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Sperm competition and sperm midpiece size: no consistent pattern in passerine birds

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Abstract

Sperm competition is thought to be a major force driving the evolution of sperm shape and function. However, previous studies investigating the relationship between the risk of sperm competition and sperm morphometry revealed inconclusive results and marked differences exist between taxonomic groups. In a comparative study of two families of passerines (Fringillidae and Sylviidae) and also across species belonging to different passerine families we investigated the relative importance of the phylogenetic background on the relationship between sperm morphometry and the risk of sperm competition. The risk of sperm competition was inferred from relative testis mass as an indicator of investment in sperm production. We found: (i) a significant positive association between both midpiece length and flagellum length and relative testis mass in the Fringillidae, (ii) a significant negative association in the Sylviidae, and (iii) no association across all species. Despite the striking difference in the patterns shown by the Sylviidae and the Fringillidae, the relationship between midpiece length and flagellum length was positive in both families and across all species with positive allometry. Reasons for the differences and similarities between passerine families are discussed.

Keywords: sperm competition, sperm size, midpiece size, Passeriformes

1. Introduction

Sperm are amongst the most diverse of all animal cells (Cohen 1977a). Three factors are thought to explain the diversity in sperm shape across species: (i) phylogeny, (ii) mode of fertilisation and (iii) postcopulatory sexual selection including sperm competition and cryptic female choice (Miller & Pitnick 2002; Snook 2005). Sperm competition (Parker 1970; Birkhead & Parker 1997) appears to be a particularly powerful force driving the diversity in sperm phenotype (Birkhead & Pizzari 2002; Pizzari & Birkhead 2002; Snook 2005), but the relationships between the size of sperm traits and the risk of sperm competition appear to differ markedly between taxa (e.g. Stockley *et al*. 1997; Balshine *et al*. 2001; Anderson & Dixson 2002; Gage & Freckleton 2003; Malo *et al*. 2006).

The role of two particular sperm traits in sperm competition is hotly debated in evolutionary biology: flagellum length (often closely correlated with total sperm length) and the size of the sperm midpiece. Theoreticians have predicted that: (a) increased flagellum length results in increased sperm velocity (Katz & Drobnis 1990) and (b) increased midpiece size, resulting from more or larger mitochondria, results in greater power output (Cardullo & Baltz 1991). In both cases therefore, we might expect species that experience high levels of sperm competition to have longer sperm and/or larger midpieces (see also Parker 1993). We also might expect on energetic grounds, all else being equal, a fixed relationship between midpiece size and flagellum length (Cardullo & Baltz 1991; but see Gage 1998). Empirical tests of these theoretical predictions have yielded mixed results. First, the relationship between overall sperm length and sperm competition is positive in some cases (Gomendio & Roldan 1991; Gage 1994; Breed & Taylor 2000; Morrow & Gage 2000; Balshine *et al*. 2001), negative in others (Stockley *et al*. 1997), or shows no relationship in yet other cases (Gage & Freckleton 2003; Anderson & Dixson 2002). Second, in terms of the midpiece, Anderson and Dixson (2002) found a pronounced positive association between midpiece volume and the risk of sperm competition in primates. However, contrary to theory (Cardullo & Baltz 1991) they found no association between flagellum length and sperm competition. Profound biological differences between taxonomic groups might be a potential explanation for these inconclusive results.

Passerine birds exhibit variation in both levels of sperm competition (Griffith *et al*. 2002) and in sperm morphometry, including sperm length and midpiece size (Retzius 1909; MacFarlane 1963; Birkhead *et al*. 2006). However, previous studies were unable

to detect a clear association between sperm length and the risk of sperm competition across passerine species belonging to different families, although there was an indirect effect mediated through the sperm storage tubules (Briskie *et al*. 1997). Previous studies of passerine birds have included a wide range of species from many different families. Passerine families have diverged markedly and exhibit profound biological differences which are likely to affect life history and reproductive traits (Bennett & Owens 2002). To test the possible influence of phylogeny on the relationship between sperm morphometry and the risk of sperm competition in passerine birds investigated how midpiece size and flagellum length covary with the risk of sperm competition within two families of passerine birds as well as across species belonging to several families.

2. Material & Methods

We focused on two passerine families, namely the finches (Fringillidae) and the Old World warblers (Sylviidae). We obtained data from 18 species of Fringilllidae and 22 species of Sylviidae (see Electronic Appendix). We chose these two passerine families because of their well resolved phylogenies and their accessibility. We also collected data from 33 other species belonging to a variety of passerine families (see electronic appendix).

Sperm morphometry

Morphometry is defined as the measurement of shape dimensions and throughout this paper 'sperm morphometry' refers to the measurement of the length of sperm traits (Gage 1998). Two different methods were used to obtain sperm samples: (i) from faeces of males in reproductive condition (Immler & Birkhead 2005); (ii) from the seminal glomera of dissected males in reproductive condition found dead (e.g. road kills), or collected under a license. Sperm collected by different methods do not differ in their morphometry (Immler & Birkhead 2005). Samples from one to ten males per species were collected. A power analysis performed at the beginning of the study on five species of Acrocephaline Sylviidae (one to 15 males per species) which show similar sperm morphometry, revealed that measuring 10 males per species allowed us to detect significant differences even between closely related species with similar sperm morphometry. Five randomly chosen sperm were measured from each male since in the zebra finch *Taeniopygia guttata*, five sperm per male provide a representative value for

individual males (Birkhead *et al*. 2005; see also Morrow & Gage 2001). Sperm were fixed in a 5% formalin solution. For analysis, a sub-sample was examined using a light microscope at x250 or x400 magnification and digital pictures were taken. Passerine sperm are typically elongate with a short helical head and a long mitochondrial helix twisted around almost the entire length of the flagellum (McFarlane 1963). The following sperm traits were measured from digital images (using analysis software Leica IM50 Image manager): (i) head length, (ii) midpiece length (along the length of the flagellum), (iii) and flagellum length were measured to the nearest 0.5μm, and (iv) the number of midpiece helix curves were counted to calculate straight helix length (= SHL i.e. the total length of the straightened midpiece twisted around the flagellum using the method described in Birkhead *et al*. 2005). Hereafter, midpiece length is used as the measurement of the straight midpiece length. Within-species repeatability (Lessels & Boag 1987) of all morphometric sperm traits was estimated.

In passerines, SHL provides a reliable measurement of the variation of midpiece size: Across five species belonging to different families, midpiece volume (cylindrical volume calculated from SHL and the mitochondrial radius obtained from transmission electron microscopy pictures; coefficient of variance CV = 97.91%) varies substantially due to variation in midpiece length (CV = 73.52%) whereas midpiece width shows little variation (CV = 14.48%). In Anderson *et al*.'s (2005) dataset on mammals, variation in midpiece volume (CV = 73.59%) was substantially larger than variation in midpiece length (CV = 23.30%). Interestingly, an earlier study of a wider range of mammals failed to show a relationship between either midpiece volume (calculated as the volume of a cylinder subtracting the volume of the inner axoneme) or midpiece length and relative testis mass (Gage & Freckleton 2003). In Gage and Freckleton's (2003) study study, the difference in variation between volume and length was smaller (midpiece length: CV = 73.34%; midpiece volume: CV = 90.62%) than in Anderson *et al*.'s (2005) study. By including the inner axoneme in their calculation of midpiece volume, Anderson *et al*. (2005) may have overestimated midpiece size and this may explain the discrepancy between their study and that of Gage and Freckleton (2003). Alternative hypotheses for the differences between the studies of mammals may be the variation in sample size and the different sources of data used.

Testis mass and body mass

We used relative testis mass as an indicator of the risk of sperm competition (Harcourt *et al*. 1981; Møller & Briskie 1995; Dunn *et al*. 2001; Pitcher *et al*. 2005). Data on testis mass and body mass were obtained from the literature (Dunn *et al*. 2001; Calhim & Birkhead, in press) and from personal observations. Testis mass was not available for all species included and accounts for varying sample sizes amongst analyses.

Comparative methods

To account for statistical non-independence of data points due to shared ancestry of species (Felsenstein 1985; Harvey & Pagel 1991) we used a generalised least-squares approach in a phylogenetic framework for our analyses (GLS: Pagel 1999; Freckleton *et al*. 2002). The GLS method allows the estimation of a phylogenetic scaling parameter λ: values of λ close to zero correspond to traits where the similarities are likely to have evolved independently of phylogeny whereas λ values close to one indicate strong phylogenetic association of the traits. A likelihood ratio test was applied to compare models including the maximum likelihood value of λ with models including λ set to either zero (no phylogenetic association) or one (complete phylogenetic association). Analyses were performed using a code for the statistical package R V.2.1.0 (R Foundation for Statistical Computing 2005). The phylogeny including all species was obtained from the literature: the deeper nodes of the phylogenetic tree were inferred from Sibley & Ahlquist (1990) and higher nodes were obtained from different sources (see electronic appendix). We assumed constant branch length for our analyses.

Multiple regression analysis

We performed multiple regression analyses in a phylogenetic framework as described above to investigate the relationship between morphometric sperm traits and relative testis mass. We conducted the following analyses: (i) across all passerine species included, (ii) and within two individual passerine families as low sample size in other families did not allow statistical analyses. The Sylviidae exhibit a wide range of different mating systems which might affect testis mass (Dunn *et al*. 2001; Griffith *et al*. 2002; Leisler *et al*. 2002). To test for a possible influence of mating system on testis mass we analysed socially monogamous Sylviidae species separately. We included individual sperm traits as dependent variables and both testis mass and body mass as

independent variables to control for allometry between the latter (Briskie & Montgomerie 1992). The highest condition index (estimated from the matrix of the independent variables to detect collinearity between independent variables; Belsley *et al*. 1980) being 14.5 for body mass allowed us to exclude collinearity between independent variables. Where necessary, data were normalised using the appropriate transformation to meet parametric requirements of the GLS model.

We performed GLS analyses to establish the relationships between individual morphometric sperm traits.

Multiple comparisons

We performed a series of comparative analyses on different subsets of the data. However, we rejected the use of Bonferroni correction as it enhances the probability of committing Type II errors, particularly in studies with small samples sizes (Nakagawa 2004). We calculated effect size to establish the strength of the relationship between sperm traits and the predicting variables (Nakagawa 2004). We calculated the effect size *r* from *t* values (Cohen 1977b) obtained from the GLS model and used Cohen's (1988) benchmarks to estimate the size of the effect. We also calculated 95% noncentral confidence limits (CL) for *r* which indicate statistical significance if zero is not included in the CLs (Smithson 2003).

3. Results

Association between sperm morphometry and relative testis mass

Striking differences existed for the relationship between sperm trait dimensions and relative testis mass between the Fringillidae and the Sylviidae. For the Fringillidae, a positive association existed between most sperm traits (except head length) and relative testis mass, whereas for the Sylviidae, the relationship between sperm trait dimensions and relative testis mass was negative (Table 1a; Figure 1).

Values of λ for the Fringillidae were close to zero for most traits indicating that phylogeny plays only a minor role in these relationships. Since the Fringillidae included in the analyses are all considered socially monogamous and only vary in the rate of extra-pair paternity (Dunn *et al*. 2001; Griffith *et al*. 2002) no further analyses were undertaken. For the Sylviidae, effect size was medium to large and values of λ varied

considerably between sperm traits (Table 1). The Sylviidae exhibit variation in mating system across species, being monogamous, polygynous and promiscuous and males are exposed to varying copulation rate. It has been argued that the risk of sperm depletion due to frequent copulation may influence testis mass and possibly confound the relationship between sperm competition risk and testis mass. We therefore performed the analyses considering only socially monogamous Sylviidae which vary in the rate of extra-pair paternity (Dunn *et al*. 2001; Leisler *et al*. 2002), negative relationships existed between sperm traits (except head length) and relative testis mass (Table 1c). Effect sizes were large. Values of λ were close to zero indicating that factors other than phylogeny explain these patterns. The Reduced Major Axis regression slopes (RMA: Ricker 1973; McArdle 1988) between all Sylviidae (v_1) and monogamous Sylviidae (v_2) differed significantly in that in monogamous Sylviidae the slope is significantly steeper than in all Sylviidae (midpiece length: $v_1 =$ -0.085 ± 0.02 s.e., $v_2 =$ -0.57 ± 0.14 s.e., $t =$ 3.46, $p < 0.01$; flagellum length: $v_1 =$ -0.11 ± 0.02 s.e., $v_2 =$ -44.10 ± 11.41 s.e., $t =$ 3.86, $p < 0.001$; total sperm length: $v_1 =$ -0.08 ± 0.02 s.e., $v_2 =$ -40.23 ± 10.23 s.e., $t =$ 3.93, $p < 0.001$). The increased effect size in monogamous Sylviidae suggests that mating system may have some influence on the relationship between sperm dimensions and relative testis mass.

Across all passerines, we found no association between any sperm trait dimensions (except head length) and relative testis mass (Table 1d). Accordingly, effect size was small and confidence limits were large, indicating a weak effect. However, there was a significant negative association between sperm dimensions and body mass (Table 1d). This negative association exists mainly due to the inclusion of the two *Corvus* species which have extremely small sperm compared to their body mass and disappears when the two *Corvus* species are excluded. Values of λ were close to one for all sperm traits except for sperm head, indicating a very strong phylogenetic component.

For all morphometric sperm traits, within-species repeatability was very high (see electronic appendix).

Relationships between sperm traits

Across all species, midpiece length was significantly positively associated with both flagellum length ($r = 0.84$, $p < 0.0001$, n = 73; Figure 2) and total sperm length ($r = 0.69$, $p < 0.0001$, n = 73) after controlling for phylogeny. To assess whether an allometric relationship exists between midpiece length and flagellum length (as a

possible indicator of the metabolic regulation of sperm) as predicted by Cardullo & Baltz (1991 - see also Gage 1998) we calculated the slope *v* of a Reduced Major Axis regression (RMA: Ricker 1973; McArdle 1988), using the standard errors (s.e.) obtained from the GLS regression as an approximation (Sokal & Rohlf 1995) and performed a *t* test of *v* against one. We found a significant positive allometric relationship between midpiece length and flagellum length across all species included, and also within the Fringillidae. Similarly, across all the Sylviidae (excluding *Locustella* spp.) and across only the monogamous Sylviidae the RMA slopes were significantly larger than one (Table 2).

Head length was positively correlated with all other sperm traits across all species: midpiece length ($r = 0.13$, $p = 0.001$, n = 73), flagellum length ($r = 0.16$, $p = 0.0003$, n = 73) and total sperm length ($r = 0.18$, $p = 0.0001$, n = 73).

4. Discussion

Our results revealed striking differences for the relationship between sperm trait dimensions and the risk of sperm competition inferred from relative testis mass between passerine families, being positive in the Fringillidae and negative in the Sylviidae. Across the passerine species belonging to different families including Fringillidae and Sylviidae, we found no association between sperm trait dimensions and the risk of sperm competition. This is consistent with previous studies of passerine birds (Briskie & Montgomerie 1992; Briskie *et al*. 1997). Our study highlights the variation across different taxonomic groups which may explain the results obtained in earlier studies. Despite the marked differences in sperm dimensions between passerine families the relationship between midpiece length and flagellum length was positive with a positive allometry. This suggests that essential biological functions (e.g. energetic principles) determine gross passerine sperm morphology.

The importance of phylogeny

Our results highlight the importance of phylogeny for the investigation of trait coevolution and emphasise that statistical analyses correcting for phylogeny sometimes deal insufficiently with differences between taxonomic groups. The size and composition of taxonomic groups used in comparative studies differ markedly. Despite rigorous control for phylogeny as applied in most comparative studies (Felsenstein

1985; Harvey & Pagel 1991) it is possible that inter-taxonomic variation in other life history traits such as breeding cycle, number of eggs per clutch or number of copulations prior and during ovulation may confound the results (see also Arnold & Owens 2002; Bennett & Owens 2002). This variation is likely to be reduced within smaller taxonomic groups such as orders and families. It is therefore important for future comparative studies to investigate trait coevolution at different taxonomic levels.

Sperm morphometry and risk of sperm competition in passerine birds

Our finding of an inconsistent pattern of the relationship between midpiece length and relative testis mass in passerines contrasts with Anderson and Dixson's (2002) finding of a positive relationship between midpiece volume and relative testis size in primates (Anderson & Dixson 2002) and across mammals in general (Anderson *et al*. 2005). We consider four possible non-exclusive reasons for the existence of different relationships between sperm trait dimensions and relative testis mass in the Fringillidae and Sylviidae:

I) Mating systems: Relative testis size is known to be a reasonable index of the risk of sperm competition as relative testis size is positively correlated with the rate of sperm production (Harcourt *et al*. 1981; Møller 1988a, b, 1991; Møller & Briskie 1995; Hosken *et al*. 2001; Pitnick *et al*. 2001). But testis size may also provide an index of increased sperm production due to polygynous or promiscuous mating systems which may entail the risk of sperm depletion (Cartar 1985; Wedell *et al*. 2002). All Fringillidae included in this study are socially monogamous with varying rate of extra-pair paternity (Dunn *et al*. 2001; Griffith *et al*. 2002), whereas the Sylviidae exhibit a range of mating systems including monogamy, polygyny and promiscuity (Dunn *et al*. 2001; Leisler *et al*. 2002) and testis mass in the two families might be subject to differential selection. However, the results including all Sylviidae did not differ from those that consider only monogamous Sylviidae and therefore variation in mating systems can be excluded as a possible explanation for the difference between the two families.

II) Trade-off between sperm size and number: Parker (1993) assumed that a trade-off might exist between sperm size and sperm number. If so the different results in the Fringillidae and the Sylviidae could be explained by a possible advantage of few larger sperm in the Fringillidae, whereas in the Sylviidae, increased sperm numbers might be favoured at the expense of sperm size. Overall, the Fringillidae produce sperm that are twice the size of Sylviidae sperm (see Electronic Appendix). In addition, there

the lack of a significant difference in testis size between the two families (see Electronic Appendix) indicates that the overall expenditure on sperm production is constant and might therefore indicate a trade-off between sperm size and number. Future studies will have to take sperm numbers produced into account to specifically investigate this issue.

III) Sperm-female coevolution: The differences in sperm morphometry between closely related taxonomic groups such as the Fringillidae and the Sylviidae may be the result of coevolution between sperm and the female reproductive tract rather than sperm competition (Briskie & Montgomerie 1992; Briskie *et al*. 1997). The anatomy of the female reproductive tract may have an equal (or stronger) impact on sperm morphometry than sperm competition (Briskie & Montgomerie 1992; Briskie *et al*. 1997; Miller & Pitnick 2002; Minder *et al*. 2005) and may interfere with and even reverse the relative impact of sperm competition, as suggested by the opposite associations between sperm morphometry and the risk of sperm competition in the Fringillidae and the Sylviidae. To test this we would need info on female reproductive anatomy.

IV) Sperm survival: A trade-off between sperm size and sperm longevity (as proposed in mammals: Cardullo & Baltz 1991; Gomendio & Roldan 1991, 1993; and fish: Stockley *et a*l. 1997) might explain the divergent results in the Fringillidae and Sylviidae: the smaller sperm of the Sylviidae might have to survive for longer after ejaculation than the larger sperm of the Fringillidae. The biological bases for any trade-off between sperm size and sperm longevity are still poorly understood. In mammals it has been suggested that the trade-off results from the negative allometry between midpiece size and flagellum length (i.e. longer sperm have a relatively shorter but absolutely longer midpiece; Cardullo & Baltz 1991; but see Gage 1998). In other words, the relatively small midpiece of longer sperm generates less power per unit length of flagellum, resulting in rapid energy consumption and early death.

In contrast to the situation in mammals, we found a positive allometry between midpiece length and flagellum length in passerines (i.e. longer sperm have a relatively and absolutely longer midpiece; Figure 2). Using the same logic as applied to mammalian sperm, all else being equal we might expect longer passerine sperm to survive longer than short sperm. However, in a preliminary *in vitro* study we found exactly the opposite pattern: shorter sperm with a smaller midpiece survived longer than longer sperm (S. Immler & T. R. Birkhead, unpubl. data). This suggests that in

passerines the increased metabolic rate of longer sperm is generated by an absolutely longer midpiece.

Conclusions

The results of this study emphasise how little we still understand about the evolution of sperm design and function. We can almost certainly exclude mating system as a possible explanation for the opposite relationship between sperm morphometry and testis size in Fringillidae and Sylviidae. The difference between Fringillidae and Sylviidae might indicate some crucial biological limitations to sperm production and a possible trade-off between sperm size against sperm number at the extremes, but no firm conclusions can be drawn at this stage. Future studies should concentrate on both broad evolutionary patterns within and across a variety of taxonomic groups and on the detailed investigation of the functional significance of specific sperm traits and their role in postcopulatory sexual selection including both sperm competition and female reproductive biology.

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Table 1a-d: Multiple regression analyses controlling for phylogeny (GLS) of sperm morphometry in relation to testis mass and body mass within families and across all species. A *t* test was used to compare the slopes against zero. The fitted model including the maximum likelihood value of λ was compared against the models including λ = 1 and λ = 0: Supersrcipts after the λ value indicate significance levels of the likelihood ratio tests (first position: against λ = 1; second position: λ = 0; significance levels: * = p <0.05; ** = p <0.01). Effect size *r* calculated from the *t* value and the noncentral 95% confidence interval are presented. CIs excluding zero indicate a significant relationship whereas CIs including zero indicate no statistical significance. The data of the monogamous Sylviidae are a subset of the data of Sylviidae.

sperm trait	predictor	slope	*t*	*p*	*λ*	*r*	CI
a) Fringillidae (n = 12)							
head	testis mass	-0.65	-2.03	0.07	<0.001 *, n.s.	-0.58	-0.83 – 0.07
	body mass	-0.21	-0.77	0.46		-0.26	-0.07 – 0.40
midpiece	testis mass	397.91	3.01	0.02	<0.001 *, n.s.	0.73	0.18 – 0.89
	body mass	-144.34	-1.30	0.23		-0.42	-0.76 – 0.27
flagellum	testis mass	365.38	3.06	0.01	<0.001 *, n.s.	0.73	0.19 – 0.89
	body mass	-144.96	-1.45	0.18		-0.46	0.78 – 0.23
total length	testis mass	355.98	3.04	0.01	<0.001 *, n.s.	0.73	0.19 – 0.89
	body mass	-148.35	-1.51	0.17		-0.47	-0.78 - 0.21
b) Sylviidae (n = 14)							
head	testis mass	0.03	1.17	0.27	<0.001 n.s., n.s.	0.35	-0.06 - 0.79
	body mass	-0.24	-2.86	0.02		-0.67	-0.85- -0.15
midpiece	testis mass	-0.10	-4.43	0.001	0.14 n.s., n.s.	-0.81	-0.91 - -0.46
	body mass	-0.25	-3.94	0.002		-0.78	-0.90 - -0.38
flagellum	testis mass	-0.09	-3.69	0.004	0.69 n.s., n.s.	-0.76	-0.89 - -0.33
	body mass	-0.21	-3.55	0.005		-0.75	-0.89 - -0.33
total length	testis mass	-0.07	-3.40	0.006	0.80 n.s., n.s.	-0.73	-0.88 - -0.27
	body mass	-0.29	-4.12	0.002		-0.79	-0.91 - -0.41
c) Monogamous Sylviidae (n = 7)							
head	testis mass	0.73	0.29	0.79	<0.001 n.s., n.s.	0.17	-0.70 – 0.79
	body mass	-13.60	-3.26	0.03		-0.88	-0.97 – 0.02
midpiece	testis mass	-0.50	-3.69	0.02	<0.001 n.s., n.s.	-0.91	-0.97 - -0.13
	body mass	-0.55	-2.49	0.07		-0.82	-0.95 – 0.18
flagellum	testis mass	-35.28	-3.09	0.04	<0.001 n.s., n.s.	-0.87	-0.96 – 0.02
	body mass	-25.93	-1.40	0.23		-0.63	-0.90 – 0.47
total length	testis mass	-33.90	-3.31	0.03	<0.001 n.s., n.s.	-0.89	-0.97 – -0.03
	body mass	-33.85	-2.04	0.11		-0.76	-0.93 - 0.30
d) across passerine species (n = 50)							
head	testis mass	0.04	2.21	0.03	0.37 *, n.s.	0.31	0.04 – 0.61
	body mass	-0.08	-3.82	<0.001		-0.49	-0.82 - -0.25
midpiece	testis mass	3.83	0.52	0.61	0.84 n.s., *	0.08	-0.21 – 0.37
	body mass	-17.68	-1.88	0.07		-0.27	-0.56 – 0.01
flagellum	testis mass	0.07	1.29	0.20	0.93 n.s., *	0.19	-0.09 – 0.48
	body mass	-0.20	-2.59	0.01		-0.26	-0.55 – 0.02
total length	testis mass	7.44	1.15	0.25	0.80 n.s., *	0.17	-0.11 – 0.46
	body mass	-17.56	-2.19	0.04		-0.31	-0.61 - -0.35

Table 2: Relationship between midpiece length and flagellum length: correlation coefficient *r* and slope *b* obtained from the GLS regression was used to calculate the RMA regression slope *v*. Standard errors (s.e.) for *b* were used to compare *v* against one and *t* and *p* are given from a one sample *t* test of the slope against one. The data of the monogamous Sylviidae are a subset of the data of Sylviidae.

Family	*b*	*v*	s.e.	*r*	n	*t*	*p*
all species	1.48	1.76	0.11	0.99	73	6.91	<0.005
Fringillidae	1.13	1.14	0.03	0.99	16	4.67	<0.005
Sylviidae	1.03	1.11	0.06	0.93	22	1.83	< 0.05
monogamous Sylviidae	1.23	1.26	0.11	0.97	7	2.36	< 0.05
Alaudidae	1.18	1.19	0.02	0.99	10	9.50	<0.005
Turdidae	1.29	1.30	0.05	0.99	8	6.00	<0.005

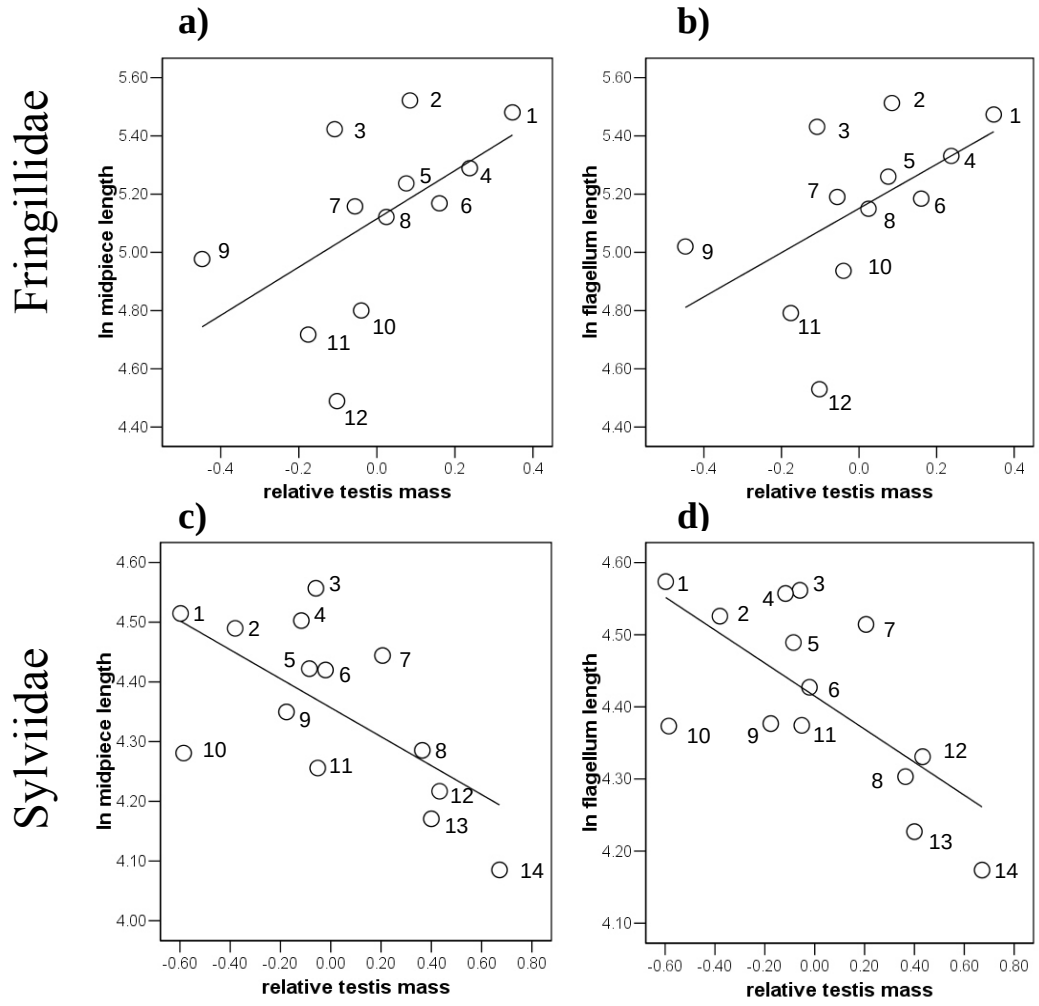

Figure 1: Association between morphometric sperm traits and relative testis mass. Figures are not controlled for phylogeny and relative testis mass indicates the use of residual values from a linear regression of testis mass on body mass: Fringillidae (n = 12): **a)** association between midpiece length and relative testis mass (b = 397.91, t = 3.01, p = 0.02, λ < 0.0001); **b)** association between flagellum length and relative testis mass (b = 365.38, t = 3.06, p = 0.01, λ < 0.0001); Sylviidae (n = 14): **c)** association between flagellum length and relative testis mass (b = -0.09, t = -3.69, p = 0.004, λ = 0.69); **d)** association between total sperm length and relative testis mass (b = -0.07, t = -3.40, p = 0.006, λ = 0.80).

Species list (in brackets: sample size): Fringillidae: 1) *Fringilla coelebs* (10), 2) *Carduelis erythrinus* (2), 3) *Serinus serinus* (1), 4) *S. flaviventris* (1), 5) *S. canaria* (10), 6) *C. flammea* (12), 7) *C. tristis* (6), 8) *C. chloris* (5), 9) *C. cannabina* (4), 10) *Carpodacus mexicanus* (1), 11) *C. carduelis* (7), 12) *Loxia curvirostra* (3), Sylviidae: 1) *Acrocephalus baeticatus* (1), 2) *Phylloscopus fuscatus* (1), 3) *A. scirpaceus* (10), 4) *A. palustris* (2), 5) *P. sibilatrix* (4), 6) *P. collybita* (5), 7) *A. melanopogon* (4), 8) *Sylvia curruca* (, 9) *P. trochilus* (5), 10) *A. arundinaceus* (1), 11) *A. schoenobaenus* (10), 12) *A. paludicola* (7), 13) *S. communis* (4), 14) *S. atricapilla* (10).

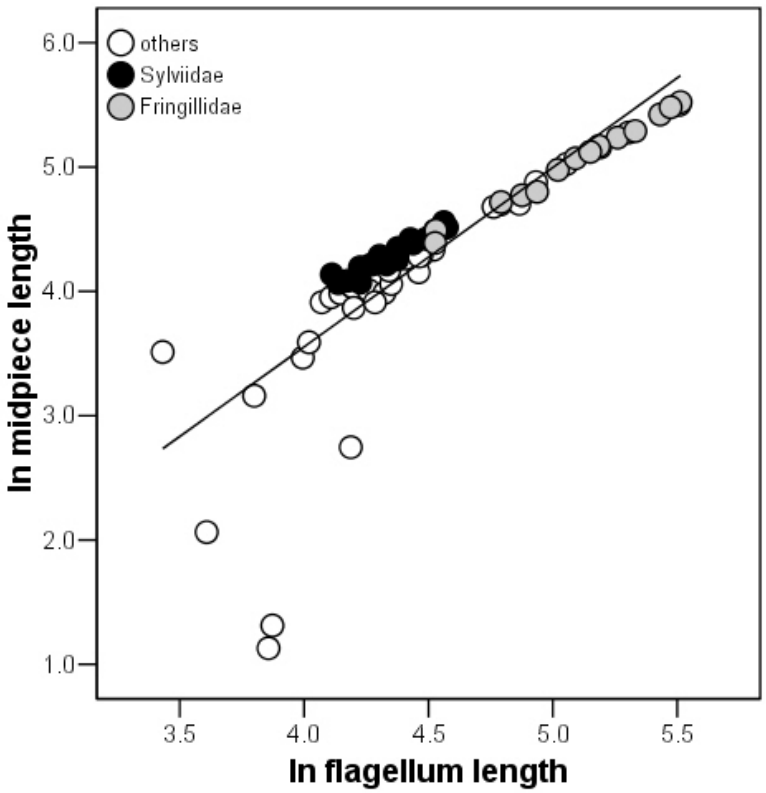

Figure 2: Association between midpiece length and flagellum length across 73 passerine species belonging to different families. The results of RMA Regression revealed a positive allometry between midpiece length and flagellum length with the slope $v = 1.76 \pm 0.11$ s.e. (t test against 1: $t = 6.91$, $p < 0.005$). Similarly, a positive allometry existed within the Fringillidae ($v = 1.14 \pm 0.03$ s.e., $t = 4.67$, $p < 0.005$) and the Sylviidae ($v = 1.11 \pm 0.06$ s.e., $t = 1.83$, $p < 0.05$).