

Sperm competition games played by dimorphic male beetles: fertilization gains with equal mating access

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Alternative mating tactics can generate asymmetry in the sperm competition risk between males within species. Theory predicts that adaptations to sperm competition should arise in males facing the greater risk. This prediction is met in the dung beetle *Onthophagus binodis* where minor males which sneak copulations have a greater expenditure on the ejaculate. In its congener *Onthophagus taurus* there is a reduced asymmetry in sperm competition risk such that both tactics have equal ejaculate expenditure. We used the irradiated male technique to test whether adaptations to sperm competition in minor males result in higher paternity. We found that for both species, on average, each of two males gained equal numbers of fertilizations, confirming the assumption that sperm compete in a raffle. There were no differences in the sperm competition success of major and minor males in *O. taurus* as predicted from their equal expenditure on their ejaculate. Contrary to expectations, there were also no differences in fertilization success between the male tactics in *O. binodis*. Thus, in *O. binodis* minor males must expend more on their ejaculate in order to obtain the same fertilization gains as major males.

Keywords: male dimorphism; sperm competition; *Onthophagus*; paternity

1. INTRODUCTION

Sperm competition occurs when the ejaculates of two or more males compete within a single female for the fertilization of her ova (Parker 1970). When a male mates with a female, there is a probability between zero and one that his ejaculate will have to compete for fertilizations with that of another male; this probability is termed the risk of sperm competition (Parker 1970; Parker *et al.* 1997). Game theoretical models have revealed the potential for sperm competition in influencing ejaculation strategies; species in which males face a high risk of sperm competition are predicted to have a higher ejaculate expenditure than species in which males face a low risk of sperm competition (Parker 1982; Parker *et al.* 1997). This theoretical expectation has been met by comparative analyses of testis mass from a number of taxa (Harcourt *et al.* 1981; Stockley & Purvis 1993; Gage 1994; Hosken 1997) although in insects the diversity of sperm competition mechanisms can make the interpretation of such analyses problematic (see Simmons & Siva-Jothy 1998). Asymmetries in sperm competition risk can arise within species as a consequence of the behavioural tactics adopted by different males (Parker 1990). For example, guarding males physically prevent other males from copulating with their mate (Thornhill & Alcock 1983) so that they face a low risk of sperm competition (Parker 1990). Sneak males, on the other hand, always face a high risk of sperm competition because, when they do achieve a mating, the female is likely to be already paired and, therefore, subject to previous and subsequent matings (Parker 1990). The degree of asymmetry in the risk of sperm competition between alternative tactics is likely to depend on the relative frequencies of the two tactics. Where the sneak tactic is rare, there will be a low probability (low risk) of sperm competition for guarding males so that the asymmetry in sperm competition risk

between tactics will be high. In contrast, if the sneak tactic is common, guarding males will suffer a high probability of sperm competition from sneaks so that the asymmetry in sperm competition risk between tactics is reduced. The variation in ejaculate expenditure between tactics depends critically on the variation in the probability of sneak matings; when there is a high asymmetry in risk, sneaks should expend more on their ejaculate but when asymmetry is low both tactics should expend equally (Parker 1990; Gage *et al.* 1995). Alternative mating strategies of this nature thus provide an empirical model with which adaptations to sperm competition risk can be examined within species.

Alternative male mating tactics are common in insects (Thornhill & Alcock 1983) and are particularly prevalent in the dung beetle genus *Onthophagus* (Cook 1987; Emlen 1994, 1996; Hunt & Simmons 1998; Moczek 1999). Large males are horned (major males) while small males are hornless (minor males) and resemble females. The distinct morphologies of dimorphic male onthophagines are associated with alternative reproductive tactics. In most onthophagines, females gather dung from the dung pad and transport it below ground to form a brood mass into which a single egg is laid (Cook 1990; Emlen 1997; Hunt & Simmons 1998). Major males guard tunnels and help females provision the brood masses during offspring production. Minor males do not assist with provisioning, but rather sneak copulations from already guarded and provisioning females (Emlen 1997; Hunt & Simmons 1998; Moczek 1999). The alternative morphologies and behavioural tactics are largely determined by the amount of dung that is provided to the larva in its brood mass (Emlen 1997; Hunt & Simmons 1998; Moczek 1999) and the two alternative reproductive tactics represent a conditional strategy (Gross 1996).

In a recent study of two species of male dimorphic onthophagine, *Onthophagus taurus* and *Onthophagus binodis*, we investigated how asymmetries in sperm competition risk were associated with adaptations likely to be

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important in sperm competition (Simmons *et al.* 1999). Minor males outnumber major males by 60% in *O. taurus* (Simmons *et al.* 1999) so that both major and minor males should face a high sperm competition risk (Gage *et al.* 1995). In contrast *O. binodis* populations are dominated by major males (minor males represent just 30% of the male population) (Simmons *et al.* 1999) so that the frequency of sneak matings and, therefore, sperm competition risk for major males should be much lower. The asymmetry in sperm competition risk between major and minor males should therefore be low in *O. taurus* and high *O. binodis*. In accordance with theoretical expectations, we found that major and minor male *O. taurus* invested equally in their testis mass and ejaculate volumes and their sperm lengths did not differ between the two morphs. In contrast, minor male *O. binodis* had larger testes relative to their body size and produced larger ejaculates and longer sperm than did major males (Simmons *et al.* 1999).

Determining the mechanism of sperm competition is of vital importance in the interpretation of patterns of testes and ejaculate expenditure (Simmons & Siva-Jothy 1998). If sperm mixing occurs so that sperm compete numerically, then the possession of relatively larger testes and ejaculates with more and perhaps longer sperm has the potential of yielding a sperm competition advantage as envisaged by Parker's (1990) game theoretical analysis. However, if sperm removal or displacement occurs, sperm will not compete numerically within the female so the relative sperm numbers should be unimportant in fertilization success. Nevertheless, large testes and ejaculate sizes might still be favoured if sperm function by flushing a rival's sperm from the female (Parker 1990; Simmons & Siva-Jothy 1998). Here we test the assumption of Parker's (1990) models that sperm compete numerically for the fertilization of ova in *O. taurus* and *O. binodis*. Furthermore, we test the hypothesis that the apparent adaptations to sperm competition found in minor male *O. binodis* result in a fertilization advantage when morphs compete on a per ejaculate basis.

2. METHODS

Both *O. taurus* and *O. binodis* were collected from fresh cattle dung in paddocks around the town of Pemberton in the south-west of Western Australia. Females were paired with males and placed in 9 cm diameter and 30 cm deep PVC tubes three-quarters filled with sand with 0.25 l of cow dung placed on the surface. The brood masses produced by these pairs were placed into individual containers and the beetles collected and sexed on emergence. To ensure reproductive maturity, virgin females were maintained on fresh dung for three weeks before the start of the experiments.

The males used in the experiments were caught in the field and housed in isolation from the females for two weeks prior to the start of the experiment. Their pronotum widths were used as a linear measure of body size and were measured with digital callipers. Their head horn heights (*O. taurus*) and pronotal horn lengths (*O. binodis*) were measured under a binocular microscope. We used the model of Eberhard & Gutierrez (1991) for discriminating between the male morphs. These species are known to be dimorphic (Simmons *et al.* 1999) and we calculated the position of the inflexion point for each population used in this study. The inflexion point is the pronotum width at which

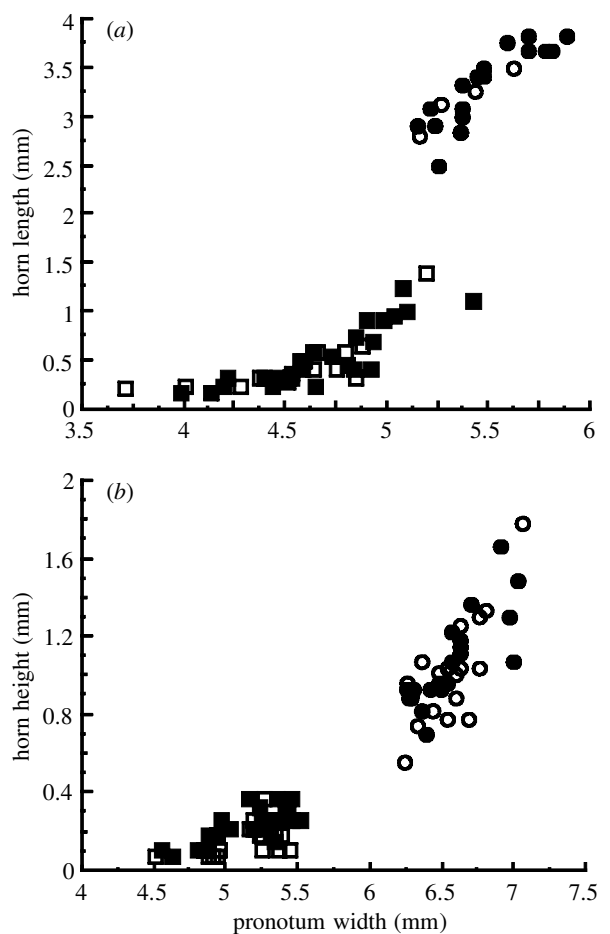


Figure 1. The relationship between the male pronotum width and (a) head horn height in *O. taurus* and (b) pronotal horn length in *O. binodis*. Data for major males (circles), minor males (squares), normal males (filled symbols) and irradiated males (open symbols) are shown. The data for *O. taurus* are incomplete due to the unexpectedly rapid decay of dead irradiated beetles prior to measurement.

the morphs change. From a range of pronotum widths, those which explained the largest proportion of the nonlinear variation in horn height were 5.15 mm ($F_{4,59} = 224.065$ and $r^2 = 0.934$) for *O. taurus* and 6.125 mm ($F_{4,97} = 129.25$ and $r^2 = 0.836$) for *O. binodis*. Males larger than these critical sizes were classified as majors and those smaller as minors. Two minor males with body sizes slightly larger than 5.15 mm (5.19 and 5.42 mm) were mistakenly included in the *O. taurus* experiment (figure 1). However, these are unlikely to have significantly influenced the results. The major and minor males selected for use in our experiments differed significantly in their pronotum widths and horn lengths (*O. taurus*, pronotum width $F_{3,74} = 50.57$ and $p = 0.0001$, and horn height $F_{3,68} = 280.87$ and $p = 0.0001$; and *O. binodis*, pronotum width $F_{3,81} = 242.86$ and $p = 0.0001$, and horn height $F_{3,81} = 106.39$ and $p = 0.0001$) (figure 1).

We used the irradiated male technique (Boorman & Parker 1976) for evaluating the fertilization success of individual males when females were allowed to copulate twice. After dividing the males by morph, half of the males from each morph were randomly assigned to be sterilized. The males were sterilized by first anaesthetizing them in nitrogen for 5 min and then exposing them to 10 krad of gamma radiation from a cobalt 60 source. Irradiation leaves the male's sperm functionally competent but results in lethal mutations which induce early embryonic

mortality. Eggs which are fertilized by irradiated males fail to hatch. Post-hoc comparisons revealed that, within the morphs, there were no differences in their pronotum widths or horn lengths between irradiated (R) and normal (N) males (figure 1).

The matings took place in plaster of Paris chambers of 1 cm width and 5 cm length. Each chamber had an observation window of clear plastic through which the beetles could be observed. The chambers were smeared with fresh dung before introducing a single virgin female and one experimental male. The duration of copula was recorded and the male removed. The second male was then introduced to the observation chamber and his duration of copula recorded. Both copulations were performed on the same day for *O. taurus*. However, the *O. binodis* pairs required a longer period of cohabitation before they would mate so the second copulations had to be performed 24 h later. Some females (26 out of 89) did not remate until between two and four days after their first copulation. Each female was mated with a major and a minor male. The male mating order was alternated such that majors mated first and minors second in 50% of the trials and minors first and majors second in the other 50%. A second experiment was performed for *O. binodis* in which a major male was competed against a second major male or a minor male was competed against a second minor male. In order to control for the influences of irradiation on the fertilization capacity of sperm, the mating order with respect to irradiation treatment (RN and NR) was alternated within the two morph mating order treatments. Control double matings (NN and RR) were performed in order to determine the natural levels of infertility among normal males and the residual fertility of sterile males.

Twice-mated females were placed into PVC tubes with sand and cow dung in order to produce brood masses (females will not lay eggs until they are able to construct brood masses). Females and their brood masses were sieved from the sand once a week for a period of three weeks. Females were given fresh dung and sand and their brood masses collected. In order to give freshly laid eggs time to hatch, the brood masses were not broken open until three days after collection. Brood masses containing larvae were recorded as fertile and unhatched rotting eggs recorded as sterile. All data are presented as means \pm s.e.

3. RESULTS

(a) Copula duration

Copula duration was analysed as a three-factor analysis of variance with morph, mating order and irradiation treatment as the main effects. There was a significant order effect in the length of copulations for *O. taurus*, with second males spending significantly less time in copula (2.49 ± 0.01 min) compared to first males (2.88 ± 0.01 min) ($F_{1,120} = 5.286$ and $p = 0.0232$). There were no other effects on copula duration. There was also an order effect on copula duration in *O. binodis*, again with first males (6.78 ± 0.16 min) copulating for significantly longer than second males (6.2 ± 0.15 min) ($F_{1,188} = 3.140$ and $p = 0.0455$). There were no other effects on copula duration. Since two males mated with the same female this analysis was also carried out as a repeated-measures ANOVA in order to determine whether females differed consistently in their copula duration across the two males. The variation in copula duration between females was not significant for either *O. taurus* ($F_{38,39} = 0.709$; and $p = 0.853$) or *O. binodis* ($F_{66,67} = 1.098$ and $p = 0.352$) although the significant variation in copula duration

between first and second males remained (*O. taurus*, $F_{1,38} = 4.52$ and $p = 0.04$; and *O. binodis*, $F_{1,66} = 6.31$ and $p = 0.014$).

(b) Paternity

The proportion of eggs which hatched when females were doubly mated to irradiated males was 0.153 ± 0.045 ($n = 10$) for *O. taurus* and 0.083 ± 0.083 ($n = 10$) for *O. binodis*. The proportion of eggs which hatched when females were doubly mated to normal males was 0.871 ± 0.048 ($n = 9$) for *O. taurus* and 0.848 ± 0.021 ($n = 17$) for *O. binodis*. The proportion of total offspring which were sired by the normal (P_N) male when the female's mating sequence was either NR or RN was calculated by controlling for the residual fertility (RR matings) and infertility (NN matings) in the treatments using the formula given in Sillen-Tullberg (1981). The proportion of offspring sired by the second male to mate is then P_N for RN matings and $1 - P_N$ for NR matings. Negative values and values greater than one can sometimes be obtained when the fertility of an experimental NR mating is lower than the estimated RR control or that from a RN mating is higher than the estimated NN control. We therefore corrected the values of P_2 using the procedure recommended by Cook *et al.* (1997).

An analysis of variance of arcsine-transformed P_2 -values was performed with the order of the male morphs and the order of the irradiation treatment as the main effects. The copula durations of males 1 and 2 were initially entered as covariates for both species. In addition, the number of days between matings was initially entered as a covariate in the *O. binodis* trial. These covariates and their interactions with the main effects were non-significant and were dropped from the final analysis. There were no significant effects of morph sequence for either *O. taurus* or *O. binodis* (tables 1 and 2) indicating that the morphs do not differ in the competitive ability of their ejaculates. There were significant effects of the order of irradiation on P_2 in *O. binodis* (table 1) which reflected the greater fertilization capacity of the sperm from normal males (table 2). The power of these tests in accepting the null hypothesis that the observed difference between the morphs in sperm competition success which is noted in table 2 is low being 0.09 for *O. taurus* and 0.05 for *O. binodis*. Our data on the differences in ejaculate size between morphs (Simmons *et al.* 1999) showed that minor males of *O. taurus* produce ejaculates which are not statistically distinguishable in volume from those produced by major males so we did not expect to see differences in fertilization success between the morphs when in competition. In contrast, minor male *O. binodis* produce ejaculates 27% larger in volume than major males (Simmons *et al.* 1999). All else being equal we might therefore expect major males to sire 27% more offspring. The power of our analysis in detecting differences in fertilization success of 27% was high at 1.00.

In our second experiment, female *O. binodis* mated with either two majors or two minors. Again, there was a significant irradiation sequence effect on P_2 (normal-irradiated, 0.315 ± 0.04 ; and irradiated-normal, 0.725 ± 0.04) ($F_{1,25} = 36.86$ and $p < 0.0001$) but no difference in P_2 due to the male morph (major-major, $0.574 < 0.07$ and $n = 15$ and minor-minor, 0.521 ± 0.07 and $n = 13$) ($F_{1,25} = 0.432$

Table 1. Analysis of variance of the arcsine-transformed P_2 -values for *O. taurus* and *O. binodis*

(The morph sequence is the order in which the minor and major males mated with the female and the irradiation sequence is the order of the males with respect to whether they were irradiated or normal. Days between matings was entered as a covariate in the *O. binodis* analysis but was not significant and was removed from the model. The interaction between the morph and irradiation sequences was non-significant in both analyses and was removed from the model. * $p = 0.06$, ** $p < 0.0001$.)

effect	<i>O. taurus</i>			<i>O. binodis</i>		
	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>
model	2	0.234	2.072	2	2.2050	32.47**
morph sequence	1	0.059	0.528	1	0.0007	0.1099
irradiation sequence	1	0.398	3.531*	1	4.4107	64.95**
error	42	0.113	—	49	0.0679	—

Table 2. Mean P_2 -values (\pm s.e.) for the two morph sequences pooled across the irradiation sequences and for the irradiation sequences pooled across the morph sequences

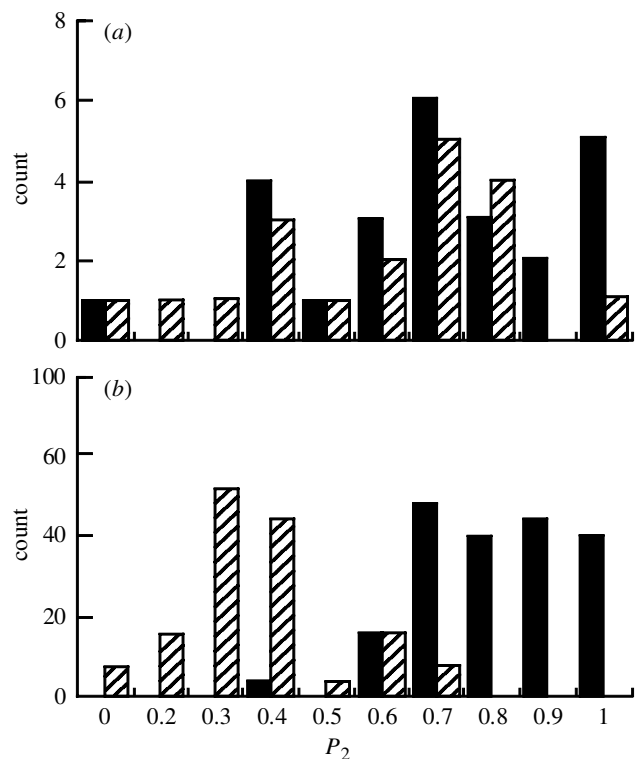
order	<i>n</i>	<i>O. taurus</i>	<i>n</i>	<i>O. binodis</i>
major–minor	24	0.564 \pm 0.06	26	0.579 \pm 0.06
minor–major	21	0.647 \pm 0.05	26	0.578 \pm 0.05
normal–irradiated	20	0.512 \pm 0.06	25	0.340 \pm 0.04
irradiated–normal	25	0.647 \pm 0.05	27	0.799 \pm 0.02

and $p = 0.517$). The covariate, i.e. the interval between the first and second copulations, was not significant and, thus, removed from the model.

Since there was no detectable difference between the morphs in their sperm competition success, the P_2 -values were pooled across morphs and tested against 0.5, the value expected if sperm mix in storage and are used in proportion to their representation, the so-called fair raffle (Parker 1998). Due to the different numbers of replicates for the NR and RN treatments the mean P_2 calculated from the pooled data would be biased towards the irradiation sequence (NR or RN) which was over-represented. To avoid bias the mean and variance were calculated for each irradiation sequence and the average mean was then compared to 0.5 using the pooled variances for estimating the standard error. The average mean for NR and RN was 0.58 ± 0.196 for *O. taurus* which was not significantly different from 0.5 ($t = 0.4088$ and d.f. = 43). The average mean for NR and RN was 0.552 ± 0.143 for *O. binodis* which was not significantly different from 0.5 ($t = 0.363$ and d.f. = 79). The P_2 -values from each of the treatment groups (NR and RN) were tested for normality. The distributions were normal for both sequences in *O. taurus* (RN, $W = 0.954$ and $p = 0.324$; and NR, $W = 0.939$ and $p = 0.246$) and for the RN sequence in *O. binodis* ($W = 0.949$ and $p = 0.086$). The NR sequence was non-normal due to a single outlying low value of P_2 ($W = 0.9201$ and $p = 0.013$) (see figure 2b). The distributions support the conclusion of mixed parentage representing both males rather than their being a principal sire.

4. DISCUSSION

Parker's (1990) sperm competition game models are based on two underlying assumptions: (i) that there is a

Figure 2. Frequency distributions of the P_2 -values (filled bars, irradiated–normal and hatched bars, normal–irradiated) for (a) *O. taurus* and (b) *O. binodis*.

trade-off between expenditure on the ejaculate and some other aspect of reproductive effort such as the number of matings obtained, and (ii) that sperm competition conforms to a raffle in which sperm are used in proportion to their representation in the female's sperm stores. Our data for onthophagines confirm the most critical of these assumptions. We found that, on average, male *Onthophagus* fertilize half of the female's clutch when in competition with another male. There was no systematic bias in the individual values of paternity towards the first or second males, no effect of the interval between the first and second male copulations and no effect of copula duration on paternity. These data show that females store sperm from both males and that sperm must compete for fertilizations under the raffle principle (Parker 1990).

Parker's (1990) sperm competition games between sneaks and guards were based on the notion of asymmetry

in the sperm competition risk between sneaks, who by nature of their tactic are always subject to sperm competition and guards, who will be subject to sperm competition with a probability which is dependent on the number of sneaks in the population. The models predict that, when sneaks are rare, they should have a greater expenditure on their ejaculate and that this increased expenditure, all else being equal, should be translated into a fertilization advantage in sperm competition. In contrast to this prediction, we found no effect of male tactics on fertilization success. We expected this outcome in *O. taurus*, since minor males are common in this species, making the asymmetry in sperm competition risk low. Thus, both minor males and major males expend equally on their ejaculates (Simmons *et al.* 1999). However, we did expect to find differences in fertilization success between minor males and major males in *O. binodis*, because in this species minor males are less abundant and expend more on their ejaculate than do major males (Simmons *et al.* 1999). One potential problem with our study was the large effect of irradiation on the fertilization capacity of male *O. binodis*. Nevertheless, both male morphs were affected equally by the irradiation; there was no irradiation sequence by male morph sequence interaction so the main effect of morph sequence can be interpreted reliably. Our results therefore suggest that minor males must expend more on their ejaculate in order to obtain the same fertilization gains as major males.

Additional asymmetries relating to the marginal costs of sperm production for sneaks and guards are built into Parker's (1990) models. He found that, as the marginal costs of sperm production increased for sneaks relative to guards, the disparity in expenditure on their ejaculates between tactics should increase; when ejaculate production is more expensive for sneaks than guards, sneaks should expend proportionally more on their ejaculate than expected when the marginal costs are equal. Importantly, the disparity in ejaculate costs can counter the expected fertilization advantage of the sneaks' increased expenditure. When ejaculates are very cheap for guards relative to sneaks and the probability of a sneak mating is high, both tactics can return equal fertilization gains despite the greater expenditure of the sneak (Parker 1990). Unlike *O. taurus* in which the testes mass adjusted for body mass is similar for both morphs, in *O. binodis* minor males have testes which are 40% larger than major males for the same body size (Simmons *et al.* 1999) suggesting that the costs of ejaculate production may indeed be far greater for minor males. Our finding that minor male *O. binodis* expend more on their testes for the same fertilization gain might therefore seem consistent with Parker's (1990) theoretical expectation when sneaks have greater costs of ejaculate production.

Nevertheless, we are still left with the paradox that minor male *O. binodis* have absolutely larger ejaculate volumes than major males (Simmons *et al.* 1999) so that, with a fair raffle, minor males should have fertilized more offspring. We found that minor males produce longer sperm than major males. We currently have no knowledge of how sperm length contributes to sperm competition in any species, but it has been suggested that longer sperm may be better able to enter storage or better able to resist displacement once in storage (Dybas & Dybas 1981;

Sivinski 1984; Ladle & Foster 1992). If there were a trade-off between sperm length and number, as assumed in Parker's (1982, 1993) models, then minor males could transfer the same number or fewer sperm than major males, despite the larger volume of their ejaculates. A sperm size or number trade-off across morphs has the potential of generating equal fertilization gains for major and minor males if the defensive properties of longer sperm counter the numerical advantages derived from smaller sperm or if both males transfer the same numbers of sperm and sperm length has no impact on fertilization success. Unfortunately because of their length, we have not yet managed to count the number of sperm contained within ejaculates accurately. Parker (1990) also assumed that the female was an inert vehicle for the evolution of male strategies and noted that there are good reasons why this may not be so (Eberhard 1996). Thus, females could favour major males as fathers by loading the raffle against minor males. Any increased ejaculate size for minors would then yield lower fertilization gains than might be expected if the raffle were truly fair. Finally, our experiments were artificial in that they examined the relative fertilization gains following just two matings. There is some evidence from pseudo-scorpions and wasps that sperm-use patterns can change following multiple matings by a female (Wilkes 1966; Zeh & Zeh 1994). Although similar patterns have not been confirmed in beetles (Eady & Tubman 1996; Lewis & Jutkiewicz 1998) it may be possible that the mechanism generating the observed patterns of sperm use changes after females have mated multiply, so that larger ejaculates may then perform better than small ejaculates. Further experiments are required using molecular markers for examining sperm use following matings by more than two males.

We found a significant variation in copula duration which was dependent on a male's position in the mating sequence; first males copulated for longer than second males. This result is consistent with studies of *Drosophila* and pentatomid bugs (Wang & Millar 1997; Snook 1998). From the perspective of sperm competition the result seems counter-intuitive since we might expect males to increase their copula duration with non-virgin females. Moczek (1999) recently found that male *O. taurus* copulate for less time in the presence of rivals, a finding which is also inconsistent with predictions from sperm competition (e.g. Gage 1991; Gage & Baker 1991). We offer two possible explanations for the variation in copula duration. First, it may be male driven, with males discriminating between virgin females and mated females. Virgin females will be of higher reproductive value than mated females because of the lack of current sperm competition. Recent models of ejaculate allocation have predicted that males should expend more with high-quality females (Galvarni & Johnstone 1998). Thus, if copula duration were correlated with ejaculate size, the longer copulations with virgin females could represent cryptic male choice. Alternatively, copula duration may be female driven, with females resistant to insemination after they have received sperm from their first mating (Linley & Hinds 1975). Female resistance to insemination was one mechanism suggested by Eberhard (1996) for cryptic female choice. We failed to find significant variation due to females in our repeated-measures analysis of copula duration.

Although this could be taken as evidence that copula duration is more strongly influenced by males than females, it could also reflect a consensus among females on how long they should copulate on their first and subsequent matings. In his study of *O. taurus*, Moczek (1999) reported an effect of male dimorphism on copula duration; minor males were found to copulate for less time than major males. Moczek (1999) suggested that this could be a possible example of cryptic female choice, with females preferring major males as mates. However, we were unable to replicate this finding in our study of *O. taurus* or in a second species, *O. binodis*. Unfortunately it is not possible to distinguish the degree to which males and females influence copula duration from our study. Nevertheless, the fact that copula duration did not influence paternity suggests that it is not related to the amount of ejaculate transferred so that explanations based on sperm competition, cryptic male choice or cryptic female choice seem tenuous at best.

In conclusion, we found that, given equal access to matings, male onthophagine dung beetles adopting alternative mating tactics gain equal numbers of fertilizations, even when sneaks have greater expenditure on their ejaculates. Recent studies of testis morphology have reported evidence consistent with theoretical expectations that sneaks should expend more on gamete production than guards (Gage *et al.* 1995; Taborsky 1998; Simmons *et al.* 1999). However, to our knowledge, our results represent the first attempt at determining whether these morphological differences translate into fertilization gains under sperm competition. Our results are not entirely consistent with theory and more studies of parentage are required in order to evaluate the biological significance of sperm competition game models fully.

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REFERENCES

- Boorman, E. & Parker, G. A. 1976 Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol. Entomol.* **1**, 145–155.
- Cook, D. 1987 Sexual selection in dung beetles. I. A multivariate study of the morphological variation in two species of *Onthophagus* (Scarabaeidae: Onthophagini). *Aust. J. Zool.* **35**, 123–132.
- Cook, D. F. 1990 Differences in courtship, mating and postcopulatory behavior between male morphs of the dung beetle *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae). *Anim. Behav.* **40**, 428–436.
- Cook, P. A., Harvey, I. F. & Parker, G. A. 1997 Predicting variation in sperm precedence. *Phil. Trans. R. Soc. Lond.* **B352**, 771–780.
- Dybas, L. K. & Dybas, H. S. 1981 Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. *Evolution* **35**, 168–174.
- Eady, P. & Tubman, S. 1996 Last-male sperm precedence does not break down when females mate with three males. *Ecol. Entomol.* **21**, 303–304.
- Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.
- Eberhard, W. G. & Gutierrez, E. E. 1991 Male dimorphisms in beetles and earwigs and the question of developmental constraints. *Evolution* **45**, 18–28.
- Emlen, D. J. 1994 Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. Lond.* **B256**, 131–136.
- Emlen, D. J. 1996 Artificial selection on horn body-length size allometry in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Evolution* **50**, 1219–1230.
- Emlen, D. J. 1997 Alternative reproductive tactics and male dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* **41**, 335–341.
- Gage, M. J. G. 1991 Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Anim. Behav.* **42**, 1036–1037.
- Gage, M. J. G. 1994 Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proc. R. Soc. Lond.* **B258**, 247–254.
- Gage, M. J. G. & Baker, R. R. 1991 Ejaculate size varies with socio-sexual situation in an insect. *Ecol. Entomol.* **16**, 331–337.
- Gage, M. J. G., Stockley, P. & Parker, G. A. 1995 Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations. *Phil. Trans. R. Soc. Lond.* **B350**, 391–399.
- Galvarni, A. & Johnstone, R. 1998 Sperm allocation in an uncertain world. *Behav. Ecol. Sociobiol.* **44**, 161–168.
- Gross, M. R. 1996 Alternative reproductive tactics: diversity within sexes. *Trends Ecol. Evol.* **11**, 92–98.
- Harcourt, A. H., Harvey, P. H., Larson, S. G. & Short, R. V. 1981 Testis weight, body weight and breeding system in primates. *Nature* **293**, 55–57.
- Hosken, D. J. 1997 Sperm competition in bats. *Proc. R. Soc. Lond.* **B264**, 385–392.
- Hunt, J. & Simmons, L. W. 1998 Strategies of parental care covary with male morphology in a horned beetle (*Onthophagus taurus*) (Coleoptera: Scarabaeidae). *Behav. Ecol. Sociobiol.* **42**, 447–451.
- Ladle, R. J. & Foster, E. 1992 Are giant sperm copulatory plugs? *Acta Oecol.* **13**, 635–638.
- Lewis, S. M. & Jutkiewicz, E. 1998 Sperm precedence and sperm storage in multiply mated red flour beetles. *Behav. Ecol. Sociobiol.* **43**, 365–370.
- Linley, J. R. & Hinds, M. J. 1975 Quantity of the male ejaculate influenced by female unreceptivity in the fly, *Culicoides melleus*. *J. Insect Physiol.* **21**, 281–285.
- Moczek, A. P. 1999 Facultative paternal investment in the polyphenic beetle *Onthophagus taurus*: the roles of male morphology and social context. *Behav. Ecol.* **10**, 641–647.
- Parker, G. A. 1970 Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *J. Insect Physiol.* **16**, 1301–1328.
- Parker, G. A. 1982 Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J. Theor. Biol.* **96**, 281–294.
- Parker, G. A. 1990 Sperm competition games: sneaks and extra-pair copulations. *Proc. R. Soc. Lond.* **B242**, 127–133.
- Parker, G. A. 1993 Sperm competition games: sperm size and sperm number under adult control. *Proc. R. Soc. Lond.* **B253**, 245–254.
- Parker, G. A. 1998 Sperm competition and the evolution of ejaculates: towards a theory base. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 3–54. London: Academic Press.

- Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. 1997 Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond. B* **264**, 1793–1802.
- Sillen-Tullberg, B. 1981 Prolonged copulation: a male 'post-copulatory' strategy in a promiscuous species, *Lygaeus equestris* (Heteroptera: Lygaeidae). *Behav. Ecol. Sociobiol.* **9**, 283–289.
- Simmons, L. W. & Siva-Jothy, M. T. 1998 Sperm competition in insects: mechanisms and the potential for selection. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 341–434. London: Academic Press.
- Simmons, L. W., Tomkins, J. L. & Hunt, J. C. 1999 Sperm competition games played by dimorphic male beetles. *Proc. R. Soc. Lond. B* **266**, 145–150.
- Sivinski, J. 1984 Sperm in competition. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 86–115. London: Academic Press.
- Snook, R. R. 1998 The risk of sperm competition and the evolution of sperm heteromorphism. *Anim. Behav.* **56**, 1497–1507.
- Stockley, P. & Purvis, A. 1993 Sperm competition in mammals: a comparative study of male roles and relative investment in sperm production. *Funct. Ecol.* **7**, 560–570.
- Taborsky, M. 1998 Sperm competition in fish 'bourgeois' males and parasitic spawning. *Trends Ecol. Evol.* **16**, 222–227.
- Thornhill, R. & Alcock, J. 1983 *The evolution of insect mating systems*. Harvard University Press.
- Wang, Q. & Millar, J. G. 1997 Reproductive behaviour of *Thyanta pallidovirens* (Heteroptera: Pentatomidae). *Annl. Entomol. Soc. Am.* **90**, 380–388.
- Wilkes, A. 1966 Sperm utilisation following multiple insemination in the wasp *Dahlbominus fuscipennis*. *Can. J. Genet. Cytol.* **8**, 451–461.
- Zeh, J. A. & Zeh, D. W. 1994 Last-male sperm precedence breaks down when females mate with three males. *Proc. R. Soc. Lond. B* **257**, 287–292.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.