

# Sperm length is not influenced by haploid gene expression in the flies *Drosophila melanogaster* and *Scathophaga stercoraria*

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Recent theoretical models have postulated a role for haploid–diploid conflict and for kin selection favouring sperm cooperation and altruism in the diversification and specialization of sperm form. A critical assumption of these models—that haploid gene expression contributes to variation in sperm form—has never been demonstrated and remains contentious. By quantifying within-male variation in sperm length using crosses between males and females from populations that had been subjected to divergent experimental selection, we demonstrate that haploid gene expression does not contribute to variation in sperm length in both *Drosophila melanogaster* and *Scathophaga stercoraria*. This finding casts doubt on the importance of haploid–diploid conflict and kin selection as evolutionary influences of sperm phenotypes.

**Keywords:** haploid selection; sperm competition; sperm variation; Diptera; *Scathophaga stercoraria*

## 1. INTRODUCTION

Throughout the Metazoa sperm are evolutionarily labile and diverge rapidly, exhibiting a bewildering array of morphological specializations (reviewed by Pitnick *et al.* 2009a). Although understanding of the adaptive significance of variation in sperm form is meager, post-copulatory sexual selection, occurring through competition to fertilize ova, is widely regarded as the primary agent for sperm diversification (reviewed by Pitnick *et al.* 2009b; Pizzari & Parker 2009). Recent theoretical treatments of sperm adaptation, with a focus on sperm conjugation and heteromorphism, have postulated roles of kin selection and multi-level selection (Immler 2008; Pizzari & Foster 2008; Pizzari & Parker 2009). Sperm conjugation occurs when two or more sperm physically unite to enhance motility and/or transport through the female reproductive tract, with sperm typically disassociating from one another only after reaching the site of sperm storage or fertilization (reviewed by Immler 2008; Pitnick *et al.* 2009a). Sperm heteromorphism is the tightly regulated production by individual males of more than one morphological class of sperm, with the distinct sperm types specialized for performing different reproductive functions and only one type typically participating in fertilization (reviewed by Buckland-Nicks 1998; Swallow & Wilkinson 2002; Till-Boutraud *et al.* 2005). Applying social evolution theory, Immler (2008), Pizzari & Foster (2008) and Pizzari & Parker (2009) consider the role of haploid–diploid conflict, arising from the disparate fitness interests of males and of individual sperm they produce, and postulate adaptive variation in sperm form arising through selection for sperm altruism, cooperation and spite at the level of individual

spermatozoa (also see Parker & Begon 1993). These authors correctly point out that critical testing of these ideas includes demonstration that some among-sperm morphological variation is attributable to haploid gene expression.

The extent to which within-ejaculate variation in sperm form and function is a consequence of haploid gene expression, and the target of haploid selection, is an open question (Joseph & Kirkpatrick 2004). It is widely held that spermatozoa are terminally differentiated cells (i.e. transcriptionally inert, Hecht 1998). Nevertheless, it is now clear that post-meiotic gene expression occurs (Erickson 1990; Wang *et al.* 2001; Schultz *et al.* 2003; Dadoune *et al.* 2004; Namekawa *et al.* 2006). The precise timing of expression has not, however, been determined for most of the numerous genes required for spermatogenesis and for sperm function (but see Barreau *et al.* 2008). Such determination is complicated by the fact that, due to DNA condensation and repackaging during spermatogenesis, some genes required for spermatid function will be transcribed in primary spermatocytes, with the mRNAs stored in spermatids to be translated later during sperm elongation (reviewed by Schäfer *et al.* 1995; Hecht 1998; Dorus & Karr 2009). Consequently, intra-male variation in sperm phenotypes may predominantly be determined by testicular gene expression and hence the diploid genome of the male (Eddy 2002).

Even with post-meiotic gene expression, it is altogether unclear how much of the phenotypic variation among sperm within an ejaculate is attributable to allelic variation among haplotypes, and hence amenable to haploid selection (Joseph & Kirkpatrick 2004). A phenomenon of incomplete cytokinesis throughout spermatogenesis, resulting in all of the cells derived from each spermatogonial cell developing within a common syncytium, is taxonomically widespread, occurring even in diploblastic

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basal metazoan lineages (Gaino *et al.* 1984). Large inter-cellular bridges connect the cells and permit the sharing of cytoplasmic constituents. This arrangement has been experimentally demonstrated with mice to result in phenotypically diploid spermatids, despite haploid expression of genetic differences between developing sperm cells (Erickson 1973; Braun *et al.* 1989). To date, no characteristic of sperm form or function has been demonstrated to exhibit natural variation resulting from haploid gene expression.

The sperm attribute most widely investigated with regard to post-copulatory sexual selection is flagellum length (reviewed by Pitnick *et al.* 2009a; Pizzari & Parker 2009). Here we use crosses between discrete laboratory populations of both *Drosophila melanogaster* and *Scathophaga stercoraria* that had been subjected to divergent experimental selection for sperm length (i.e. long-sperm and short-sperm populations, Miller & Pitnick 2002; Dobler & Hosken in press) to definitively determine whether or not haploid gene expression contributes to sperm length in these two species. Specifically, given the segregation of alleles at multiple loci contributing to sperm length (Miller *et al.* 2003), the hypothesis of haploid gene expression generates the prediction that sperm length should be more variable within 'hybrid' among-line males than within males from either pure (long-sperm or short-sperm) population. On the other hand, if sperm length is strictly determined by each male's diploid genome, then no difference between hybrid and 'pure' crosses in within-male variation should be observed.

## 2. MATERIAL AND METHODS

### (a) *Experimental populations and culturing*

All flies were from experimentally evolved populations of *D. melanogaster* and *S. stercoraria* selected bi-directionally for sperm length, with one replicate pair of short-sperm and long-sperm populations for *D. melanogaster* and three replicates for *S. stercoraria*. Control sperm length selection lines were not used in the present investigation. Details of the source populations and selection protocols are provided in Miller & Pitnick (2002) and Dobler & Hosken (in press).

#### (i) *Drosophila melanogaster*

This investigation was conducted in the 40th generation following the inception of selection on sperm length. Although experimental selection for sperm length ceased following generation 17, as demonstrated by data presented herein, no appreciable regression of sperm length had occurred in the experimental lines. All flies, including sires and dams to be crossed and their experimental progeny, were reared at moderate density on standard cornmeal molasses agar medium at 25°C and a 12L:12D light cycle. Flies were collected as virgins following light ether anaesthesia and stored 10 flies per 8-dram vial with medium inoculated with live yeast until reaching experimental age. For each of the four crosses (♀:♂—long × long, long × short, short × long, short × short), 30 pairs were mated and eight sperm were measured from one son per pair. Results are thus based on measures of 960 sperm: eight sperm per male × 30 males per line cross × four line crosses.

#### (ii) *Scathophaga stercoraria*

This investigation was conducted in the sixth generation after the inception of selection on sperm length. Although experimental sperm selection only lasted for four generations, sperm length had significantly diverged between treatments and this difference remained at generations five and six. All flies were kept at low densities, with 20 eggs placed on approximately 75 ml of cow dung in each 100 ml plastic container. Emerged flies were housed singly with ad libitum food in 100 ml glasses until maturation. Rearing conditions were 20°C, 66 per cent relative humidity and a 12L:12D light cycle. Dams and sires originated from three independent replicates for both the short-sperm and long-sperm populations. Results are based on measures of 1155 sperm: 15 sperm per male × 77 males, with 15–22 males for each of the four line crosses.

### (b) *Sperm morphometry*

#### (i) *Drosophila melanogaster*

Sperm of each anaesthetized male were measured following dissection of the seminal vesicles into phosphate-buffered saline (PBS) on a subbed slide. After passively releasing a few hundred sperm into the saline, preparations were dried in a 60°C oven, fixed in methanol:acetic acid (3:1) and then mounted with glycerol:PBS (9:1) under a glass coverslip. Using digital images of sperm viewed under dark-field optics at a magnification of 200×, the total length of each sperm was measured using NIH IMAGE public domain software (<http://rsb.info.nih.gov/nih-image>).

#### (ii) *Scathophaga stercoraria*

Sperm of each male (killed by freezing at –20°C) were measured following thawing and dissection of the testis into distilled water on a microscope slide. To obtain only mature sperm, the testis was pierced at the proximate end (close to the ejaculatory duct). Released sperm were gently diluted in the droplet of distilled water and dispersed on the slide. After the slide was air-dried, sperm length was measured using microscope images (magnification 160×) conveyed to a PC running ZEISS KS300 software (Carl Zeiss AG, Switzerland).

### (c) *Statistical analyses*

Means, s.d. and coefficients of variation (CVs) in sperm length were calculated independently for each male based on  $n = 8$  and 15 sperm per male for *D. melanogaster* and *S. stercoraria*, respectively. Data were analysed with ANOVA and post hoc multiple range tests (Bonferroni Dunn and Tukey's, where appropriate) using a generalized linear measures approach using SAS (SAS Institute Inc.). All analyses compared values among four 'genotypes' (S × S, S × L, L × S, L × L). Analyses of sperm length variation additionally compared between two 'cross types' (within line: S × S and L × L versus among line: S × L and L × S). Because dams and sires of *S. stercoraria* originated from multiple replicate sperm-length selection lines, all analyses of this species were by mixed model ANOVAs with male nested within 'replicate' (the unique combination of dam and sire source populations) and replicate nested within genotype or cross type. For both species, statistically identical results were obtained from analyses of within-male standard deviations in sperm length as with CVs in sperm

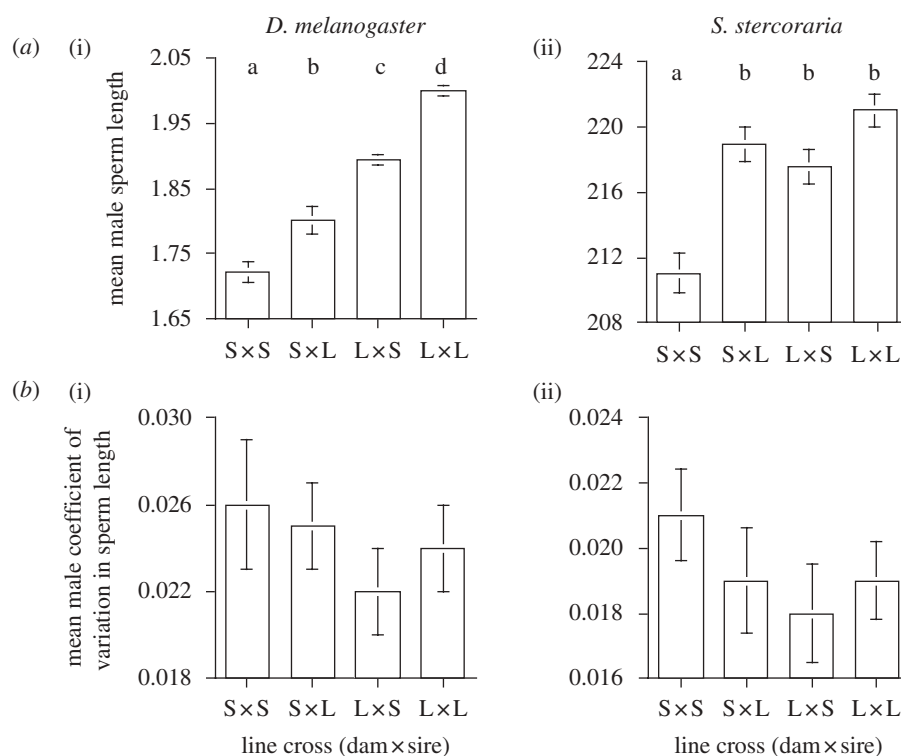


Figure 1. (a) Mean sperm length and (b) mean CVs in sperm length for males of (i) *D. melanogaster* and (ii) *S. stercoraria* from each of the four different genotypic crosses between dams and sires from experimental sperm length evolution lines (S, short-sperm line; L, long-sperm line). Error bars = 1 s.e. Cross means with the same letters (above bars) are not significantly different.

Table 1. Analysis of variance comparing mean male sperm length and mean male CV in sperm length among crosses using short- and long-sperm selection lines of *D. melanogaster* and *S. stercoraria*.

dependent variable	model component	MS (error)	F	d.f. <sup>a</sup>	p-value
<i>D. melanogaster</i>					
mean sperm length	genotype	0.43 (0.006)	73.19	3,116	<0.0001
CV sperm length	genotype	14.55 (15.64) <sup>b</sup>	0.93	3,116	0.429
	cross type	0.65 (15.74) <sup>b</sup>	0.04	1,118	0.839
<i>S. stercoraria</i>					
mean sperm length	genotype	272.55 (49.28)	5.53	3,12.23	<0.05
	replicate (genotype)	54.99 (17.01)	3.23	11,62	<0.005
CV sperm length	genotype	2.52 (3.54) <sup>b</sup>	0.71	3,13.94	0.561
	replicate (genotype)	3.70 (2.66) <sup>b</sup>	1.39	11,62	0.200
	cross type	2.96 (3.40) <sup>b</sup>	0.87	1,16.96	0.364
	replicate (cross type)	3.61 (2.63) <sup>b</sup>	1.37	12,63	0.203

<sup>a</sup>Degrees of freedom (model component, error term).

<sup>b</sup>MS and error are both  $\times 10^{-05}$ .

length; thus, only analyses of the latter are presented. Analyses of both species revealed no significant differences ( $p > 0.10$ ) between populations in body size (i.e. thorax length for *D. melanogaster* and left hind tibia length for *S. stercoraria*) and no significant main effects or interaction effects when entering body size as a covariate into models of sperm length. Hence, body size was not included in the final analyses presented.

### 3. RESULTS

As expected from previous analyses of the divergence in sperm length among the experimental evolution populations (Miller & Pitnick 2002; Dobler & Hosken in press),

ANOVAs examining variation in mean male sperm length revealed a significant effect of population genotype in both *D. melanogaster* and *S. stercoraria* (table 1; figure 1). In both species, males with hybrid cross genotypes had sperm that were intermediate in length relative to pure short- and long-sperm line males (figure 1). However, Bonferroni (Dunn) and Tukey's Studentized range tests both indicated that all four genotypes differed significantly in mean sperm length only in *D. melanogaster*. In *S. stercoraria*, only the S x S cross males differed in mean sperm length from all other crosses, with males from the S x L, L x S and L x L crosses not being statistically different from one another (figure 1).

Contrary to the haploid gene expression hypothesis, there were no statistically significant differences among either genotypes ( $S \times S$ ,  $S \times L$ ,  $L \times S$ ,  $L \times L$ ) or cross type (within line:  $S \times S$  and  $L \times L$  versus among line:  $S \times L$  and  $L \times S$ ) in CV in sperm length in either *D. melanogaster* or *S. stercoraria* (table 1; figure 1). For the analyses of cross type, the difference between mean CVs in *D. melanogaster* sperm length (within line–among line) was  $-0.00047$ , with effect size 95 per cent confidence limits overlapping zero but having a narrow range (95% CIs =  $-0.0050$  to  $0.0041$ ). For *S. stercoraria*, the same difference in CVs was  $0.0017$ , with 95 per cent confidence limits also overlapping zero and having a narrow range (95% CIs =  $-0.0007$  to  $0.0040$ ).

Whenever there is a failure to reject the null hypothesis (e.g. no haploid gene expression contribution to variation in sperm length), it is prudent to consider the statistical power of the tests and hence the probability of committing a type II error. Such consideration is frequently based upon *post hoc* power analyses and determination of detectable effect size. However, these approaches have been shown to be flawed (Hoenig & Heisey 2001; Colegrave & Ruxton 2003). In support of this study's failure to find a significant difference in sperm-length variation in our treatments, we point out that the difference in mean CVs in sperm length between within-line and among-line crosses was very small (2.12%) in *D. melanogaster* and in a direction opposite to the *a priori* prediction ( $-7.00\%$ ) in *S. stercoraria*. As a consequence, *p*-values were high (*D. melanogaster*: 0.8389; *S. stercoraria*: 0.3637). Further, confidence intervals (see above) were of only moderate breadth.

It is possible, however, that an insufficient number of sperm were measured per male (i.e.  $n = 8$  in *D. melanogaster* and  $n = 15$  in *S. stercoraria*) to accurately estimate variation in sperm length, a particularly important consideration given that within-male variation in sperm length tends to be low in nearly all taxa examined (reviewed by Morrow & Gage 2001; Pitnick *et al.* 2009a). To explore this possibility with *D. melanogaster*, we analysed data on within-male variation in total sperm length, for males from the long-sperm and short-sperm selection lines, collected two generations prior to the hybrid cross experiment and using identical protocol (Pattarini *et al.* 2006);  $n = 20$  sperm per male were measured for  $n = 15$  males per line. Of the resulting 600 sperm measures, six measures were statistical outliers (presumed to be broken sperm), causing five males to be excluded from the final analysis. For each male, sperm measures were randomly re-sampled ( $N = 10\,000$  runs per male) to determine mean estimates of the CVs in sperm length based on  $n = 8$  sperm. These estimates were found not to statistically differ from estimates based on  $n = 20$  sperm (CV  $\pm$  s.e.,  $n = 8$  sperm:  $1.964 \pm 0.142$ ;  $n = 20$  sperm:  $2.015 \pm 0.150$ ;  $t = -1.003$ ,  $p = 0.326$ ). This result suggests that measuring more sperm per male, at least up to 20 sperm, would not alter the conclusions of this study.

#### 4. DISCUSSION

Transcription of numerous genes, presumably encoding components of the mature spermatozoan, has been shown to occur in the spermatids of mammals (e.g.

Schultz *et al.* 2003). Although post-meiotic transcription may be less common in *Drosophila* (based on the relative lack of empirical evidence), there is convincing evidence for its occurrence (Barreau *et al.* 2008). Nevertheless, no variation in sperm form or function in any species has been demonstrated to result from haploid gene expression. On the other hand, there is no empirical evidence that haploid gene expression does not contribute to variation in sperm phenotypes, either within ejaculates or among males.

Sperm length is a trait of great interest as it varies dramatically across species (reviewed by Pitnick *et al.* 2009a), and comparative studies have demonstrated associations between sperm dimension and sperm function (e.g. Lüpold *et al.* 2009; Fitzpatrick *et al.* 2009). Although this relationship within species may be more complex (e.g. Birkhead *et al.* 2005), investigations of several taxa, including *D. melanogaster*, have shown a relationship between sperm size and competitive fertilization success (Radwan 1996; LaMunyon & Ward 1998; Miller & Pitnick 2002; Malo *et al.* 2006), although this is not always the case (Hosken *et al.* 2001). Nonetheless, the results of the present study suggest, at least for the two very distantly related species of flies examined, that sperm length is strictly a male genome-mediated trait, with no contribution from haploid gene expression. Consistent with this conclusion, analysis of segregation ratios of offspring of heterozygous males, derived from chromosome-extracted *D. melanogaster* lines with a range of sperm precedence phenotypes, found that sperm competition success depends on the diploid male genome and is not a property of the haploid sperm (Clark *et al.* 2000).

Moore & Moore (2002) postulated that morphological variation in the pronounced apical hook on the sperm head of mice, which appears to have diversified in response to post-copulatory sexual selection (Immler *et al.* 2007) and is critical to the formation of sperm trains (Moore *et al.* 2002), is a result of haploid gene expression. In support of this claim, some genes involved in the patterning of the hooked heads of mouse sperm have been shown to exhibit post-meiotic expression (Kim *et al.* 1989; Xu *et al.* 1999; Kleene 2001). However, it is not clear whether any variation among sperm in apical hook form or function is influenced by haploid allelic variation, and thus whether or not this trait may be subject to haploid selection (Keller 2002). Although haploid–diploid conflict and a role for kin selection and social selection in the diversification of sperm form as adaptations for evolutionary cooperation and altruism present interesting intellectual fodder (Immler 2008; Pizzari & Foster 2008; Pizzari & Parker 2009), we suggest caution until the influence of haploid gene expression on relevant variation in sperm form has been demonstrated.

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## REFERENCES

- Barreau, C., Benson, E., Gudmannsdottir, E., Newton, F. & White-Cooper, H. 2008 Post-meiotic transcription in *Drosophila* testes. *Development* **135**, 1897–1902. (doi:10.1242/dev.021949)
- Birkhead, T. R., Pellatt, E. J., Brekke, P., Yeates, R. & Castillo-Juarez, H. 2005 Genetic effects on sperm design in the zebra finch. *Nature* **434**, 383–387. (doi:10.1038/nature03374)
- Braun, R. E., Behringer, R. R., Peschon, J. J., Brinster, R. L. & Palmiter, D. 1989 Genetically haploid spermatids are phenotypically diploid. *Nature* **337**, 373–376. (doi:10.1038/337373a0)
- Buckland-Nicks, J. 1998 Prosobranch parasperm: sterile germ cells that promote paternity? *Micron* **29**, 267–280. (doi:10.1016/S0968-4328(97)00064-4)
- Clark, A. G., Dermitzakis, E. T. & Civetta, A. 2000 Non-transitivity of sperm precedence in *Drosophila*. *Evolution* **54**, 1030–1035.
- Colegrave, N. & Ruxton, G. D. 2003 Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. *Behav. Ecol.* **14**, 446–450. (doi:10.1093/beheco/14.3.446)
- Dadoune, J.-P., Siffroi, J.-P. & Alfonsi, F. 2004 Transcription in haploid male germ cells. *Int. Rev. Cytol.* **237**, 1–56. (doi:10.1016/S0074-7696(04)37001-4)
- Dobler, R. & Hosken, D. J. In press. Response to selection and realized heritability of sperm length in the yellow dung fly (*Scathophaga stercoraria*). *Heredity*. (doi:10.1038/hdy.2009.93)
- Dorus, S. & Karr, T. L. 2009 Sperm proteomics and genomics. In *Sperm biology, an evolutionary perspective* (eds T. R. Birkhead, D. J. Hosken & S. Pitnick), pp. 433–459. London, UK: Academic Press.
- Eddy, E. M. 2002 Male germ cell gene expression. *Rec. Prog. Horm. Res.* **57**, 103–128. (doi:10.1210/rp.57.1.103)
- Erickson, R. P. 1973 Haploid gene expression versus meiotic drive: the relevance of intercellular bridges during spermatogenesis. *Nature: New Biol.* **243**, 210–212.
- Erickson, R. P. 1990 Post-meiotic gene expression. *Trends Genet.* **6**, 264–269. (doi:10.1016/0168-9525(90)90209-O)
- Fitzpatrick, J., Montgomerie, R., Desjardins, J. K., Stiver, K. A., Kolm, N. & Balshine, S. 2009 Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proc. Natl Acad. Sci. USA* **106**, 1128–1132.
- Gaino, E., Burlando, B., Zunino, L., Pansini, M. & Buffa, P. 1984 Origin of male gametes in *Spongia officinalis* (Porifera, Demospongiae). *Int. J. Invert. Reprod.* **7**, 83–93.
- Hecht, N. B. 1998 Molecular mechanisms of male germ cell differentiation. *Bioessays* **20**, 555–561. (doi:10.1002/(SICI)1521-1878(199807)20:7<555::AID-BIES6>3.0.CO;2-J)
- Hoenig, J. M. & Heisey, D. M. 2001 The abuse of power: the pervasive fallacy of power calculations for data analysis. *Am. Stat.* **55**, 1–6.
- Hosken, D. J., Garner, T. W. J. & Ward, P. I. 2001 Sexual conflict selects for male and female characters. *Curr. Biol.* **11**, 489–493. (doi:10.1016/S0960-9822(01)00146-4)
- Immler, S. 2008 Sperm competition and sperm cooperation: the potential role of diploid and haploid expression. *Reproduction* **135**, 275–283. (doi:10.1530/REP-07-0482)
- Immler, S., Moore, H. D. M., Breed, W. G. & Birkhead, T. R. 2007 By hook or by crook? Morphometry, competition and cooperation in rodent sperm. *PLoS One* **2**, e170. (doi:10.1371/journal.pone.0000170)
- Joseph, S. B. & Kirkpatrick, M. 2004 Haploid selection in animals. *Trends Ecol. Evol.* **19**, 592–597. (doi:10.1016/j.tree.2004.08.004)
- Keller, L. 2002 Marsupial sperm pairing: a case of ‘sticky’ green beards? *Trends Ecol. Evol.* **17**, 113. (doi:10.1016/S0169-5347(01)02420-X)
- Kim, E., Waters, S. H., Hake, L. E. & Hecht, N. B. 1989 Identification and developmental expression of a smooth muscle gamma-actin in post-meiotic male germ cells of mice. *Mol. Cell. Biol.* **9**, 1875–1881.
- Kleene, K. C. 2001 A possible meiotic function of the peculiar patterns of gene expression in mammalian spermatogenic cells. *Mech. Dev.* **106**, 3–23. (doi:10.1016/S0925-4773(01)00413-0)
- LaMunyon, C. & Ward, S. 1998 Larger sperm outcompete smaller sperm in the nematode *C. elegans*. *Proc. R. Soc. Lond. B* **265**, 1997–2002. (doi:10.1098/rspb.1998.0531)
- Lüpold, S., Calhim, S., Immler, S. & Birkhead, T. R. 2009 Sperm morphology and sperm velocity in passerine birds. *Proc. R. Soc. B* **276**, 1175–1181. (doi:10.1098/rspb.2008.1645)
- Malo, A. F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A. J. & Roldan, E. R. S. 2006 Sperm design and sperm function. *Biol. Lett.* **2**, 246–249. (doi:10.1098/rsbl.2006.0449)
- Miller, G. T. & Pitnick, S. 2002 Sperm–female coevolution in *Drosophila*. *Science* **298**, 1230–1233. (doi:10.1126/science.1076968)
- Miller, G. T., Starmer, W. T. & Pitnick, S. 2003 Quantitative genetic analysis of among-population variation in sperm and female sperm-storage organ length in *Drosophila mojavensis*. *Genet. Res.* **81**, 213–220. (doi:10.1017/S0016672303006190)
- Moore, T. & Moore, H. D. 2002 Marsupial sperm pairing: a case of ‘sticky’ green beards? *Trends Ecol. Evol.* **17**, 112–113. (doi:10.1016/S0169-5347(01)02425-9)
- Moore, H., Dvořáková, K., Jenkins, N. & Breed, W. 2002 Exceptional sperm cooperation in the wood mouse. *Nature* **418**, 174–177. (doi:10.1038/nature00832)
- Morrow, E. H. & Gage, M. J. G. 2001 Consistent significant variation between individual males in spermatozoal morphology. *J. Zool. Lond.* **254**, 147–153. (doi:10.1017/S0952836901000656)
- Namekawa, S. H., Park, P. J., Zhang, L.-F., Shima, J. E., McCarrey, J. R., Griswold, M. D. & Lee, J. T. 2006 Post-meiotic sex chromatin in the male germline of mice. *Curr. Biol.* **16**, 660–667. (doi:10.1016/j.cub.2006.01.066)
- Parker, G. A. & Begon, M. E. 1993 Sperm competition games: sperm size and sperm number under gametic control. *Proc. R. Soc. Lond. B* **253**, 255–262. (doi:10.1098/rspb.1993.0111)
- Pattarini, J. M., Starmer, W. T., Bjork, A. & Pitnick, S. 2006 Mechanisms underlying the sperm quality advantage in *Drosophila melanogaster*. *Evolution* **60**, 2064–2080.
- Pitnick, S., Hosken, D. J. & Birkhead, T. R. 2009a Sperm morphological diversity. In *Sperm biology, an evolutionary perspective* (eds T. R. Birkhead, D. J. Hosken & S. Pitnick), pp. 69–149. London, UK: Academic Press.
- Pitnick, S., Wolfner, M. F. & Suarez, S. S. 2009b Ejaculate– and sperm–female interactions. In *Sperm biology, an evolutionary perspective* (eds T. R. Birkhead, D. J. Hosken & S. Pitnick), pp. 245–302. London, UK: Academic Press.
- Pizzari, T. & Foster, K. R. 2008 Sperm sociality: cooperation, altruism, and spite. *PLoS Biol.* **6**, e130, 925–931.
- Pizzari, T. & Parker, G. A. 2009 Sperm competition and sperm phenotype. In *Sperm biology, an evolutionary perspective* (eds T. R. Birkhead, D. J. Hosken & S. Pitnick), pp. 205–244. London, UK: Academic Press.
- Radwan, J. 1996 Intraspecific variation in sperm competition success in the bulb mite: a role of sperm size. *Proc. R. Soc. Lond. B* **263**, 855–859. (doi:10.1098/rspb.1996.0126)

- Schäfer, M., Nayernia, K., Engel, W. & Schäfer, U. 1995 Translational control in spermatogenesis. *Dev. Biol.* **172**, 344–352. (doi:10.1006/dbio.1995.8049)
- Schultz, N., Hamra, F. K. & Garbers, D. L. 2003 A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc. Natl Acad. Sci. USA* **100**, 12 201–12 206. (doi:10.1073/pnas.1635054100)
- Swallow, J. G. & Wilkinson, G. S. 2002 The long and short of sperm polymorphisms in insects. *Biol. Rev.* **77**, 153–182. (doi:10.1017/S1464793101005851)
- Till-Bouttraud, I., Joly, D., Lachaise, D. & Snook, R. R. 2005 Pollen and sperm heteromorphism: convergence across kingdoms? *J. Evol. Biol.* **18**, 1–18. (doi:10.1111/j.1420-9101.2004.00789.x)
- Wang, P. J., McCarrey, J. R., Yang, F. & Page, D. C. 2001 An abundance of X-linked genes expressed in spermatogenesis. *Nat. Genet.* **27**, 422–426. (doi:10.1038/86927)
- Xu, X., Toselli, P. A., Russell, L. D. & Seldin, D. C. 1999 Globozoospermia in mice lacking the casein kinase II  $\alpha'$  catalytic subunit. *Nat. Genet.* **23**, 118–121. (doi:10.1038/12729)