Sperm midpiece length predicts sperm swimming velocity in house mice

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Evolutionary biologists have argued that there should be a positive relationship between sperm size and sperm velocity, and that these traits influence a male's sperm competitiveness. However, comparative analyses investigating the evolutionary associations between sperm competition risk and sperm morphology have reported inconsistent patterns of association, and *in vitro* sperm competition experiments have further confused the issue; in some species, males with longer sperm achieve more competitive fertilization, while in other species males with shorter sperm have greater sperm competitiveness. Few investigations have attempted to address this problem. Here, we investigated the relationship between sperm morphology and sperm velocity in house mice (*Mus domesticus*). We conducted *in vitro* sperm velocity assays on males from established selection lines, and found that sperm midpiece size was the only phenotypic predictor of sperm swimming velocity.

Keywords: sperm motility; sperm design; sperm competition; ejaculate quality

1. INTRODUCTION

When females mate with more than one male in a single reproductive event, sperm from rival males are forced to compete for fertilization (Parker 1998). Theory predicts that sperm competition will favour increased spermatogenic investment, and thus can be an influential force in the evolution of testes size and sperm number (Parker 1998). Consistent with theory, experimental evolution studies have shown that sperm competition selects for larger (Hosken & Ward 2001) and more efficient testes (Firman & Simmons *in press*). Comparative studies across taxa have used testes size (relative to body size) as a proxy for sperm competition risk, and investigated the evolutionary associations between sperm competition and sperm morphology. These studies have provided conflicting evidence for the role of sperm competition in the evolution of sperm size. Sperm competition has been shown to have no influence on sperm length (Gage & Freckleton 2003), and to select for increased sperm length (Gomendio & Roldan 1991; Briskie *et al.* 1997; Byrne *et al.* 2003). Thus, the influence of sperm competition on sperm morphology and function remains controversial (Humphries *et al.* 2008).

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For vertebrates, it has been suggested that increased sperm length results in greater energy reserves and faster swimming speeds, and thus may confer an advantage in sperm competition, either by reaching the site of fertilization quicker or by having better ova penetrating ability (Gomendio & Roldan 1991; Byrne *et al.* 2003; Fitzpatrick *et al.* 2009). In mammals, variation in overall sperm length is due primarily to differences in the length of the midpiece and flagellum (Cummins & Woodall 1985). The sperm midpiece contains a dense helical array of mitochondria that provides energy to propel the cell, the volume of which determines the flagellar beat frequency (Cardullo & Baltz 1991). Thus, the size of the sperm midpiece may be important in determining the outcome of sperm competition. Indeed, comparative studies among vertebrates have shown that sperm size correlates positively with sperm swimming speed (Fitzpatrick *et al.* 2009; Lüpold *et al.* 2009). Among primates, males of polygamous species have sperm with larger midpieces, and presumably higher densities of mitochondria, compared with monogamous species, suggesting that increases in midpiece volume may translate to greater swimming velocities and thus lead to an advantage in sperm competition (Anderson & Dixon 2002). This evolutionary association extends across a variety of mammalian taxa (Anderson *et al.* 2005). In contrast, a study of red deer (*Cervus elaphus*) revealed a negative association between sperm midpiece length and sperm swimming speed (Malo *et al.* 2006), highlighting the need for further within-species investigations of this relationship in other mammals. Here, we performed sperm velocity assays, obtained linear measurements of sperm and analysed the relationship between sperm morphology and sperm velocity in house mice populations subject to selection from sperm competition and populations subject to enforced monogamy.

2. MATERIAL AND METHODS

In 2005, wild-derived house mice ($n = 120$) were obtained from an animal resources centre (Murdoch, Western Australia, Australia) and subjected to laboratory evolution (Firman & Simmons *in press*). Eight selection lines were established and maintained under either a polygamous (P) or a monogamous (M) mating regime. Historical information on the source population used to establish the selection lines, the mating design and selection regime has been described previously (Firman & Simmons *in press*). Following five generations of selection, eight-week-old males ($n = 68$) were sacrificed via lethal injection and used for sperm analyses. Males from four M lines and three P lines were used in this experiment. Sperm from the caudal epididymides were incubated at 37°C for 90 min in a culture medium (Firman & Simmons 2008a). A detailed description of the medium and culture conditions is described elsewhere (Murase & Roldan 1996). Sperm velocity was quantified using the CEROS computer-assisted sperm analysis system (CASA) (v.10, Hamilton & Thorne Research) using standard mouse parameters (Nayernia *et al.* 2003). An approximately 10 µl aliquot of sperm suspension was loaded into a haemocytometer and five fields of view were scanned. The CASA gave measures of total sperm number and the percentage motile sperm, and provided velocity parameters including: average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), beat cross frequency, amplitude of head displacement, linearity and straightness. Samples were maintained at 37°C and CASA scans were repeated at set intervals over 5 h. These data were used to construct a 'percentage motile sperm' decay curve, the magnitude of which was used as a measure of sperm longevity.

Following the initial 90 min incubation period, a 50 µl aliquot of the incubated sperm suspension was fixed in a 4 per cent formaldehyde solution. Sperm smears were stained with Coomassie brilliant blue. Images of stained sperm enabled linear measurements

Table 1. Nested ANOVA of sperm traits for house mice, to test (a) which traits influence sperm swimming velocity, (b) whether male selection history influenced sperm number, and (c) the relationship between sperm velocity and sperm longevity. To account for non-independence of males, replicate selection line was nested within selection history (monogamous/polygamous). Significant *p*-values are given in *italic*.

term	mean square	d.f.	<i>F</i> ratio	prob > <i>F</i>
(a) <i>PC1</i>				
whole model	7.07	11, 53	1.88	<i>0.048</i>
sperm head length	4.03	1, 53	1.07	0.303
sperm head width	0.11	1, 53	0.03	0.353
sperm midpiece length	22.31	1, 53	5.92	<i>0.037</i>
sperm flagellum length	0.65	1, 53	0.17	0.451
total sperm length	0.62	1, 53	0.17	0.795
selection history	2.49	1, 5	0.66	<i>0.024</i>
line [selection history]	23.70	5, 53	1.26	0.780
(b) <i>sperm number</i>				
whole model	66.86	6, 58	3.18	<i>0.009</i>
selection history	34.37	1, 5	0.53	0.497
line [selection history]	66.73	5, 58	3.18	<i>0.013</i>
(c) <i>sperm longevity</i>				
whole model	18.63	7, 54	3.36	<i>0.044</i>
PC1	92.41	1, 54	16.65	<i>0.004</i>
selection history	0.16	1, 5	0.07	0.457
line [selection history]	1.91	5, 54	0.34	0.806

of sperm components, including *sperm flagella length* and *total sperm length* (400× magnification), and *sperm head length*, *sperm head width* and *sperm midpiece length* (1000× magnification) using the image analysis application IMAGEJ (v.132). Ten cells per individual were measured.

3. RESULTS

Preliminary ANOVAs showed that there was significantly more variation between individuals than within individuals in sperm number, sperm morphology measurements and sperm velocity traits ($p < 0.001$). As such, repeatability estimates were high ($r > 0.966$; Becker 1984), reflecting that we had sufficient replication to capture individual variation (table S1 in the electronic supplementary material). To summarize the variation among the highly correlated sperm velocity traits, we performed a principal component analysis, which produced a single principal component that accounted for 50 per cent of the total variation, and had a corresponding eigenvalue of 3.47 (table S2 in the electronic supplementary material). VAP, VSL and VCL contributed significantly to PC1 (hereafter referred to as composite sperm velocity) (Mardia *et al.* 1979) (table S2 in the electronic supplementary material). We performed statistical analyses to investigate the relationship between sperm morphology and composite sperm velocity. Our independent unit of replication was the number of lines for each selection treatment ($M = 4$, $P = 3$). Thus, we conducted nested ANOVAs with replicate line nested within selection history as a random factor. Of the five sperm components, sperm midpiece length was the only significant predictor (table 1); sperm with longer midpieces had faster swimming velocities (figure 1a). The selection history of the lines from which males were sampled also had a significant effect on sperm velocity; males from the polygamous lines had, on average, greater PC1 values ($0.73 \pm$

0.27) compared with males from the monogamous lines (-0.67 ± 0.25) (table 1). Males from the P lines had on average higher mean values compared with males from the M lines for many of the sperm performance traits (table S1 in the electronic supplementary material). However, there was no statistically significant divergence in the total number of sperm (table 1) or sperm morphology (table S3 in the electronic supplementary material). There was a significant negative relationship between composite sperm velocity and sperm longevity (table 1; figure 1b).

4. DISCUSSION

Analyses across mammal taxa have lead to the intuitive conclusion that sperm competition may select for larger, faster sperm (Gomendio & Roldan 1991; Anderson & Dixon 2002; Fitzpatrick *et al.* 2009). However, there have been few direct empirical investigations to support this hypothesis. Here, we provide conclusive data on the relationship between sperm form and sperm function, and show that sperm midpiece length predicts sperm swimming velocity in house mice.

A similar investigation on red deer sperm revealed the opposite relationship between sperm midpiece size and sperm swimming velocity. Malo *et al.* (2006) showed that sperm with elongated heads and shorter midpieces swam faster, and found no relationship between sperm velocity and flagellum or total sperm lengths. The inconsistencies between these two mammalian species may be due to variation in sperm head morphology, a factor that may also account for the absence of a general relationship between sperm competition risk and sperm length among mammals (Gage & Freckleton 2003). Red deer sperm have an ovoid head shape, while mouse sperm bear a hooked acrosomal cap that is characteristic of muroid rodents.

Figure 1. (a) The relationship between composite sperm velocity and sperm midpiece length ($y = 1.12x - 24.29$, $r^2 = 0.070$), and (b) sperm longevity ($y = -0.64x - 4.52$, $r^2 = 0.143$) in house mice. The black circles represent data from males of the monogamous selection lines, and the grey circles represent data from males of the polygamous selection lines.

Thus, hydrodynamic forces imposed on morphologically varied sperm would be markedly different, and different sperm components might be expected to contribute to sperm swimming performance (Humphries *et al.* 2008). Mouse sperm may require large amounts of energy to overcome the drag generated by the hooked sperm head, and to propel them through the viscous microenvironment. In house mice, sperm with larger midpieces may have greater mitochondrial loads and higher ATP content, which translates into the faster velocity as we have observed here.

Our results have important implications for the role of sperm competition in the evolution of sperm quality in rodents. We found that males with a polygamous selection history had significantly greater sperm velocities, but not longer sperm, compared with males with a monogamous selection history. This result implies that sperm velocity (independent of midpiece size) responds rapidly to sperm competition during the early stages of selection, and suggests that changes in sperm size may arise further along the evolutionary

pathway. Our sperm velocity data also revealed a trade-off between sperm longevity and sperm velocity. Successful fertilization is dependent upon sperm reaching the oviduct and maintaining their fertilizing ability while the ova are viable. In mice, it has been reported that as much as eight hours may lapse between copulation and fertilization (Rugh 1968). Therefore, it would be detrimental if sperm expended their ATP supplies and lost their fertilizing ability prior to ova maturation. Our own studies of house mice suggest that shorter sperm, which would have greater longevity, have a selective advantage when in competition for fertilization (Firman & Simmons 2008b). Thus, selection via sperm competition is likely to be stabilizing around an optimum morphology that maximizes sperm velocity and longevity.

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