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Superior sperm competitors sire higher-quality young

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The evolution of polyandry remains controversial. This is because, unlike males, in many cases multiple mating by females does not increase fecundity and inevitably involves some costs. As a result, a large number of indirect benefit models have been proposed to explain polyandry. One of these, the good sperm hypothesis, posits that high-quality males are better sperm competitors and sire higher-quality offspring. Hence, by mating multiply, females produce offspring of superior quality. Despite being potentially widely applicable across species, this idea has received little attention. In a laboratory experiment with yellow dung flies (*Scathophaga stercoraria*) we found that males that were more successful in sperm competition also had offspring that developed faster. There was no relationship between paternal success in sperm competition and the ability of offspring to survive post-emergence starvation. Since faster development times are likely to be advantageous in this species, our data provide some support for polyandry evolving as a means of producing higher-quality offspring via sperm competition.

Keywords: sperm competition; good genes; polyandry; *Scatophaga*

1. INTRODUCTION

Polyandry (females mating with multiple males) remains one of the most controversial topics in evolutionary biology (Yasui 1997). This is primarily because in most species, females derive no direct benefits from mating with many males, but frequently incur direct costs (Chapman *et al.* 1995; Blanckenhorn *et al.* 2002). Nevertheless, polyandry is widespread, with females of most taxa mating with more than one male (Birkhead & Møller 1998). In species where females do obtain direct benefits from males, for example via nuptial gifts, the evolution of polyandry presents no great conundrum (Arnqvist & Nilsson 2000; Hosken & Stockley 2003). Moreover, a recent meta-analysis suggested that direct benefits drive polyandry in many insects (Arnqvist & Nilsson 2000), although this type of study cannot distinguish between adaptations to polyandry and benefits that could explain its prevalence. For instance, in species where females mate repeatedly, females are not selected to store significant quantities of sperm, hence experiments where females are prevented from remating will reveal lower fecundities of monandrous females. However, this does not demonstrate that repeated mating has evolved owing to the need for sperm replenishment.

Several hypotheses have been proposed to explain the evolution of polyandry in the absence of direct female benefits (Harvey & May 1989; Birkhead *et al.* 1993; Keller & Reeve 1995; Zeh & Zeh 1996; Yasui 1997; Hosken & Blanckenhorn 1999; Jennions & Petrie 2000; Tregenza & Wedell 2000, 2002; Hosken & Stockley 2003). One of these, the good sperm hypothesis (Harvey & May 1989; Birkhead *et al.* 1993; Yasui 1997), suggests that a male's success in sperm competition correlates with other aspects of his genetic quality. Therefore, males that are more successful during sperm competition are of higher quality, and hence sire high-quality offspring (i.e. represent indirect benefits to females). The fact that sperm-competitive ability correlates with diploid, not haploid genotype bodes well for this idea (Clark *et al.* 2000). However, while there is evidence that some males produce high-quality ejaculates and are consistently better in sperm competition (Dzuik 1996), there have been few investigations of the good sperm hypothesis. Madsen *et al.* (1992) reported an association between polyandry and offspring survival. They suggested that this was owing to correlations between sperm-competitive ability, male quality and offspring quality (and see Parker 1992). While these associations are theoretically plausible (Yasui 1997), subsequent findings indicate that the effect originally reported was owing to genetic compatibility rather than genetic quality *per se* (Olsson *et al.* 1996). In addition to this work, the only other study, to our knowledge, to test the good sperm hypothesis experimentally failed to find any association between sperm competitiveness and offspring quality in field crickets (Simmons 2001).

Recent work on *Drosophila melanogaster* suggests explanations for why data consistent with the good sperm hypothesis may be rare (Chippindale *et al.* 2001; reviewed in Pizzari & Birkhead 2002). Selection can operate in opposite directions in each gender, hence the optimal phenotype of each sex differs and alleles enhancing fitness in one sex may reduce fitness when expressed in the other sex (Chippindale *et al.* 2001). In addition to this intralocus conflict, sexually antagonistic genes, which increase the fitness of one sex at a cost to the other, are expected to accumulate on the X-chromosome (Rice 1992). When males are the heterogametic sex (XY), they cannot pass their X-chromosome to their sons. Therefore, if sexually antagonistic genes do indeed accumulate on the X-chromosome, and these at least partly determine reproductive quality, fathers cannot influence the quality of their sons at these loci, and high-quality males may produce low-quality daughters owing to intralocus sexually antagonistic effects. Furthermore, and everything else

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being equal, females whose offspring are sired by the highest-quality males could at best produce average daughters, and sons whose quality is inversely related to hers. Cloned *Drosophila* genotypes provide some evidence for much of the above (Rice 1992; Chippindale *et al.* 2001; Rand *et al.* 2001).

Here, we investigated the good sperm hypothesis in the yellow dung fly, *Scathophaga stercoraria*. Sperm competition has been extensively studied in this species (Parker 1970; Ward & Simmons 1991; Parker & Simmons 1994; Ward 2000). Sperm competitiveness has an additive genetic component (Hosken *et al.* 2001), but there is no evidence that genetic similarity has a detectable effect on sperm competition in this taxon (Hosken *et al.* 2002*a*). Furthermore, although P2 (the proportion of offspring sired by the second of two males to mate) in typical laboratory settings is *ca*. 0.8, the variance around this mean is enormous (P2 ranges from 0.02 to 1), and typically unexplained (Simmons & Siva-Jothy 1998). For example, body size has no effect on P2 (Parker & Simmons 1994), although it is favoured by selection at other levels (Borgia 1981). It therefore appears plausible that male genetic quality influences paternity in competitive situations. In addition, there are no direct benefits to polyandry in these flies (Tregenza *et al.* 2003), even though females mate multiply in nature (Parker 1970) and multiple mating decreases female longevity (Hosken *et al.* 2002*b*). We carried out a sperm-competition experiment and determined whether fertilization success and offspring quality were associated. Adult survival in the absence of food and development time from egg to adult were the two measures of offspring quality. Food deprivation ensured that conditions were stressful, as this is when survival differences are most likely to be manifest (e.g. Wilkinson 1984; Hoffmann & Parsons 1991; Moret & Schmid-Hempel 2000). Flies were kept at low temperature to increase the variance in survival. Yellow dung flies are cold-adapted and this treatment reflects problems faced by *S. stercoraria* in nature where flies emerging on rainy days have to rely solely on fat reserves until the rain stops and they can hunt successfully. Development time has large fitness consequences in taxa inhabiting ephemeral habitats (Newman 1992). This is likely to be especially true in species like *S. stercoraria* where body size and development time are largely uncoupled (i.e. growth rate is highly plastic), but that still experience severe time limitations owing to resource depletion (i.e. dung is limited during development by strong intra- and interspecific competition), predation and strong seasonal effects (i.e. larva must complete development to over-winter or prior to dung desiccation) (summarized in Amano 1983; Blanckenhorn 1998). Faster development times are also likely to be favoured because *S. stercoraria* are multivoltine. In addition to testing the good sperm hypothesis, we also assess the possibility that the accumulation of sexually antagonistic alleles and/or intersexual developmental antagonism results in negative correlations between the fitness of sibling males and females, because under sexual conflict genes producing good males may generate poor females.

2. MATERIAL AND METHODS

Field-captured females ($n = 60$) were brought to the laboratory and allowed to lay in a portion of dung. Offspring were reared under control conditions with ample dung, reducing larval competition (more than 2 g per larva; Amano 1983). On emergence, a random sample of offspring (about two males and three females per clutch) was reared under controlled conditions (15 °C and 60% relative humidity) with *ad libitum* water, sugar and *Drosophila* until maturity (*ca*. 18 days).

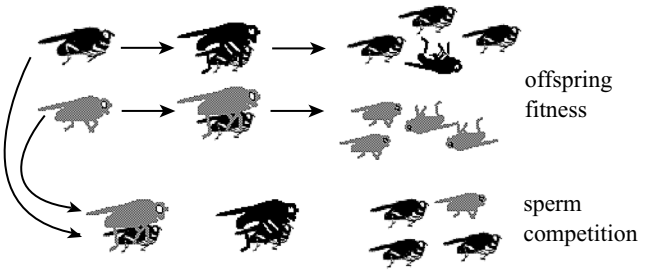

Figure 1. The experimental design. A pair of males were each mated to a different female and the development time and post-emergence survival of the offspring were measured. The same males were also mated to one single female and their success in sperm competition was assessed by determining offspring paternity using microsatellite markers. A total of 46 replicates of this scheme were performed.

One male from each family was randomly assigned a male from another family to form a competitive pair ($n = 46$ pairs of males). These males copulated once in succession with an unrelated but randomly assigned female, with male roles (first or second—representing sperm defence (the ability to resist displacement) and offence (the ability to pre-empt rival sperm), respectively) randomly assigned and no interference during copula. Copula durations were recorded to the nearest minute. After both copulations, these doubly mated females were allowed to lay their clutch of eggs. The males were then singly mated to another female. Where possible, these second females were the sisters of the female in which their sperm competed, and where related females were not available, unrelated females were used (17 out of 46 pairs, and note that males never mated with their own sisters) (figure 1). These singly mated females were then also allowed to lay eggs. Subsequently, all members of the competitive triad (i.e. the doubly mated females and the two males that copulated with them) were frozen at −80 °C.

All clutches were reared under controlled conditions (as above) and all emerging offspring ($n = 1081$) from the competitive matings were frozen at −80 °C. DNA was extracted from the frozen sperm-competition dams, sires and a random sample of offspring ($n = 12$ per female, *ca*. 50% of emerging offspring; mean number of young emerging = 25 per triad). Paternity was assigned (blind) using PCR of microsatellite markers and examining the products using an Elchrom (SEA 2000) electrophoresis system with Spreadex gels (Garner *et al.* 2000). Parents were scored at loci sequentially until one or more were found that allowed us to assign paternity unequivocally (mean number of loci required was 1.7).

For the non-competitive matings, offspring development times were recorded (laying day till emergence ± 2 hours), and upon emergence, a sample of *ca*. five members of each sex per family were housed under constant conditions at 10 °C without food ($n = 720$). The survival of this group was measured, with flies checked for death every 4 hours, and body size (length of the hind tibia) was measured at death. We assessed offspring quality from the non-competitive matings to alleviate problems associated with data loss owing to difficulties in DNA analysis

from dead flies. We also had no way of knowing *a priori* which male sired which offspring in the sperm-competition assay. So, to ensure that all males' offspring were assessed with a reasonable sample size, offspring quality and sperm competitiveness were measured in different females. Associations between sperm-competition success and offspring survival were then assessed with a multivariate general linear model (MGLM) that included whether or not singly mated females were sisters to sperm-competitive females as a factor. This analysis included mean offspring body size as a covariate since size influences survival. Note that when we used models with P1 (the proportion of offspring sired by the first male in our competitive mating examining sperm defence) or P2 (the proportion of offspring sired by the second male in our competitive mating examining sperm offence) as the *dependent* variables and all offspring traits (including size) and relatedness of tester females as predictors, identical results were obtained. We used family means in the paternity–offspring quality analyses rather than repeated measures because there is no really satisfactory way to deal with missing values or unequal sample sizes in repeated measures analysis. We also examined whether there were any associations between the mean size of male and female offspring, and their survival and development times. The accumulation of sexually antagonistic alleles may lead to no or negative correlations between male and female fitness measures (Chippindale *et al.* 2001). All data were normally distributed (Kolmogorov–Smirnov tests all $p > 0.13$) except for P1 and P2 (sperm defence and offence) data. These variables became normal after arcsin square-root transformation (both $p > 0.05$), and in any case, residuals from our analyses were all normally distributed ($p > 0.9$). Finally, sample sizes vary somewhat because not all females laid eggs.

3. RESULTS

Copulation duration did not differ between first and second copulations (d.f. = 41; $t = 0.91$; $p = 0.37$), and because differences in copula duration did not explain any of the variance in the sperm-competition success (P1 or P2) ($p = 0.88$ and 0.35, respectively) or offspring quality (all $F < 3.3$; all $p > 0.08$), we did not include it in our final models.

A MGLM of offspring survival and development time, with whether or not the female used to measure P1/P2 and that used to produce offspring for fitness assays were related as a factor, and mean offspring size and P2 (sperm offence) as covariates, indicated that offspring size had a significant effect on the multivariate combination of offspring survival and development time (Wilks' Lambda here and throughout, $F_{2,31} = 30.0$; $p = 0.0001$), P2 had a marginally non-significant multivariate effect ($F_{2,31} = 2.89$; $p = 0.07$) and relatedness of tester females had no significant multivariate effect ($F_{2,31} = 0.87$; $p = 0.43$). Univariate analyses showed that the strong effect of size was owing to its positive effect on survival (figure 2; $F_{1,32} = 54.1$; $p = 0.0001$), whereas size was not associated with development time ($F_{1,32} = 2.44$; $p = 0.13$). These analyses also showed that P2 was significantly negatively associated with offspring development time (figure 3; $F_{1,32} = 5.86$; $p = 0.02$), but had no effect on offspring survival ($F_{1,32} = 0.51$; $p = 0.47$). The relatedness of tester females was not significantly associated with either dependent variable in univariate tests ($F_{1,32} < 1.72$; $p > 0.19$).

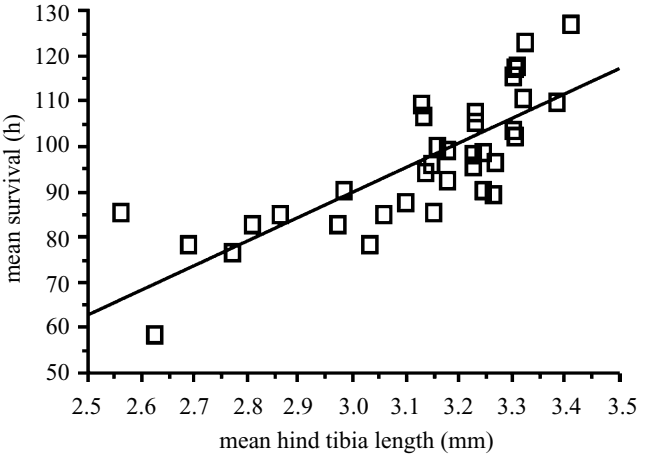

Figure 2. The positive association between mean post-emergence survival and mean offspring size in the absence of food.

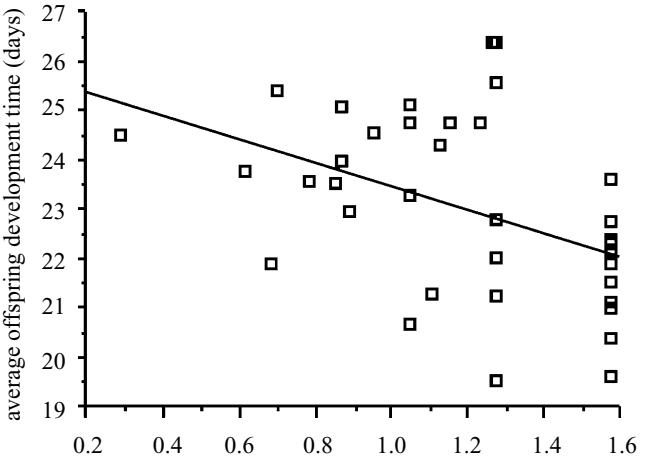

Figure 3. The negative association between mean offspring development time and sperm offence, measured as P2, the proportion of offspring sired by the second of the two males to mate.

An identical analysis of the offspring of first males to mate in the competitive setting (with relatedness of tester females as a factor and mean offspring size and P1 (sperm defence) as covariates) indicated that only mean offspring size had a significant multivariate effect ($F_{2,36} = 23.2$; $p = 0.0001$; all other $p > 0.30$). Univariate analyses indicated that this was again driven by the positive association between body size and survival ($F_{1,36} = 41.5$; $p = 0.0001$), and no other significant associations were found (all $F < 2.4$; all $p > 0.13$).

Because a male's competitive performance may depend on the quality of his competitor(s), we also looked at P1 and P2 and relative offspring quality. Relative quality was measured as the mean of the focal male's offspring minus the mean values of their competitors (although analyses with other relative measures (i.e. in the form $a/(a + b)$) produce identical results). A MGLM (with P2 as a predictor and relative development time and relative residual (controlling for size) survival as dependents) indicated that there was a significant multivariate association

between offspring performance and P2 ($F_{2,26} = 5.43$; $p = 0.015$). Univariate analysis indicated that this was again driven by the negative association between relative development time and P2 ($F_{1,27} = 10.6$; $p = 0.004$), as the association between relative residual survival and P2 was not significant ($F_{1,27} = 0.54$; $p = 0.82$). In contrast to the analysis of the absolute fitness measures presented above, multivariate analysis of associations between P1 and relative development time and relative residual (controlling for size) survival also indicated a significant multivariate effect ($F_{2,26} = 5.19$; $p = 0.013$). Univariate analysis indicates that this was driven by a negative association between relative development time and P1 ($F_{1,27} = 10.2$; $p = 0.004$), because the association between relative residual survival and P1 was not significant ($F_{1,27} = 0.67$; $p = 0.44$). Relatedness of tester females had no significant effects at any level (all $F < 0.5$; all $p > 0.48$).

It is possible that these associations are owing to correlations between offspring survival to emergence and development time, even in our benign laboratory environment. If this were true then flies sampled in the P2 experiment would be more likely to be those that had developed faster, and hence P2 would be falsely inflated. While this should not occur because larvae were supplied with *ad libitum* food, one way to assess this possibility is to look at the association between P2 and the number of offspring emerging. If faster-developing flies were more likely to survive, and hence be sampled, then there should be a negative association between P2 and number of offspring emerging. Regression analysis indicated that there was no association between these two variables ($r = 0.19$; $F_{1,42} = 1.58$; $p = 0.22$).

To look at associations between male and female characters, we used family means for each sex across all single matings, and regressed female values on male values. These analyses revealed significant positive associations in all comparisons (figure 4, all $n = 74$; all $t > 9.63$; all $p < 0.0001$), indicating that body size, development time and survival of males and females were strongly correlated at the family level. We also looked to see if development time and survival traded-off at the family level but there was no significant association ($F_{1,72} = 0.001$; $p = 0.98$).

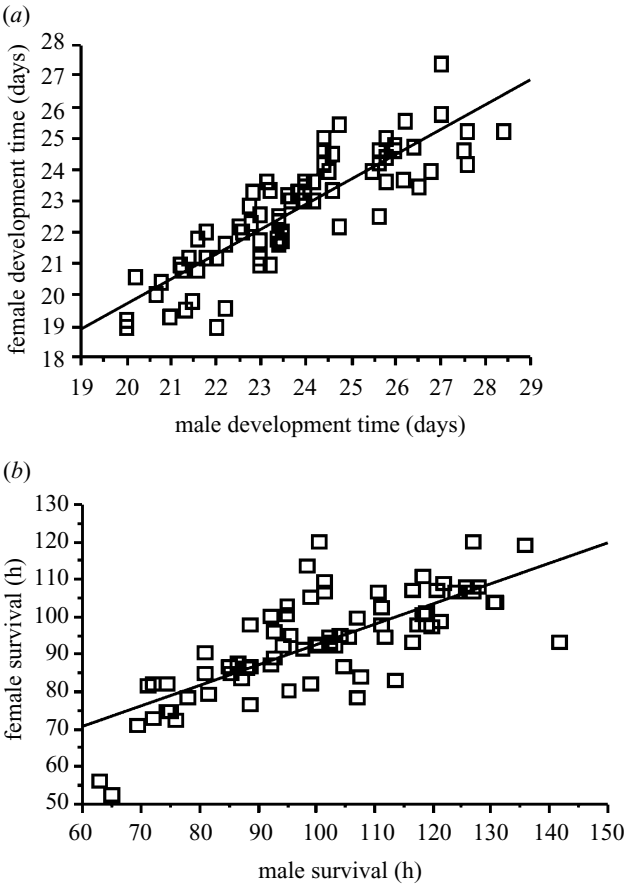

Figure 4. Family-level correlations between male and female offspring in (*a*) development time and (*b*) survival. Family level correlations imply additive genetic variation for these traits.

4. DISCUSSION

A male's ability to pre-empt previously stored rival sperm (offence), measured by P2 (the proportion of offspring sired by second males), was negatively associated with offspring development time. This association holds whether we used absolute or relative development time as the dependent measure. Therefore the offspring of males with superior sperm offence developed faster. Since faster development time is advantageous in dung flies (for competitive, predation and environmental reasons), especially because size and development time are not associated (Blanckenhorn 1998; the present study), our study provides some support for the good sperm hypothesis as a selective force favouring polyandry (also see Simmons & Kotiaho 2002). That the relatedness of tester females had no significant effect on our results further corroborates this claim, and suggests that male genetic quality rather than genetic compatibility underlies the result. This is important because the results of the only other previous study supporting the good sperm hypothesis (Madsen *et al.* 1992), were subsequently shown probably to be owing to genetic incompatibility from costs of inbreeding rather than male quality *per se* (Olsson *et al.* 1996). In addition, our results support previous findings that genetic similarity does not influence sperm-competition success in yellow dung flies (Hosken *et al.* 2002*a*). The lack of association between paternity and number of offspring emerging in the sperm-competition experiment also supports our claim (and see Ward 2000). Sperm defence (measured by P1), however, was only associated with offspring development time when we used the relative development time of the offspring of males whose sperm had competed against one another. Once again, the association was negative. However, this analytical approach makes it inevitable that either both offence and defence will be associated with development time or neither will be, because both sperm-competitive success and development time are relative to the other male in the pair. Nevertheless, the significant result of the analysis employing non-relative measures suggests a stronger association between sperm offence and speed of offspring development than between sperm defence and development time. This contrasts somewhat with findings from *Drosophila melanogaster*, where there appears to be more heritable variation for sperm defence than offence (Clark *et al.* 2000). We previously failed to find any support for the good sperm

hypothesis in yellow dung flies, although the protocol employed was not as powerful (Tregenza *et al.* 2003). There, we sampled random offspring from multiply mated females, which incidentally increases the variance in quality measures (as offspring from good and bad males are pooled), making detecting differences more difficult. We suggest that future investigations employ more powerful designs such as used in the current study.

There were no associations between survival and sperm competitiveness, either in offence or defence. This may simply have been for the reason that our experimental conditions were too harsh because we starved flies after emergence, although as we argued earlier, flies experience similar conditions in nature. In addition, there was variation in adult survival, and although much of it was associated with fly size, our model left more than 50% of the variation unexplained. There was also no evidence for a significant development time/survival trade-off. Why sperm competitiveness was associated with only one measure of offspring quality remains unclear. It may simply be that selection is stronger at the larval stage and hence with development time it is easier to detect an effect (Hellriegel 2000). The survival results are, however, in partial agreement with previous work which found that better sperm competitors have slightly weaker immune systems (Hosken 2001). This may be because, with longer development time, offspring of poor sperm competitors are likely to suffer increased exposure to nematode parasites in the dung and hence require more investment in immune function. Nonetheless, because the immune system difference was modest and the development time association quite strong, it appears that the net quality of offspring will be greater for better sperm competitors even after taking this previous result into account. It should also be noted that this previous work (Hosken 2001; Hosken *et al.* 2001) involved flies that were forced to evolve under novel selection. Under conditions closer to the natural state (as here), beneficial adaptations may be easier to detect, but sexually antagonistic coevolution more difficult (Rice 2000).

We also found strong positive associations between male and female fitness measures at the family level. Although this appears to challenge the ontogenetic antagonism hypothesis, negative associations are not predicted to be manifest until flies are adult, because the phenotype and behaviour of larvae are probably not sexually dimorphic (Chippindale *et al.* 2001). This is what was found in *D. melanogaster* experiments (Chippindale *et al.* 2001). Here, we investigated adult flies, but survivorship had to be largely determined by resources accrued during the larval stage, and similarly, fly development time is partly spent as a larva. Hence, our assay may not have been appropriate for the detection of potential developmental antagonism, even though yellow dung flies are extremely sexually size-dimorphic on emergence. In addition, we only measured components of fitness. What we can say, however, is that there is likely to be additive genetic variation for development time and survival in dung flies given the strong family-level associations (see also Blanckenhorn 2002). Finally, our finding that survival under starvation conditions was largely determined by size supports starvation resistance-based arguments for Bergman's rule in ectotherms (discussed in McNab 2002, pp. 89–90).

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REFERENCES

Amano, K. 1983 Studies on the intraspecific competition in dung-breeding flies. I. Effects of larval density on yellow dung fly, *Scathophaga stercoraria* L. Diptera: Scathophagidae. *Jpn. J. Sanit. Zool.* **34**, 165–175.

Arnqvist, G. & Nilsson, T. 2000 The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**, 145–164.

Birkhead, T. R. & Møller, A. P. 1998 *Sperm competition and sexual selection*. London: Academic.

Birkhead, T. R., Møller, A. P. & Sutherland, W. J. 1993 Why do females make it so difficult for males to fertilize their eggs? *J. Theor. Biol.* **161**, 51–60.

Blanckenhorn, W. U. 1998 Adaptive phenotypic plasticity in growth, development and body size in the yellow dung fly. *Evolution* **52**, 1394–1407.

Blanckenhorn, W. U. 2002 The consistency of quantitative genetic estimates in field and laboratory in the yellow dung fly. *Genetica* **114**, 171–182.

Blanckenhorn, W. U., Hosken, D. J., Martin, O. Y., Reim, C., Teuschl, Y. & Ward, P. I. 2002 The costs of copulating in the dung fly *Sepsis cynipsea*. *Behav. Ecol.* **13**, 353–358.

Borgia, G. 1981 Mate selection in the fly *Scathophaga stercoraria*: female choice in a male-controlled system. *Anim. Behav.* **29**, 71–80.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995 Costs of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**, 241–244.

Chippindale, A. K., Gibson, J. R. & Rice, W. R. 2001 Negative genetic correlations for adult fitness between the sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 1671–1675.

Clark, A. G., Dermitzakis, E. T. & Civetta, A. 2000 Nontransitivity of sperm competition in *Drosophila*. *Evolution* **54**, 1030–1035.

Dzuik, P. J. 1996 Factors that influence the proportion of offspring sired by a male following heterospermic insemination. *Anim. Reprod. Sci.* **43**, 65–88.

Garner, T. W. J., Brinkmann, H., Gerlach, G., Meyer, A., Ward, P. I., Sporri, M. & Hosken, D. J. 2000 Polymorphic DNA microsatellites identified in the yellow dung fly *Scathophaga stercoraria*. *Mol. Ecol.* **9**, 2207–2208.

Harvey, P. H. & May, R. M. 1989 Copulation dynamics—out for the sperm count. *Nature* **337**, 508–509.

Hellriegel, B. 2000 Single- or multistage regulation in complex life cycles: does it make a difference? *Oikos* **88**, 239–249.

Hoffmann, A. A. & Parsons, P. A. 1991 *Evolutionary genetics and environmental stress*. Oxford University Press.

Hosken, D. J. 2001 Sex and death: microevolutionary tradeoffs between reproductive and immune investment in dung flies. *Curr. Biol.* **11**, R379–R380.

Hosken, D. J. & Blanckenhorn, W. U. 1999 Female multiple mating, inbreeding avoidance and fitness: it's not only the magnitude of costs and benefits that count. *Behav. Ecol.* **10**, 462–464.

Hosken, D. J. & Stockley, P. 2003 Benefits of polyandry: a life history perspective. *Evol. Biol.* **33**, 173–194.

Hosken, D. J., Garner, T. W. J. & Ward, P. I. 2001 Sexual conflict selects for male and female reproductive characters. *Curr. Biol.* **11**, 489–493.

Hosken, D. J., Blanckenhorn, W. U. & Garner, T. W. J. 2002*a*

Heteropopulation males have a fertilization advantage during sperm competition in the yellow dung fly (*Scathophaga stercoraria*). *Proc. R. Soc. Lond.* B **269**, 1701–1707. (DOI 10.1098/rspb.2002.2094.)

Hosken, D. J., Uhía, E. & Ward, P. I. 2002*b* The function of female accessory reproductive gland secretion and a cost to polyandry in the yellow dung fly. *Physiol. Entomol.* **27**, 87–91.

Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**, 21–64.

Keller, L. & Reeve, H. K. 1995 Why do females mate with multiple males? The sexually selected sperm hypothesis. *Adv. Study Behav.* **24**, 291–315.

McNab, B. K. 2002 *The physiological ecology of vertebrates: a view from energetics*. Ithaca, NY: Cornell University Press.

Madsen, T., Shine, R., Loman, J. & Hakansson, T. 1992 Why do female adders copulate so frequently? *Nature* **355**, 440–441.

Moret, Y. & Schmid-Hempel, P. 2000 Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**, 1166–1168.

Newman, R. A. 1992 Adaptive plasticity in amphibian metamorphosis. *Bioscience* **42**, 671–678.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelstrom, H. 1996 Sperm selection by females. *Nature* **383**, 585.

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. 1992 Snakes and sexuality. *Nature* **355**, 395–396.

Parker, G. A. & Simmons, L. W. 1994 Evolution of phenotypic optima and copula duration in dungflies. *Nature* **370**, 53–56.

Pizzari, T. & Birkhead, T. R. 2002 The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. *Biol. Rev.* **77**, 183–189.

Rand, D. M., Clark, A. G. & Kann, L. M. 2001 Sexually antagonistic cytonuclear fitness interactions in *Drosophila melanogaster*. *Genetics* **159**, 173–187.

Rice, W. R. 1992 Sexually antagonistic genes—experimental evidence. *Science* **256**, 1246–1439.

Rice, W. R. 2000 Dangerous liaisons. *Proc. Natl Acad. Sci. USA* **97**, 12 953–12 955.

Simmons, L. W. 2001 The evolution of polyandry: an examination of the genetic compatibility and good-sperm hypotheses. *J. Evol. Biol.* **14**, 585–594.

Simmons, L. W. & Kotiaho, J. S. 2002 Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits. *Evolution* **56**, 1622–1631.

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.

Tregenza, T. & Wedell, N. 2000 Genetic compatibility, mate choice and patterns of parentage. *Mol. Ecol.* **9**, 1013–1027.

Tregenza, T. & Wedell, N. 2002 Polyandrous females avoid costs of inbreeding. *Nature* **415**, 71–73.

Tregenza, T., Wedell, N., Hosken, D. J. & Ward, P. I. 2003 Maternal effects on offspring depend on female mating patterns and offspring environment in yellow dung flies. *Evolution* **57**, 297–304.

Ward, P. I. 2000 Cryptic female choice in the yellow dung fly *Scathophaga stercoraria*. *Evolution* **54**, 1680–1686.

Ward, P. I. & Simmons, L. W. 1991 Copula duration and testes size in the yellow dung fly, *Scathophaga stercoraria* L.: the effects of diet, body size, and mating history. *Behav. Ecol. Sociobiol.* **29**, 77–85.

Wilkinson, G. S. 1984 Reciprocal food sharing in the vampire bat. *Nature* **308**, 181–184.

Yasui, Y. 1997 A 'good-sperm' model can explain the evolution of costly multiple mating by females. *Am. Nat.* **149**, 573–584.

Zeh, J. A. & Zeh, D. W. 1996 The evolution of polyandry. I. Intragenomic conflict and genetic incompatibility. *Proc. R. Soc. Lond.* B **263**, 1711–1717.