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ORIGINAL ARTICLE

Relationships between sperm morphological traits and sperm swimming performance in wild Great Tits (*Parus major*)

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Abstract

Sperm competition, the competition among rival males' sperm for the fertilization of a given female's set of ova, is a powerful selective force shaping male reproductive traits such as sperm performance. Sperm morphology, the size and shape of the different parts of a spermatozoon, plays a major role in sperm swimming performance with consequences for a male's sperm competitive ability and reproductive success. However, despite important implications for the evolution of sperm traits and associated reproductive strategies, the intraspecific relationships between sperm morphology and sperm swimming performance remain unclear. Using wild Great Tits (*Parus major*), we quantified the among-male relationships between sperm morphological components and sperm swimming performance measured as sperm motility, sperm velocity, sperm swimming endurance, and sperm longevity. We also examined the within- and among-male relationships across sperm morphological traits. Sperm motility was positively correlated with sperm head length and sperm total length while sperm velocity was positively related to sperm midpiece length. In contrast, sperm swimming endurance and longevity were unrelated to any sperm morphological trait. We also observed positive among-male correlations among sperm morphological traits and substantial within-male variation in those traits, which potentially reflects antagonistic selection pressures acting on sperm morphology. Our study shows that sperm morphological components predict different aspects of sperm swimming performance in passerine birds though these relationships were rather weak. Overall, longer sperm morphological components were associated with faster and more motile sperm, which may transfer into higher reproductive success.

Keywords *Parus major* · Reproductive success · Sexual selection · Sperm competition · Sperm morphology · Sperm performance

Zusammenfassung

Zusammenhang zwischen Morphologie und Schwimmvermögen von Spermien bei Kohlmeisen *Parus major*

Spermienkonkurrenz, die Konkurrenz zwischen den Samenzellen rivalisierender Männchen um die Befruchtung eines Weibchens, ist eine sehr starke selektive Kraft bei der Ausbildung erfolgreicher Reproduktions-Eigenschaften wie zum Beispiel der Leistungsfähigkeit von Spermien. Die Spermien-Morphologie, also die Größe und Form der unterschiedlichen Teile einer Samenzelle, spielt für ihre Schwimmfähigkeit eine große Rolle, mit Auswirkungen auf die Konkurrenzfähigkeit der

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Samenzellen und damit auf den Fortpflanzungserfolg des betreffenden Männchen. Aber ungeachtet der großen Bedeutung für die Evolution der Eigenschaften von Spermien und der damit verbundenen Fortpflanzungsstrategien, ist der innerartliche Zusammenhang zwischen Spermien-Morphologie und ihrem Schwimmvermögen nach wie vor unklar. Für Männchen von Wildfängen der Kohlmeise quantifizierten wir Zusammenhänge zwischen den morphologischen Eigenheiten der Spermien und ihrer Schwimmfähigkeit, gemessen anhand ihrer Beweglichkeit, Geschwindigkeit, Schwimm-Ausdauer und ihrer Lebensspanne. Ferner erfassten und verglichen wir die morphologischen Eigenschaften der Spermien eines einzigen Männchen sowie die innerhalb der ganzen Gruppe der getesteten Männchen. Die Spermien-Beweglichkeit korrelierte positiv mit der Länge des Spermienkopfs sowie mit der gesamten Länge der Spermien, während die Geschwindigkeit positiv mit der Länge des mitt leren Abschnitts der Spermien korrelierte. Im Gegensatz dazu konnte kein Zusammenhang zwischen der Schwimmausdauer und Langlebigkkeit der Spermien und ihren morphologischen Eigenschaften gefunden werden. Wir stellten außerdem für die Männchen der Gruppe eine positive Korrelation zwischen den morphologischen Eigenschaften der Spermien fest, wobei diese Eigenschaften innerhalb der einzelnen Individuen stark variierten. Das deutet auf einen möglichen antagonistischen Selektionsdruck auf die Spermien-Morphologie hin. Unsere Untersuchung zeigt, dass bei Sperlingsvögeln von morphologi schen Gegebenheiten eines Spermiums auf dessen Schwimmfähigkeit geschlossen werden kann, wobei diese Zusammenhänge jedoch recht schwach ausgeprägt waren. Generell kann man sagen, dass längere Spermien und Spermienabschnitte schnellere und beweglichere Spermien bedeuten, was zu einem größeren Fortpflanzungserfolg von diesen führen könnte.

Introduction

Sperm competition, the post-copulatory competition among different males' sperm for the fertilization of the same set of ova, is a powerful force shaping male reproductive traits (Parker 1970; Birkhead and Møller 1998, Birkhead 2009; Fitzpatrick and Lüpold 2014). Sperm morphology, the size and shape of the different parts of a spermatozoon, may influence male sperm competitive ability (Immler and Birkhead 2007; Hemmings et al. 2016, reviewed in Simmons and Fitzpatrick 2012) and hence is likely under post-copulatory sexual selection (Humphries et al. 2008; Pitnick et al. 2009; Fitzpatrick and Lüpold 2014). The substantial phenotypic variance that remains in sperm morphological traits has been hypothesized to reflect differential selection patterns acting upon them, but the causes of such substantial variance remain unclear (Fitzpatrick and Lüpold 2014). Directional selection for longer sperm flagellum, which generates the thrust to propel spermatozoa, has long been suggested to operate because longer spermatozoa presumably provide higher sperm competitive ability through faster swimming (Gomendio and Roldan 1991; Simmons and Fitzpatrick 2012). The length of the sperm midpiece, which contains mitochondria that provide energy to the flagellum, has similarly been hypothesized to positively affect sperm swimming ability (Humphries et al. 2008) while the sperm head, by causing drag counteracting the flagellum thrusting force, presumably negatively affects sperm velocity (Humphries et al. 2008). In addition, ratios among morphological traits rather than absolute sperm traits, e.g., lengths, have been suggested as primary morphological modulators of sperm velocity (Humphries et al. 2008). However, despite the potential major influence of sperm morphology on a male's sperm performance and hence competitive ability, the relationships between sperm morphological traits and sperm swimming performance are still unclear, particularly at the intraspecific level (reviewed in Simmons and Fitzpatrick 2012; Fitzpatrick and Lüpold 2014).

Interspecifically, positive relationships between sperm length and sperm swimming velocity have been demonstrated across mammals (Gomendio and Roldan 2008), fish (Fitzpatrick et al. 2009), and birds (Lüpold et al. 2009), corroborating the hypothesis that sperm competition exerts positive directional selection on sperm size. However, at the intraspecific level, functional relationships between sperm morphological traits and sperm performance, and in turn patterns of selection acting on such traits, remain less clear. Although some studies reported a positive relationship between sperm velocity and morphology (e.g., flagellum: Mossman et al. 2009; midpiece: Firman and Simmons 2010; head-to-flagellum ratio: Helfenstein et al. 2010), several did not detect any such relationship (Mossman et al. 2009; Fitzpatrick et al. 2012) and some reported negative ones (e.g., flagellum: Lüpold et al. 2012; Cramer et al. 2015; midpiece and total length: Cramer et al. 2015). Recently, Simpson et al. (2014) showed that sperm with longer flagella and shorter heads displayed higher swimming speeds in externally fertilizing species while the opposite was true in internally fertilizing species, highlighting the context-dependence of those relationships. To make progress in our understanding of relationships between sperm morphology and performance, intraspecific studies undertaken in wild populations experiencing natural variation are hence required.

So far, studies that examined such relationships have mostly focused on sperm velocity because it is an important predictor of males' fertilizing and competitive abilities (e.g., Birkhead et al. 1999; Fitzpatrick and Lüpold 2014). However, sperm performance is multidimensional and other key sperm traits may also depend on the absolute size of,

and ratios between, sperm morphological components. As an example, a negative correlation was observed between head-to-flagellum ratio and sperm longevity in the House Sparrow (*Passer domesticus*) (Helfenstein et al. 2010), hence advocating the need to consider additional sperm traits. Nevertheless, relationships among sperm morphological components and other metrics of sperm performance such as sperm longevity or sperm swimming endurance have rarely been quantified. In addition, theoretical developments and empirical evidence showed that major sperm traits are underlined by trade-offs (e.g., trade-off between sperm velocity and longevity, Levitan 2000), which might themselves be mediated by the length of sperm morphological components (Humphries et al. 2008).

Irrespective of their association with sperm swimming traits, sperm morphological components are themselves of primary interest in sperm competition contexts. In fact, despite the hypothesized directional selection exerted on those traits by post-copulatory competition, substantial within-species and within-individual variation in sperm morphological traits persists (Pitnick et al. 2009; Helfenstein et al. 2010; Fitzpatrick and Lüpold 2014; Rojas Mora et al. 2017). The evolutionary maintenance of such variation in species with significant sperm competition is paradoxical because sperm competition has been hypothesized to exert selection towards an optimal sperm design (Parker 1998; Birkhead and Pizzari 2002; Birkhead 2009), and comparative analyses accordingly show a decrease in within-ejaculate variance with increasing intensity of sperm competition (Kleven et al. 2008; Lifjeld et al. 2010). In contrast, a recent experiment on House Sparrows showed that within-male variation in sperm morphology depended on male social status, suggesting the existence of selective agents promoting the maintenance of within-ejaculate variation in sperm morphology (Rojas Mora et al. 2017). The maintenance of substantial within-individual variance might also stem from phenotypic synergies and/or trade-offs among sperm morphological traits. To make progress in our understanding of these selection patterns, one must quantify the within- and among individual relationships among sperm morphological traits at the phenotypic level.

We used a wild population of Great Tits, a passerine bird for which sperm morphological components (i.e., sperm head, midpiece, and flagellum) can be clearly distinguished, to quantify the among-male relationships between sperm morphological components (i.e., absolute lengths and ratios among components) and sperm swimming performance measured as sperm motility, velocity, longevity, and swimming endurance. Further, we quantified the within- and among-male relationships across sperm morphological components.

Materials and methods

This study was conducted in spring (April–June) of 2008 and 2009 in a natural population of Great Tits breeding in nest boxes in a forest near Bern, Switzerland (46°7′N, 7°8′E). Nest boxes were regularly visited from the beginning of the breeding season to determine hatching dates in 65 (2008) and 67 (2009) nests. In both years, all adult males were captured at their nest 7 days post-hatch. For each male, two sperm samples (ca. 0.5 µl) were collected by gentle cloacal massage (Wolfson 1952); one sample was immediately used for sperm swimming performance analyses and the second one was fixed in 100 µl formalin (5%) for later sperm morphological measurements. In 2008, as part of another experiment, brood size was manipulated on day 2 post-hatch [i.e., either augmented with two nestlings ($n = 31$ broods) or kept as original ($n = 34$ broods)] to quantify the effect of increased workload on male oxidative status (Losdat et al. 2011). Here, since the brood size manipulation may have influenced male sperm performance because of increased workload and energy expenditure, data from males whose brood size was augmented ($n = 31$) were excluded from all analyses. Altogether, this study included sperm data from 75 males.

Sperm performance

The first sperm sample was mixed with 50 µl of pre-warmed Dulbecco's Modified Eagle's Medium (4500 mg glucose/l, 110 mg sodium pyruvate/l, 4 mM L-glutamine, Sigma-Aldrich, Switzerland). Then 9 µl of sperm/buffer solution was immediately transferred under a dark-field phase contrast microscope. Sperm motion was video recorded for 2 min at 40 °C (using a microscope thermoplate) and analyzed after 0, 60, and 120 s of video-recording using a Computer Assisted Sperm Analysis plug-in implemented in ImageJ (Wilson-Leedy and Ingermann 2007). We assessed sperm performance as the percentage of motile sperm at time 0 (i.e., sperm motility) and as sperm straight line velocity (measured across motile sperm) at time 0 because those traits are primary predictors of fertilizing and competitive abilities (Pizzari et al. 2008; Pizzari and Parker 2009). We additionally used the rate at which sperm motility declines across the 0–2 min of video-recording (hereafter referred to as "sperm longevity") and the rate at which sperm velocity declines across the 0–2 min of video-recording (hereafter referred to as "sperm swimming endurance"). To estimate the repeatability of sperm motion analyses, we reanalyzed different fractions of the sperm videos briefly after ejaculation for 66 males, which yielded very high measurement repeatability (sperm motility: $r = 0.94$, $p < 0.0001$; sperm velocity: $r = 0.97$, $p < 0.0001$).

Sperm morphological components

A drop of sperm/formalin solution was deposited on a microscope slide, which was subsequently air-dried, rinsed with distilled water, and fixed with Glycogel (Roth, AG). Photographs of individual spermatozoa were then taken with an Olympus E-520 digital camera mounted on a phase contrast Olympus BX43 microscope at ×400 magnification. We aimed at selecting ca. ten intact spermatozoa (no broken tail, midpiece correctly coiled around the flagellum, parts not separated from each other) for each individual male. Measuring ten spermatozoa per male to assess sperm morphology provides a representative sample of sperm morphology for each male (Laskemoen et al. 2007, 2013; Rowe et al. 2015). For each spermatozoon, we measured total length, head length, midpiece length (straight length of the part twisted around the flagellum), and flagellum length using ImageJ software at a resolution of 0.119 µm/pixel. To assess measurement repeatability, all spermatozoa were measured a second time in a distinct measurement session. In addition to absolute sizes, we calculated the ratios among all three sperm trait lengths. We initially aimed at summarizing sperm morphological design in a single measurement by computing a principal component analysis including sperm head, midpiece, and flagellum lengths, as recently done in a passerine bird (Rojas Mora et al. 2017). However, since the first principal component was correlated with midpiece and flagellum lengths but uncorrelated with head length, it only described midpiece and flagellum length, irrespective of head length, and hence did not characterize a sperm "design". We therefore used the three absolute lengths and ratios as sperm morphological traits. SL did all the morphological measurements blindly with respect to bird identity.

Statistical analyses

To partition the total phenotypic variance in each sperm morphological component, we used random-effect models with no fixed effect, but with male identity and spermatozoa identity nested within male identity as random effects to account for the two measurements taken per spermatozoon and for the multiple spermatozoa per male.

To quantify among-male relationships between sperm morphological traits and sperm motility and velocity, we used linear models including sperm motility or velocity as dependent variables. Independent variables were head length, midpiece length, flagellum length, total length, and the three associated ratios (head/flagellum, head/midpiece, and midpiece/flagellum). We also included Julian date as a covariate to control for seasonal effects. We also originally included the time of day as a covariate but since it was never significant we chose not to include it in the final models to avoid overparameterization.

To quantify the relationships between morphological traits and sperm longevity and swimming endurance, we used mixed-effect models including sperm motility across time (i.e., sperm longevity) and sperm velocity across time (i.e., sperm swimming endurance) as dependent variables. Independent variables were head length, midpiece length, flagellum length, total lengths, and the three associated ratios. Those repeated models included fixed effects of time since the start of video recording and the morphological trait by time interaction. Male identity was fitted as a random effect to control for the three values per male. Sperm motility was logit-transformed to achieve normality of the residuals.

To quantify within- and among-individual relationships between the three sperm morphological traits, we ran three linear mixed-effect models (i.e., head–flagellum, head–midpiece, and midpiece–flagellum). To disentangle within- versus among-individual male effects, we used the "within-subject centering" method through which within- and between-male effects are quantified separately by deriving two new predictors (Van De Pol and Wright 2009). The within-male-effect predictor is calculated for each male by subtracting its mean trait value (e.g., a male's mean sperm head length) from each of its observations for that trait (e.g., each of a male's individual sperm head lengths), thereby getting rid of any among-individual variation. The between-male-effect predictor is the mean trait value for each male (e.g., a male's mean sperm head length) such that all observations for a given male are given the same value. Both predictors, calculated for sperm head, midpiece, and flagellum lengths, were then included as fixed effects in three separate models. We modeled random male identity effects to account for the multiple spermatozoa measured within a single sperm sample.

Given the multiplicity of tests conducted, we used false discovery rate (FDR) correction for multiple testing (Benjamini and Hochberg 1995), a method that maintains higher statistical power than the Bonferroni correction but still controls for type I errors. All analyses were run in R software version 3.3.2 (R Core Team 2016) using the "nlme" package (Pinheiro et al. 2011) for linear mixed-effect models.

Results

Data structure

We collected sperm samples from 75 males (54 measurements in 2009 and 21 in 2008) and the mean number of individual spermatozoa measured per sperm sample was 8 (range 4–10). For two males sampled in both years we randomly chose sperm values from a single sampling event to simplify models.

Variance partitioning in sperm morphological traits

Measurement error was low for all four morphological traits, accounting for a small proportion of the total within-ejaculate variance (head length, 14%; midpiece length, 4%; flagellum length, 4%; total sperm length, 4%). Within-ejaculate variance in sperm morphology was substantial, approximately accounting for half of the total variance in each sperm morphological trait (head, 60%; midpiece, 48%; flagellum, 47%; total length, 44%; Fig. S1) and the same pattern was observed for between-male variance, except for sperm head length (head, 26%; midpiece, 48%; flagellum, 49%; total length, 52% of the total variance; Fig. S1).

Among-male relationships between sperm performance and sperm morphological traits

Sperm velocity was positively related to sperm midpiece length ($\beta = 1.46 \pm 0.71$, $F_{1,72} = 6.24$, $p = 0.015$; Fig. 1), and this relationship remained significant after FDR correction. All other sperm trait lengths or ratios were not significantly associated with sperm velocity (all $F_{1,72} < 0.14$, $p > 0.12$). Sperm velocity was generally positively associated with Julian date (all $F_{1,72} > 8.0$, $p < 0.01$). Sperm motility was significantly positively related to sperm total length ($\beta = 0.07 \pm 0.03$, $F_{1,72} = 5.13$, $p = 0.027$; Fig. 1) and to sperm head length ($\beta = 0.47 \pm 0.17$, $F_{1,73} = 7.96$, $p = 0.006$; Fig. 1), which were both still significant after FDR correction. Sperm motility was unrelated to midpiece or flagellum or to any of the ratios (all $F_{1,72} < 0.14$, $p > 0.14$). The linear mixed-effect models testing for effects on the change in sperm velocity/motility with time (Table 1) showed that sperm swimming endurance (i.e., velocity across time) and sperm longevity (i.e., motility across time) were both unrelated to any sperm morphological trait or ratio, as shown by the non-significance of all interactions with time (Table 1).

Within- and among-individual relationships between morphological traits

Among males, we found significant positive linear relationships between all three sperm morphological traits

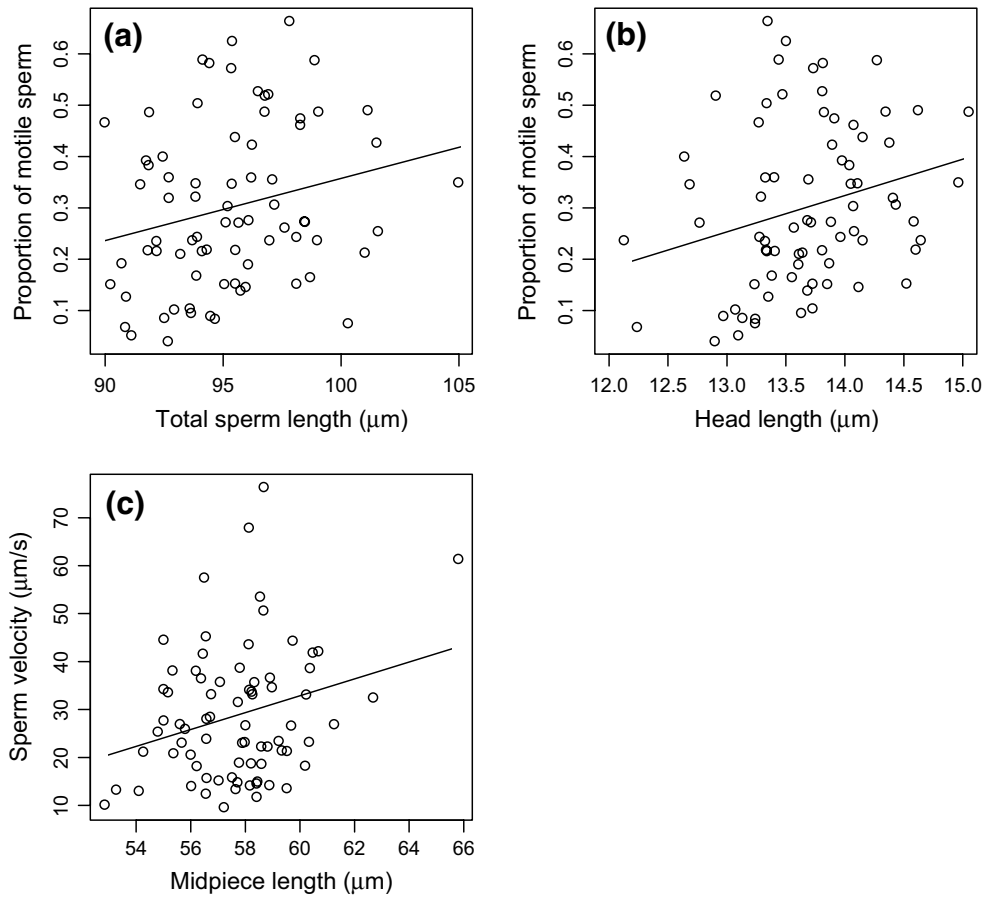

Fig. 1 Among-male relationships between **a** sperm motility and total sperm length, **b** sperm motility and sperm head length, and **c** sperm velocity and sperm midpiece length. Each dot corresponds to the mean value for each individual male. Lines represent linear regression lines across all 75 sperm samples collected from 75 males

Table 1 Linear mixed-effect models testing for among-individual relationships between sperm performance (sperm swimming endurance and longevity) and sperm morphological traits

Effect	**Sperm swimming endurance**			**Sperm longevity**		
	Estimate ± SE	F_{df}	p	Estimate ± SE	F_{df}	P
Total length	0.39 ± 0.60	$3.55_{1,72}$	0.06	**0.02 ± 0.06**	$\mathbf{5.96_{1,72}}$	**0.017**
Time	**− 0.40 ± 0.40**	$\mathbf{8.57_{1,146}}$	**0.004**	**− 0.14 ± 0.06**	$\mathbf{69.6_{1,146}}$	**< 0.001**
Julian date	**0.86 ± 0.21**	$\mathbf{16.80_{1,72}}$	**0.0001**	**0.036 ± 0.018**	$\mathbf{4.41_{1,71}}$	**0.04***
Total length × time	0.004 ± 0.004	$0.84_{1,146}$	0.36	0.001 ± 0.001	$0.15_{1,147}$	0.07
Head length	0.54 ± 3.15	$0.61_{1,72}$	0.44	0.25 ± 0.32	$\mathbf{3.35_{1,72}}$	0.07
Time	**− 0.42 ± 0.30**	$\mathbf{8.59_{1,146}}$	**0.004**	**− 0.06 ± 0.05**	$\mathbf{67.8_{1,146}}$	**< 0.001**
Julian date	**0.91 ± 0.21**	$\mathbf{18.90_{1,72}}$	**0.0001**	**0.04 ± 0.02**	$\mathbf{6.27_{1,72}}$	**0.01**
Head length × time	0.03 ± 0.02	$1.66_{1,146}$	0.20	0.003 ± 0.004	$0.69_{1,146}$	0.41
Midpiece size	**1.40 ± 0.85**	$\mathbf{6.25_{1,72}}$	**0.015**	**0.04 ± 0.09**	$\mathbf{5.80_{1,72}}$	**0.02**
Time	**− 0.08 ± 0.34**	$\mathbf{8.53_{1,146}}$	**0.004**	**− 0.11 ± 0.06**	$\mathbf{69.4_{1,146}}$	**< 0.001**
Julian date	**0.85 ± 0.21**	$\mathbf{16.86_{1,72}}$	**0.0001**	**0.04 ± 0.02**	$\mathbf{4.72_{1,72}}$	**0.03***
Midpiece size × time	0.0007 ± 0.0060	$0.01_{1,146}$	0.91	0.002 ± 0.001	$2.89_{1,146}$	0.09
Flagellum length	0.45 ± 0.66	$3.56_{1,72}$	0.06	**0.01 ± 0.07**	$\mathbf{5.11_{1,72}}$	**0.03***
Time	**− 0.31 ± 0.37**	$\mathbf{8.56_{1,146}}$	**0.009**	**− 0.13 ± 0.06**	$\mathbf{69.7_{1,146}}$	**< 0.001**
Julian date	**0.86 ± 0.21**	$\mathbf{16.59_{1,72}}$	**0.0001**	**0.04 ± 0.02**	$\mathbf{4.36_{1,72}}$	**0.04***
Flagellum length × time	0.003 ± 0.005	$0.54_{1,146}$	0.46	0.001 ± 0.001	$3.34_{1,146}$	0.07
Head/flagellum ratio	− 74.5 ± 246.4	$0.45_{1,72}$	0.50	16.34 ± 25.30	$\mathbf{0.001_{1,72}}$	0.98
Time	**− 0.22 ± 0.28**	$\mathbf{8.52_{1,146}}$	**0.004**	**0.013 ± 0.05**	$\mathbf{67.9_{1,146}}$	**< 0.001**
Julian date	**0.91 ± 0.21**	$\mathbf{18.31_{1,72}}$	**0.0001**	**0.04 ± 0.02**	$\mathbf{6.09_{1,72}}$	**0.02**
Head/flagellum × time	1.12 ± 1.70	$0.43_{1,146}$	0.51	− 0.18 ± 0.28	$0.40_{1,146}$	0.53
Head/midpiece ratio	− 166.8 ± 162.9	$1.24_{1,72}$	0.27	7.02 ± 16.80	$\mathbf{0.05_{1,72}}$	0.82
Time	**− 0.33 ± 0.27**	$\mathbf{8.58_{1,146}}$	**0.004**	**0.01 ± 0.04**	$\mathbf{67.9_{1,146}}$	**< 0.001**
Julian date	**0.89 ± 0.40**	$\mathbf{18.01_{1,72}}$	**0.0001**	**0.04 ± 0.02**	$\mathbf{5.92_{1,72}}$	**0.017**
Head/midpiece × time	1.24 ± 1.12	$1.22_{1,146}$	0.27	− 0.12 ± 0.19	$0.37_{1,146}$	0.54
Midpiece/flagellum ratio	105.6 ± 85.4	$0.83_{1,73}$	0.36	3.16 ± 8.84	$0.20_{1,73}$	0.66
Time	**0.24 ± 0.42**	$\mathbf{8.51_{1,146}}$	**0.004**	**− 0.02 ± 0.07**	$\mathbf{67.6_{1,146}}$	**< 0.001**
Julian date	**0.91 ± 0.21**	$\mathbf{19.36_{1,72}}$	**0.0001**	**0.04 ± 0.02**	$\mathbf{6.11_{1,72}}$	**0.015**
Midpiece/flagellum × time	− 0.39 ± 0.59	$0.43_{1,146}$	0.51	0.005 ± 0.10	$0.003_{1,146}$	0.96

Significant terms are highlighted in bold

*$p > 0.05$ after testing for false discovery rates

(head–flagellum, head–midpiece, midpiece–flagellum; Table 2, Fig. 2). Within males, midpiece length was significantly positively correlated with flagellum length but there was no significant relationship between head length and midpiece length, or between head length and flagellum length (Table 2, Fig. 2).

Table 2 Linear mixed effect models testing for within- and between-individual relationships among morphological traits

Model	Within-individual effect			Between-individual effect		
	Estimate ± SE	F_{df}	p	Estimate ± SE	F_{df}	p
Head–midpiece	− 0.002 ± 0.02	$0.015_{1,525}$	0.90	**0.08 ± 0.03**	$\mathbf{7.15_{1,75}}$	**0.01**
Head–flagellum	− 0.02 ± 0.01	$2.45_{1,525}$	0.12	**0.07 ± 0.02**	$\mathbf{10.0_{1,75}}$	**0.002**
Midpiece–flagellum	**0.31 ± 0.03**	$\mathbf{101.8_{1,525}}$	**< 0.0001**	**0.49 ± 0.07**	$\mathbf{53.7_{1,75}}$	**< 0.0001**

Significant terms (highlighted in bold) all remained significant after correcting for multiple testing using the Benjamini–Hochberg false discovery rate correction

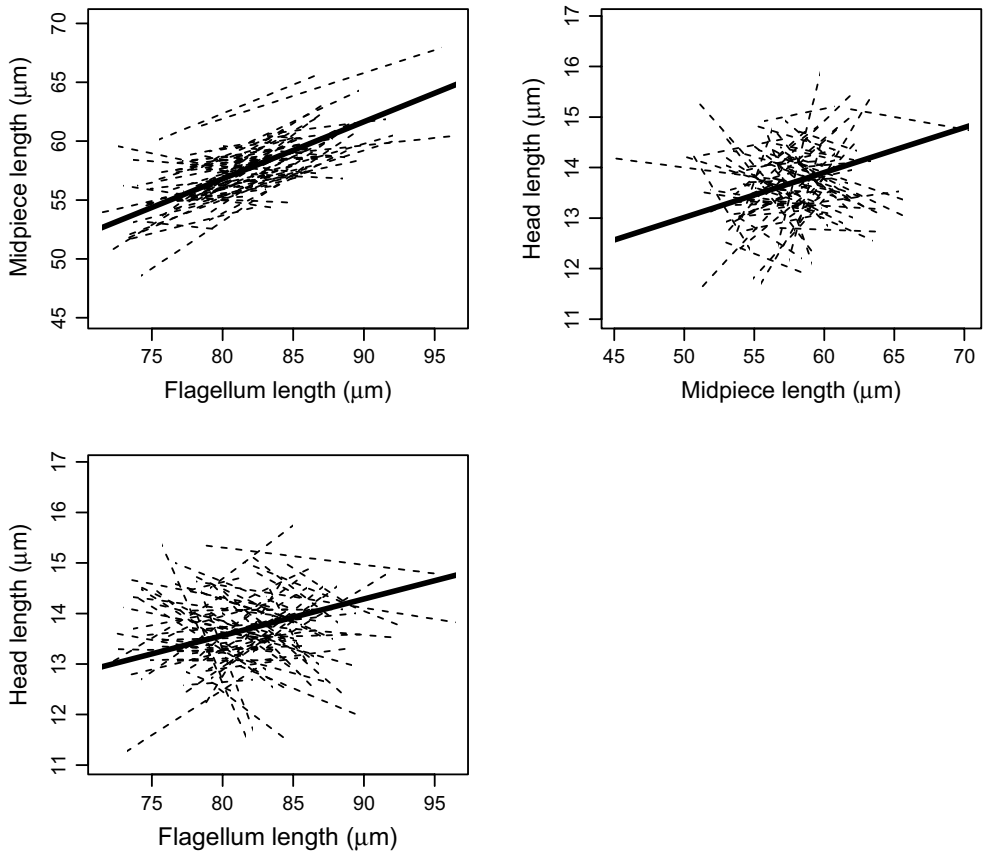

Fig. 2 Within- and between-individual relationships among morphological traits (total of 75 sperm samples from 75 males). Among-males, regression slopes (solid lines) are all significantly different from zero. Within males (dashed lines; each line represents the linear regression line fitted within one sperm sample), only the linear regression between midpiece and flagellum is significant

Discussion

We observed weak but positive relationships between sperm morphological components and sperm swimming performance, generally showing that longer sperm components presumably provide higher sperm swimming ability in wild Great Tits. In contrast, sperm swimming endurance and longevity were unrelated to morphological components. Among males, sperm midpiece, flagellum, and head were all positively correlated with each other but those relationships were mostly absent within males, suggesting the existence of antagonistic selective pressures fueling within-male variation in sperm morphology.

Sperm swimming performance and morphology

Sperm total length and head length were positively correlated with sperm motility, which is in line with some previous studies (e.g., Mossman et al. 2009; Firman and Simmons 2010, but not others, see "Introduction"). We also report a positive correlation between midpiece length and sperm velocity, therefore corroborating the assumption that longer sperm are faster (Gomendio and Roldan 1991; Simmons and Fitzpatrick 2012) and supporting the hypothesis that directional positive selection may act on the length of sperm morphological components at the sperm population level (i.e., among males). Our results have prime evolutionary implications since male Great Tits producing longer sperm, which is in turn faster and/or more motile, may achieve higher fertilization success (Snook 2005; Gomendio et al. 2007; Fitzpatrick and Lüpold 2014). In fact, a recent study empirically demonstrated the fitness benefit of producing longer sperm in Zebra Finches (*Taeniopygia guttata*); longer sperm fertilized more eggs (Bennison et al. 2015). Therefore, in species with a similar mode of fertilization (i.e., internal) and similar female reproductive tract environment (i.e., sperm storage tubules) such as Great Tits and other passerines, males producing longer sperm could similarly achieve higher Darwinian fitness by siring more offspring. Interestingly, sperm velocity and motility were positively related to different morphological components. For instance, sperm velocity was only positively correlated with sperm midpiece length, as observed in mice (Fisher et al. 2016), suggesting that increased energy production in sperm cells

is primarily beneficial to sperm velocity. In contrast, sperm motility was positively correlated with both total sperm length and head length (not with sperm midpiece length), suggesting that sperm length itself is the primary determinant of motility. Importantly, we investigated the relationships between sperm velocity/motility and sperm morphology among males, i.e., using mean trait values per male, but could not test such relationships within males, i.e., using actual trait values for each individual sperm cell, because this would require high-resolution videos allowing direct morphometric measurements of sperm cells. Simpson et al. (2014) and Fitzpatrick et al. (2010) stressed the importance of considering intra-male variation because it could mask among-male relationships, as observed in Fitzpatrick et al. (2010) where sperm velocity–morphology relationships were absent among males but significant within males. In our study, however, we observed significant among-male velocity–morphology relationships and hence do not face this potential issue.

In general, the inconsistent patterns of sperm performance–morphology relationships observed among species, as well as the relatively weak correlations observed here, likely stem from different sources. First, the fertilization environment may strongly influence the existence and direction of those relationships. Recently, Simpson et al. (2014) observed that sperm with longer flagella and shorter heads were faster in externally fertilizing species but slower in internally fertilizing species, hence providing evidence of opposite patterns of sperm velocity–morphology relationships between modes of fertilization, potentially due to differences in fluid viscosity (Simpson et al. 2014). Second, in internal fertilizing species, differences among species may also reflect differences among taxa in the female reproductive tract's microenvironment. In passerine birds, inseminated sperm is stored in sperm storage tubules until it is released for fertilization (Birkhead and Møller 1998; Froman 2003). In this context, higher sperm velocity supposedly predicts the number of sperm being initially stored in the female storage tubules but high velocity may come at the expense of longevity, which predicts the risk of sperm getting flushed out of the storage tubules and not reaching the site of fertilization (Froman 2003). Therefore, selection pressures acting on sperm morphological components may strongly differ between species with vs. without sperm storage tubules. Third, in species with sperm storage tubules, sperm length has been suggested to co-evolve with female sperm storage tubules' length leading to a general positive relationship between sperm size and the size of the sperm storage organs (Briskie and Montgomerie 1992; Briskie et al. 1997), a process that may also be expected to exert selection on sperm morphological traits.

Within-male variation in sperm morphological components

Despite the positive relationships we report, within-male variation in all sperm morphological components was substantial, accounting for a striking 44–60% of the total variance in those components. Such ample within-male variance has been observed repeatedly in several species exhibiting various levels of sperm competition (e.g., Immler et al. 2008; Helfenstein et al. 2010; Calhim et al. 2011; Blengini et al. 2014) but yet remains paradoxical since within-male variance might be expected to erode because of the selection acting on those traits. The evolutionary maintenance of this substantial variance remains puzzling in light of the general negative relationship between within-male variance in sperm morphology and the risk of sperm competition observed in comparative studies (Immler et al. 2008; Lifjeld et al. 2010). One explanation for this pattern could be the strategic production by males of sperm with variable total length and/or with variable length of sperm components. In fact, short or long sperm may be expected to be more successful depending on the current fertilizing situation or yet on the inseminated female, such that producing sperm with highly variable morphological component lengths might be adaptive in sperm competition contexts and hence selected for (Helfenstein et al. 2010). Illustrating this, Rojas Mora et al. (2017) experimentally showed in House Sparrows that within-male variation in sperm morphology depended on male social status. Such context-dependent modulation of within-ejaculate variance may hence promote the evolutionary maintenance of within-individual variation in sperm morphology. However, a recent study showed that within-male variance in morphological components was lower for sperm trapped in the perivitelline layer (i.e., sperm that successfully reached the site of fertilization) than in sperm not reaching the site of fertilization (Hemmings et al. 2016), thereby illustrating the higher fertilizing ability of ejaculates with lower morphological variance and suggesting stabilizing selection on sperm morphology. Lastly, a recent study by Rojas Mora et al. (2017) suggests that variation in sperm competition levels within species and among males could also explain the evolutionary maintenance of within-ejaculate variation in sperm morphology.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

Ethical approval This work was conducted under license of the Ethical Committee of the Agricultural Office of the Canton Bern (Switzerland). Ringing permits were provided by the Swiss Federal Agency for Environment, Forests and Landscapes and the Swiss Ornithological Institute (Sempach, Switzerland).

Data availability Data used in this study is available in the Excel file provided as supplementary material.

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