

SHORTER SPERM CONFER HIGHER COMPETITIVE FERTILIZATION SUCCESS

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Spermatozoa exhibit taxonomically widespread patterns of divergent morphological evolution. However, the adaptive significance of variation in sperm morphology remains unclear. In this study we examine the role of natural variation in sperm length on fertilization success in the dung beetle *Onthophagus taurus*. We conducted sperm competition trials between males that differed in the length of their sperm and determined the paternity of resulting offspring using amplified fragment length polymorphism (AFLP) markers. We also quantified variation in the size and shape of the female's sperm storage organ to determine whether female morphology influenced the competitiveness of different sperm morphologies. We found that fertilization success was biased toward males with relatively shorter sperm, but that selection on sperm length was dependent on female tract morphology; selection was directional for reduced sperm length across most of the spermathecal size range, but stabilizing in females with the smallest spermathecae. Our data provide empirical support for the theory that sperm competition should favor the evolution of numerous tiny sperm. Moreover, because sperm length is both heritable and genetically correlated with condition, our results are consistent with a process by which females can accrue genetic benefits for their offspring from the incitement of sperm competition and/or cryptic female choice, as proposed by the "sexy sperm" and "good sperm" models for the evolution of polyandry.

KEY WORDS: Evolution of polyandry, fertilization success, *Onthophagus taurus*, sperm competition, sperm length, sperm morphology, sperm-female coevolution.

Sperm competition, the competition between the ejaculates of several males for the fertilization of a female's ova (Parker 1970), is recognized as a major evolutionary force generating selection on male reproductive biology (Birkhead and Møller 1998; Simmons 2001). Theoretical models have generated a number of predictions regarding the evolution of ejaculate features in response to sperm competition (Parker 1990a,b, 1993, 1998; Parker et al. 1997; Ball and Parker 1998), and empirical data have generally supported these predictions, in particular those dealing with the evolution of sperm numbers (Parker et al. 1997; Birkhead and Møller 1998; Simmons 2001; Wedell et al. 2002). However, whether sperm morphology is affected by selection imposed by sperm competition is a subject of considerable debate (Simmons 2001; Hosken 2003; Snook 2005).

Sperm show widespread variation in their morphology. For example, across mammalian species sperm can vary more than two thousand fold in length (Gage and Freckleton 2003 and references therein). Within the genus *Drosophila*, the sperm of *D. bifurca* at 5.8 cm (Pitnick et al. 1995), are more than 250 times longer than those of *D. suboscuro* (Snook 1997). Within-species variation in sperm length is also widespread (Ward and Hauschteck-Jungen 1993; Ward 1998; Simmons et al. 1999, 2003; Morrow and Gage 2001; Snook and Markow 2001). Yet one of the fundamental predictions from sperm competition theory is that competition between the ejaculates of different males should favor the evolution of numerous and tiny sperm (Parker 1982). Positive and negative effects of selection on sperm size are theoretically possible, depending on the underlying assumptions of the models (Parker

1993, 1998; Ball and Parker 1996). For instance, longer sperm could be selected if the competitive benefits of sperm size increase with the number of sperm in competition, or if sperm size has an effect on survival, and sperm competition risk increases with female remating interval (Parker 1993). Collectively, it has been hypothesized that longer sperm might increase the competitive potential of an ejaculate or promote female sperm choice because longer sperm may, among other reasons, swim faster, live longer, be more effective in sperm displacement within the female reproductive tract, or indicate higher male quality (Snook 2005).

Comparative analyses of the influence of sperm competition on sperm length have yielded mixed results. Sperm length has been reported to be positively associated with levels of sperm competition in some taxa including butterflies and moths, frogs, fish, and primates (Gomendio and Roldan 1991; Gage 1994; Morrow and Gage 2000; Balshine et al. 2001; Byrne et al. 2003), but negatively associated with levels of sperm competition across fish generally (Stockley et al. 1997), and not associated to sperm competition risk across mammals (Hosken 1997; Gage and Freckleton 2003). These studies vary enormously in a number of ways, including the method used to assess levels of sperm competition, or whether suitable controls for phylogeny are made. Moreover, comparative and correlational studies are often influenced by uncontrolled variables, one of the most obvious being that characteristics of the fertilization arena or sperm storage organs can interact with sperm traits (Gomendio and Roldan 1993; Briskie et al. 1997; Pitnick et al. 1999; Presgraves et al. 1999; Morrow and Gage 2000; Miller and Pitnick 2002; Snook 2005).

Direct experimental manipulation of sperm competition offers a powerful means of determining whether selection is currently acting on sperm morphology. As for comparative studies, this empirical approach has yielded mixed results. Experimental removal of selection via sperm competition did not result in changes in sperm length in *Drosophila melanogaster* (Pitnick et al. 2001) or *Scathophaga stercoraria* (Hosken et al. 2001). Of the four studies that have examined the outcome of sperm competition between males known to differ in the length of their sperm, two found no role for sperm length in determining paternity (Simmons et al. 2003; Gage et al. 2004), one found a selective advantage for shorter sperm (Gage and Morrow 2003), and one an advantage for longer sperm, but only under special conditions dictated by female reproductive tract morphology (Miller and Pitnick 2002; Pattarini et al. 2006). Finally, in bulb mites (Radwan 1996) and nematodes (LaMunyon and Ward 1998, 1999), where sperm lack a flagellum, larger sperm have a higher competitive fertilization success. Thus, although sperm size is a male trait predicted to be under strong sexual selection (Hosken 2003; Snook 2005), the selective advantage to different sperm sizes remains largely unclear, and additional studies on the adaptive significance of variation in sperm morphology are greatly needed.

The dung beetle *Onthophagus taurus* is an ideal model system with which to explore the adaptive significance of variation in sperm length. Sperm competition is an important selection pressure in this species. Males exhibit dimorphic morphology: large major males develop head horns whereas small minor males lack horns. Importantly, dimorphism in body plan is associated with alternative mate securing tactics. Major males monopolize females whereas minor males sneak copulations with females guarded by major males (Emlen 1997; Hunt and Simmons 1998; Moczek and Emlen 2000). The frequency of minor males in Australian populations of *O. taurus* is high, with about 60% of males adopting the minor male morphology (Simmons et al. 1999). The risk of sperm competition for both major and minor males is therefore expected to be high, and phenotypic studies of ejaculate traits show that major and minor males have equal expenditure on the ejaculate (Simmons et al. 1999). As such, fertilization success is not associated with male morph (Tomkins and Simmons 2000; Simmons et al. 2004). Rather, the outcome of sperm competition conforms to a fair raffle (Tomkins and Simmons 2000; Simmons et al. 2004). Quantitative genetic analyses of ejaculate traits suggest that the production of short sperm is both heritable and covaries with heritable variation in male condition (Simmons and Kotiaho 2002). This later finding holds important implications for postcopulatory cryptic female choice (Eberhard 1996). If males with shorter sperm were better sperm competitors, then females could accrue indirect genetic benefits for their offspring from the incitement of sperm competition because of the collective inheritance of sperm competitiveness and condition (Keller and Reeve 1995; Yasui 1997).

In this study we prescreened F1 male *O. taurus* from parentals collected in nature, and characterized individuals on the basis of their sperm length. Subsequently, we sperm-competed males that differed in the length of their sperm, and determined paternity for the offspring of these males using amplified fragment length polymorphism (AFLPs) markers (Vos et al. 1995). We also examined the morphology of the sperm storage organ, the spermatheca, to determine whether females play a role in determining which sperm fertilize their eggs.

Material and Methods

BEETLES

Beetles were collected from fresh cattle dung from pastures near Perth (Western Australia), and maintained for one week in a mixed sex culture with a constant access to fresh dung. We placed 250 females into individual breeding chambers (PVC piping, 25 cm in length and 6 cm in diameter, three-quarters filled with moist sand topped with approx. 200 mL of dung). Chambers were sieved after eight days to collect brood masses. A brood mass provides the resources for the development to adulthood of a single offspring. Brood masses were buried en masse in moist sand in 6-l containers

and incubated at 28°C for 21 days. On emergence, adult beetles were placed in single sex populations and provided access to fresh dung for eight days prior to use in experiments.

SPERM LENGTH MEASUREMENTS

One hundred and nineteen haphazardly chosen virgin males were mated to virgin females. Matings took place under red light in artificial tunnels constructed from clear, rectangular plastic vials (6 cm × 3.6 cm × 1.3 cm). Vials were half filled with plaster-of-Paris to create a tunnel in which fresh dung was smeared. A pair of beetles was placed into each tunnel and matings were observed. After mating, males were kept in isolation in small plastic containers (7 cm × 7 cm × 5 cm) filled with moist sand and fed dung ad libitum, and females were dissected to collect the spermatophore from the bursa copulatrix. The spermatophore was placed on a slide and ruptured in 20 μL of saline. Sperm was smeared across the slide and left to dry. The slide was rinsed with distilled water and air-dried. Using a Hitachi HV-C20E/K-S4 (Hitachi Ltd., Hong Kong, China) camera attached to a Leica DMLS microscope (Leica Microsystems GmbH, Wetzlar, Germany), we captured the images of five haphazardly chosen sperm from each male, subject to the criteria that these sperm showed no signs of damage. Sperm were measured using the Optimas Image Analysis package (Media Cybernetics, Silver Spring, MD, USA).

Eight days after their initial copulation, males were weighed to an accuracy of 0.01 mg and the width of their pronotum measured with digital calipers to an accuracy of 0.01 mm.

SPERM COMPETITION EXPERIMENT

For sperm competition trials we selected pairs of males at opposite extremes of the distribution of sperm lengths for the 119 males that were measured. To check whether sperm lengths changed across successive ejaculates, we examined variation in sperm length in a sample of 10 nonexperimental males. Each male was mated to a virgin female, allowed to replenish its sperm reserves for 38.24 ± 3.44 h (mean ± SE), before being mated to a second virgin female. Sperm length measurements were thus obtained for both first and second ejaculates (mean number of intact sperm measured per ejaculate = 10 ± 1.1). A male's mean sperm length did not differ between his two ejaculates (matched pairs *t*-test, $t_9 = 0.78$, $P = 0.456$), so that a male characterized as having long or short sperm should have produced sperm of similar length in his experimental mating.

A total of 43 virgin females were each mated to a short-spermed (S) and a long-spermed (L) male. Mating order was alternated: in 23 cases the order was S–L, and in 20 the order was L–S. Matings were conducted as described above. After their first copulation, females were housed in small plastic boxes (7 cm × 7 cm × 5 cm) filled with moist sand and fresh dung until the following day, when the second male was introduced

to the female for her second copulation. The interval between copulations was therefore held constant (mean ± SE = 22.01 ± 0.27 h; range 20–26.5 h, $n = 43$). Immediately after copulation, males were frozen at –20°C for DNA extraction. Doubly mated females were placed in individual breeding chambers and left to construct brood masses for eight days. After this period, the brood masses of each female were incubated at 28°C for 21 days. On emergence, adult beetles were placed individually in 1.5-mL Eppendorf vials and frozen for DNA extraction. Doubly mated females were weighed to an accuracy of 0.01 mg, and the width of their pronotum measured with digital calipers to an accuracy of 0.01 mm, before being dissected to remove the spermatheca. Following dissection females were also frozen.

ANALYSIS OF SPERMATHECAL SIZE AND SHAPE

Images from the spermatheca of each female were captured with an Hitachi HV-C20E/K-S4 camera attached to a Leica MZ6 stereomicroscope and the Optimas Image Analysis package. To examine variation in spermathecal shape we analyzed the spatial relationship between spermathecal landmarks using a geometric morphometric approach (see reviews by Adams et al. 2004; Zelditch et al. 2004). Landmark-based geometric morphometric analysis preserves all information about shape differences among specimens while removing information unrelated to shape, such as position and orientation. Twelve landmarks describing the shape of the spermatheca were established and superimposed onto the image of each spermatheca (see Fig. 1) using tpsDig v2.04 (2005: F. James Rohlf, Department of Ecology and Evolution, SUNY at Stony Brook. Available at <http://life.bio.sunysb.edu/morph/>). The same program was used to digitize these images, following which a relative warp analysis was performed using tpsRelw v1.42 (2005: F. James Rohlf, Department of Ecology and Evolution, SUNY at Stony Brook. Available at <http://life.bio.sunysb.edu/morph/>). This software produces partial warps scores, which describe variation in spermathecal morphometry among females as deviations in shape from a consensus shape, and displays a visualization of variation in shape as deformations of the thin-plate spline (Adams et al. 2004; Zelditch et al. 2004). Relative warp analysis corresponds to a principal components analysis of variation in shape and provides the relative warp scores, which we used as factors in our models examining the effects of spermatheca shape on paternity (see below). As a measure of spermathecal size we used centroid size, the square root of the summed squared distances between each landmark and the centroid of the form, which, in the absence of allometry, is the sole size variable that is independent of shape (Zelditch et al. 2000). A more detailed explanation of geometric morphometrics can be found in Adams et al. (2004), Zelditch et al. (2004), and at <http://life.bio.sunysb.edu/morph/>. Geometric morphometric analysis of the spermatheca yielded 20 relative warps.

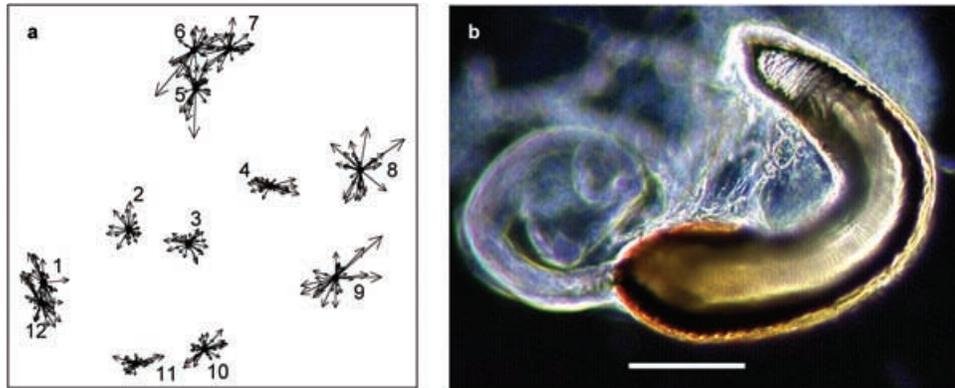


Figure 1. (a) The consensus spermatheca from geometric morphometric analysis is shown (black dots indicate the different landmarks along the spermathecal perimeter) along with spermathecal variation in shape (vectors with origin in the landmarks). Twelve landmarks were established in each spermatheca (note that landmarks 1 and 12 determine the beginning of the proximal section of the spermatheca, where sperm enters for storage and exits for fertilization). (b) Spermatheca of *O. taurus*. The scale bar represents 100 μm .

PATERNITY DETERMINATION

We used AFLP fingerprinting to determine paternity. The AFLP fingerprinting is a powerful multilocus DNA-fingerprinting technique based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (Vos et al. 1995). The utility of AFLP for parentage analysis has been demonstrated in insects (García-González et al. 2003, 2005), including *O. taurus* (Simmons et al. 2004). We used nonmaternal AFLP markers within offspring to assign paternity to each of the two males mated to a female, by excluding the nonsire. A diagnostic exclusion marker is defined by the situation in which, for a given locus, the male being screened as the potential sire and the mother both lack the allele, whereas the particular offspring has the allele (nonmaternal allele). The existence of these diagnostic fragments is used to exclude a male as the sire. In other words, for each offspring, nonmaternal markers must have been inherited from the true sire, and thus an absence of one or more of these markers in a given male is used to identify the male as a nonsire.

Genomic DNA was isolated from the six legs of adult beetles using the MasterPure DNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA), with the following modification: RNase treatment was performed post DNA precipitation, and re-suspension using Riboshredder (Epicentre) at 0.5 unit per 50 μL DNA sample incubated at 37°C for 1 h. The genomic DNA was analyzed by agarose gel electrophoresis (0.8% agarose gel in 0.5 \times TBE) to confirm integrity and estimate yield. We used the AFLP Core Reagent Kit and AFLP Pre-amp Primer Mix I (Invitrogen Life Technologies, Invitrogen Australia Pty Ltd, Mount Waverly, Victoria, Australia), but the reactions were performed with half the volume described in the protocol, using between 200 and 300 ng of sample DNA in the initial digestion. Fragment analysis was conducted on an ABI 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA), and multilocus profiles were visualized

using Genemapper v3.7 (Applied Biosystems). Further details, including information about the preselective and selective primers used and PCR details have been described elsewhere (Simmons et al. 2004).

To optimize the cost-effectiveness of AFLP analysis, a criterion of at least five offspring per family was set for paternity analysis: we thus excluded two families producing only four and three offspring, respectively, prior to DNA fingerprinting. The sample size for parentage analysis was further reduced because of DNA extraction failures and/or ambiguity of AFLP markers for one or more of the parental individuals (seven families), or for offspring within families for which we could score the parental individuals (see Results section for final sample sizes).

ANALYSIS

The proportion of offspring sired by the second male to mate with a female (P_2) was analyzed with generalized linear models (GLIM) with binomial error structure and logit link function (Wilson and Hardy 2002) in Genstat 8.2.0.158 (VSN International Ltd, Hemel Hempstead, UK). The number of offspring sired by the second male was fitted as the response variable, and the total number of offspring produced by each female as the binomial denominator. We included in the maximal model explanatory variables that could potentially explain paternity and all the second-order interactions between them: sperm length of the first male, sperm length of the second male, spermatheca centroid size, and the first three relative warps that explained 79.8% of the variance in the shape of the spermatheca (51.28, 19.55, and 8.96% respectively). We then dropped terms sequentially until the model included only terms whose elimination would decrease significantly the explanatory power of the model. We corrected for overdispersion with Williams' procedure (Williams 1982). The significance of each term was inferred from changes in the deviance after dropping

Table 1. Significance of each term in the minimal GLIM model explaining P_2 , and standardized effect sizes. SCA: spermatheca. Standardized effect sizes (Φ) estimated according to Rosenthal 1991.

	Change in deviance χ^2_1	Deviance explained (%)	P	Effect size (Φ)
Second male sperm length	3.90	10.7	.048	.34
SCA size	4.14	11.4	.042	.35
Third SCA relative warp	4.72	12.9	.030	.38
Second male sperm length is \times SCA size	4.27	11.7	.039	.36

the term from the model, which distributes as χ^2 with degrees of freedom equal to the difference in degrees of freedom between the models compared. Preliminary analysis of the influence of body size or body weight (for first males, second males, and females) on paternity yielded no effects of these factors or the interactions involving them. Body size was therefore excluded from the analysis. Means are given with \pm 1SE.

Results

Mean sperm length across the 119 males screened was 0.974 ± 0.002 mm (range 0.893–1.049). Variation in sperm length between males was considerably greater than within males ($F_{118,468} = 35$, $P \ll 0.0001$; repeatability (R) after Becker (1984) = 0.87).

A total of 655 adult offspring were obtained from 43 double-mated females (mean offspring per family = 15.35 ± 0.75 ; hatching success across the 43 double-mated females was high = $89.74 \pm 1.96\%$). Paternity analysis was possible for a total of 372 individuals distributed across 33 families (33 females, 33 short-spermed males, 33 long-spermed males, and 273 offspring; Mean number of offspring per family = 8.27 ± 0.53). Mating order was evenly distributed across these 33 families: in 18 families the order was S–L, and in 15 the order was L–S. Sperm length differed significantly between the two competing males in the 33 mating trials for which paternity was determined (matched pairs t -test, $t_{32} = -11.48$, $P \ll 0.0001$; mean sperm length (mm) for S males = 0.953 ± 0.002 , range 0.925–0.969; and for L males = 0.997 ± 0.003 , range 0.979–1.049).

The variables affecting the proportion of offspring sired by the second male were analyzed with a full generalized linear model that included 21 terms (six factors and 15 second-order interactions). The full model including these 21 terms explained 72.18% of deviance. The minimal model (the model including only significant factors and interactions) included four terms that accounted for 35.3% of deviance. The four terms that significantly explained the proportion of offspring sired by the second male were the second male's sperm length, the size of the spermatheca (centroid size), the third spermathecal relative warp, and the interaction between the second male sperm length and the size of the spermatheca (see Table 1). The shorter the sperm of the second male the higher the P_2 . This is graphically appreciated if we plot the

simple correlation between P_2 and the sperm length of the second male (see Fig. 2, $r = -0.378$, $P = 0.030$, $n = 33$). Figure 3 reveals the nature of the interaction between the second male's sperm length and the size of the spermatheca on the proportion of offspring he sired. The advantage of shorter sperm was greater in large spermathecae than in small spermathecae (see Fig. 3).

Finally, the relationship between P_2 and the third relative warp of the spermatheca is such that lower P_2 values were obtained with more positive values. Note however that the third relative warp explained only 9.00% of the variation in spermathecal shape, and that the differences in shape described by this relative warp were slight (see Fig. 4).

The distribution of P_2 values indicates a degree of bimodality. Values of P_2 between 0.4 and 0.6 are absent (see Fig. 2), such that paternity was generally biased towards one of the two competing males. We selected for our trials males that were at opposite extremes of the distribution of sperm lengths. If sperm length is a determinant of paternity, as the data in Figure 2 suggest, we should expect a degree of bimodality in the P_2 distribution due to our experimental protocol, because values of $P_2 \approx 0.5$ would be expected for pairs of males that were matched for sperm length.

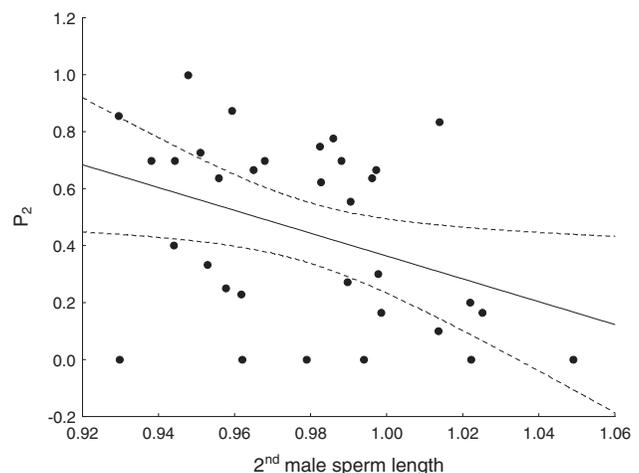


Figure 2. P_2 in relation to sperm length. The correlation between the proportion of offspring sired by the second male to mate a female (P_2) and his sperm length is negative and significant. The dotted line represents 95% confidence limits.

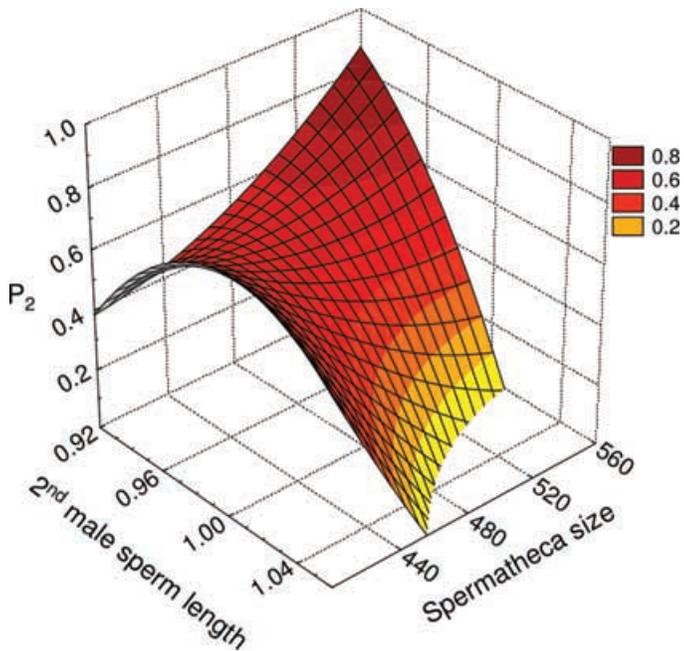


Figure 3. Sperm–female interaction on fertilization success. Three-dimensional surface plot using quadratic smoothing reveals the interaction between the sperm length (mm) of the second male to mate a female and the size of the spermatheca (centroid size) upon the proportion of offspring sired by the second male to mate a female (P_2). For most of the spermatheca size range the fitness function is directional toward shorter sperm lengths, whereas in the smallest spermathecae the fitness function is stabilizing.

The data in Figure 2 also show six cases of $P_2 = 0$ and one of $P_2 = 1$. Extreme P_2 values can result from nonsperm representation (García-González 2004). It is unlikely that nonsperm representation explains all the cases of complete sperm precedence in

our data because the beetles used in the sperm competition trials exhibited successful sperm transfer in their first matings (the matings carried out to categorize the males according to their sperm length). A further analysis of sperm transfer across two different matings for each of 10 males showed 100% success in sperm transfer. Nonetheless, the fact that a male is able to transfer sperm does not imply that the sperm has fertilization ability (reviewed in García-González 2004), and thus nonsperm representation might still be responsible for these extreme P_2 values. Of the seven cases with complete sperm precedence, short-spermed males sired all the offspring in five of them. This may suggest that these cases are just the result of variation in the competitiveness of sperm with different lengths such as our general results indicate. It is however difficult to interpret whether extreme P_2 values in our study are the result of genuine sperm competition or a random response because of the low number of cases with absence of mixed paternity, and the low numbers of offspring per family for these cases (7.85 ± 1.74). Analyses excluding extreme P_2 values yield qualitatively similar results, although P values for the factors included in our final model range from 0.044 to 0.103, probably due to the reduction in sample size. More importantly, the negative correlation between P_2 and second male sperm length shown in Figure 2 remains significant if we exclude extreme P_2 values ($r = -0.4$, $P = 0.042$, $n = 26$).

Discussion

Sperm morphology exhibits a pattern of rapid and divergent evolution, typical of traits subject to directional sexual selection (Simmons 2001). However, our knowledge of the selective pressures operating on sperm is limited at best. Here we have provided

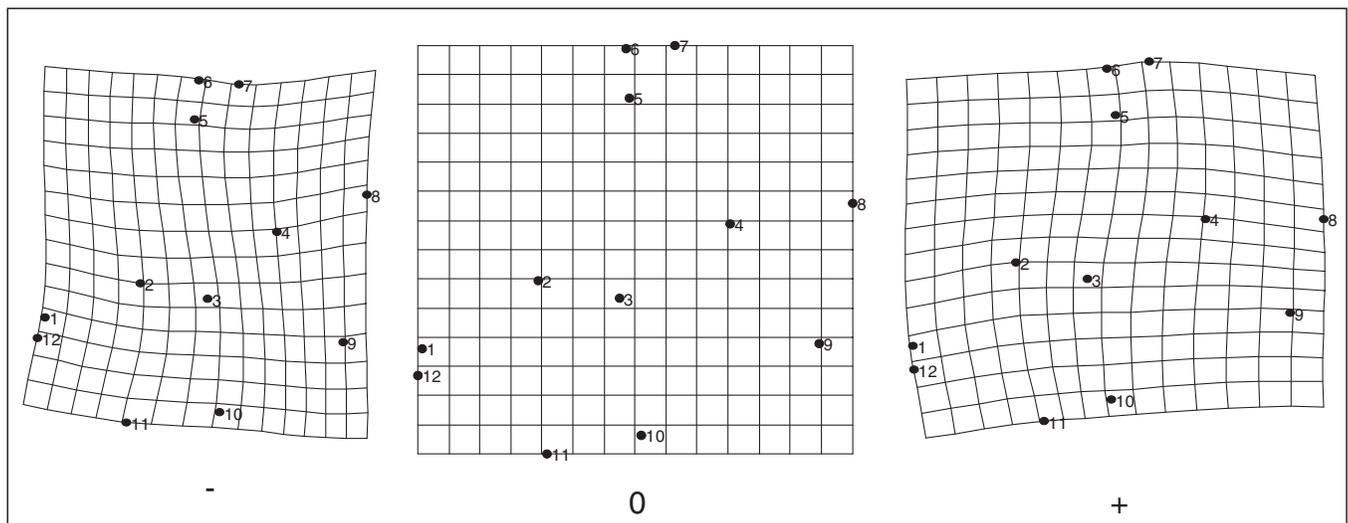


Figure 4. Changes in the shape of the spermatheca along the third relative warp. Negative values for the third relative warp are associated with higher P_2 values, whereas positive values for the third relative warp are associated with lower P_2 values (see text).

evidence from a species of beetle, *O. taurus*, that sperm competition selects for shorter sperm. Our finding is consistent with early theoretical models for the evolution of male gametes, which predicted that sperm competition should favor the evolution of numerous tiny sperm (Parker 1982).

Our study provides an important addition to what is currently a limited database on the adaptive significance of sperm morphology. Gage and Morrow (2003) obtained similar results to ours, in that male crickets, *Gryllus bimaculatus*, selected over five generations to have a short sperm, had a fertilization advantage when in competition with males artificially selected to have long sperm. No effect of natural variation in sperm length was found on competitive fertilization success in a related cricket, *Teleogryllus oceanicus* (Simmons et al. 2003), but in mites and nematodes that produce aflagellate sperm, larger sperm cells are associated with higher competitive fertilization success (Radwan 1996; LaMunyon and Ward 1998). For mammals, recent work with red deer, *Cervus elaphus*, has found that sperm swimming speed, a key determinant of male fertility in noncompetitive inseminations (Malo et al. 2005), is positively correlated with head and tail length but negatively correlated with mid-piece length (Malo et al. 2006), but in house mice, *Mus musculus*, males producing sperm with long mid-pieces and short tails are more likely to obtain fertilizations in mixed paternity litters than those producing sperm with short mid-pieces and long tails (R. Firman and L. W. Simmons, pers. comm.). These divergent patterns of selection on sperm morphology are likely to contribute to the widespread divergence in sperm morphology across species, and account for the lack of consensus in broader scale comparative analyses of the role of sperm competition in the evolution of sperm length (Gomendio and Roldan 1991; Gage 1994; Hosken 1997; Stockley et al. 1997; Morrow and Gage 2000; Balshine et al. 2001; Byrne et al. 2003; Gage and Freckleton 2003).

Our data highlight the importance of considering the environment within which sperm compete, which for internal fertilizers means the role that females play in influencing sperm function. We found that 9% of the variation in spermathecal shape (the third warp) explained an equivalent proportion of the variation in paternity to that explained by differences in sperm length (Table 1). We also found that the size of the female's spermatheca influenced the pattern of selection operating on sperm length. For most of the spermatheca size range the fitness function was directional toward shorter sperm lengths. However, in the smallest spermathecae the fitness function was stabilizing. These results warrant further research into the mechanisms by which spermathecal morphology interacts with sperm length to influence paternity in *O. taurus*. Miller and Pitnick (2002) likewise found an interaction between female sperm storage organ morphology and sperm length on male competitive fertilization success in their study of *D. melanogaster*. Males artificially selected for long sperm had a competitive advantage

over those selected for short sperm, but only when they competed within females artificially selected for long rather than short seminal receptacle lengths. Subsequently, Pattarini et al. (2006) have shown how sperm are distributed across two populations within the seminal receptacle, and that longer sperm are more likely to reside in the population closest to the seminal receptacle's exit. These data show that changes in the morphology of female sperm storage structures can drive the evolution of sperm morphology. Accordingly, covariation between the dimensions of the female reproductive tract and sperm has been reported from a number of taxa (Dybas and Dybas 1981; Briskie and Montgomerie 1992; Pitnick et al. 1999; Presgraves et al. 1999; Morrow and Gage 2000).

The mechanism(s) by which small sperm gain a competitive advantage in *O. taurus* are unclear. In Parker's (1982, 1998) theoretical models it is assumed that males face a trade-off between the number and size of gametes they can produce. Thus, if males producing short sperm also produce more sperm, our result could be explained by a simple lottery. A number of factors make such an explanation unlikely. First, with the exception of the giant sperm producing *Drosophila* (Pitnick 1996), there is generally little empirical evidence for a size number trade-off in sperm production. For example Gage and Morrow (2003) found no such trade-off in their study of *G. bimaculatus*, in which sperm size and number had statistically independent effects on competitive fertilization success. Second, although counting individual sperm has thus far proved impossible in *O. taurus*, quantitative genetic analyses of ejaculate production have revealed no or slightly positive genetic covariances between sperm length and testes size, and sperm length and ejaculate volume, rather than the negative covariances that might be predicted by a size-number trade-off (Simmons and Kotiaho 2002). Third, the interaction between spermathecal morphology and sperm length indicate a more active role for females in determining paternity.

Patterns of genetic variation in sperm length in *O. taurus* suggest that females can gain indirect genetic benefits for their offspring by biasing paternity toward males producing short sperm (Simmons and Kotiaho 2002). The sexy sperm hypothesis proposes that multiply mating females will produce sons who are more successful in sperm competition, because of heritable variation in the trait(s) that contribute to their fathers' sperm competitive success (Keller and Reeve 1995). The good sperm hypothesis goes further in proposing that the offspring of successful sperm competitors have higher viability (Yasui 1997). Sperm length has a very high level of heritability in *O. taurus* (1.14 ± 0.61 assuming autosomal inheritance, or 0.57 ± 0.31 assuming Y-linkage; Simmons and Kotiaho 2002). The sexy sperm hypothesis is more likely to work in species in which the traits that contribute to fertilization success exhibit Y-linked inheritance (Pizzari and Birkhead 2002), which seems likely for sperm length in *O. taurus* (Simmons and

Kotiaho 2002). There is also significant negative genetic covariance between male condition and sperm length ($r_g = -0.897 \pm 0.255$); males of greater mass than would be expected for their body size have shorter sperm (Simmons and Kotiaho 2002). These patterns of genetic (co)variance thus provide an avenue through which females can enhance the genetic quality of their offspring; females that mate with multiple males will have their offspring sired by those with shorter sperm who are of a higher genetic quality. In turn, changes in female sperm storage structures that bias paternity toward males with short sperm are likely to be favored in the same way that female preferences evolve via precopulatory processes of mate choice (Andersson 1994; Andersson and Simmons 2006). Directional postcopulatory selection on sperm length might be expected to reduce the levels of additive genetic variation in this trait, and indeed the coefficient of additive genetic variation in sperm length is low relative to other traits (Simmons and Kotiaho 2002). The fact that selection is stabilizing rather than directional in a subset of the female population could contribute to the maintenance of genetic variance in sperm length. Moreover, a recent survey of the literature on the quantitative genetics of sperm (Simmons and Moore 2007) shows that, as with condition generally (Rowe and Houle 1996), large numbers of genes regulate sperm form and function, presenting a large mutational target for the maintenance of genetic variation in these fitness-related traits (Houle 1998).

In conclusion, we have found that the production of shorter sperm allows *O. taurus* males to achieve a higher fertilization success. These findings provide direct experimental evidence that sperm morphology is subject to postcopulatory sexual selection. We have identified an interaction between female tract morphology and sperm length on fertilization success implicating selection by female sperm choice. Given the genetic association between male condition and sperm length in this beetle, our results support the view that polyandrous *O. taurus* females may accrue genetic benefits for their offspring via the incitement of sperm competition.

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