

Crowding, sex ratio and horn evolution in a South African beetle community

Joanne C. Pomfret and Robert J. Knell*

*School of Biological and Chemical Sciences, Queen Mary, University of London,
Mile End Road, London E1 4NS, UK*

Sexually selected ornaments and weapons are exceptionally variable, even between closely related species. It has long been recognized that some of this diversity can be explained by differences in mating systems between species, but there remains substantial variation between species with similar mating systems. We investigated the roles of sex ratio (measured as operational sex ratio, OSR) and population density (measured as mean male crowding, a measure indicating the average number of conspecific males that an individual male animal will encounter) in determining horn presence in a community of South African dung beetles. Analysis of data from 14 species using a generalized least-squares model incorporating phylogenetic influences found that both OSR and mean crowding were significant predictors of horn presence, with hornless species tending to show female-biased sex ratios and high levels of crowding. The influence of mean crowding on horn diversity between species probably reflects the difficulty of guarding and monopolizing females when many competitors are present, meaning that males who adopt ‘scramble’ tactics tend to be favoured.

Keywords: sexual selection; mean crowding; population density; sexual weaponry; Onthophagini; operational sex ratio

1. INTRODUCTION

One of the most striking features of sexually selected traits is the tremendous variation that exists in the size and shape of such traits between species. The distribution of these traits is often unrelated to phylogeny: in some taxa sexually selected traits are expressed in some species but not in other closely related species (Andersson 1994). The first attempt to understand these patterns was by Darwin (1871), who realized that interspecific differences in mating systems can explain some of the diversity of sexual ornamentation in birds: plumage is more exaggerated in polygynous birds, and monogamous birds tend to be monomorphic. More recently, there has been substantial research interest in how ecological factors such as the operational sex ratio can influence mating systems and sexual ornamentation (Emlen & Oring 1977; Hamilton 1979; Kvarnemo & Ahnesjö 1996, 2002; Reynolds 1996; Shuster & Wade 2003). Most research to date, however, has focused on individual species: despite some interspecific studies, notably those on agamid lizards (Stuart-Fox & Ord 2004; Ord & Stuart-Fox 2006), the factors that lead some species to gain or lose sexually selected traits, or to grow larger or smaller ones, are generally poorly understood (Wiens 2001). Here, we focus on the effects of operational sex ratio (OSR) and population density in determining patterns of presence or absence of weaponry in a community of Southern African dung beetles. We measure population density as the number of conspecific male competitors that an individual

male is likely to encounter per patch of resource, which can be estimated using a quantity called ‘mean crowding’.

(a) Mean crowding

Lloyd (1967) proposed that a measure he called mean crowding should be used as an estimate of the number of conspecifics that an individual animal is likely to encounter within a particular patch of habitat. Mean crowding is a better estimate of crowding than simple abundance because it includes a measure of how aggregated the population is: individuals from more aggregated populations are more likely to encounter conspecifics than are individuals from more evenly dispersed populations.

Crowding could affect the evolution of weaponry by a variety of mechanisms. More crowded species will experience more intraspecific competition for resources such as food and mates, and therefore reproductive skew and the strength of sexual selection will be greater (Zeh 1987; Kemp 2001; Tomkins & Brown 2004; Bertin & Cézilly 2005; Kokko & Rankin 2006). If this is the case, then more competition between males for access to females could lead to selection favouring investment by males into weapons that aid them in obtaining females (Tomkins & Brown 2004). Alternatively, more competition could favour males that are successful in scramble-type competition for females, because the costs of defending females or territories may become greater than the benefits if there are many rival males present (Otte & Joern 1975; Borgia 1980; Warner & Hoffman 1980; Alcock & O’Neill 1986; Connor 1989; Rutowski 1991; Radwan 1993a; Moczek 2003). Under these circumstances, males that invest their resources in characters that allow them to locate females and enhance

* Author for correspondence (r.knell@qmul.ac.uk).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2007.1498> or via <http://journals.royalsociety.org>.

their sperm competition ability will be selected (Warner & Hoffman 1980; Mills & Reynolds 2003). Thus, increasing population density (and therefore crowding) can be predicted to either enhance or reduce selection for characters that increase success in intrasexual contests (Jirotkul 1999a).

(b) *Operational sex ratio*

The OSR can be calculated as the ratio of the number of sexually active males to the sum of sexually active males and receptive females at a given time in a population and is usually expressed as a percentage, ranging from 0, when only females are prepared to mate, to 100%, when only males are ready to mate (Kvarnemo & Ahnesjö 1996). This method of measuring competition is preferable to the simple sex ratio because it takes the proportion of each sex that is not available to prospective mates into account (Emlen 1976; Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996; Hardy 2002).

OSR has been identified as an important factor in determining the nature of sexual selection in a species because the OSR can determine the strength of competition for mates (Emlen & Oring 1977; Arak 1983; Gwynne & Simmons 1990; Clutton-Brock & Parker 1992; Hardy 2002). Biased OSRs have been shown to drive sexual selection for secondary sexual traits in many organisms (Kvarnemo & Ahnesjö 1996; Reynolds 1996; Kvarnemo & Ahnesjö 2002). Both male–male competition and female mate choice can be influenced by OSR; for example, both of these alter with changing OSR in the guppy, *Poecilia reticulata* (Jirotkul 1999b). In this species, when the OSR becomes more male biased, the females' preference for orange-coloured males is stronger and there are more male interference behaviours.

(c) *Onthophagine dung beetles*

The Onthophagini is a tribe of dung beetles consisting of the genus *Onthophagus*, itself with over 2000 described species (Emlen & Nijhout 2000), and a group of related genera. All of the onthophagine dung beetles studied to date have approximately similar mating systems, with females tunnelling under dung pats and male beetles guarding and mating with these females, and in those species with horned males, the horns are used as weapons during intrasexual contests (Emlen 1997; Moczek & Emlen 2000). Even though the purpose of the horns carried by male onthophagines does not seem to vary much between species, there has been prolific evolution of horns within this taxon, with an extraordinary variability of size, shape, position and number now being known (Emlen & Nijhout 2000). In the case of the genus *Onthophagus*, the ancestral state appears to be the possession of a single horn at the base of the head in male beetles, and in a phylogeny based on 48 species there were 25 separate losses and gains of horns (Emlen *et al.* 2005). The obvious question posed by the extraordinary evolutionary lability of horn presence and location is what the selective forces are that lead these animals to gain or lose horns. Emlen *et al.* were able to answer this question to some extent by looking for correlations between gains and losses of horns and ecological characteristics of the species in question and found, for example, that species that were particularly abundant were more likely to gain horns on the thorax than less abundant species, and that

increased horn length was associated with increases in population density.

The study by Emlen *et al.* (2005) represents a broad survey of the diversity of horn presence and location across five continents and numerous habitats; here we present a finer grained survey of the ecological factors associated with horn presence and absence in a community of dung beetles sampled at Nylsvley research station, Northern province, South Africa. By sampling beetles from the same savannah habitat, at the same time of year and from the same location, and by using dung from the same animals (cattle and horses) as bait, we were able to control for a wide variety of environmental factors and concentrate on the influence of mean male crowding (Lloyd 1967) and the operational sex ratio (Emlen & Oring 1977) on horn presence.

2. MATERIAL AND METHODS

Fieldwork was carried out at Nylsvley research station, Northern province, South Africa in 2002 and 2003. Dung beetle abundance in this region peaks during the summer rainfall period that lasts roughly from November to March (Davis 1996), and sampling was performed during this period. In 2002 beetles were trapped from 5 January 2002 to 15 January 2002 and then again from 1 February 2002 to 9 February 2002. In 2003 the field season was from 15 January 2003 to 6 February 2003. Ten pitfall traps were put out at 10 m intervals, five baited with cow dung and five with horse dung. After 24 hours the beetles caught in the traps were collected and the traps were set up again. Cow and horse dung were used in an attempt to sample a wider range of species (cattle farming is widespread in the area and horses are commonly kept), but in practice there was no apparent difference between the catch rates of the beetle species caught by either type of bait.

The beetles were returned to the laboratory, where they were sorted into species and counted. Mean crowding was calculated as the arithmetic mean number of male beetles per trap per day adjusted by the variance to mean ratio – 1, (equation six of Lloyd 1967)

$$\bar{x}^* = \bar{x} + \left(\frac{\sigma^2}{\bar{x}} - 1 \right).$$

An aggregated distribution will have a variance to mean ratio of greater than 1, so mean crowding will be greater than the arithmetic mean, and an overdispersed distribution will have a variance to mean ratio of less than 1, so mean crowding will be less than the arithmetic mean. Male mean crowding was transformed by adding one and taking logs before analysis: this produced more acceptable distribution of the data than simply log transforming it.

Beetles were killed with ethyl acetate, and the genitalia of each beetle were dissected out to enable each one to be definitively sexed. Each beetle was scored for the hardness of the elytra, which is a good indicator of sexual maturity (J. C. Pomfret 2004, unpublished data): newly emerged, immature beetles have soft cuticles and the cuticle hardens during the beetle's period of maturation feeding. It was assumed that all sexually mature beetles were available for mating, and OSR was calculated on this basis. It can be argued that this measure of OSR is rather crude in that it does not take account of effects such as refractory period after mating, but given the difficulty in measuring such things for a large

Table 1. Summary data for 14 species of Onthophagini, pooled across 2002 and 2003.

species	total number caught	mean male crowding	OSR (% males)	average size (mm)
horned males				
<i>Hyalonthophagus alcyonides</i>	82	2.57	55.4	7.35
<i>Onthophagus aeruginosus</i>	871	6.76	51.0	5.30
<i>Onthophagus albipodex</i>	42	0.281	50.0	5.56
<i>Onthophagus leroyi</i>	180	4.63	54.1	5.73
<i>Onthophagus obtusicornis</i>	21	0.938	59.1	5.97
<i>Onthophagus quadrinodosus</i>	31	1.31	54.5	6.58
<i>Onthophagus rhodesianus</i>	122	2.95	47.5	5.96
<i>Onthophagus vinctus</i>	949	13.8	45.1	5.21
<i>Proagoderus tersidorsis</i>	60	0.002	48.0	8.28
hornless males				
<i>Euonthophagus carbonarius</i>	1340	19.7	46.6	7.13
<i>Onthophagus pallidipennis</i>	67	1.55	27.7	4.36
<i>Onthophagus quadraticeps</i>	2779	17.2	46.7	6.32
<i>Onthophagus signatus</i>	280	2.7	37.7	4.76
<i>Proagoderus sappharinus</i>	1075	7.87	44.5	7.85

number of species a more precise estimate would not be practicable. Body size was estimated as total body length by measuring with vernier callipers. Species identifications were confirmed by Dr Adrian Davis of the Department of Zoology and Entomology at Pretoria University.

(a) Comparative analysis

There is currently no available phylogeny of the species studied here, so we constructed one using sequences of the mitochondrial DNA COX1 gene (see electronic supplementary material for methods). To investigate the relationship between horn presence and absence as a response variable, and mean crowding, OSR and size as explanatory variables while taking account of phylogeny, we used a generalized least-squares (GLS) technique as implemented in CONTINUOUS v. 1.4 (Pagel 1997, 1999; NB: CONTINUOUS is now distributed as part of a larger package called BAYES TRAITS <http://www.evolution.rdg.ac.uk/BayesTraits.html>). This takes phylogenetic relations between species into account for the across-species analysis of comparative data, and the regression is an estimate of the relationship between the variables as they evolved along the branches of the phylogenetic tree; the method is described in Pagel (1997, 1999). In the case of horn presence or absence, this treats our discrete response variable as a continuous variable: statistical techniques for the analysis of relationships between discrete and continuous variables in comparative studies are not currently well developed.

We used the standard constant variance random walk model of evolution as implemented in CONTINUOUS (model A) because models using the directional model (model B) did not give significantly better fits to the data. Maximum-likelihood estimates of the scaling parameters lambda (λ) and kappa (κ) were used. λ indicates the maximum-likelihood estimate of the parameter that adjusts the variance-covariance matrix used in the analysis depending on how strong the phylogenetic signal of the correlation is, and κ adjusts the length of the branches in the phylogeny (Pagel 1997, 1999; Freckleton *et al.* 2002). Models were fitted with all explanatory variables, and partial correlation coefficients were calculated from the correlation coefficients produced by CONTINUOUS and tested for significance using a *t*-test (*t* calculated as $r_{12}\sqrt{(n-p-1)/\sqrt{(1-r_{12}^2)}}$ distributed on $n-p-1$ d.f., where *p* is the number of partial correlations):

model simplification was done by removal of non-significant terms. Models were also fitted with single explanatory variables and tested for significance using a likelihood-ratio test that compares the log likelihood of fitted models with and without the correlation between the two variables set to zero.

3. RESULTS

Fourteen species were caught in both 2002 and 2003 (table 1) and we restrict our analysis to these. Nine of these have horned males and five do not. Of the horned species, one (*Onthophagus vinctus*) has both horned males and females, although the female's horn is very small (average horn length for males is 0.19 mm and for females is 0.05 mm). Abundance ($r=0.634$, d.f. = 12, $p=0.015$), mean male crowding ($r=0.698$, d.f. = 12, $p=0.005$) and size ($r=0.989$, d.f. = 12, $p<0.001$) were all significantly correlated between years, but OSR was not ($r=-0.011$, d.f. = 12, $p=0.97$). The lack of correlation between years in OSR is probably a consequence of small sample sizes for some species in one or the other year leading to error in the estimate for OSR in that year: in 2002, for example, three species were represented by fewer than 10 individuals in total. Owing to the general consistency between years in abundance, mean crowding and size, and in order to ensure sample sizes large enough to give a reasonable estimate of OSR, a pooled dataset from both years was used in subsequent analyses.

Beetles of a number of species of *Onthophagus* have been reported to show polyphenism in the male populations, with horned 'major' males and hornless 'minors' (Eberhard & Gutierrez 1991; Emlen 1994; Moczek & Emlen 2000; Emlen *et al.* 2005). Only *Onthophagus aeruginosus* from our sample showed clear evidence of such polyphenism (J. C. Pomfret & R. J. Knell 2004, unpublished data), and so we do not consider this particular aspect of onthophagine horns further in our study.

(a) Phylogenetic analysis

For the COX1 gene, a total of 674 base pairs (bp) were sequenced for 13 species of Onthophagini and compared with the sequence obtained for *Proagoderus tersidorsis* from Dr D. J. Emlen (2003 personal communication) and the

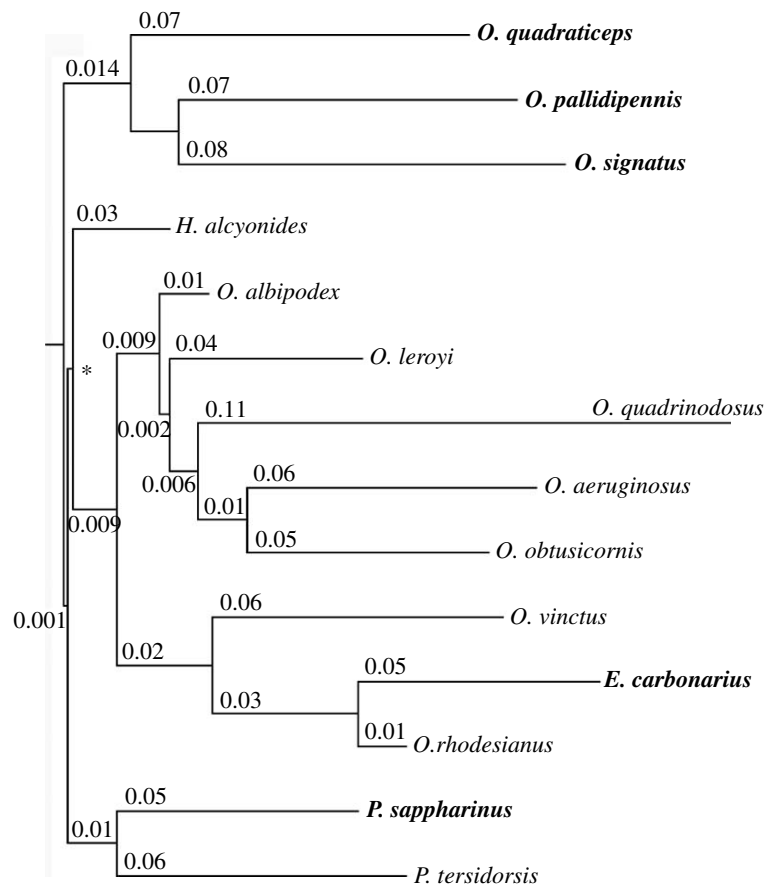


Figure 1. Phylogram showing the maximum-likelihood tree for 14 species of Onthophagini based on an analysis of 674 bp of the COX1 gene and rooted using a sequence from *A. verna* as an outgroup. The numbers indicate genetic distances for each branch. The genetic distance for the branch indicated by an asterisk is 0.001. Hornless species are indicated in bold.

Table 2. Results of GLS analysis of correlates of horn presence and absence using CONTINUOUS. See §2a for an explanation of the various parameters estimated. Numbers in brackets after estimates for λ and κ indicate CIs. These are constrained to the range 0–1 for λ and 0–3 for κ .

model with two variables

variable	λ	κ	partial correlation coefficient	<i>t</i>	<i>P</i>
log mean crowding + 1	0 (0–1)	1.23 (0.15–2.20)	–0.53	–2.34	0.037
OSR	n.a.	n.a.	0.698	3.52	0.004

single variable models

variable	λ	κ	correlation coefficient	change in log likelihood	<i>P</i>
log mean crowding + 1	0.56 (0–1)	0.77 (0–2.18)	–0.543	2.35	0.03
OSR	0 (0–1)	1.69 (0–3)	0.672	2.59	0.023

outgroup sequence from *Aleochara verna*. A combined analysis of all the data produced a single most parsimonious cladogram with strong bootstrap support at the majority of nodes (6 of the 12 nodes have bootstrap values greater than or equal to 95% and 5 have values over 75%). This tree was then used to produce a maximum-likelihood tree, which is shown in figure 1.

(b) Correlates of horn presence

Body size was not related to horn presence either when a model was fitted with all three explanatory variables

(partial correlation coefficient = –0.04, $p > 0.5$), or in a model with only size as an explanatory variable (change in log likelihood = 0.038, $p > 0.5$) or when phylogeny was not taken into account (generalised linear model with binomial errors, $p > 0.5$). For this reason size was removed from the model and a simplified model with only male mean crowding and OSR as explanatory variables was fitted (table 2). When both log mean crowding + 1 and OSR were entered into a model both partial correlation coefficients were statistically significant, and both variables were also significantly correlated with horn presence when fitted as

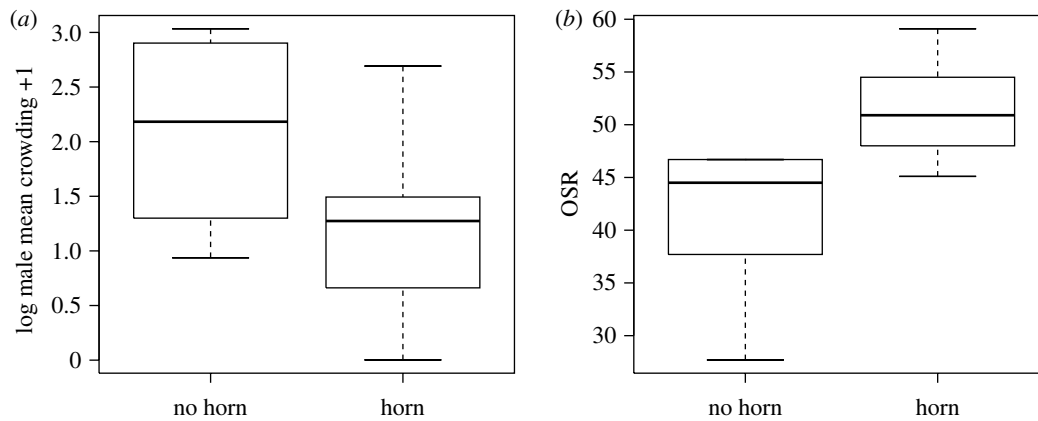


Figure 2. Boxplots showing (a) log male mean crowding + 1 and (b) OSR for horned and hornless species. The bold line is the median, the box the interquartile range and the whiskers extend to the furthest point less than 1.5 times the interquartile range from the box. Points beyond this would be indicated as outliers, if there were any.

single explanatory variables. OSR was positively correlated with horn presence, and log mean crowding was negatively correlated (figure 2). Qualitatively similar results were obtained when overall mean crowding or simple population density for each species was entered into the model instead of mean male crowding.

4. DISCUSSION

Ecological factors have been recognized as important in the evolution of mating systems, and thereby in the evolution of sexually selected characters, since the 1970s (Emlen & Oring 1977; Hamilton 1979). Much of the research in the field since then has concentrated on the role of OSR, which is now indeed recognized as being one of the fundamental determinants of mating system diversity (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996; Reynolds 1996; Kvarnemo & Ahnesjö 2002), although Shuster & Wade (2003) have recently put forward an alternative view. Our data support a role for both OSR and crowding in determining horn diversity in this community of beetles: hornless beetles tend to experience high levels of crowding and to have female-biased OSRs.

It has been argued that increases in crowding should select both for and against male weaponry. An increase in the total number of individuals in an area could lead to an increase in male–male competition intensity and consequently favour males who are able aggressively to monopolize females (Zeh 1987; Tomkins & Brown 2004; Bertin & Cézilly 2005). Alternatively, as appears to be the case here, crowding could cause reduced levels of direct competition because females are not defensible, leading to ‘scramble’ competition between males (Otte & Joern 1975; Borgia 1980; Alcock & O’Neill 1986; Connor 1989; Rutowski 1991; Mills & Reynolds 2003).

Negative relationships between the size, presence or use of male weaponry and crowding (or measures related to crowding such as population density) have been found in three other studies that concentrated on patterns within individual species. Firstly, male mites of the species *Sancassania berlessei* that are reared at low densities will develop into ‘fighter’ morphs, with armoured and pointed legs that they use to kill rivals, whereas males reared at high densities develop into ‘scrambler’ morphs that are unarmed and do not fight (Timms *et al.* 1980; Radwan

1993a,b). Secondly, Moczek (2003) found that there was a lower proportion of horned major morphs of males of the dung beetle *Onthophagus taurus* in higher-density populations. Finally, Connor (1989) compared male mating success in the forked fungus beetle, *Bolitotherus cornutus* between high- and low-density trees, and found that horn length was positively correlated with mating success on two out of three low-density trees but not on any high-density trees. Our data indicate that these intraspecific patterns are reflected in interspecific patterns of weapon diversity.

If male dung beetles in crowded species are competing via scramble competition for matings, then high fitness will be related to mobility, the ability to find females and success in sperm competition. Of these, the last is likely to be of considerable importance: in the frenetic world of the Southern African dung pat, females of these species are likely to encounter, and mate with, a large number of males, leading to high levels of sperm competition. There is strong theoretical and empirical evidence that sperm competition selects for males that invest heavily in sperm production (Parker 1998; Simmons 2001), and importantly two recent studies have indicated a link between investment in horns and investment in testes in onthophagine beetles. Firstly, in the species *Onthophagus nigriventris*, males that were prevented from growing horns grew relatively larger testes (Simmons & Emlen 2006), and secondly a comparison of testis size across 16 species of *Onthophagus* with dimorphic males found that horned major morphs tended to invest less in testis mass than hornless minor morphs (Simmons *et al.* 2007). One plausible explanation for the loss of horns in more crowded species is therefore that males of these species experience high levels of sperm competition and trade off weapon size against testis size: this is a clear direction for future research into this system.

By contrast with these studies that have shown the evolution of male weaponry to be favoured in less-crowded conditions, both Zeh (1987) and Tomkins & Brown (2004) have reported positive relationships between population density and weapon size, in pseudoscorpions and the earwig *Forficula auricularia*, respectively. In these cases the benefits of aggression and investment in weaponry may outweigh the costs even at the highest densities: whether this occurs in a specific system will depend on details of the intrasexual contests such as how costly the weapon is to

produce; how long contests take to resolve; how serious the risk of injury is; and on details of the reproductive system such as patterns of sperm precedence and whether reproduction is iteroparous or semelparous.

Operational sex ratio has been shown to be an important force influencing the intensity of sexual selection in a wide variety of species (Kvarnemo & Ahnesjö 1996; Reynolds 1996; Kvarnemo & Ahnesjö 2002), and it is perhaps not particularly surprising that it is related to horn presence in these animals: hornless species show more female-biased OSRs than horned ones, most probably because intrasexual competition between males is reduced in these species. Nonetheless, the correspondence between possession of a horn and OSR is striking: all of the hornless species had female-biased sex ratios, and two of them had OSRs of less than 40%, whereas two-thirds of the horned species had slightly male-biased or even sex ratios. We do not currently know the reasons behind these biased sex ratios, and this is another direction for future research.

As with any field study, our conclusions rest on a number of assumptions, and as a consequence some caution must be exercised in interpreting these data. Most obviously, we are assuming that sampling these animals by using baited pitfall traps gives a good estimate of the density and aggregation experienced by these animals in the wild. This assumption is, we feel, a reasonable one: these beetles locate dung pats by odour, and the mechanism by which they arrive in a pitfall trap is therefore the same as the mechanism by which they would arrive at a dung pat under natural conditions. We are also assuming that the degree of crowding that we measured reflects the most important crowding that these species have experienced during their evolution, and clearly we cannot rule out the possibility that the crowding measured during the two field seasons was exceptional: nonetheless, we have no particular reason to believe that this is the case either.

We are grateful to the staff of Nylsvley research station for their assistance during the fieldwork, to Dr Adrian Davis of Pretoria University for help identifying the beetles to Dr Chris Faulkes of Queen Mary, University of London and Dr Doug Emlen of the University of Montana for advice and assistance with the phylogenetic analysis. We are also grateful to Lotta Kvarnemo and Leigh Simmons for their constructive criticism of an earlier draft.

REFERENCES

- Alcock, J. & O'Neill, K. M. 1986 Density-dependent mating tactics in the Grey Hairstreak, *Strymon melinus* (Lepidoptera: Lycaenidae). *J. Zool.* **209**, 105–113.
- Andersson, M. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
- Arak, A. 1983 Male–male competition and mate choice in anuran bullfrogs. In *Mate choice* (ed. P. P. G. Bateson), pp. 181–210. Cambridge, UK: Cambridge University Press.
- Bertin, A. & Cézilly, F. 2005 Density-dependent influence of male characters on mate-locating efficiency and pairing success in the waterlouse *Asellus aquaticus*: an experimental study. *J. Zool.* **265**, 333–338. (doi:10.1017/S0952836905006400)
- Borgia, G. 1980 Sexual competition in *Scatophaga stercoraria*: size- and density-related changes in male ability to capture females. *Behaviour* **75**, 185–206.
- Clutton-Brock, T. H. & Parker, G. A. 1992 Potential reproductive rates and the operation of sexual selection. *Q. Rev. Biol.* **67**, 437–456. (doi:10.1086/417793)
- Connor, J. 1989 Density-dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* **43**, 1378–1386. (doi:10.2307/2409454)
- Darwin, C. 1871 *The descent of man and selection in relation to sex*. London, UK: John Murray. (Reprinted by Princeton University Press 1981.)
- Davis, A. L. V. 1996 Seasonal dung beetle activity and dung dispersal in selected South African habitats: implications for pasture improvement in Australia. *Agr. Ecosyst. Environ.* **58**, 157–169. (doi:10.1016/0167-8809(96)01030-4)
- Eberhard, W. G. & Gutierrez, E. E. 1991 Male dimorphisms in beetles and earwigs and the question of developmental constraints. *Evolution* **45**, 18–28. (doi:10.2307/2409478)
- Emlen, S. T. 1976 Lek organisation and mating strategies in the bullfrog. *Behav. Ecol. Sociobiol.* **1**, 285–313. (doi:10.1007/BF00300069)
- Emlen, D. J. 1994 Environmental-control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera, Scarabaeidae). *Proc. R. Soc. B* **256**, 131–136. (doi:10.1098/rspb.1994.0060)
- Emlen, D. J. 1997 Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* **41**, 335–341. (doi:10.1007/s002650050393)
- Emlen, D. J. & Nijhout, H. F. 2000 The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.* **45**, 661–708. (doi:10.1146/annurev.ento.45.1.661)
- Emlen, S. T. & Oring, L. W. 1977 Ecology, sexual selection and the evolution of mating systems. *Science* **197**, 215–223. (doi:10.1126/science.327542)
- Emlen, D. J., Marangelo, J., Ball, B. & Cunningham, C. W. 2005 Diversity in the weapons of sexual selection: horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). *Evolution* **59**, 1060–1084.
- Freckleton, R. P., Harvey, P. H. & Pagel, M. 2002 Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**, 712–726. (doi:10.1086/343873)
- Gwynne, D. T. & Simmons, L. W. 1990 Experimental reversal of courtship roles in an insect. *Nature* **346**, 172–174. (doi:10.1038/346172a0)
- Hamilton, W. D. 1979 Wingless and fighting males in fig wasps and other species. In *Reproductive competition, mate choice and sexual selection in insects* (eds M. S. Blum & N. A. Blum), pp. 167–220. New York, NY; London, UK: Academic Press.
- Hardy, I. C. W. (ed.) 2002 *Sex ratios: concepts and research*. Cambridge, UK: Cambridge University Press.
- Jirotkul, M. 1999a Population density influences male–male competition in guppies. *Anim. Behav.* **58**, 1169–1175. (doi:10.1006/anbe.1999.1248)
- Jirotkul, M. 1999b Operational sex ratio influences female preference and male–male competition in guppies. *Anim. Behav.* **58**, 287–294. (doi:10.1006/anbe.1999.1149)
- Kemp, D. J. 2001 Investigating the consistency of mate-locating behavior in the territorial butterfly *Hypolimnas bolina* (Lepidoptera: Nymphalidae). *J. Insect Behav.* **14**, 129–147. (doi:10.1023/A:1007809915296)
- Kokko, H. & Rankin, D. J. 2006 Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Phil. Trans. R. Soc. B* **361**, 319–334. (doi:10.1098/rstb.2005.1784)
- Kvarnemo, C. & Ahnesjö, I. 1996 The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* **11**, 404–408. (doi:10.1016/0169-5347(96)10056-2)
- Kvarnemo, C. & Ahnesjö, I. 2002 Operational sex ratios and mating competition. In *Sex ratios: concepts and research methods* (ed. I. C. W. Hardy), pp. 366–382. Cambridge, UK: Cambridge University Press.

- Lloyd, M. 1967 Mean crowding. *J. Anim. Ecol.* **36**, 1–30. (doi:10.2307/3012)
- Mills, S. C. & Reynolds, J. D. 2003 Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behav. Ecol. Sociobiol.* **54**, 98–104.
- Moczek, A. P. 2003 The behavioral ecology of threshold evolution in a polyphenic beetle. *Behav. Ecol.* **14**, 841–854. (doi:10.1093/beheco/arg062)
- Moczek, A. P. & Emlen, D. J. 2000 Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* **59**, 459–466. (doi:10.1006/anbe.1999.1342)
- Ord, T. J. & Stuart-Fox, D. M. 2006 Ornament evolution in dragon lizards: multiple gains and widespread losses reveal a complex history of evolutionary change. *J. Evol. Biol.* **19**, 797–808. (doi:10.1111/j.1420-9101.2005.01050.x)
- Otte, D. & Joern, A. 1975 Insect territoriality and its evolution: population studies of desert grasshoppers on creosote bushes. *J. Anim. Ecol.* **44**, 29–54. (doi:10.2307/3850)
- Pagel, M. 1997 Inferring evolutionary processes from phylogenies. *Zool. Scr.* **26**, 331–348. (doi:10.1111/j.1463-6409.1997.tb00423.x)
- Pagel, M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884. (doi:10.1038/44766)
- Parker, G. A. 1998 Sperm competition and the evolution of ejaculates: towards a theory base. In *Sperm competition and sexual selection* (eds T. R. Birkhead & A. P. Møller), pp. 3–54. London, UK: Academic Press.
- Radwan, J. 1993a Kin recognition in the acarid mite, *Caloglyphus berlesei*: negative evidence. *Anim. Behav.* **45**, 200–202. (doi:10.1006/anbe.1993.1022)
- Radwan, J. 1993b The adaptive significance of male polymorphism in the acarid mite *Caloglyphus berlesei*. *Behav. Ecol. Sociobiol.* **33**, 201–208. (doi:10.1007/BF00216601)
- Reynolds, J. D. 1996 Animal breeding systems. *Trends Ecol. Evol.* **11**, 68–72. (doi:10.1016/0169-5347(96)81045-7)
- Rutowski, R. L. 1991 The evolution of male mate-locating behavior in butterflies. *Am. Nat.* **138**, 1121–1139. (doi:10.1086/285273)
- Shuster, S. M. & Wade, M. J. 2003 *Mating systems and strategies*. Monographs in behavior and ecology. Princeton, NJ; Oxford, UK: Princeton University Press.
- Simmons, L. W. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ; Oxford, UK: Princeton University Press.
- Simmons, L. W. & Emlen, D. J. 2006 Evolutionary trade-off between weapons and testes. *Proc. Natl Acad. Sci. USA* **103**, 16 346–16 351. (doi:10.1073/pnas.0603474103)
- Simmons, L. W., Emlen, D. J. & Tomkins, J. L. 2007 Sperm competition games between sneaks and guards: a comparative analysis using male beetles. *Evolution* **61**, 2684–2692. (doi:10.1111/j.1558-5646.2007.00243.x)
- Stuart-Fox, D. M. & Ord, T. J. 2004 Sexual selection, natural selection and the evolution of dimorphic colouration and ornamentation in agamid lizards. *Proc. R. Soc. B* **271**, 2249–2255. (doi:10.1098/rspb.2004.2802)
- Timms, S., Ferro, D. N. & Waller, J. B. 1980 Suppression of production of pleomorphic males in *Sancassania berlesei* (Michael) (Acari: Acaridae). *Int. J. Acarol.* **6**, 91–96.
- Tomkins, J. L. & Brown, G. S. 2004 Population density drives the local evolution of a threshold dimorphism. *Nature* **431**, 1099–1103. (doi:10.1038/nature02918)
- Warner, R. R. & Hoffman, S. G. 1980 Population density and the economics of territorial defense in a coral reef fish. *Ecology* **61**, 772–780. (doi:10.2307/1936747)
- Wiens, J. J. 2001 Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends Ecol. Evol.* **16**, 517–523. (doi:10.1016/S0169-5347(01)02217-0)
- Zeh, D. W. 1987 Aggression, density and sexual dimorphism in chernetid pseudoscorpions (Arachnida: Pseudoscorpionida). *Evolution* **41**, 1072–1087. (doi:10.2307/2409192)