Sperm competition in bats

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SUMMARY

Sperm competition is a widespread phenomenon influencing the evolution of male anatomy, physiology and behaviour. Bats are an ideal group for studying sperm competition. Females store fertile sperm for up to 200 days and the size of social groups varies from single animals to groups of hundreds of thousands. This study examines the relationship between social group size and investment in spermatogenesis across 31 species of microchiropteran bat using new and published data on testis mass and sperm length. In addition to male competition, I examined the effects of female reproductive biology on characteristics of spermatogenesis.

Comparative studies indicate that relative testis mass is positively related to sperm competition risk in a wide range of taxa. Social group size may also influence the level of sperm competition, and one of the costs of living in groups may be decreased confidence of paternity. I used comparative analysis of independent contrast (CAIC) to control for phylogeny. Using two possible phylogenies and two measures of social group size, I found a significant positive relationship between social group size and testis mass. There was no relationship between testis mass and the dimension of the female reproductive tract or oestrus duration. Sperm length was not significantly related to body mass or group size, nor was it related to oestrus duration.

1. INTRODUCTION

Parker (1970) first suggested that competition to fertilize ova will occur between ejaculates when females mate with more than one male within a period of sexual receptivity. Parker (1970, 1982, 1990a, b) envisaged sperm competition as analogous to a raffle, where males that deliver the greatest number of sperm into a competition win the highest number of fertilizations. However, sperm production is costly (Dewsbury 1982; Nakatsuru & Kramer 1982). Therefore males should optimize their investment in ejaculates according to the risk of sperm competition (Parker 1982, 1990a, b). As predicted by sperm competition theory, a positive relationship between relative testis mass and sperm competition risk is found among diverse taxa (primates, Harcourt *et al.* 1981; birds, Møller 1988a, 1991a; amphibians, Jennions & Passmore 1993; butterflies Gage 1994; fish Stockley *et al.* 1997), and sperm production is positively related to testes' mass in many species, with large testes producing more sperm (Møller 1988a, b, 1989; Gage 1994). Comparative studies also indicate that sperm competition risk is positively related to social group size in many taxa (insects, Gage 1995; birds, Birkhead & Møller 1992; mammals, Møller & Birkhead 1989), suggesting that one cost of group living may be decreased confidence of paternity (Alexander 1974; Thornhill & Alcock 1983).

While a growing number of comparative studies suggest that increased investment in gonadal tissue relative to somatic tissue occurs under conditions of sperm competition, the effects of sperm competition on sperm size have received less attention (Parker 1993). Parker (1993) predicted that sperm size should evolve independently of sperm competition risk, except under special conditions, such as when sperm size increases survivorship and the risk of sperm competition increases as the delay between mating and fertilization increases. Some studies have reported a positive correlation between sperm size and risk of sperm competition across species (Gage 1994; Gomendio & Roldan 1991). This relationship may result from selection for faster swimming speed or power of longer sperm in competition (Gomendio & Roldan 1991). Sperm size has also been reported to correlate positively with body mass across the Chiroptera (Cummins & Woodall 1985). However, this study did not control for phylogenetic associations.

Factors other than sperm competition may influence male investment in spermatogenesis (Gomendio & Roldan 1993; Stockley *et al.* 1996). Female reproductive biology potentially influences male resource allocation to gonadal tissue and how resources are invested in individual spermatozoa. Dimensions of the female reproductive tract and hence dilution of ejaculates after insemination, could influence testis mass (Brown *et al.* 1995; Harcourt *et al.* 1981).

Sperm competition occurs in many mammals (Ginsberg & Huck 1989; Møller & Birkhead 1989), and probably occurs in bats (Fenton 1984). Aspects of

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the reproductive biology of microchiropteran bats make them unique among mammals and an ideal group to examine sperm competition and its consequences. They are one of few mammalian groups which display prolonged female sperm storage (up to 200 days; Racey 1979), which may promote sperm competition (Birkhead & Møller 1993). Similarly, oestrus duration, which is highly variable in microchiroptera, may affect the intensity of sperm competition (Møller 1991*b*). Bat mating patterns are variable and include promiscuity (although few detailed studies of bat mating patterns are available). Moreover, bat social and reproductive groups vary in size from solitary individuals to colonies numbering hundreds of thousands, leading to tremendous variation in sperm competition risk. Males may also copulate with torpid females during the hibernal period (e.g. Strelkov 1969; Gebhard 1995). However, although sperm competition is likely to be widespread and variable across microchiropteran bats, no studies have examined its evolutionary consequences.

Here, after controlling for body mass and phylogeny, I examined the relationships between testis mass and group size, dimensions of the female reproductive tract and oestrus duration across microchiropteran bats. In addition, I examined relationships between sperm length and body mass, group size and oestrus duration.

2. METHODS

Data on the mean maximum testis mass, mean body mass, mean group size, oestrus duration, sperm length, female sperm storage, female tract dimensions and sex ratio of microchiropteran bats were collected from published studies and unpublished results. Data from a maximum of 31 species, in 20 genera and seven families were included in the analysis (Appendix 1). The sample sizes presented vary depending on the availability of data. After controlling for phylogeny, sample sizes represent the number of independent contrasts and not the number of species used in the comparisons.

Where possible, the mass of the animals from which the testis mass data were obtained were used. Alternatively, body mass data used were median or mean mass values for the species, or median or mean male mass for the species. Since testis mass changes cyclically (Gustafson 1979), the mean testis mass during the period of spermatogenesis was used. Testis mass data include mostly fixed and some fresh material. Fixing has no detectable effect on testis mass in at least one bat species (D. J. Hosken, unpublished data).

Since group size may vary within a species, where possible I used the size of groups from which male testis data were obtained. Alternatively, data were obtained by averaging reports of group size from published or unpublished observations. In species where evidence suggests that matings occur within hibernacula (e.g. Aubert 1963; Thomas *et al.* 1979; Gebhard 1995; E. Jansen, personal communication), the colony size during the hibernal period was used as the group size. This is because female choice is constrained by torpor and males copulate with torpid females. Thus, maximum levels of sperm competition are likely to occur during hibernation, when females can potentially be inseminated by any male in a hibernal group.

In some species' subdivisions, clustering occurs within colonies, (*P. pipistrellus*, Kruetzschmar & Heinz 1995; *Miniopterus australis* and *M. schreibersii*, Dwyer 1966; *Myotis lucifugus*, Guthrie 1933; *Coleura afra*, McWilliam 1987), and
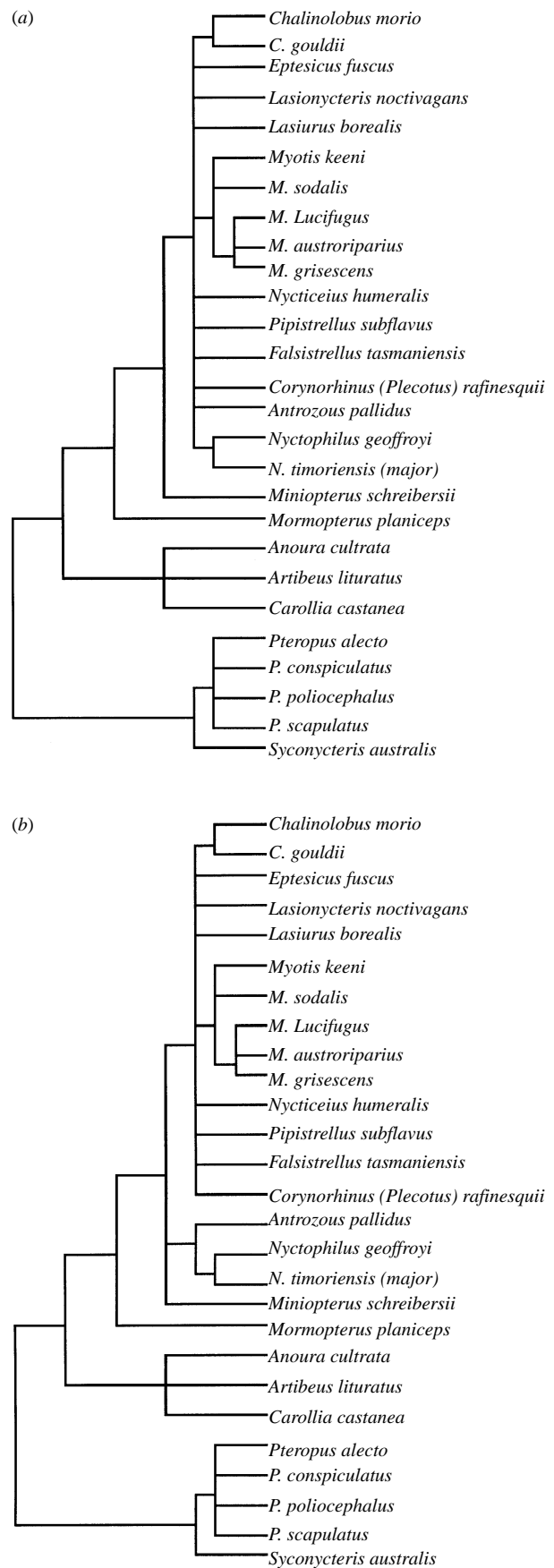

Figure 1. The two phylogenies used in CAIC analysis of variation in sperm length; (*a*) based on Koopman (1984), (*b*) based on Hill & Smith (1984).

clusters may be stable groupings (McWilliam 1987). Therefore, with these species, data were also analysed using the mean or median size of clusters within the colony (Appendix 1).

Oestrus duration was defined as the approximate time between the first recording of copulation or of sperm in the female tract, and the first recording of ovulation. Since assumptions that female reproductive tract volume varies directly with body size (Harcourt *et al.* 1981) are often incorrect (Brown *et al.* 1995), associations between testis mass and female tract dimensions were examined. The tract dimension used in the analysis was the mean diameter or width of the uterine horn of non-pregnant, non-inseminated females (this was the most widely available measure).

Sperm length data obtained from Cummins & Woodall (1985) were reanalysed controlling for phylogeny, to evaluate the need to include body mass as a covariate in sperm length analyses. This allometry analysis included megachiropteran bats (figure 1), but subsequent analysis was concerned only with microchiropteran bats. Two phylogenies were used (see below) and other data (group size and oestrus duration) were collected from published and new results (Appendix 2).

Data were analysed as independent contrasts to minimize problems associated with taxonomic relatedness and phylogenetic inertia (Harvey & Pagel 1991). I used the Comparative Analysis by Independent Contrasts (CAIC) program (Purvis & Rambaut 1994) to estimate the contrasts for each node in the phylogeny for which there was variation in the independent variable. In sex ratio (male or female biased) and female sperm storage (present or absent) analyses, insufficient data were available to generate independent contrasts and data were analysed at a species level.

In CAIC analyses, branch lengths were assumed to be equal, thereby assuming a punctuated model of evolution. Equal branch lengths were used because a sub-sample of the total number of bat species were used in the phylogeny, and some of the phylogenetic relationships are debatable. The phylogenies used in the analysis (Hill & Smith 1984; Koopman 1984), are based on morphological evidence (figure 2). In all phylogenies, *Hipposideros cervinus* was synonymized with *H. galeritus*, following Koopman (1982).

As organ size usually scales allometrically with body size (Calder 1984), testis mass, body mass and uterine width data were log transformed. Likewise, the group size and oestrus duration data were log transformed to normalize their distributions.

Data were analysed using least squares multiple regression analysis forced through the origin (Harvey & Pagel 1991), or, for the dichotomous data, non-parametric Mann–Whitney U-tests.

3. RESULTS

To test for biases in branch length estimates and for heterogeneity of variance in CAIC residuals, absolute contrast values were plotted against the variances of the raw contrasts (Purvis & Rambaut 1994). No significant relationships were found.

Simple regression analysis revealed that testis mass and body mass were not significantly related at a species level (testis mass $= 1.148 + 0.489$ body mass; $n = 31$; $p = 0.06$; $r^2 = 0.12$). However, body mass was included as a covariate in all multiple regressions. This is because of the strong trend detected, since organ size usually scales allometrically with body mass (Calder 1984; Moller 1988 *a*, *b*), and since the true relationship
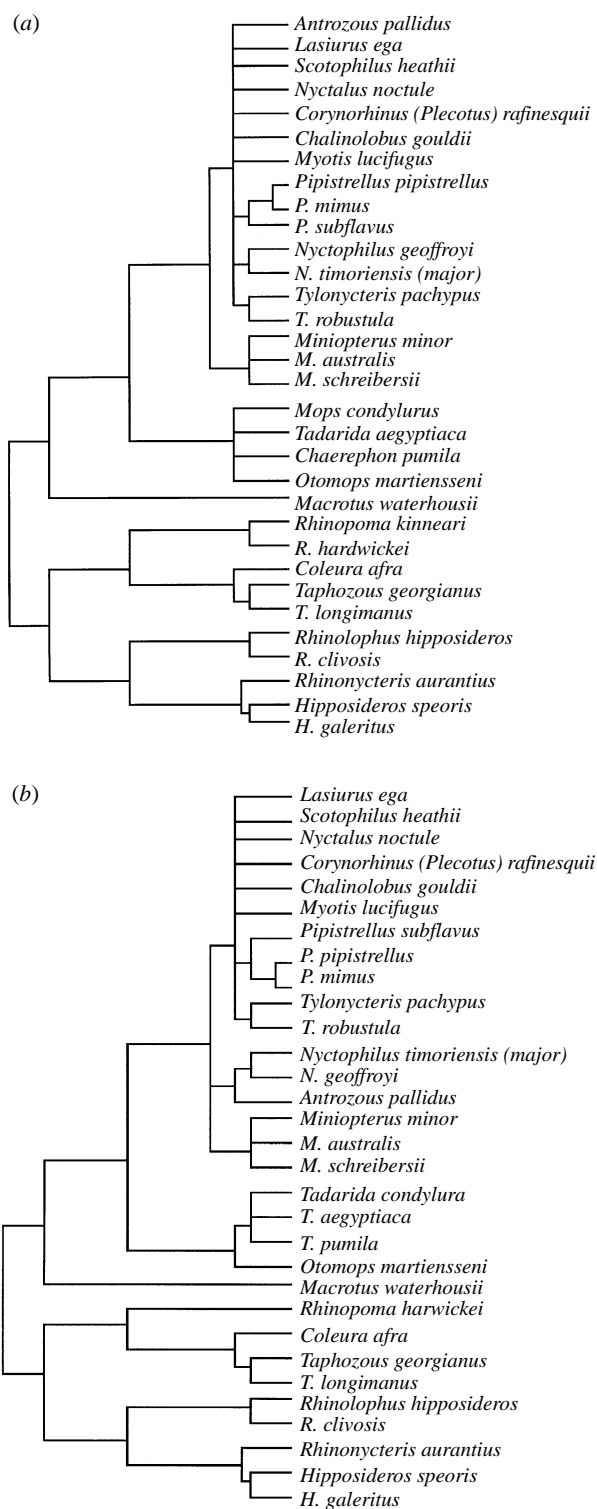

Figure 2. The two phylogenies used in CAIC analysis of variation in testis mass; (*a*) based on Koopman 1984, (*b*) based on Hill and Smith 1984.

between variables is underestimated in least squares regression (Harvey & Pagel 1991).

After controlling for phylogeny, the relationship between partial residual testis mass and partial residual group size was significant and positive. The same relationship was significant in all phylogenetic analyses, regardless of the phylogeny used and regardless of the measure of group size (i.e. with or without the inclusion of cluster data). Furthermore, analysis using
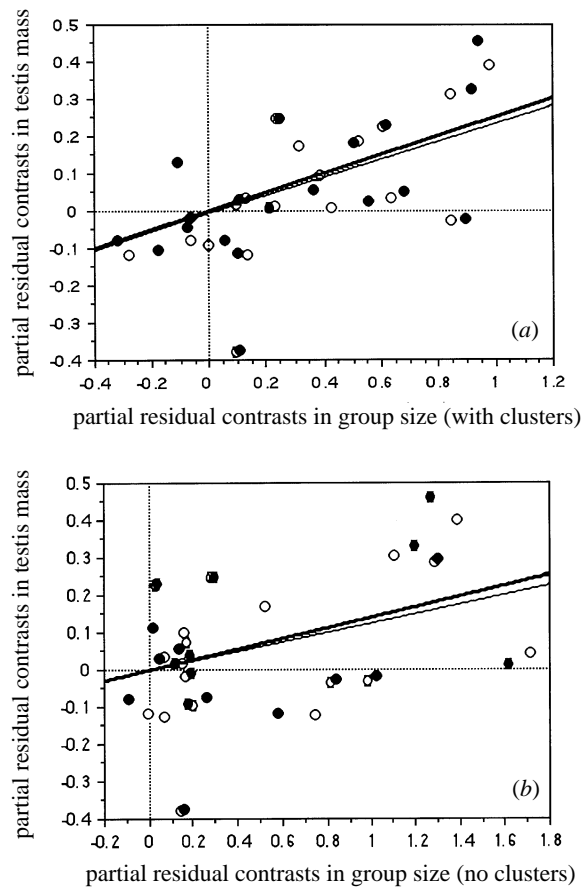
Figure 3. Plots of partial residuals generated using multiple regression analysis forced through the origin. Testis mass was regressed against body mass and group size (all log transformed). Plot (*a*) shows residuals generated using independent contrasts that included cluster data (solid symbols and heavy regression line are data based on Koopman's (1984) phylogeny, open symbols and light regression line are based on Hill & Smith's (1984) phylogeny). Plot (*b*) is based on contrasts without cluster data (solid symbols and heavy regression line are data based on Koopman's (1984) phylogeny, open symbols and light regression line are based on Hill & Smith's (1984) phylogeny). The relationship between partial residual testis mass and partial residual group size was significant in all analyses (Hill & Smith (with clusters) d.f. = 1, 18; $r^2 = 0.56$; $\beta = 0.233$; $F = 11.8$; $p = 0.004$; Hill & Smith (no clusters), d.f. = 1,18 $r^2 = 0.44$; $\beta = 0.125$; $F = 5.48$; $p = 0.036$; Koopman (with clusters), d.f., 1,17; $r^2 = 0.52$; $\beta = 0.242$; $F = 10.8$; $p = 0.006$; Koopman (no clusters), d.f. = 1, 17; $r^2 = 0.39$; $\beta = 0.131$; $F = 5.11$; $p = 0.043$).

clusters always increased the strength of the relationship between testis mass and group size. The statistical significance of the slope of partial residual contrasts in testis mass regressed against partial residual contrasts in group size (figure 3), indicates a significant evolutionary association between testis mass and social group size (Grafen 1989).

There was no significant relationship between partial residual testis mass and partial residual oestrus duration or partial residual uterine horn diameter, regardless of the phylogeny used (table 1). Likewise, no significant relationship was found between residual testis mass and sex ratio or female storage at the species level, although testis mass tended to be greater in

species where there was a male-biased sex ratio ($p = 0.097$, $n = 18$). Likewise in species where females store sperm, residual testis mass tended to be greater ($p = 0.089$, $n = 27$). Analysis at a phylogenetic level could not be undertaken as only three contrasts could be generated.

There was no significant relationship between body size and sperm length after controlling for phylogeny, regardless of the phylogeny used ($\beta \leqslant 0.031$; $p \geqslant 0.59$). There was also no significant relationship between sperm length and group size, regardless of the phylogeny or the group size measure used (i.e. with or without cluster data) (table 2). No relationship was found between sperm length and oestrus duration (table 2), regardless of the phylogeny used.

4. DISCUSSION

Testis mass is positively related to social group size in microchiropteran bats. Social group size is known to influence the risk of sperm competition (e.g. in mammals, the close proximity of conspecifics leads to an increased risk of multi-male copulations, Møller & Birkhead (1989)), and testis mass is known to be positively related to sperm competition risk in a wide range of taxa (e.g. Møller 1988*a*; Gage 1994). Thus a likely explanation for the positive association found between testis mass and social group size in microchiropteran bats is sperm competition risk. That is, as the risk of sperm competition (social group size) increases, males are investing more in gonadal relative to somatic tissue. This result is consistent with Parker's (1970, 1982, 1984, 1990*a*, *b*) predictions based on a raffle-based mode of sperm competition for mammals, and supports Fenton's (1984) suggestion that sperm competition is likely to be an important phenomena in microchiropteran bats. The finding of a relationship between social group size and male investment in spermatogenesis in bats is also consistent with studies of other taxa (e.g. Møller 1991*a*; Gage 1995). Specifically, Møller (1991*a*) defined the risk of sperm competition as the number of neighbouring males and found a relationship between the risk of sperm competition and residual testis size in birds. The trend of greater testis size in species with a male-biased sex ratio is also consistent with sperm competition theory and a similar relationship was found in birds (Møller 1991*a*).

No significant relationship between diameter of the uterine horn and testis mass was detected. This indicates that selection from sperm competition on testis mass has possibly overridden female dilution effects. Alternatively, uterine horn diameter of un-inseminated females may be an inappropriate measure of female tract size since some tracts are capable of tremendous distention (e.g. Racey 1975). However, female sperm storage may increase the probability and/or intensity of sperm competition (Birkhead & Møller 1993). The trend ($p = 0.09$) of greater testis mass in microchiropteran species which experience female sperm storage suggests that female sperm storage may lead to an increase in the risk of sperm competition. Interestingly, to date the only report of

Table 1. *Results of multiple regression analysis of independent contrasts in testis mass and uterine horn diameter and oestrus length for both phylogenies*

phylogeny	predictor variable	d.f.	β	r^2	F	p
Koopman	oestrus duration	1, 13	0.07	0.005	0.071	0.80
	uterine horn	1, 7	−0.08	0.00	< 0.001	0.88
Hill & Smith	oestrus duration	1, 14	0.001	0.10	1.42	0.51
	uterine horn	1, 6	0.004	0.00	< 0.001	0.99

Table 2. *Results of multiple regression analysis of independent contrasts in sperm length and oestrus length and the risk of sperm competition (group size), with and without cluster data*

phylogeny	predictor variable	d.f.	β	r^2	F	p
Koopman	group size	1, 10	−0.002	0.003	0.032	0.86
	group size (clusters)	1, 10	−0.01	0.06	0.65	0.44
	oestrus duration	1, 6	−0.04	0.04	0.224	0.65
Hill & Smith	group size	1, 11	−0.008	0.03	0.31	0.59
	group size (clusters)	1, 11	−0.002	0.002	0.02	0.89
	oestrus duration	1, 7	−0.03	0.03	0.19	0.68

mixed paternity within a bat litter comes from a sperm storing species (Mayer 1995).

Using the sperm length data set of Cummins & Woodall (1985) and controlling for phylogeny, sperm length was not found to be related to body mass. This suggests that the positive relationship between sperm length and body mass Cummins & Woodall (1985) reported is the result of phylogenetic effects, rather than some underlying sperm length/body mass relationship.

No relationship was found between sperm length and group size (risk of sperm competition). Assuming the raffle principle, evolutionarily stable strategy (ESS) modelling of sperm competition in internal fertilizers such as mammals indicates that, unless special conditions apply, sperm size should evolve independently of sperm competition risk (Parker 1993). However, sperm number should be positively related to the intensity of sperm competition (Parker 1993). Findings of the presented study are consistent with these predictions: investment in spermatogenesis relates to group size (sperm competition risk) but sperm length is independent of group size. In the ESS model (Parker 1993), a special condition which would select for increased sperm length occurs when increased sperm size leads to increased survivorship, and the risk of sperm competition increases with an increase in the time between mating and fertilization. In microchiropteran bats, sperm longevity appears to be related to oestrus duration (e.g. Racey 1979; Hosken *et al.* 1997). Therefore, sperm length does not appear to be related to sperm longevity (= oestrus duration), and testis mass is not significantly affected by oestrus duration.

Gomendio & Roldan (1993; see also Parker 1984) reported that sperm length and oestrus length were related across 11 species of mammal, but after controlling for phylogeny, the relationship was not significant. Bats display enormous variation in oestrus

duration (at least 16–200 days; Racey 1979). If a negative relationship between sperm length and oestrus length exists in mammals, a relationship should be clear in microchiropteran bats. In this study, no significant relationship was found, suggesting that sperm length is not related to sperm longevity (at least in bats).

In conclusion, after appropriate phylogenetic control, sperm length in bats was not associated with body mass, the risk of sperm competition, or with oestrus duration. However, there was a positive relationship between relative testis mass and social group size which was independent of other measured variables. Thus, it appears that testis mass in microchiropteran bats varies in accordance with sperm competition theory: increases in the risk of sperm competition have resulted in selection for increased investment in spermatogenesis across this suborder.

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APPENDIX 1.

Summary of species data used in analysis of testis mass variation

species	group numbers (with cluster)	testis mass (mg)	body mass (g)	size of uterine horn (mm)	prolonged female sperm storage (y/n)	sex ratio bias (m/f)	references
Antrozous pallidus	60	181.2	23		y		5; 6; 19; 58; 75; 85
Chalinolobus gouldii	20	14.5	14	1.0	y		16; 43; 80; I. Schlawe, unpublished data
Coleura afra	15 850 (12)	15	10		n		32; 41; 61; 62; 83
Hipposideros galeritus	1	30	6.5		n	f	3; 11; 68
H. speoris	300	40	10		n	f	11; 26; 27; 45; 76
Lasiurus ega	3	12.5	11	1.0	y		56; 72; 81
Macrotus waterhousii	120	18.5	16		n		1; 34; 54; 73
Miniopterus australis	4900 (5)	20	7.5	0.95	n		3; 18; 66; 82
M. minor	170	25	7.5	0.3	n	m	61; 63; 65
M. schreibersii	4040 (150)	40	13		n	f	9; 17
Myotis lucifugus	37 500 (500)	54.1	6.8		y	m	4; 28; 29; 73; 84; 87; A. Kurta, personal communication; J. Kennedy, personal communication; J. Senulis, personal communication
Nyctalus noctula	500	270	27.5		y	m	21; 23; 78; 86; 2
Nyctophilus geoffroyi	1	23	6.5		y		33; D. J. Hosken, unpublished data
N. major (timoriensis)	1	30	13.5		y		33; D. J. Hosken, unpublished data
Otomops martiensseni	200	85	36				2; 41; 70; 83
Pipistrellus mimus	20	26.5	3.6	1.0	y	f	11; 35; 51; 52
P. pipistrellus	3020 (50)	112.5	5.0		y	f	46; 79; 74; P. A. Racey, personal communication; E. Jansen, personal communication;
P. subflavus	5	28.6	5.0		y	m	15; 24; 30; 53
Plecotus rafinesquii	144	377	9.0	1.2	y	m	14; 38; 77
Rhinolophus clivosis	1000	177	16.2	0.8	y		7; 41; 83
R. hipposideros	13	19	7.0		y	f	20; 22; 32; 60
Rhinonycteris aurantius	64	6	9.8	1.2	n		12; 13
Rhinopoma hardwickei	1050	125.5	19.0	3.0		f	11; 39; 40; 57
Scotophilus heathii	15	148	37.5		y	m	47; 48; 59
Tadarida aegyptiaca	36	66	14.9	0.95	n		8; 11; 41; 83
T. condylura	60	60	40.0				31; 41; 70
T. pumila	12	50	8.6			f	31; 64; 70
Taphozous georgianus	8	38	30.0	1.5	n	f	36; 37; 42; 44
T. longimanus	7	38.5	36.0		n	f	25; 49; 50; 55
Tylonycteris pachypus	5	70	4.1	0.45	y	f	10; 67; 69
T. robustula	4	100	8.4	0.5	y	f	10; 67; 69

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APPENDIX 2.

Summary of species data used in analysis of variation in sperm length

(Note that all sperm length data were obtained by Cummins & Woodall (1985) *J. Reprod. Fert.* **75**, 153–175.)

species	mass (g)	group numbers (with cluster)	sperm length (µm)	oestrus duration (days)	references
Mormopterus planiceps	12	10	66	61	2; 13; 19
Anoura cultrata	20.4	10	62.58		5; 13; 18
Artibeus lituratus	66.5	20	85.75		5; 18
Carrolia castanea	14.55		64.19		5; 18
Pteropus alecto	600		65.6		6
P. conspiculatus	500		103.0		6
P. poliocephalus	677		60.1		6
P. scapulatus	358				17
Syconycteris australis	15		80.6		6
Antrozous pallidus	23	60	58.54	198	14
Chalinolobus morio	10	150	45	61	9; 17
C. gouldii	14	20	58	122	10; 17
Eptesicus fuscus	15.5	75	72	182.5	1
Lasionycteris noctivagans	8	1	73.4		7
Lasiurus borealis	12.2	1	213	67.1	7
Miniopterus schreibersii	13	4040 (150)	36	65	4
Myotis keeni	9	150 (12)		75.22	7
M. sodalis	6.5		182	65.5	7
M. lucifugus	8	37 500 (500)	213	51	7
M. austroriparius	6	44 000		49	7
M. grisescens	7.2	500 000	30	72.55	7
Nycticeius humeralis	5.5		213	77.3	7
Nyctophilus geoffroyi	6.5	1	122	53	8; D. J. Hosken, unpublished data
N. timoriensis (major)	12.5	1	122	52	8; D. J. Hosken, unpublished data
Pipistrellus subflavus	4.8	5	213	79.08	3; 7; 11
Falsistrellus tasmaniensis	20	15		66	16
Plecotus rafinesquii	9	144	103	54.65	15

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