

Experimental evidence for testis size evolution via sperm competition

David J. Hosken and Paul I. Ward
Zoological Museum, University
of Zürich, Winterthurerstr 190,
Zürich 8057, Switzerland. E-mail:
hosken@zoolmus.unizh.ch

Abstract

Sperm competition theory predicts increased spermatogenic investment with increased sperm competition risk when competition is numerical. There is ample correlational evidence for this relationship in a wide range of taxa. However, as with all correlations, this does not establish cause and effect. Nevertheless, there are no published experimental studies of the evolutionary influence of sperm competition on testis size. We report here on evolutionary responses of testis size to variation in sperm competition intensity in the yellow dung fly. Experimental flies were divided across two treatments, polyandrous or monogamous, with four replicates of each. There was a rapid evolutionary response in testis size resulting from selection via sperm competition, with larger testes found when sperm competition intensity was greatest. These results provide direct experimental evidence of evolutionary change consistent with macro-evolutionary patterns found across a wide range of taxa.

Keywords

Polyandry, Scathophaga, Scatophaga, sperm competition, testes.

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INTRODUCTION

Sperm competition (Parker 1970a) theory predicts that when competition is fundamentally analogous to a raffle, relative sperm number in competition will be the primary determinate of success (Parker 1970a, 1982, 1990a, b). Thus when sperm competition risk is high, theory suggests selection will favour increased investment in spermatogenesis. Correlational evidence indicates this is the case both within and across species (e.g. Harcourt *et al.* 1981; Kenagy & Trombulak 1986; Gage 1994; Hosken 1997; Stockley *et al.* 1997; Simmons *et al.* 1999a), and larger testes typically produce ejaculates containing more sperm (e.g. Amann 1970; Møller 1988, 1989; but see Pitnick 1996). However, as with all correlational studies, results are indicative rather than firmly establishing cause and effect. Nevertheless, direct selection on testis size can lead to rapid size divergence, as well as causing correlated responses in other characteristics such as male body size (e.g. Pitnick & Miller 2000), and the only study to date that has experimentally varied sperm competition intensity across generations found evolutionary responses in testis size, which decreased in response to the removal of sperm competition (Pitnick *et al.* unpublished).

The yellow dung fly, *Scathophaga stercoraria* (= *Scatophaga*), has become a model system for the study of sperm competition (e.g. Parker 1970b; Parker *et al.* 1990;

Simmons & Parker 1992; Ward 1993, 1998, 2000; reviewed in Hosken 1999). Typically, males that copulate second (or last) fertilize about 80% of the subsequent clutch, at least under common laboratory conditions (Parker 1970b). In addition, male fertilization success is often dependent on copula duration, and the number of sperm stored by females is not thought to increase with successive copulations (Parker 1970b; Parker *et al.* 1990). Thus it appears that the last males to copulate displace approximately 80% of the sperm stored in the female storage organs, the spermathecae, with sperm mixing also occurring. Therefore, if ejaculate size is an important component of success in these flies, testis size should also respond to selective variation in sperm competition risk. Here we test this prediction with an experimental study of the evolutionary effects of the sperm competition on spermatogenic investment in yellow dung flies.

MATERIALS AND METHODS

Thirty-five female flies were collected at Fehraltorf, Switzerland in Autumn 1998 and brought to the laboratory. They were allowed to lay eggs and the flies that emerged from these were used as the parental generation of the experimental flies. Experimental flies were divided into two treatments, polyandrous (P) or monogamous (M), with four replicates of each (i.e. eight

lines). In the P treatment, females copulated with three males in succession before laying a clutch (i.e. they were placed with one male, then upon completion of copula, with the next and so on), while in the M treatment females copulated only once. Since male yellow dung flies can copulate successfully more than five times in succession and copulate on average with four females per day in the field (Ward & Simmons 1991), our protocol is unlikely to be influenced by sperm depletion. Brother/sister matings were avoided, all copulations were observed to ensure they were of normal duration, and at least 12 females were reproduced per generation, except once when one polyandrous line had only eight families. After copulations, females were left alone to lay, then a sample of eggs were transferred to plastic bottles containing over-abundant dung (> 2 g/larva; Amano 1983). These were subsequently housed at constant conditions and three females and four males per family were collected from the next generation. Flies were collected over approximately the first week of emergence, and those selected for subsequent copulations were a subset of these. Flies were fed with sugar, water and *Drosophila* for 3 weeks, and then mated (as described above). After 10 generations of selection, body size (hind tibia length-HTL) and testes size (area) were measured in samples of virgin male flies with a binocular microscope that conveyed images to a PC running OPTIMAS software. To measure testis area, testes were dissected in a ringer and placed on a microscope slide, orientated in approximately the same way, and images were transferred to the PC. Testis outlines were traced on the PC screen, and their area automatically calculated by OPTIMAS. We used the mean area of the two testes in the analysis presented here (note: using total area gives identical results).

RESULTS

Linear regression on a subsample of HTLs and testes measured twice indicated these measures were highly repeatable ($F_{1,20} = 18256.8$ and $F_{1,24} = 551.6$; $r^2 = 0.99$ and 0.96 , respectively; both $P < 0.0001$). ANOVA, with selection (M or P) as a factor and HTL as the dependent variable, indicated there were no differences in male body size due to our treatments (mean $m = 3.30$ mm, mean $p = 3.32$ mm; $F_{1,6} = 0.062$; $P = 0.82$). ANCOVA was used to investigate the effects of monogamy or polyandry on testis size, with selection (M or P) as a factor and male body size a covariate. Treatment and body size influenced testes size, with polyandrous lines having significantly larger testes than monogamous lines, but there was no interaction between body size and treatment (Table 1; Fig. 1). Comparison of testes allometry in the two treatments indicated the allometric slope in the M-lines (β of log

Table 1 ANCOVA results of evolutionary response in testis size in eight experimental lines as a result of variation in sperm competition intensity. The ANCOVA included selection (monogamy or polyandry) as a factor and body size as a covariate

Source	d.f.	MS	F	P
Selection	1	0.022	51.7	0.002
Male size	1	0.004	9.89	0.035
Selection \times male size	1	0.0004	0.91	0.39
Error	4	0.0004		

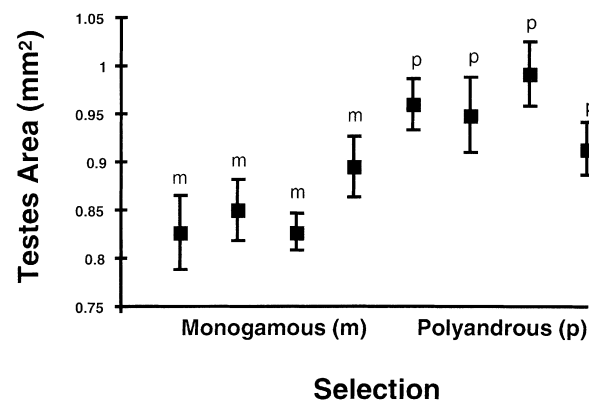


Figure 1 Mean (\pm SE) testes size (area) of the four lines from each treatment (polyandry or monogamy) after 10 generations of selection. Testis size was significantly greater in polyandrous flies. Body size also independently and positively influences testes size ($\beta = 0.43 \pm 0.19$).

transformed data = 0.84 ± 0.23) was significantly less than that in the P-lines (β of log transformed data = 0.96 ± 0.17) (d.f. = 59; $t = 7.19$; $P < 0.001$).

DISCUSSION

As predicted by sperm competition theory, our experiments clearly show an evolutionary response in testes size resulting from selection via sperm competition, with larger testes selected for when sperm competition intensity was greatest. Moreover, the responses were rapid, with testis size differing after only 10 generations. Responses this fast are typical of many morphological traits (Roff 1997; pp 153), and similarly, with direct selection on testes size, Pitnick & Miller (2000) were able to cause statistically significant divergence in testis size in another fly, *Drosophila hydei*, after only six generations. However, unlike them, we found no change in body size caused by our treatments, which indicates that males from P lines were investing relatively more in spermatogenesis than M males. Two other studies of *Drosophila melanogaster* also reported a reduction in male body size when sexual selection intensity was reduced (Promislow *et al.* 1998;

Pitnick *et al.* unpublished), although this may have solely been due to reduced precopulatory selection (Pitnick *et al.* unpublished). Nevertheless, our results are consistent with macro-evolutionary patterns that show that relative testis mass increases with sperm competition risk across a wide range of taxa (e.g. Gage 1994; Hosken 1997). The differences we observed appear to be largely due to larger males in the M lines investing less in testes, as the allometric slope in M lines is shallower than in P lines (i.e. the whole curve had not simply moved up or down). This is arguably expected, as some minimum testis size must be required to attain fertility, and similar increases in allometry were found after 10 generation of direct selection on eye-stalk scaling in diopsid flies (Wilkinson 1993). The responses we report are probably due to a combination of removal of sperm competition in the M lines, which relaxes selection for large testes, and an increase in the intensity of sperm competition in the P lines (selecting for larger testes) since females are very unlikely to copulate with three males per clutch in nature (e.g. Parker 1970b). One selective pressure that may have driven the evolution of smaller testes in the M lines was our collecting flies that emerged relatively early for subsequent copulations. It is possible selection for fast development (which both P and M lines experienced) plus removal of sperm competition selection led to smaller testes evolving in M line males. As noted above, we do not think sperm depletion selected for larger testes in P lines, as in the field males copulate with four females per day on average, which is greater than the number of copulation they had to perform in our P treatments (see also Møller & Briskie 1995). Finally, it would have been more satisfactory if females from both treatments received equal numbers of copulations per generation, but we had no way to sterilize males. Moreover, to us it seems that any differential selection on females via their mating patterns producing such a rapid correlated response in male spermatogenic characters is much less parsimonious than the straight-forward sperm competition argument presented.

Our results also confirm previous studies that indicated sperm number can largely influence sperm competition in yellow dung flies (e.g. Parker 1970b). Direct and indirect evidence indicate that male dung flies use their ejaculates to flush rival sperm from the female tract (e.g. Simmons *et al.* 1999b; Hosken & Ward 2000), and that numerical representation can determine paternity (e.g. Simmons & Parker 1992). Under these conditions, testes size evolution in response to variation in sperm competition intensity is expected, and was found. It is important to note, however, that females facilitate sperm displacement (Ward 1993; Hosken *et al.* 1999; Simmons *et al.* 1999b; Hosken & Ward 2000) and are able to influence paternity based on male PGM genotype (e.g. Ward 2000). Thus as with many other

taxa (e.g. Otronen & Siva-Jothy 1991; Rice 1996; see Eberhard 2000), males and females and their interactions determine reproductive outcomes in yellow dung flies.

In conclusion, our results provide clear evidence of a rapid evolutionary response in testis size resulting from selection via sperm competition, with larger testes selected for when sperm competition intensity was greatest, as predicted by sperm competition theory. In addition, they provide direct experimental evidence of evolutionary change consistent with macro-evolutionary patterns found across a wide range of taxa

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BIOSKETCH

David Hosken's main research interest is in sexual selection, particularly sperm competition, and sperm and genital morphology, although he is broadly interested in evolutionary biology. He is also interested in the influence of stress on development and genetic variability, and mechanistic aspects of sperm storage, reproduction and metabolism.

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