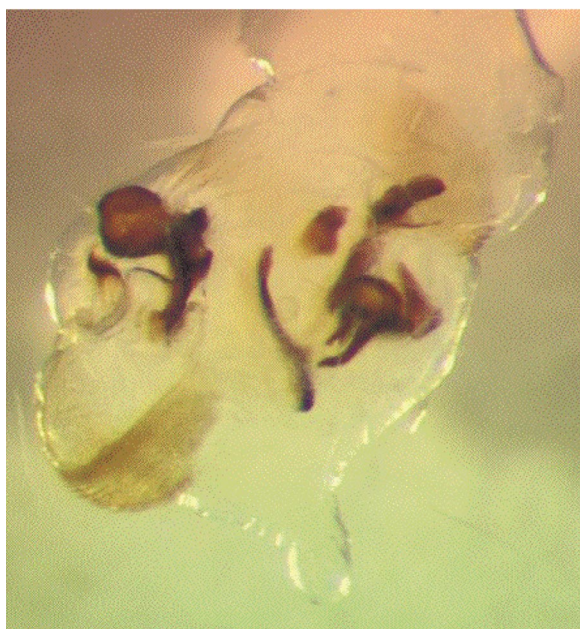
Figure 1. Orientation of the genital sclerites within a partially inflated endophallus, captured *in situ*. Magnification, ×60.
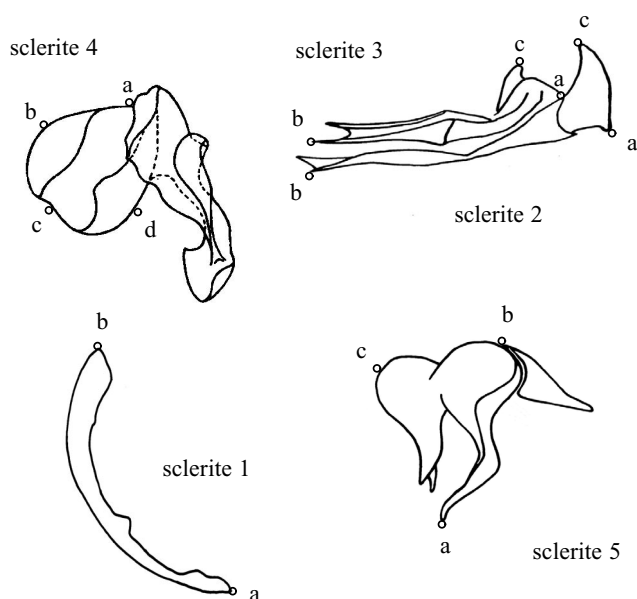

Figure 2. Diagrammatic views of five of the genital sclerites removed from the endophallus. Sclerites 2 and 3 are closely associated in the endophallus, as drawn, but were separated for measurement. Sclerite size was characterized by measuring the linear distance between fixed landmarks: a–b on sclerites 1–3; b–d on sclerite 4; and a–b and a–c on sclerite 5. The relative positions of the landmarks were characterized by measuring the perimeter of and area bound by the landmarks a–b–c on sclerites 2, 3 and 5 and the landmarks a–b–c–d on sclerite 4.

ous lactic-acid solution for 2 h before being mounted on slides with Eukitt fixative.

Distinctive landmarks on five of the sclerites were selected, and the distance between and the perimeter and/or area bound by these points were extracted using the measurement explorer function of the Optimas Image Analysis package (figure 2). Male and female pronotum widths were also measured using the eyepiece graticule of a binocular microscope. Repeated measures of the genital traits were taken from a cohort of 20 beetles. Variation between subjects was significantly greater than that within subjects for the measures of five of the sclerites (range of repeatability estimates 0.578–0.968; $F_{1,19} = 2.371$–30.809, $p = 0.031$–0.0001).

Statistical analyses were performed using procedures in the JMP statistical package and all data are presented as mean ± 1 s.e.

3. RESULTS

(a) *Morphometrics*

The statistical properties of and correlations between morphological measures of the five genital sclerites measured are presented in table 1. Coefficients of variation (CVs) for area measures were larger than for linear measures as would be expected from the differences in dimensionality. After adjusting for dimensionality, some traits stand out as having higher CVs than others. For example, the length of sclerite 1, area of sclerite 4, length (a–c) of sclerite 5 and area of sclerite 3 each have approximately twice the variation of any of the other traits, which were more in line with the CV for body size. Not surprisingly different measures of the same trait were often correlated. However, there were few significant between-sclerite correlations and only measures of sclerite 4 showed significant correlations with body size.

To reduce the dataset and describe the variance in genital morphology we conducted a principal components

(PC) analysis on the correlation matrix. The analysis generated 12 PCs. The first five components explained *ca.* 74% of the total variance in genital morphology. The remaining components had eigenvalues of less than one and were excluded from further analysis (table 2). A rotation of the first five components was performed to determine which of the original variables contributed most to each PC (table 2). Variables that were 0.7 times as large as the largest rotation vector were considered to have contributed significantly to that PC (Mardia *et al.* 1979). All measures of sclerite 2 contributed equally and strongly to PC1 (cut-off point = 0.545), length and area of sclerite 5 contributed equally strongly to PC2 (cut-off point = 0.652), area and width of sclerite 4 contributed to PC3 (cut-off point = 0.656), area and perimeter of sclerite 3 contributed to PC4 (cut-off point = 0.648) and the length of sclerite 1 contributed to PC5 (cut-off point = 0.655). PC scores were extracted from the analysis to examine how variation in the genital morphology of competing males influenced their paternity.

We used a generalized linear model to analyse the effects of irradiation order, copula duration, female remating interval, male body size and genital morphology on the fertilization success of the second male to copulate (P_2). Consistent with previous studies (Tomkins & Simmons 2000), there were no significant effects of female remating interval ($F_{1,57} = 0.065$, $p = 0.799$), the copula duration of the first male (2.41 ± 0.08 min) ($F_{1,57} = 0.251$, $p = 0.619$) or second male (2.45 ± 0.12 min) ($F_{1,57} = 0.912$, $p = 0.345$), or the body size of the first male (4.42 ± 0.05 mm) ($F_{1,57} = 0.198$, $p = 0.659$) or second male (4.29

Table 1. Correlation matrix and sample mean and variances for the morphological measures of sclerites 1–5 (see figure 2) of the male endophallus of *Onthophagus taurus*. ($n = 124$, Pearson $rs > 0.174$ are individually significant at $p < 0.05$.)

variable	1 a–b	2 area	2 a–b–c	2 a–b	3 a–b	3 area	3 a–b–c	4 b–d	4 a–b–c–d	5 a–b	5 a–c	5 area	pronotum
1 a–b													
2 area	0.062												
2 a–b–c	0.025	0.465[a]											
2 a–b	0.026	0.481[a]	0.376[a]										
3 a–b	−0.131	0.471[a]	0.264	0.361[a]									
3 area	0.131	0.298	0.168	0.156	0.230								
3 a–b–c	0.039	0.474[a]	0.239	0.272	0.582[a]	0.823[a]							
4 b–d	−0.021	0.025	0.029	0.060	0.267	0.029	0.053						
4 a–b–c–d	0.015	0.130	0.126	0.118	0.325[a]	0.146	0.136	0.747[a]					
5 a–b	0.047	0.276	0.124	0.156	0.186	0.179	0.173	0.027	0.039				
5 a–c	0.046	−0.006	0.090	0.036	0.096	−0.038	−0.009	0.058	0.135	−0.016			
5 area	0.059	0.197	0.158	0.076	0.206	0.051	0.090	0.134	0.223	0.089	0.718[a]		
pronotum	0.096	0.262	0.237	0.260	0.250	0.244	0.218	0.423[a]	0.432[a]	0.292	0.158	0.242	
mean (in mm)	0.799	0.123	1.842	0.690	0.661	0.087	1.499	0.427	0.112	0.551	0.476	0.083	4.353
(±s.e.)	(0.008)	(0.001)	(0.011)	(0.003)	(0.004)	(0.002)	(0.009)	(0.003)	(0.001)	(0.002)	(0.005)	(0.001)	(0.031)
CV[b]	10.79	6.19	6.62	5.48	7.38	9.51	6.52	7.60	14.16	4.95	10.88	7.11	7.84

[a] Significant after Bonferroni correction $\alpha = 0.05$.
[b] CVs for area measures are divided by two to account for their dimensionality and allow direct comparison with linear measures, see Houle (1992).

Table 2. PC analysis of the morphology of genital sclerites in *Onthophagus taurus*.

PC	1	2	3	4	5
eigenvalue	3.26	1.88	1.51	1.22	1.04
per cent	27.16	15.65	12.56	10.17	8.66
cumulative per cent	27.16	42.82	55.38	65.56	74.21
rotated factor pattern[a]					
sclerite 1					
a–b	0.070	−0.047	−0.017	−0.062	**0.936**
sclerite 2					
area	**0.779**	−0.052	−0.019	−0.298	−0.014
a–b–c	**0.730**	−0.109	−0.015	−0.012	0.006
a–b	**0.770**	0.015	−0.060	−0.034	−0.052
sclerite 3					
area	0.089	0.019	−0.025	**−0.916**	0.156
a–b–c	0.279	−0.019	−0.047	**−0.927**	−0.074
a–b	0.470	−0.131	−0.325	−0.427	−0.383
sclerite 4					
area	0.088	−0.120	**−0.912**	−0.090	0.016
b–d	−0.000	−0.021	**−0.937**	0.006	−0.018
sclerite 5					
area	0.128	**−0.909**	−0.117	−0.054	0.025
a–c	−0.011	**−0.932**	−0.029	0.038	0.007
a–b	0.388	0.013	−0.008	−0.157	0.182

[a] Factor loadings in bold contribute significantly to the PC scores following the method of Mardia *et al.* (1979).

± 0.04 mm) ($F_{1,57} = 0.635$, $p = 0.430$). As is typical (Simmons 2001), the irradiation sequence had a significant effect on P_2, reflecting the decrease in the competitive ability of irradiated sperm (NR, 0.49 ± 0.63; RN, 0.74 ± 0.48) ($F_{1,57} = 18.104$, $p < 0.0001$). Thus, paternity of the second male to mate was underestimated in NR matings and inflated in RN matings. As remating interval, copula duration and male size were non-significant, these factors were excluded from the final model.

Analysis of the reduced model indicated that a significant proportion of the variance in P_2 was explained by variation in male genital morphology (whole model: $F_{11,50} = 5.26$, $p < 0.001$, $r^2 = 0.536$; see table 3). P_2 decreased, or the fertilization success of the first male increased, as the first male's score on PC2 (sclerite 5) became more positive ($F_{1,61} = 9.66$, $p = 0.003$) and the score on PC3 (sclerite 4) became more negative ($F_{1,61} = 7.405$, $p = 0.009$). Thus, high paternity for the first male corresponds with an enlarged sclerite 5 and a small narrow sclerite 4 (table 3; figure 3a). Paternity of the second male increased as his score on PC1 (sclerite 2) became more positive ($F_{1,61} = 10.58$, $p = 0.002$) and PC5 (sclerite 1) became more negative ($F_{1,61} = 4.27$, $p = 0.04$). Thus, high paternity for the second male corresponds with an enlarged sclerite 2 and a short sclerite 1 (table 3; figure 3b).

4. DISCUSSION

We have demonstrated that a significant proportion of the variation in fertilization success in *O. taurus* is the result of differences between males in the morphology of their intromittent genitalia. While it has long been recognized that male genital morphology is important for sperm removal and/or repositioning by male dragonflies and damselflies (Waage 1979; Córdoba-Aguilar 2002; see review in Simmons 2001), there have been few attempts to assess the general role of male genital morphology in influencing fertilization success. Prior to our study the only other published work, to our knowledge, was that of Arnqvist & Danielsson (1999) and Danielsson & Askenmo (1999) who showed that variation in the relative shapes of two sclerites on the endophallus of waterstriders influenced fertilization success (but see also Rodriguez 1994). Our study thereby provides empirical support for the general hypothesis that male genital morphology can evolve under sexual selection mediated through variation in fertilization success (Eberhard 1985; Arnqvist 1997).

The flaccid primary male genitalia consist of a sclerotized phallotheca and endophallus (see also Palestrini *et al.* 2000). The endophallus is folded within the phallotheca and is membranous, but contains several sclerotized parts. During copulation the vagina is widened by insertion of the apex of the parameres of the phallotheca, which itself remains external. The force of body fluids causes the endophallus to extrude through the apex of the phallotheca into the bursa, into which the male ejaculates (Palestrini *et al.* 2000). Four out of the five sclerites of the endophallus that we could measure influenced a male's fertilization success. Undoubtedly our simple two-dimensional measures of the genital sclerites grossly underestimate the overall variation in genital morphology, but these simple measures explained over 50% of the variance in male fertilization success. It is worth noting that Arnqvist & Thornhill (1998) concluded that simple linear measures and more complex measures of shape gave essentially the same results when comparing genital and general morphology. Interestingly, we found that the fourth and fifth sclerites improved the first male's ability to withstand lost paternity to the second male and the first and second

Table 3. General linear model of the effects of irradiation sequence and first and second male genital morphology on the proportion of offspring sired by the second male to mate, P_2.

source	estimate	s.e.	t_{50}	p
intercept	0.963	0.043	22.52	< 0.0001
irradiation sequence	−0.199	0.044	−4.52	< 0.0001
first male				
PC1 (sclerite 2)	−0.045	0.041	−1.08	0.285
PC2 (sclerite 5)	0.133	0.043	3.11	0.003
PC3 (sclerite 4)	−0.137	0.051	−2.72	0.009
PC4 (sclerite 3)	0.008	0.041	0.19	0.846
PC5 (sclerite 1)	0.054	0.039	1.39	0.171
second male				
PC1 (sclerite 2)	0.161	0.049	3.25	0.002
PC2 (sclerite 5)	−0.080	0.044	−1.82	0.074
PC3 (sclerite 4)	−0.033	0.039	−0.84	0.403
PC4 (sclerite 3)	−0.058	0.050	−1.16	0.251
PC5 (sclerite 1)	−0.107	0.052	−2.07	0.044

sclerites improved the second male's ability to pre-empt first-male paternity. Sexual selection via sperm competition thereby selects for both 'defensive' and 'offensive' adaptations in males. Remarkably, similar patterns were found in waterstriders, where the dorsal and ventral sclerites contribute to second-male paternity and the lateral sclerites contribute to first-male paternity (Arnqvist & Danielsson 1999). Sexual selection under sperm competition has long been predicted to generate antagonistic adaptations in males (Parker 1984). For example, mechanisms for gaining paternity with non-virgin females and behavioural strategies of mate guarding are widely recognized antagonistic adaptations to sperm competition (Simmons 2001). Work with *Drosophila* accessory-gland products has indicated that different peptides in the seminal fluid serve 'defensive' (first male) and 'offensive' (second male) roles, and may likewise represent antagonistic adaptations (Clark *et al.* 1995). Moreover, 'offensive' and 'defensive' abilities in *Drosophila* seem to be under the control of alleles located on different chromosomes and show no sign of correlation (Clark *et al.* 1995). Likewise, we found no evidence for phenotypic correlations between measures of sclerites involved in first-male and second-male paternity so that, like *Drosophila*, males who are successful as first males may not necessarily be successful as second males. The independent evolution of multiple genital traits that serve different functions during copulation is likely to contribute greatly to the structural complexity seen in male genitalia.

The genital sclerites of *O. taurus* could influence paternity if they enable males to remove rival sperm physically from the female's genital tract (an offensive tactic) or induce sperm mobilization so that the ejaculate is safe from manipulation (a defensive tactic). Females are likely to be active participants in both of these mechanisms because sperm are stored in a spermatheca to which males have no access during copulation; the male's endophallus lies in the bursa copulatrix during copula. Thus, although it is beyond the scope of this study, it is likely that variance in female morphology also influences male fertilization success, as found for waterstriders (Arnqvist & Danielsson 1999). During copulation the male is orientated vertically to the female and is supported by his phallotheca. There

is no evidence that the male stimulates the female externally as the hind tibiae are folded up close to the abdomen and although the front tibiae are rhythmically raised and lowered, they do not contact the female. External stimulation of the female by the male is therefore unlikely to influence a male's fertilization success, a conclusion supported by our finding that copula duration did not influence paternity. Nevertheless, if genital sclerites stimulate the female's genital traits in such a way as to influence female-mediated sperm transfer (Simmons *et al.* 1999a; Bloch Qazi *et al.* 1998; Edvardsson & Arnqvist 2000) or storage (Rodriguez 1995; Otronen & Siva-Jothy 1991; Otronen 1997), then variation among females in their sensitivity to stimulation and/or the dimensions of their reproductive tracts, for example, could be important sources of variation in paternity.

We found levels of phenotypic variation in the genital sclerites of male *O. taurus* that are comparable with the findings of previous studies. The average coefficients of phenotypic variation were 5.9% across seven genital traits in the waterstrider *G. incognitus* (Arnqvist & Thornhill 1998), 8.1% for the length of the gonopodium of *Poecilia reticulata* (Kelly *et al.* 2000), 10.8% for the length of the gonopodium of *Brachyrhaphis episcopi* (Jennions & Kelly 2002) and a mean of 6.5% for a variety of genital traits measured across 20 species of insect and spider (Eberhard *et al.* 1998). Genital traits were at least as variable as general body size, a finding also consistent with Arnqvist & Thornhill's (1998) study of waterstriders.

The magnitude of the CVs for genital sclerites and the finding that this variation contributes to fitness via variation in fertilization success are counter to the predictions of the lock-and-key hypothesis and consistent with the sexual-selection hypothesis (see also Arnqvist & Thornhill 1998). However, Pomiankowski & Møller (1995) found that, on average, traits subject to sexual selection had higher CVs (*ca.* 20%) than traits subject to natural selection (*ca.* 7%). We can make similar comparisons within *O. taurus*. The CV in size of head horns was considerably larger than those of general aspects of body size such as pronotum width and elytra length in Hunt & Simmons' (1998b) study of body morphology (pronotum width 8%, elytra length 7%, head horns 113%). Horn length is under
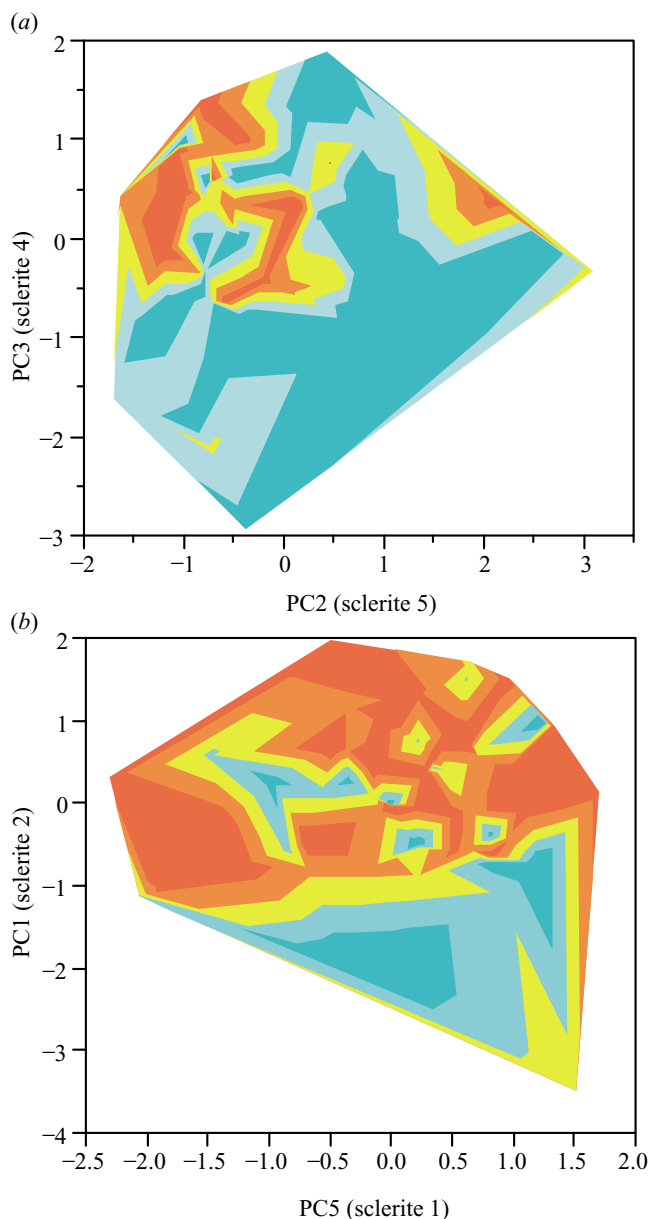
Figure 3. Contour plots of the proportion of offspring sired by a male against variation in the morphology of his genital sclerites. (*a*) For the first male to mate (male 1) paternity (P_1) is plotted against variation in the morphology of sclerites 4 and 5. (*b*) For the second male to mate (male 2) paternity (P_2) is plotted against variation in the morphology of sclerites 1 and 2. Paternity: turquoise blue, 0.20 or less; light blue, 0.40 or less; yellow, 0.6 or less; orange, 0.8 or less; red, more than 0.8. The figure illustrates the defensive role of sclerites 4 and 5 and the offensive role of sclerites 1 and 2 (see table 3 for statistical analyses).

sexual selection, being positively associated with male mating success and reproductive success (Moczek & Emlen 2000; Hunt & Simmons 2001). Palestrini *et al.* (2000) also reported considerably lower CVs for general body traits than for head horns in their study of *O. taurus*. Like us, they found that CVs of measures of the phallotheca were more in line with those of general body traits than with the sexually selected horns. Despite these apparently low levels of phenotypic variation, genital morphology can have considerable influence on fertilization success and be subject to sexual selection (Arnqvist &

Danielsson 1999; Danielsson & Askenmo 1999; this study). Thus, the patterns of phenotypic variation in *O. taurus* that we report, and those for genitalia in general (Eberhard *et al.* 1998), are inconsistent with the observation that sexually selected traits show higher levels of phenotypic variation (Pomiankowski & Møller 1995).

Eberhard *et al.* (1998) proposed that the interaction between male and female genital-trait sizes might explain why genital traits have generally low CVs. When males stimulate a female internally, her perception of the stimuli is influenced by her own size. Unless there is size-assortative mating, males should benefit by having intermediate genital sizes that appropriately stimulate the most typical female size. The same argument is unlikely to apply to secondary sexual traits that signal in the visual or auditory channels (Eberhard *et al.* 1998). Such an argument predicts a pattern of stabilizing sexual selection on male genital traits, with those males having intermediate morphologies experiencing, on average, greater fertilization success. However, our data are more consistent with a pattern of directional sexual selection on the genital sclerites.

We suggest that levels of phenotypic variation might be better understood in terms of the balance between natural and sexual selection acting on a trait. For example, the mechanisms of sound production and the variability of acoustic properties are well described in insects and anurans. Signal frequency and pulse rate (static properties) tend to have low variability (CVs less than 4%), whereas call duration and rate (dynamic properties) tend to have high variability (CVs more than 10%) (Gerhardt & Huber 2002). Static properties show limited variation, as they are dependent on the biophysics of sound-producing structures, which are determined by body size, a trait that is itself likely to be under strong natural selection. Dynamic properties can vary more widely in response to selection because they do not suffer the same constraints. A parallel example comes from the barn swallow where the outer tail streamers are subject to strong directional selection via female choice (Møller 1988). At the same time, the tail streamers are subject to natural selection because of their role in the aerodynamics of flight (Norberg 1994; Evans & Thomas 1997). The CV for tail-streamer length is just 8%, much lower than is typical for a sexually selected trait (Pomiankowski & Møller 1995).

A similar balance between natural and sexual selection might influence the evolution of genital morphology. Natural selection should favour structures of an appropriate size and shape to facilitate the basic mechanics of coupling and sperm transfer. Directional sexual selection via differential fertilization success has the potential to elaborate on these basic structures, leading to variation in genital morphology, but only to the extent that the mechanics of copula are not compromised. Opposing forces of natural and sexual selection result in a net pattern of stabilizing selection, which has been shown to be characterized, on average, by levels of phenotypic variation of a magnitude similar to those reported for genitalia (Pomiankowski & Møller 1995).

In conclusion, we have found that significant variance in fertilization success for male *O. taurus* is attributable to differences in male genital morphology. This adds to the growing number of empirical studies that have reported evidence for sexual selection as a pervasive force, shaping

the evolution of genitalia. An investigation into the anatomy of the female genitalia and the correspondence between female and male genitalia during copula may provide insight into the mechanics and effects of male genital stimulation.

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