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Mammalian sperm morphometry

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Understanding the adaptive significance of sperm form and function has been a challenge to biologists because sperm are highly specialized cells operating at a microscopic level in a complex environment. A fruitful course of investigation has been to use the comparative approach. This comparative study attempts to address some fundamental questions of the evolution of mammalian sperm morphometry. Data on sperm morphometry for 445 mammalian species were collated from published sources. I use contemporary phylogenetic analysis to control for the inherent non-independence of species and explore relationships between the morphometric dimensions of the three essential spermatozoal components: head, mid-piece and flagellum. Energy for flagellar action is metabolized by the mitochondrial-dense mid-piece and these combine to propel the sperm head, carrying the male haplotype, to the ovum. I therefore search for evolutionary associations between sperm morphometry and body mass, karyotype and the duration of oestrus. In contrast to previous findings, there is no inverse correlation between body weight and sperm length. Sperm mid-piece and flagellum lengths are positively associated with both head length and area, and the slopes of these relationships are discussed. Flagellum length is positively associated with mid-piece length but, in contrast to previous research and after phylogenetic control, I find no relationship between flagellum length and the volume of the mitochondrial sheath. Sperm head dimensions are not related to either genome mass or chromosome number, and there are no relationships between sperm morphometry and the duration of oestrus.

Keywords: gamete, comparative, spermatozoa, evolution, flagellum

1. INTRODUCTION

Despite the wide and detailed information on sperm structure (e.g. Bedford & Hoskins 1990), there has been less successful effort directed towards understanding the adaptive significance of the 'puzzling diversity of form' in sperm structure (Bedford & Hoskins 1990). This comparative study addresses some basic questions concerning the evolution of sperm morphometry in mammals. Detailed descriptions of sperm illustrate the specialized nature of spermatozoa and have advanced understanding in specific species (e.g. Bedford & Hoskins 1990), however a comparative approach is instructive in attempting to answer more general questions of spermatozoal evolution. Here, I use modern comparative analysis techniques (Felsenstein 1985; Harvey & Pagel 1991; Purvis & Rambaut 1994) to investigate evolutionary associations between and within mammalian sperm morphometry.

The first major study to examine general sperm size relationships was a pioneering analysis of mammals by Cummins and Woodall (Cummins 1983; Cummins & Woodall 1985). In addition, smaller comparative studies have investigated sperm morphometry and parameters such as body size, female tract dimensions, and sperm competition (insects: Gage 1994; Pitnick & Markow 1994; fish: Stockley *et al*. 1996, 1997; birds: Birkhead & Møller 1992; Briskie & Montgomerie 1992; mammals: Parker 1984; Gomendio & Roldan 1991, 1993; Harcourt 1991; Hosken 1997). Cummins & Woodall (1985) analysed a data set of 232 species (excluding non-classified species or data suspect in accuracy), and found a general negative relationship between sperm length and body mass across mammals (except Chiroptera where the relationship was positive but lost significance after phylogenetic control (Hosken 1997)). The negative relationship was interpreted to be due to a trade-off between sperm size and number (Cummins & Woodall 1985; Parker 1982, 1993), such that smaller sperm enable greater numbers to be produced in species where female tract size, and hence sperm dilution, is greater. However, Cummins & Woodall's study was performed prior to the advent of analysis techniques for rigorous phylogenetic control (Felsenstein 1985; Harvey & Pagel 1991), and their findings may be confounded by phylogenetic association because species were analysed as independent data points. Here, I use contemporary comparative analysis (Felsenstein 1985; Harvey & Pagel 1991) and control for the inherent non-independence of species to examine associations between sperm morphometry and body mass. Furthermore, I increase representation and include data on spermatozoal parameters from 445 mammal species. Since body mass is likely to scale with female reproductive tract size (Harcourt *et al*. 1981), I also determine specific relationships between body mass and mid-piece size and flagellum length.

I measure how dimensions of the three discrete components of sperm architecture (head, mid-piece and flagellum) relate to one another. The sperm head carries the haploid genotype in a uniquely condensed organization (Ward & Coffey 1991), but it is not known whether

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sperm head design is determined by the size of the species' haplotype. I therefore examine how sperm head dimensions are associated with two measures of genome size: genome mass and chromosome number (Olmo 1983). The head is capped by an acrosome containing proteolytic enzymes which interact with mechanical thrust from the flagellum to penetrate the cumulus oophorus and zona pellucida at fertilization (Green 1988; Katz *et al*. 1989). Head size may therefore dictate spermatozoal swimming characteristics. I examine how head size is associated with both mid-piece size and flagellum length, in the absence of confounding phylogenetic effects.

Sperm motility is essential for normal fertilization (Katz *et al*. 1989) which demands migration to the ovum (Green 1988). Motility is generated by a propulsive flagellum, energy for which is provided from metabolism of cyclic AMP by the mitochondrion-dense mid-piece (Bedford & Hoskins 1990). Catalysed ATP is shunted along the elongate flagellum and generates motility through myosin–actin interaction. Mammalian flagella appear consistently 9 + 9 + 2 in axonemal design (Bedford & Hoskins 1990). However, flagellar length, which influences motility characteristics (Katz & Drobnis 1990), varies more than ten-fold (22–245 μm). Longer flagella may enable greater velocities and thrusting forces to be achieved (Katz & Drobnis 1990; Gomendio & Roldan 1991), and a trade-off between this sperm activity and sperm longevity has been predicted such that longer sperm swim faster but survive for briefer periods (Gomendio & Roldan 1991; Stockley *et al*. 1997). If mid-piece size increases with diminishing returns with flagellum length (Cardullo & Baltz 1991), then the increased velocity and/or activity achieved by longer flagella may be traded against decreased sperm lifespan (Stockley *et al*. 1997). I therefore examine the relationship between flagellum length (the kinetic component) and both mid-piece length and mid-piece volume (the energy supply). I calculate mid-piece volume as that portion solely occupied by mitochondrial gyres, subtracting the volume occupied by the central axoneme which varies significantly in diameter due to the arrangement and size of the outer, dense fibres (Fawcett 1970). Cardullo & Baltz (1991) performed similar analyses but in the absence of phylogenetic control and showed a positive linear relationship between mitochondrial volume and flagellum length across 36 mammalian species. I control for phylogeny and calculate mid-piece volumes for 50 species.

Parker (1984) first demonstrated a positive relationship between sperm fertile lifespan and oestrus length across nine mammal species. Since then Gomendio & Roldan (1993) have shown a relationship between sperm fertile lifespan and the duration of oestrus to ovulation across 11 species, while controlling for phylogeny. More recent analyses show no relationship between sperm length and sperm longevity across bats (Hosken 1997). Gomendio & Roldan (1991, 1993) predict that sperm lifespan is inversely correlated with sperm length because longer flagella may demand greater metabolic energy which is used up more rapidly. Gomendio & Roldan (1993) found that smaller sperm remained fertile for longer periods than longer sperm, and that there was an inverse correlation between sperm length and the time from oestrus to ovulation. However, these relationships lost significance after phylogenetic control. I therefore examine how sperm design is associated with the duration of oestrus or sperm storage (Asdell 1964; Hayssen *et al*. 1993; Hosken 1997), examining associations between mid-piece length and volume, and flagellum length in relation to the temporal opportunity for fertilization, while controlling for phylogeny.

2. MATERIALS AND METHODS

Data on sperm dimensions, body mass, oestrous length, and karyotype were collated from published sources. Where more than one source existed, the character's mean was calculated. Where a range of species' values were presented the mode was taken. Sperm morphometry data included measurements of: total sperm length; head length, width and area; mid-piece length, diameter and volume; and flagellar length and diameter. There is variation in sperm head shape. However, for simplification I treated the sperm head as a flattened, ovoid disc (Bedford & Hoskins 1990), and determined mean head diameter (from length and width measures) to calculate head area. I treated the mid-piece as a cylinder (Cardullo & Baltz 1991) and calculated the volume occupied by mitochondrial gyres by subtracting the volume occupied by the central axoneme from the total mid-piece volume. I measured the diameter of the outer, dense fibre arrangement and the diamater of the mid-piece from published transmission electron micrographs of mid-piece sections and calculated volume accordingly. Sperm morphometry sources regarded as suspect in accuracy were excluded (see Cummins & Woodall 1985). I included data on a single species when only the generic name is reported (*Stenocephalemys* spp.), but discard studies where different unclassified species are documented from the same genus.

Body mass was derived from Nowak (1991), Strahan (1991) and Macdonald (1995), and mean species adult mass was calculated when male and female values were given separately. Genome mass was picograms of DNA per diploid nucleus and collated without priority by Olmo (1983) and Manfredi Romanini (1985). Chromosome numbers were derived from Altmann & Dittmer (1972) and Hsu & Benirschke (1967–1976). Oestrus length was the period of behavioural oestrus or 'heat'. It should be noted that oestrus lengths within species can be sensitive to a number of biotic and abiotic factors and, with the exception of some bats (Hosken 1997), I therefore collated data solely from Asdell's reviews (Asdell 1964; Hayssen *et al*. 1993). All data were $\log_{10}$ transformed prior to analysis (Harvey 1982).

Species comparisons may be meaningless because of phylogenetic non-independence (Felsenstein 1985; Harvey & Pagel 1991). To overcome this confounding effect, I analysed comparisons between common phylogenetic ancestors using comparative analysis by independent contrasts (CAIC, Purvis & Rambaut 1994). CAIC outputs statistically independent parameter values at independent evolutionary events (standardized linear contrasts), so that correlated evolution between continuous (or dichotomous) traits can be measured. Contrast trait values were examined for associations using regression forced through the origin to determine positive associations (Harvey & Pagel 1991).

The mammal phylogeny was combined from Novacek's (1992) higher level relationships, and Corbet & Hill's (1991) lower level classifications. I used two models of evolutionary change: (i) a speciational (or punctuational) model where all branch length segments are set equal to one (Harvey & Pagel 1991); and (ii) a gradualistic (Brownian motion) model with branch lengths set to

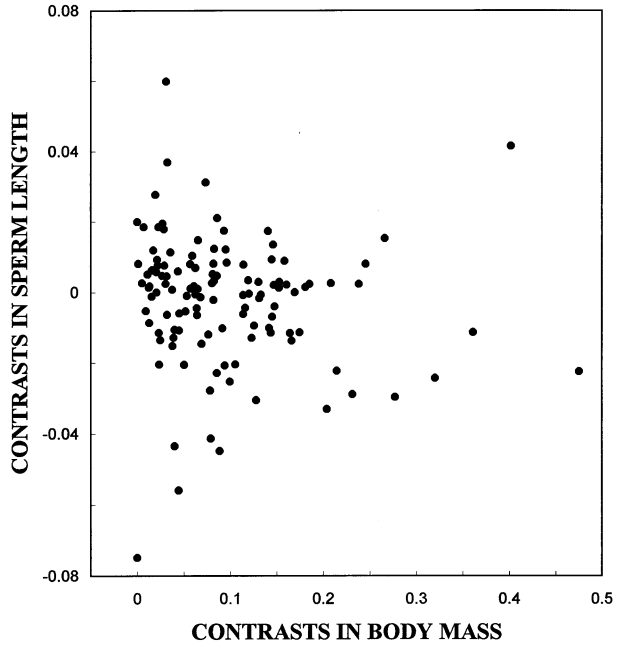

Figure 1. There is no relationship between sperm length and body mass across 300 species ($r^2=0.01$, $n=120$ contrasts).

estimated times since present (Felsenstein 1985). However, the results did not depend on the model of evolutionary change. In an attempt to maximize the phylogenetic information in the analysis I combined both speciational and gradualistic models of evolutionary change. I present the results from analyses where branch length information is provided for higher nodes, while total length is estimated for equal division between lower nodes. Higher level branching relationships and lengths were as Novacek's phylogeny (1992) and lower level relationships were derived from Corbet & Hill (1991). Branch length estimates were obtained from Novacek (1992), setting the root at 150 Ma BP. Where Novacek's (1992) branch length information was not detailed to lower level phylogenies (typically in the early Cenozoic), I assumed a gradualistic model of change, by dividing the lower lineages' total remaining branch length by the number of nodes to the present day. Thus, the lower level internodal segment value is relatively equal along each lineage, but the values relate to measured values from higher nodes. I included two subspecies divisions in the analysis and extend classification to one further level. Here, I split species to the present day segment and included an extra node.

To check for assumptions concerning the rate of evolutionary change in character values and that I had applied appropriate transformations (Purvis & Rambaut 1994), I regressed absolute values of contrasts on their estimated nodal values. I found that all regression slopes were not significantly different from zero.

3. RESULTS

All raw data and their references are lodged with the Royal Society as an electronic appendix on the web site at (`http://www.pubs.royalsoc.ac.uk/publish/pro_bs/jan98pb2.htm`) and are available directly from the author.

(a) *Body weight relations*

In contrast to previous findings, I found no relationship between total sperm length and body mass across 300 species for analyses with ($r^2=0.01$, $p=0.22$, $n=120$ contrasts, figure 1) and without ($r^2=0.002$, $p=0.31$, $n=120$) branch length information.

There were no relationships between body mass and either mid-piece length ($r^2=0.013$, $p=0.25$, $n=105$ (254 species)), mid-piece volume ($r^2=0.005$, n.s., $n=36$ (50 species)), or flagellar length ($r^2=0.005$, n.s., $n=101$ (239 species)).

(b) *Karyotype relations*

Chromosome number was not associated with either sperm head length ($r^2=0.009$, n.s., $n=58$ (106 species)) or head area ($r^2=0.003$, n.s., $n=22$ (45 species)). Genome mass was also not associated with either head length ($r^2=0.003$, n.s., $n=44$ (68 species)), or head area ($r^2=0.015$, n.s., $n=13$ contrasts (18 species)).

(c) *Spermatozoal component relations*

There was a significant relationship between mid-piece length and flagellar length ($r^2=0.195$, $p<0.0001$, $n=104$ (275 species); figure 2*a*).

Mid-piece volume was not associated with flagellar length ($r^2=0.03$, $p=0.3$, $n=33$ (46 species); figure 2*b*), head length ($r^2=0.003$, n.s., $n=34$ (48 species)), or head area ($r^2=0.072$, $p=0.267$, $n=18$ (22 species)).

There were significant relationships between sperm head length, and both mid-piece length ($r^2=0.05$, $p<0.017$, $n=113$ contrasts (303 species); figure 3*a*) and flagellar length ($r^2=0.34$, $p<0.0001$, $n=105$ (278 species); figure 3*b*).

There was a significant relationship between head area and flagellar length ($r^2=0.2$, $p<0.001$, $n=61$ (158 species); figure 4), but not between head area and mid-piece length ($r^2=0.029$, $p=0.17$, $n=66$ (176 species)).

(d) *Oestrus relations*

There was no association between oestrus length and either mid-piece length ($r^2=0.003$, $p=0.7$, $n=48$ (77 species)), mid-piece volume ($r^2=0.0001$, n.s., $n=16$ (21 species)), or flagellar length ($r^2=0.0001$, n.s., $n=48$ (75 species)). Oestrus length was also not associated with total sperm length ($r^2=0.001$, $p=0.82$, $n=51$ (84 species)).

4. DISCUSSION

Mammalian sperm are characteristically tiny, but vary over 12-fold in length from 28 μm in the porcupine *Hystrix africaeaustralis* to 349 μm in the honey possum *Tarsipes rostratus*. (Sperm size is in contrast to many invertebrate sperm where lengths are generally much greater, attaining 58.290 mm in *Drosophila bifurca* (Pitnick *et al*. 1995).) Across mammals, I found no evidence that this total sperm size variance was associated with body mass (figure 1), in contrast to earlier studies (Cummins 1983; Cummins & Woodall 1985). There were no associations between mid-piece sizes or flagellar length and body mass. Cummins & Woodall (1985) originally suggested that the inverse correlation they found between sperm size and body mass may have evolved as a response to female tract volume and sperm dilution. Evolution of smaller sperm size may enable greater sperm number to evolve, and in larger females with larger tracts, selection may have operated to overcome sperm dilution by evolving greater

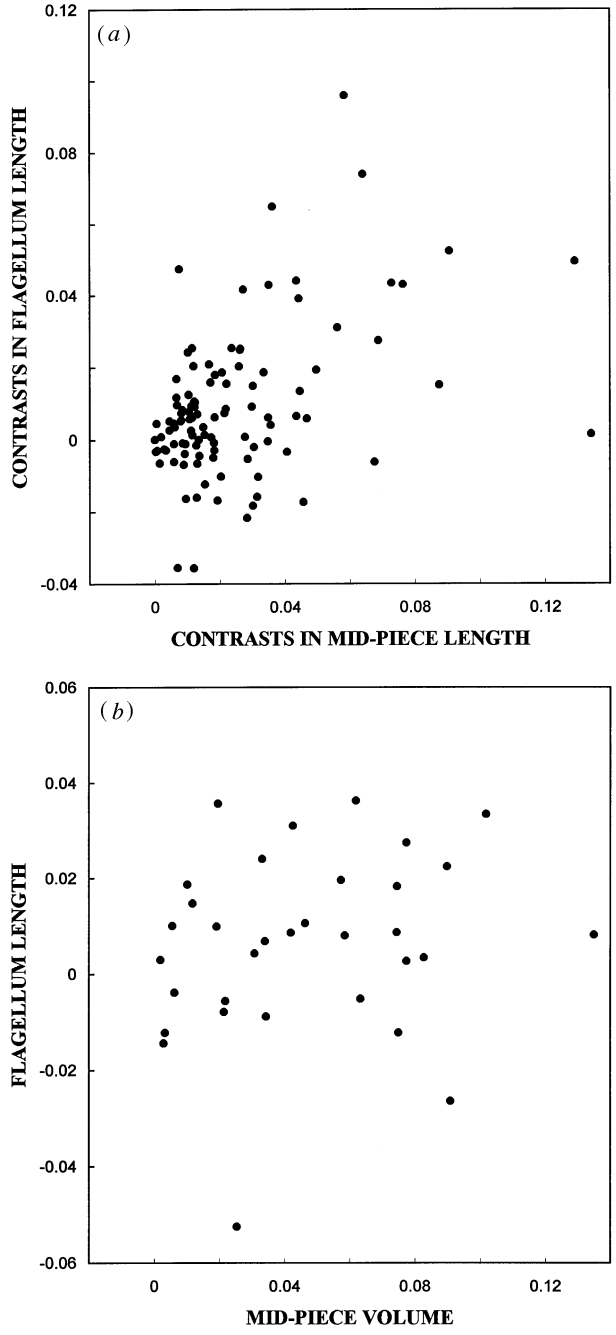

Figure 2. (*a*) Flagellum length is associated with mid-piece length across 275 species ($r^2=0.195$, $n=104$ contrasts). Slope exponent $=0.372\pm0.052$ s.e. (*b*) Flagellum length is not associated with mid-piece volume across 46 species ($r^2=0.03$, $n=33$).

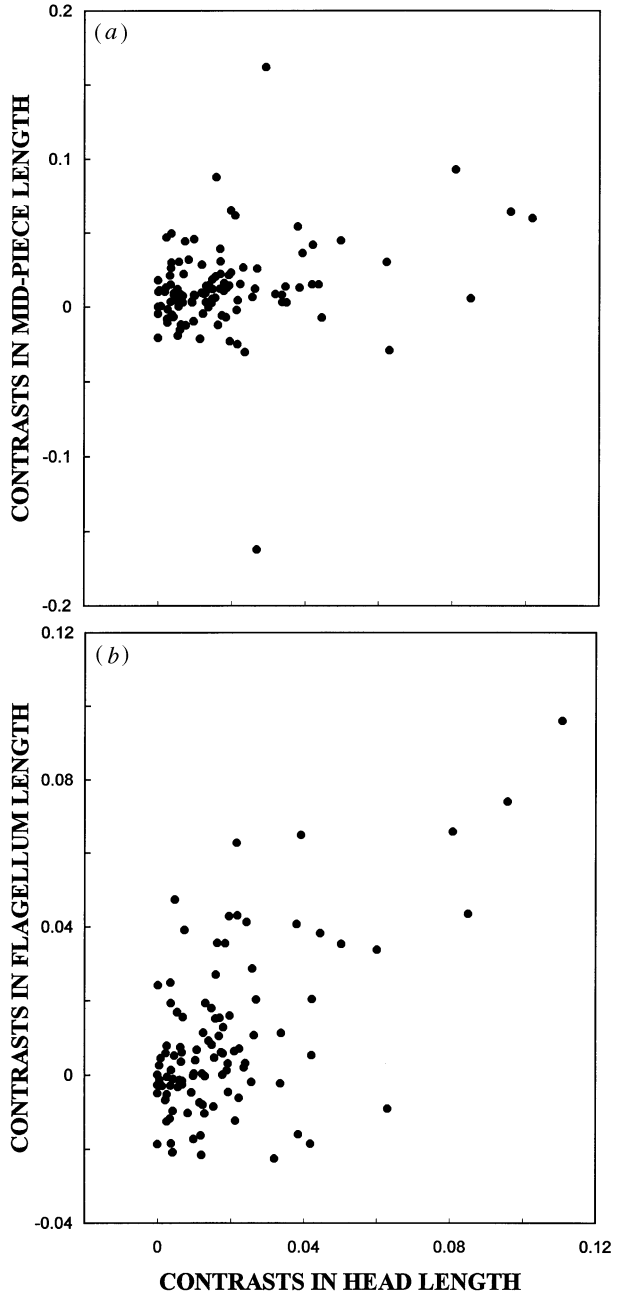

Figure 3. (*a*) Mid-piece length is associated with head length across 303 species ($r^2=0.05$, $n=113$). Slope exponent $=0.54\pm0.107$ s.e. (*b*) Flagellum length is positively associated with head length across 278 species ($r^2=0.34$, $n=105$). Slope exponent $=0.58\pm0.064$ s.e.

numbers of smaller sperm in larger species (Short 1981). Although these interpretations are logically intuitive, phylogenetic control and a more representative data set reveals that no such inverse relationship has evolved. Despite this, it still seems likely that larger species produce greater numbers of sperm since (1) testis size increases with body mass (Kenagy & Trombulak 1986), (2) larger testes enable the production of larger numbers of sperm (e.g. Møller 1989), and (3) I find no positive relationship between sperm length and body mass (figure 1). Future analyses will test for relationships or trade-offs between sperm size and sperm number (Parker 1982, 1993).

Across mammals, female tract dimensions are likely to scale with body mass (Harcourt *et al.* 1981), however figure 1 suggests no coevolution between sperm size female tract dimensions, despite representation from an enormous range of body sizes (4 to $>30\times10^6$ g—this wide range means that sexual size dimorphism is not likely to significantly confound analysis). Across butterflies (Gage 1994) there is a positive association between sperm lengths and body size. This relationship may be driven by coevolution with the female reproductive tract (e.g. Pitnick & Markow 1994), particularly in species where females store sperm in specialized spermathecae for prolonged periods. In mammals, mechanisms of sperm transport

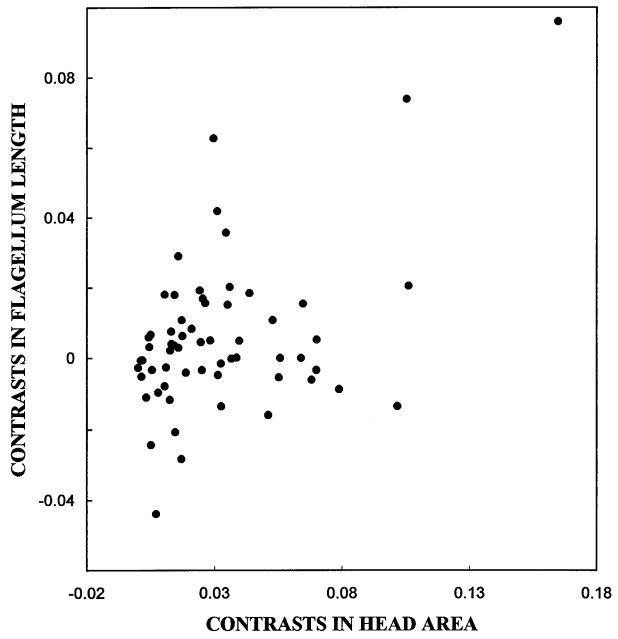

Figure 4. Flagellum length is positively associated with head area across 158 species ($r^2=0.201$, $n=66$). Slope exponent $=0.25\pm0.056$ s.e.

after ejaculation are poorly understood and the female's active role in sperm migration remains controversial (Overstreet & Katz 1990). Furthermore, the site of insemination varies between taxa (Overstreet & Katz 1990). Sperm have been recovered from the oviduct within minutes of ejaculation in many mammalian species (Overstreet 1983) and flagellar motility has evidently played a minor role in such migration. However, some of the more detailed studies show that sperm which are rapidly transported by the female are almost all immotile or structurally disrupted (Overstreet & Katz 1990) and it is suggested that this rapid transport does not move fertilizing sperm. After this initial transport there follows more prolonged migration and sequential build-up of sperm which have migrated through the cervix and lower uteri (Overstreet & Katz 1990). Flagellar motility is necessary to penetrate and migrate through this distance but I find that the variance in sperm morphometry is not associated with the huge variance in body mass (and therefore to an extent female tract dimensions (Harcourt *et al*. 1981)) across mammals.

Parker (1984) first demonstrated a positive association between oestrus duration and sperm fertile lifespan and this finding has been re-examined recently (Gomendio & Roldan 1993). Furthermore, it has been suggested that there is an inverse relationship between sperm length and sperm fertile lifespan (Gomendio & Roldan 1993). Longer sperm may swim more actively and are therefore predicted to expire more quickly (Gomendio & Roldan 1991). Across fish, Stockley *et al*. (1997) showed a negative relationship between sperm size and longevity, supporting this prediction. In externally fertilizing animals, sperm may be under different selection pressures (Levitan 1996; Ball & Parker 1996). However, across 65 mammal species, I find no evidence for a negative relationship between flagellar length (or total sperm length) and the wide variance in oestrus duration (2 to >5000 h). Furthermore, energy for sperm activity is metabolized by the mitochondrial sheath in the mid-piece, but the length or volume of this sheath was not associated with the duration of oestrus. The possibility exists for some species, however, that active female glycolytic support of spermatozoa may confound these relationships (Cardullo & Baltz 1991).

A negative relationship between sperm lifespan and sperm length is predicted to arise because of a trade-off with sperm activity (Gomendio & Roldan 1991, 1993; Stockley *et al*. 1997). Most of the variation in sperm length is due to variance in flagellar length and the trade-off between flagellar activity and mid-piece energy provision may occur because of negative allometry between the length of the mitochondrial sheath and the length of the flagellum. I find a significant positive relationship between mid-piece length and flagellar length across 179 species (figure 2*a*), and the relationship shows negative allometry (slope coefficient <1.0 ($=0.372\pm0.052$ s.e.), figure 2*a*). The slope value is less than the 0.67 exponent determined by Cardullo & Baltz (1991) using species as independent data points. This slope <1.0 supports the prediction for a trade-off between sperm activity and longevity. However, when Cardullo & Baltz (1991) refined their comparisons to examining specifically the volume of the mid-piece sheath occupied by mitochondrial gyres in relation to flagellum length, they found a single linear correlation between these two parameters. Cardullo & Baltz (1991) suggested that this relationship provided a universally constant energy balance along the flagellum for mammals. After controlling for phylogeny and increased representation of data, I find no significant relationship between flagellum length and mid-piece volume across 46 species (figure 2*b*). The relationship is neither inclined, nor declined, but decribes a variable scatter (slope $=0.13\pm0.056$ s.e., figure 2*b*). This result therefore lends no support to general predictions or findings concerning relationships between mid-piece volume, flagellar length, motility and longevity across mammals.

Ultimately, spermatozoa function to unite the male haploid genotype with the ovum. Male haplotype DNA is the most tightly compacted eukaryotic DNA (Ward & Coffey 1991). Although selection has driven the most highly efficient DNA packaging, I find no association between sperm head dimensions and either chromosome number or genome mass. Genome mass is significantly correlated with somatic cell size across 166 vertebrate species (Olmo (1983), although in the absence of phylogenetic control), but here not with sperm head dimensions. The absence of a nucleotypic effect upon spermatozoal head dimensions may be confounded by alternative sperm head functions. Mammalian sperm heads are capped by an acrosome for ovum fusion, and the size and shape of the sperm head may also be under hydrodynamic selection for optimal swimming and egg penetration efficiency (Bedford & Hoskins 1990). There are significant positive relationships between both sperm head length and mid-piece and flagellar length (figures 3*a*,*b*), and head area and flagellar length (figure 4). Selection appears to have shaped sperm motility characteristics according to the size of the head to be propelled, however the relationships have slope exponents less than 1.0 (see figure legends) and r^2 values show that only 5–34% of the

variation in flagellar length is explained by these parameters. Additional agents may therefore be responsible for determining the degree of flagellar elongation.

This comparative study therefore demonstrates that, after phylogenetic control, there is no inverse correlation between sperm size and body size across mammals (Cummins 1983; Cummins & Woodall 1985). Sperm head, mid-piece and flagellum sizes are significantly associated with each other, suggesting coevolution between these fundamental components of mammal sperm architecture. I find no evidence for relationships between the morphometry of these sperm components and either karyotype or the duration of oestrus. What agents explain the variance in mammalian sperm morphometry remain puzzling and demand further investigation. It is interesting to note how tightly sperm morphometry of individual species appears to be conserved, even under excessive nutritional limitation during spermatogenesis (e.g. in a moth, Gage & Cook (1994)). Contrastingly, spermatozoal dimensions appear to be genetically plastic and workers who have attempted to select for the sizes of spermatozoal components have achieved remarkably rapid responses in divergence within very few generations (e.g. Woolley & Beatty (1967) with mouse mid-piece lengths). Mammalian sperm are relatively tiny and range between 28 and 356 microns and are produced in great numbers (e.g. Møller 1989). Two theories (which are not mutually exclusive) currently explain general sperm characteristics for internal fertilizers. Numerical sperm competition (Parker 1970, 1982, 1993), and haploid expression of imperfect gametes (Cohen 1967; see later analyses by Manning & Chamberlain (1994)), both predict selection for the production of huge numbers of tiny sperm. When sperm competition follows the scenario of a raffle, males are selected to produce maximal numbers of minimally sized gametes to win fertilizations (Parker 1982). Sperm competition is widespread across mammals (Ginsberg & Huck 1989), and appears to have been an important selective agent in the evolution of testis size (e.g. Harcourt *et al*. 1981; Short 1981). Across mammals, chiasma frequency in meiosis is relatively high (Cohen 1967; Manning & Chamberlain 1994), which may explain why mammalian sperm are frequently abnormal and so few are able to reach the site of fertilization (Cohen 1967; Overstreet & Katz 1990). If haplotype genetic integrity is expressed in terms of sperm fitness, males may be under selection to maximize sperm number to ensure that genetically and functionally perfect gametes are produced. Selection will also operate upon females to evolve selective mechanisms which filter sub-perfect spermatozoa from the fertilization contest. Recent advances in molecular genetics show increasing evidence for post-meiotic gene expression in mammalian testes (Erickson 1990). Future analyses will investigate both potential effects of sperm competition and gamete redundancy upon mammalian sperm morphometry.

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