Short Review

Intraspecific variation in sperm size characters

PAUL I. WARD*

Zoologisches Museum der Universität Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland

Interspecific variation in quantitative characters of sperm is considerable and can sometimes be related to aspects of sperm competition. In contrast, continuous intraspecific variation is often ignored; for example, many studies concentrate on measuring a species-typical sperm size. However, statistically significant variation amongst males in a variety of sperm size characters, particularly total length and a number of head characters, has been reported in at least 13 species. Furthermore, sperm size has been shown to influence the outcomes of processes involved in sperm competition in two species, with larger sperm being more successful in both cases. It is suggested here that intraspecific variation may be a widespread phenomena, further examination of which could contribute considerably to our understanding of sperm evolution. Size differences between the sperm of competing males could be used in controlled matings to investigate the fates of sperm within female tracts and storage organs, but care should be taken that size characters may not be neutral markers. Furthermore, the genetic determination of sperm size is considerable ($h^2 = 0.56$–0.92 for total length and a number of head characters) and determinants are at least sometimes sex-linked, with the result that particular care must go into experimental design if genetic effects are to be fully elucidated. Studies combining genetical and behavioural effects of intraspecific variation in sperm morphology should be particularly rewarding.

Keywords: fertilization, heritability, heterospermic insemination, sex linkage, sperm competition, sperm storage.

Introduction

Sperm competition occurs whenever the sperm from different males compete within a female for access to her eggs, is widespread in animals and has an enormous influence on many mating systems (Parker, 1970; Smith, 1984; Birkhead & Møller, in press). (In the literature on breeding in mammals, particularly that of domestic and lab species, the term 'heterospermic insemination' is often used to describe the conditions under which sperm competition occurs (see Dziuk, 1996)). Interspecific variation in quantitative characters of sperm morphology is considerable, even between closely related species, and this variation has been associated with species differences in sperm competition risk (e.g. Gage, 1994; Pitnick & Markow, 1994; Briskie *et al.*, 1997), though not in all groups (Hosken, 1997). Although many mammalian sperm characters are strongly correlated with each other, the relationships are far from fixed and certain characters are uncorrelated, e.g. flagellum length and volume of the mitochondrial sheath (Gage 1998). Sperm morphology is thus extremely plastic evolutionarily, as might be expected of a male character intimately associated with mating and reproduction (Eberhard, 1985).

Given the interest in sperm competition and its consequences, it is surprising that few detailed studies on the extent, causes and consequences of intraspecific variation in sperm morphology have been conducted. This is especially so as it is becoming increasingly clear that there may be a close relationship between sperm morphology, especially total length, and features of the female tract or storage organ(s) (e.g. Pitnick & Markow, 1994; Briskie *et al.*, 1997). For example, Briskie *et al.* found that in birds the main causal relationship was between sperm length and the length of females' sperm storage tubules, with the positive relationship between sperm length and sperm competition risk being indirect. There are also a number of species where a male produces two size classes of sperm as in some *Drosophila* species (e.g. Snook, 1997), Lepidoptera (e.g. Sait *et al.*, 1998) and snails (e.g. *Viviparus ater*; Oppliger, Hosken & Ribi, pers. comm.). Males in some of these species alter the proportions of the different size classes of sperm in different ejaculates but this type of variation is not the subject of this review. The main purposes of this short review are to collate evidence on continuous intraspecific variation in sperm morphology within a size class of sperm and to suggest opportunities for further study.

Studies on intraspecific variation

In most studies, especially comparative ones, sperm are measured from a small number of males, typically fewer than 10 per species and the data combined to obtain species-specific sperm measures. However, a number of studies have identified statistically significant variation amongst males of a single species for a variety of quantitative characters of sperm (Table 1). Beatty (1970) is the only study I have found where formal test gave nonsignificant differences amongst individual

*Correspondence: E-mail: pward@zoolmus.unizh.ch; Fax:+41 1635 6826

males (in mice) for a variety of sperm morphological characters. However, he did report highly significant intraspecific effects between different strains. In addition to these studies, Radwan (1996) also examined differences in the surface area of ameboid sperm in the bulb mite *Rhizoglyphus robini* but did not present a formal test of the differences. However, his Fig. 1 strongly suggests the differences would be highly significant. Alvarez-Fuster *et al.* (1991) found significant intraspecific individual differences in sperm DNA content in seven out of eight *Tribolium* beetle species and a positive interspecific relationship between DNA content and spermatid area. Although there was no formal phylogenetic control for the latter result, these data suggest there would be individual differences in sperm size in at least some of these beetles. Roldan *et al.*, (1998) found a significant relationship between the degree of inbreeding and midpiece length amongst 11 *Gazella cuvieri* males. Again, their Fig. 1(b) suggests differences amongst males would be significant if formally tested. This collation suggests the phenomenon of significant individual differences in continuous sperm characters could be widespread amongst animals.

The sample sizes of the studies in Table 1 are often quite small. This suggests that much of the total variation in these characters is as yet undetected. One obvious consequence of this is that many of the species-specific values collected may not be very accurate. For example, my own data on the yellow dung fly *Scathophaga stercoraria* suggest that a sample of less than 10 males would be very unlikely to capture the population variation and could also give a misleading figure for the population mean sperm length. Figure 1 shows variation in the estimates from different-sized subsamples from a field sample of males (as in Otronen *et al.* 1997). The sperm measurements in Fig. 1 were assessed using a different method to those from the same population originally measured (Ward & Hauschteck-Jungen, 1993). The mean lengths are $\approx 5\ \mu m$ different, due to different shrinkages of the sperm using the different preparation techniques (see also Snook, 1997). At least in dung flies there is no correlation between sperm length and body size (Ward & Hauschteck-Jungen, 1993) but this relationship has not been examined in the other studies.

Differences between studies in sample size and measurement method will thus introduce additional error into comparative work, making it less likely that subtle effects can be identified. However, this may not be a serious problem as species differences are often much larger than the variation in effect size suggested by Fig. 1. However, this conclusion may have to be altered when the full range of variation in each species has been quantified. Nonetheless, comparative studies are helpful in revealing broad patterns in variation across groups while single-species studies must be used to understand the details of sperm evolution in particular species.

Consequences of intraspecific variation

There is at present almost no evidence that intraspecific sperm size variation has fitness consequences, but two studies do exist. Radwan (1996) has shown that there is a positive relationship between sperm size and fertilization success in the bulb mite *R. robini*. Otronen *et al.* (1997) have shown in the yellow dung fly that sperm total length influences the proportions of sperm from competing males which gain access to a female's sperm storage organs, the spermathecae. The latter study shows that variation amongst males in sperm characters can be used to track sperm from different males inside females. This could prove to be a very powerful technique for examining the ways sperm from different males are differentially treated within females between insemination

Table 1 Species where sperm characters have been shown to be statistically significantly different amongst (N) individuals

Species	Sperm character	N	Reference
Enchytraeus albidus and *E. bulbosus* (Oligochaeta)	Periodicity of the nuclear flange below the acrosome (a head character)	4 and 5	Westheide *et al.* (1991)
Viviparus ater (Mollusca)	Total length (eupyrene and apyrene sperm)	88	Oppliger *et al.* (pers. comm.)
Plodia interpunctella (Hexapoda)	Total length (apyrene sperm only)	41	Sait *et al.* (1998)
Drosophila melanogaster (Hexapoda)	Nucleus length	82	Beatty & Sidhu (1967)
Scathophaga stercoraria (Hexapoda)	Total length	32	Ward & Hauschteck-Jungen (1993)
Taeniopygia gutta (zebra finch)	Total length	10	Birkhead & Fletcher (1995)
Struthio camelus (ostrich)	Head, tail and total lengths	10	Soley & Roberts (1994)
Bos taurus (cattle)	Head area	29	Steinholt *et al.* (1994)
	Head length, head shape, midpiece length, acrosome cap width	13	Kant & Chakravarty (1993)
Capra hircus (goat)	Midpiece breadth, tail length	17 and 9	Joshi & Sidhu (1989)
Oryctolagus cuniculus (rabbit)	Head length	18	Napier (1961)
Canis familiarus (dog; various breeds)	Head area, length, width and degree of roundness	10	Dahlbom *et al.* (1997)
Equus caballus (horse; various breeds)	Head area and perimeter, total length	5	Ball & Mohammed (1995)
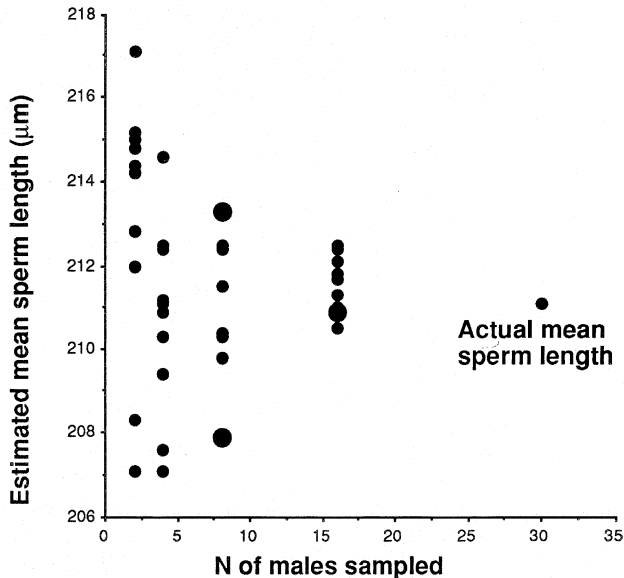
Fig. 1 The estimated mean sperm length from a population of 30 dung flies. The sets of males were randomly chosen from the population at each of the sample sizes. The larger circles indicate overlap of two data points.

and fertilization, which is of much current interest (e.g. Eberhard, 1996; Hosken & Stockley, 1998). A particular attraction of the method is that the sperm do not have to be manipulated experimentally and are thus more likely to reveal natural behaviour and its consequences. The drawback is that the sperm of competing males must first be examined before mating with a female. However, this should not be an insurmountable problem as sperm can either be obtained directly from males in many species or from females mated to the males before the experimental pairings. Care should also be taken to ensure that the effects of other ejaculate characters, such as sperm number, and male characters, such as body size or age, are controlled for in the experimental design and/or analyses. The dung fly (Otronen *et al.*, 1997) and bird data (Briskie *et al.*, 1997) suggest that sperm length may not be a neutral marker for sperm in storage, so care should be taken to examine other possible fates of sperm, such as mortality, expulsion or displacement from the female tract. Hellriegel & Ward (1998) show that such effects can markedly influence which inseminated sperm are later in the fertilization set, i.e. which may be later moved from storage sites and used to fertilize eggs (see also Parker *et al.*, 1990; Parker & Simmons, 1994).

There are also very few data on the relationship between intraspecific variation in sperm morphology and that in sperm motility. Motility is surely one of the most important characters determining which sperm actually reach an egg (e.g. Birkhead *et al.*, 1995). Interspecific comparisons again suggest looking for relationships between morphological differences and swimming performance would be worthwhile. Gomendio & Roldan (1991) suggested that longer mammalian sperm can swim faster, but this conclusion was based on

very few data and there was no correction for possible phylogenetic effects. Levitan (1993) also found interspecific differences in swimming velocity amongst three sea urchin species. Interestingly, Levitan also found significant individual differences with a total sample size of 15, again suggesting closer examination of the factors causing the variation would be worthwhile. Gee & Zimmer-Faust (1997) reported differences amongst five sea urchins of one species in the swimming speeds of their sperm. Furthermore, they also considered some experimental difficulties in making such measurements reliably. These difficulties could be even more serious for species where the sperm move in narrow ducts within a female. It will be a real challenge to estimate the relative importances of the interactions between male characters, such as a sperm's intrinsic motility and longevity, and female characters, such as the chemical milieu provided and muscle movements which may help or hinder a sperm's passage to an egg. However, it seems likely that major events leading to fertilization will be determined by male/female interactions rather than being solely determined by either male or female factors.

Genetic determination

The genetic determination of sperm size characters is also a relatively unexplored area. Beatty (1970) reviewed early work in this area and found heritability estimates were very high for a number of (mostly sperm head) characters in mice and rabbits (h^2 from 0.56 to 0.97; see Roff, 1997). Selection for midpiece size in mice was also successful (Woolley, 1968, as cited in Beatty). Joly *et al.* (1997) found that sperm length was a sex-linked trait in hybrids of *Drosophila simulans* and *D. sechellia*, with the Y chromosome having a large effect depending on the genetic background. I have also found sex linkage, in this case to the X chromosome, for determinants of total sperm length in the yellow dung fly, as well as a strong additive effect (Ward, unpublished). There is clearly a large, and sometimes complex, genetic element in the determination of sperm size in a variety of species. This is also consistent with the scattered reports that the environmental influence on sperm size variation is never very great (e.g. Beatty, 1970; Gage & Cook, 1994).

It is somewhat premature to speculate about the evolutionary mechanisms responsible for the maintenance of the observed variation in sperm characters (see Roff, 1997, for the range of mechanisms) but one attractive possibility is environmental heterogeneity in the sense of different females providing different environments for sperm of different sizes. As females do seem to vary genetically in their mate preferences for precopulatory characters (Bakker & Pomiankowski, 1995), it would be a simple extension to expect similar variation for postcopulatory choice.

It is now almost 30 years since Parker (1970) defined the field of sperm competition with his classic review. It seems paradoxical that we now have a vast amount of information on the consequences of sperm competition, especially for the evolution of animal mating systems, but so little on the biology of intraspecific variation in morphological sperm characters. Male fertilization success is highly variable and

this variation could be due, at least partly, to differences amongst males in their sperm morphologies. The time is ripe for the combination of the methods and ideas of behaviour, ecology and genetics to advance our understanding of the evolution of these very special cells.

Acknowledgements

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