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Postcopulatory sexual selection is associated with accelerated evolution of sperm morphology

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Rapid diversification of sexual traits is frequently attributed to sexual selection, though explicit tests of this hypothesis remain limited. Spermatozoa exhibit remarkable variability in size and shape, and studies report a correlation between sperm morphology (sperm length and shape) and sperm competition risk or female reproductive tract morphology. However, whether postcopulatory processes (e.g., sperm competition and cryptic female choice) influence the speed of evolutionary diversification in sperm form is unknown. Using passerine birds, we quantified evolutionary rates of sperm length divergence among lineages (i.e., species pairs) and determined whether these rates varied with the level of sperm competition (estimated as relative testes mass). We found that relative testes mass was significantly and positively associated with more rapid phenotypic divergence in sperm midpiece and flagellum lengths, as well as total sperm length. In contrast, there was no association between relative testes mass and rates of evolutionary divergence in sperm head size, and models suggested that head length is evolutionarily constrained. Our results are the first to show an association between the strength of sperm competition and the speed of sperm evolution, and suggest that postcopulatory sexual selection promotes rapid evolutionary diversification of sperm morphology.

KEY WORDS: **Birds, evolutionary diversification, evolutionary rate, passerine, sperm competition.**

Understanding the processes that promote trait diversification is a central theme in evolutionary biology research. Considerable attention has been directed toward understanding the selective processes underlying phenotypic variation, and such variation is often attributed to differences in the strength and direction of sexual selection among populations (Price and Whalen 2009; Rodríguez et al. 2013; Seddon et al. 2013). Spermatozoa exhibit remarkable levels of morphological diversity across all levels of organization: among species, among populations of the same species, among males within a population, as well as both among and within ejaculates from a single individual (Pitnick et al. 2009). Differences in sperm length between populations or closely related taxa suggest that sperm size can evolve rapidly (Landry et al. 2003; Pitnick et al. 2009; Hogner et al. 2013). Moreover, artificial selection experiments show that sperm length responds swiftly to selection in a range of animal groups (Woolley 1971; Morrow and Gage 2001; Miller and Pitnick 2002; Dobler and Hosken 2010, but see Firman and Simmons 2010). Thus sperm size appears to be evolutionarily highly labile. The evolutionary processes driving the diversification of sperm form, however, remain poorly understood.

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When females mate with multiple males during a single reproductive episode, ejaculates from rival males may overlap in the female reproductive tract generating competition among males for fertilization success (i.e., sperm competition, Parker 1970) and the potential for female control over paternity (i.e., cryptic female choice, Thornhill 1983). Selection imposed through sperm competition and cryptic female choice (i.e., postcopulatory sexual selection) is thought to influence the evolution of sperm morphology in many taxa. For example, numerous comparative studies have documented an association between sperm length and sperm competition risk or female reproductive tract morphology (reviewed in Snook 2005; Pizzari and Parker 2009; Simmons and Fitzpatrick 2012). More generally, sexual selection is credited with promoting rapid diversification of sexual traits (e.g., plumage, genitalia, Price and Whalen 2009; Fitzpatrick et al. 2012; Seddon et al. 2013), and thus playing a role in the process of speciation, especially under a competitive mating scenario (Coyne and Orr 2004). Rapid divergence in sperm size has also been putatively linked to sexual selection in the form of sperm competition (Hogner et al. 2013). However, critical tests of the relationship between sexual selection and evolutionary diversification in reproductive traits are limited, and whether the rate of sperm evolution varies across taxa in response to variation in the strength of postcopulatory sexual selection is currently unknown.

In this study, we investigated how sperm competition influences the speed of evolutionary change in sperm size using data from passerine birds. We used relative testes mass (rTM) (i.e., testes mass corrected for body mass) as our index of sperm competition because it is associated with both increases in the number of mating partners per female and the incidence of multiple paternity in birds (Møller and Briskie 1995; Pitcher et al. 2005), as well as a range of other taxa (Harcourt et al. 1995; Hosken and Ward 2001; Soulsbury 2010). More specifically, we tested the hypothesis that the rate of evolutionary diversification of sperm phenotypic traits is associated with the strength of sperm competition. Using recently developed comparative methods and data on phenotypic divergence and evolutionary age for phylogenetically independent species pairs, we quantified rates of evolution in sperm morphological traits under two evolutionary models: Brownian motion (BM), or "random" evolution that is proportional to branch length; and Ornstein–Uhlenbeck (OU), or "constrained" evolution. We compared the fit of BM and OU models with a single evolutionary rate applied to all species pairs to models in which the rate of evolution varied with the strength of sperm competition.

Materials and Methods

SPERM MORPHOLOGY

We identified all available species of passerine bird from the sperm collection database at the Natural History Museum in Oslo (NHMO) for which we could obtain measures of sperm morphology from three or more males. Because measuring few individuals per species increases the probability that species values will be estimated with error, and thus increases the risk of type I error in comparative studies (Harmon and Losos 2005), we attempted to maximize intraspecific sample size. However, we chose to include species for which the mean data were based on as few as three males, as the risk of inflated type I errors in our dataset was negligible. More specifically, following the recommendations of Harmon and Losos (2005), we performed an ANOVA on all sperm components and sperm total length, and found that ≥93% of variation in our dataset was distributed among species (head: $F_{113,1453} = 171.3$, $P < 0.001$, $R^2 = 0.93$; midpiece: $F_{113,1453} = 5980$, $P < 0.001$, $R^2 = 0.99$; flagellum: $F_{113,1453} = 4458$, $P < 0.001$, $R^2 = 0.99$; total: $F_{113,1453} = 4584$, $P < 0.001$, $R^2 = 0.99$), which suggests that the type I error rate is satisfactorily low (Harmon and Losos 2005). Thus, in total we used data on sperm morphology from 1567 males belonging to 114 species of passerine birds from 30 families (see Table S1).

Sperm (approx. 1–5 μl) was collected from adult male birds using cloacal massage (Wolfson 1952) and fixed in 300 μl of 5% buffered formaldehyde solution. To assess sperm morphology, a subsample of the fixed sperm was placed on a microscope slide and allowed to air dry before being gently rinsed with distilled water and allowed to air dry again. Digital images of sperm were then captured at 160× or 320× magnification using a camera (Leica DFC420, Leica Microsystems, Heerbrugg, Switzerland) connected to a digital light microscope (Leica DM6000B), and sperm traits were measured using digital image analysis (Leica Application suite version 2.6.0 R1). Following Laskemoen et al. (2012), we obtained measures (±0.1 μm) of the following sperm traits: (1) head length, (2) midpiece length, (3) flagellum length, and (4) total sperm length. For each individual, 10 morphologically normal and undamaged sperm were analyzed to obtain measurements, which sufficiently captures mean trait values for an individual (Immler et al. 2007; Laskemoen et al. 2007). For each sperm trait, we used the means within individuals to calculate the mean for each species (mean = 14 individuals per species, range = 3–100).

PHYLOGENY

We generated a phylogeny for the 114 species included in our dataset (see Fig. S1) from the recently published time-calibrated molecular phylogeny of all extant avian species (Jetz et al. 2012). Specifically, we downloaded 1000 randomly selected phylogenetic trees for our species from those available at www.birdtree.org using the Hackett sequenced species backbone. We then summarized the sample of trees onto a single maximum clade credibility (MCC) tree with mean node heights using TreeAnnotator version 1.8.0 (BEAST, Drummond et al. 2012).

INDEX OF SPERM COMPETITION

We used rTM as a proxy measure for the strength of sperm competition following previous authors (e.g., Immler et al. 2011; Lüpold et al. 2011; Tourmente et al. 2011). However, because this analysis required a single continuous variable as our unit of measure, we obtained the residuals from a PGLS regression (implemented in the R package "caper") of combined testes mass (CTM) on body mass (both log-transformed) using the full 114 species phylogeny. Data on CTM and body mass were obtained from the literature (Haftorn 1971; Dunning 1993; Calhim and Birkhead 2007; Laskemoen et al. 2008; Øigarden et al. 2010; Rowe and Pruett-Jones 2013) from males collected (under license) during the breeding season (own data) or from museum sources and personal communications with researchers (see Table S1 for details).

We acknowledge that rTM is not a perfect index of sperm competition, both because estimates of testes mass can be subject to error (Calhim and Birkhead 2007) and because evolutionary increases in testes size may also occur in response to factors other than sperm competition (e.g., male mating rate, Vahed and Parker 2011). Moreover, selection has been shown to favor adaptations in testes that influence sperm production beyond that of simple increases in testes size (e.g., Lüpold et al. 2009a). Thus rTM may in some instances underestimate the intensity of postcopulatory sexual selection, and should therefore be used with some caution (see also Simmons and Fitzpatrick 2012). However, in the absence of more direct measures of sperm competition (e.g., female multiple mating rate), rTM is the best proxy currently available for our study. Moreover, rTM was significantly, positively associated with extra-pair paternity levels in the subset of our data for which extra-pair paternity data were available (extra-pair young: $r = 0.56$ [95% confidence interval (CI) = 0.36–0.70], df = 56, $t = 5.05$, $P < 0.0001$, $\lambda = 0.34^{0.26,\ <0.0001}$; extra-pair broods: $r = 0.58$ [95% CI = 0.37–0.71], df = 52, $t = 5.08$, $P < 0.0001$, $\lambda = 0^{1,\ <0.0001}$), supporting our use of rTM as a proxy for the strength of sperm competition.

EVOLUTIONARY RATES ANALYSIS

In our dataset, total sperm length, as well as sperm midpiece and flagellum length, was positively associated with rTM, whereas sperm head length was not (see Supporting Information text and Table S2). Our main aim, however, was to determine whether the speed of evolutionary diversification of sperm length varied with the strength of sperm competition. We therefore quantified evolutionary rates of trait divergence using a recently developed species pairs approach (Weir and Lawson 2014). For these methods, the unit of analysis is the degree of phenotypic divergence between species in a lineage (i.e., species pair). Thus, from the full dataset of 114 passerine species, we identified 38 phylogenetically independent (i.e., non-nested) species pairs (see Fig. S1). For each sperm trait (head, midpiece, flagellum, and total sperm length), we estimated phenotypic divergence for paired taxa as the Euclidean distance between their log-transformed trait values. As it is important to consider estimates of trait divergence in the context of evolutionary time (i.e., rates of trait divergence), we estimated the evolutionary age (i.e., node age) of each pair using the branch length separating the species, which we obtained from the time-calibrated phylogeny for all 114 species.

We used rTM as a proxy measure for the strength of sperm competition. Specifically, our index of sperm competition for each lineage was the mean of the two rTM values for each member of the species pair. We added a constant to all values such that our lowest value of rTM was zero. Finally, to avoid characterizing the strength of sperm competition incorrectly for a lineage, we excluded species pairs for which rTM values differed between the two species by two or more standard deviations ($n = 2$ pairs) of the total range of rTM values. Thus only 36 of the 38 possible species pairs were included in our analyses (see Fig. S1).

Next, we modeled change in trait divergence between species pairs under two evolutionary models: a random walk model (modeled as BM); and a random walk model within a constrained trait space (modeled as an OU process), whereby trait values are evolutionarily constrained and have a greater tendency to return to a central starting value than expected under BM. More specifically, we modeled trait evolution using BM and OU models with a constant rate of evolution (β; BM null, OU null) and BM and OU models in which β was allowed to vary linearly with rTM (BM linear, OU linear). OU models also include an evolutionary constraint parameter (α), which was either constant (OU null model) or assumed to be a linear function of rTM (OU linear model). This parameter, α, reflects the "attraction" toward an optimal phenotypic value (i.e., the midpoint value between each member of the species pair), and as α approaches 0, the model collapses to a BM model. Thus, in total we quantified evolutionary rates of sperm length divergence under four models: BM null, OU null, BM linear, and OU linear. We used simulation to show that these models provided robust parameter estimates with essentially no bias for our dataset (see Supporting Information text and Tables S3, S4).

Models were compared using the Akaike Information Criterion corrected (AICc) for small sample size; the model with the lowest AICc value best explains the data. For each trait, we used simulation to calculate the threshold level of difference in AICc scores required to reject a null model without the effect of rTM while maintaining a type I error rate ≤ 0.05 (see Supporting Information text and Table S5). We also calculated Akaike weights for all models and used both AICc values and Akaike weights to assess model support. Finally, for midpiece, flagellum, and total sperm length we used profile likelihood to estimate the 95% CI for the slope parameters describing the relationship between evolutionary rate (β) and rTM under the best-fit model (BM linear). The 95% CI includes all slope values that lie within

Table 1. ΔAICc scores (AICc − AICc score for best-fit model) and Akaike (AICc) weights showing support for evolutionary models in which the rate of evolutionary divergence in sperm traits is either independent of sperm competition (null model) or linearly associated with the strength of sperm competition (linear model).

	Brownian motion (BM) models						Ornstein–Uhlenbeck (OU) models						
	BM null			BM linear			OU null			OU linear			Threshold
	N	ΔAICc	AICc weight	*N*	ΔAICc	AICc weight	*N*	ΔAICc	AICc weight	*N*	ΔAICc	AICc weight	ΔAICc
Head length	1	1.18	0.2526	2	3.30	0.0876	2	**0***	0.4560	4	1.61	0.2038	2.2
Midpiece length	1	3.62	0.1248	2	**0***	0.7630	2	5.56	0.0473	4	4.93	0.0649	2.6
Flagellum length	1	7.70	0.0187	2	**0***	0.8784	2	9.95	0.0061	4	4.41	0.0968	2.5
Total sperm length	1	4.78	0.0742	2	**0***	0.8097	2	7.03	0.0241	4	4.35	0.0920	2.5

For each sperm trait, the model with the lowest AICc value (i.e., ΔAICc = 0) is considered the best-fitting model (bold with *). *N* = number of parameters in each model. Threshold ΔAICc is the minimum ΔAICc required to reject models without the effect of sperm competition (BM null and OU null) while maintaining a type I error of 0.05 or less.

1.92 log-likelihood units of the maximum-likelihood estimate of slope. For sperm head length, we estimated 95% CI for slope of α and β under the OU linear model as this model also received moderate values of support. Slope parameters for which the CI did not include 0 were considered statistically significant. Analyses were performed using R 3.0.2 (R Core Team 2013) and the package "EvoRAG" (version 2.0, Weir and Lawson 2014).

Results

For both sperm midpiece and flagellum length, the best-fit model was a BM model that included an effect of rTM (BM linear; Table 1), with other models receiving little support (as indicated by AICc and Akaike weights; Table 1). For these traits we found that evolutionary rate (β) increased significantly with increasing values of rTM (midpiece: slope = 0.0025, 95% CI = 0.0007–0.0049; flagellum: slope = 0.0017, 95% CI = 0.001–0.003; Figs. 1A, B and 2A, B).

For sperm head length, the best-fit model was an OU model that did not include the effect of rTM (OU null; Table 1). Two other models also received moderate values of support: a BM model that did not include rTM (BM null) and an OU model that included the effect of rTM (OU linear; Table 1). The OU linear model found that evolutionary constraint (α) declined as rTM increased (α slope = −0.1001); 95% CIs for this parameter, however, included 0 (95% CI = −3.6 to 10.0). Furthermore, the effect of rTM on evolutionary rate (β) was weak: the maximum-likelihood estimate of β was extremely low ($\beta = 0.4 \times 10^{-322}$) and 95% CIs (95% CI = 0.0–10.0) enveloped both positive and zero slopes, suggesting a nonsignificant relationship between the evolution of sperm head length divergence and postcopulatory sexual selection imposed via sperm competition.

Our findings for total sperm length were similar to those for both midpiece and flagellum length, that is, the best-fit model was a BM model that included the effect of rTM (BM linear, Table 1), and in which the evolutionary rate (β) for total sperm length divergence increased significantly with increases in rTM (slope = 0.0012, 95% CI = 0.0005–0.0022; Figs. 1C and 2C). Other models received low support (Table 1).

Discussion

Analysis of evolutionary rates provides strong support for the idea that sperm length has diverged more rapidly in taxa experiencing stronger postcopulatory sexual selection in the form of sperm competition (sensu lato). rTM was positively associated with rates of evolutionary divergence in sperm midpiece and flagellum length, as well as total sperm length. To date, studies concerning the evolution of sperm size have focused on the correlation between sperm competition and sperm length (e.g., Byrne et al. 2003; Fitzpatrick et al. 2009; Immler et al. 2011; Tourmente et al. 2011), a finding that we also document in the dataset used in the current study (see Supporting Information text and Table S2). Extending this body of work, we show that postcopulatory sexual selection imposed via sperm competition also influences the speed of evolutionary change in sperm size in passerine birds.

Rapid diversification of phenotypic traits is frequently attributed to sexual selection. Direct tests of this hypothesis, however, are limited to a few examples, such as faster divergence in color patterns (Price and Whalen 2009) and male plumage traits (Seddon et al. 2013) in birds, and a higher rate of phenotypic divergence in male genitalia (i.e., baculum length) in pinnipeds (Fitzpatrick et al. 2012). Such rapid diversification of sexual traits is thought to play a role in the formation and maintenance of

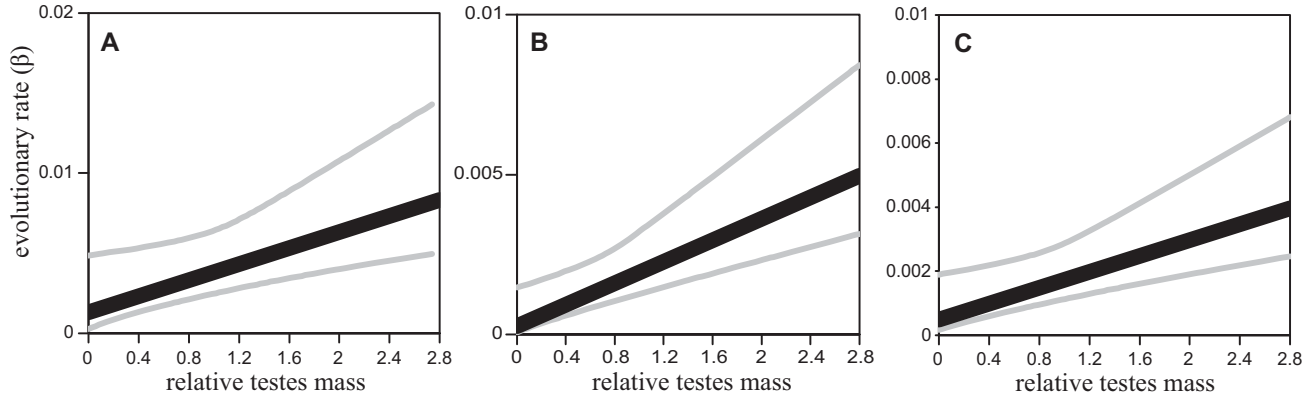

Figure 1. Evolutionary rate (β) of sperm length divergence in relation to the strength of sperm competition (i.e., relative testes mass) under the best-supported evolutionary model (BM linear). Maximum-likelihood values of β are shown across the range of values estimating the strength of sperm competition for (A) sperm midpiece length, (B) sperm flagellum length, and (C) total sperm length. Maximum-likelihood estimates are shown in black and 95% confidence bands in gray.

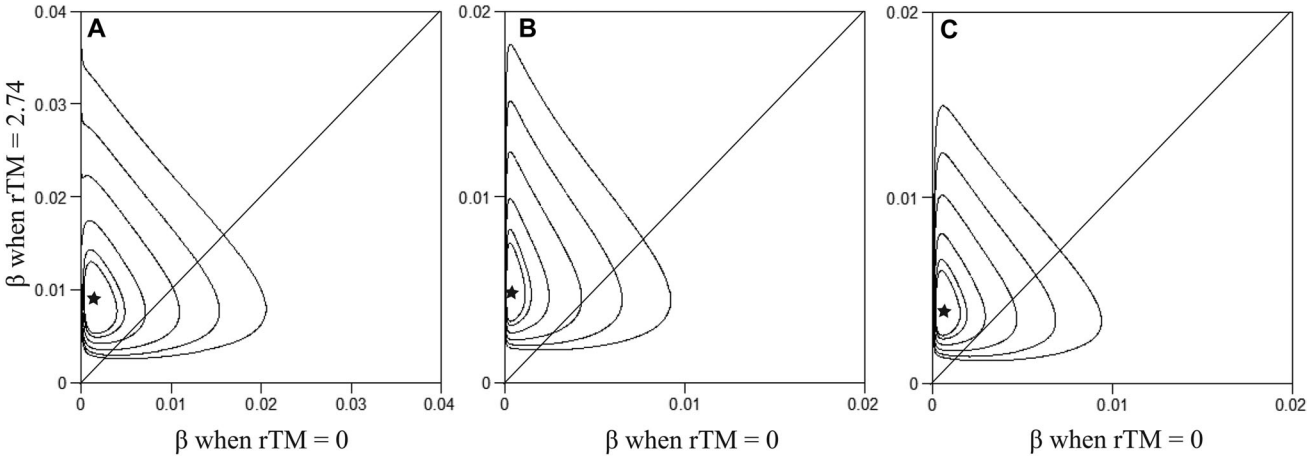

Figure 2. Likelihood surfaces of evolutionary rate (β) when relative testes mass (rTM) is 0 and 2.74 (the extent of our dataset) for the best-supported models in Table 1. (A) Sperm midpiece length, (B) sperm flagellum length, and (C) total sperm length. Maximum-likelihood values are shown by stars. Successive contours around maximum-likelihood values indicate confidence intervals with increasing values (i.e., 90%, 95%, 99%, 99.9%, 99.99%, 99.999%). Diagonal line indicates equal rates across all values of relative testes mass.

reproductive barriers between species (Swanson and Vacquier 2002; Coyne and Orr 2004), leading to the contested hypothesis that sexual selection is an "engine" of speciation (see, e.g., Coyne and Orr 2004; Ritchie 2007; Kraaijeveld et al. 2011). Support for this hypothesis comes in part from the observation that closely related species often differ in sexual traits (e.g., plumage, male genitalia, Ritchie 2007). One important source of potential bias in such studies is that taxonomists often rely on these same traits in determining species boundaries (Panhuis et al. 2001). Here, we show that the strength of sperm competition correlates with the speed of phenotypic diversification in traits unrelated to taxonomic decisions, and therefore not influenced by such a bias. Thus our evidence contributes strong, and in some ways unique, support to the hypothesis that sexual selection can drive rapid diversification of reproductive characters.

Although the role of sperm morphology in reproductive isolation is not well understood, it has been suggested that divergence in sperm traits between allopatric populations can lead to compromised ejaculate–female interactions upon secondary contact and, ultimately, postcopulatory prezygotic reproductive isolation (Howard et al. 2009). Recently, several sophisticated studies on *Drosophila* build support for this idea by demonstrating that variation in sperm traits (e.g., sperm length) that influence within-species competitive mating success via ejaculate–female interactions also lead to conspecific sperm precedence (Lüpold et al. 2012; Manier et al. 2013a,2013b). Divergence in sperm traits is also thought to have implications for the generation and maintenance of reproductive barriers in mice (Dean and Nachman 2009; Albrechtová et al. 2012). In birds, the role of postcopulatory sexual selection in speciation has

received little attention (Birkhead and Brillard 2007; Price 2008); although one recent study tests the role of sperm phenotype in postcopulatory prezygotic barriers in birds using a novel in vitro approach (Cramer et al. 2014). Our finding of rapid divergence in sperm morphology under greater levels of sperm competition highlights the potential for variation in sperm size to contribute to reproductive isolation between closely related taxa, and suggests that investigations into the role of postcopulatory sexual selection and sperm morphology in avian speciation are warranted.

In line with a previous study of passerine birds suggesting that the midpiece and flagellum exhibit a concerted response to selection (Immler et al. 2012), our results suggest that selection may act in a similar manner on both sperm midpiece and flagellum lengths, but that this selective force differs from that influencing the evolution of sperm head size. The correlated evolutionary response of sperm midpiece and flagellum lengths has been attributed to both extrinsic factors selecting on physical and metabolic sperm performance and intrinsic mechanical constraints (Immler et al. 2012). In birds, comparative studies show that midpiece length is positively associated with sperm swimming speed (Lüpold et al. 2009b) and sperm ATP levels (Rowe et al. 2013), highlighting the importance of this trait for sperm performance and metabolism. Sperm flagellum length is also positively associated with swimming speed across species (Lüpold et al. 2009b, but see Kleven et al. 2009), though longer flagella do not appear to have greater ATP levels (Rowe et al. 2013). Thus increases in flagellum length may be a response to selection for increased thrust or enable sperm to overcome drag generate by the head (Lüpold et al. 2009b). Alternatively (or additionally), given that in passerine sperm the midpiece is elongated and twisted around the flagellum (Jamieson 2007), increases in flagellum length may be linked to a support function for increasing midpiece length (cf. Lüpold et al. 2009b who proposed a stabilizing function for the elongated midpiece). Finally, although evidence from zebra finch (*Taeniopygia guttata*) indicates a negative genetic correlation between sperm midpiece and flagellum lengths (Birkhead et al. 2005), it is perhaps too early to rule out the possibility of positive genetic correlations between these traits in birds more generally as too few studies have been conducted to allow firm conclusions to be made and genetic correlations may be variable across species (Simmons and Moore 2009).

In contrast to our findings for sperm midpiece and flagellum, our analysis suggested sperm head length is evolutionarily constrained, which may be interpreted as stabilizing selection. One plausible explanation for this result is that sperm head size is constrained due to natural selection acting on the functional interaction between the sperm head and female ova at fertilization. In passerines, the sperm head is composed of the acrosome and nucleus (Jamieson 2007). Both structures are integral to sperm–egg interactions, a process that is generally conserved (Karr et al. 2009). Thus changes in sperm head length, due to, for example, alterations in the structural organization of the nucleus, may lead to a loss of function in the fertilization process. Increases or decreases in sperm head size would therefore be selected against.

In addition, selection acting on sperm performance may limit increases in sperm head length. Passerine sperm are filiform (Jamieson 2007), and recent theoretical work stresses the impact of sperm head shape and length on sperm swimming speed taking into account the Reynolds number (i.e., ratio of inertial forces to viscous forces, $Re = vl/\mu$, where v is object velocity, l is object length, and μ is the kinematic viscosity of the fluid the object operates in) characterizing the environment in which sperm operate (Humphries et al. 2008). Specifically, given the low Reynolds number environment sperm experience, sperm swimming speed is thought to be proportional to the balance between drag from the head and thrust from the flagellum, and as head shape becomes more elongate, drag is expected to increase (Humphries et al. 2008). Moreover, given that drag due to the head is related to its surface area (Humphries et al. 2008), increases in sperm head length (without appropriate increases in flagellum length) would be expected to reduce the speed attained by sperm. Thus a longer sperm head length is predicted to negatively impact sperm performance, which is consistent with recent empirical work in passerine birds documenting a negative relationship between sperm head length and swimming velocity (Lüpold et al. 2009b, but see Kleven et al. 2009 for an example of no relationship between these traits). Thus sperm head length may be evolutionarily constrained because increases in head length negatively impact sperm swimming speed and thus reduce the competitive ability of a male's sperm. It should be noted, however, that in passerine birds the sperm head is helical (Jamieson 2007, see Birkhead et al. 2006; Lifjeld et al. 2013 for exceptions), which is likely to be functionally related to the rapid spinning motion exhibited by swimming sperm (i.e., sperm rotate around the longitudinal axis, Vernon and Woolley 1999). Thus there is likely to be considerable variation in the form of the sperm head in passerines (e.g., amplitude of helical membrane, acrosome:nucleus ratio, etc.) beyond that of simple length, and future investigations of such variation may reveal interesting and novel patterns of sperm head evolution in passerines.

Conclusions

In summary, we used recently developed comparative methods to determine whether sperm competition influences the speed of evolutionary change in sperm morphology using data for passerine birds. We found that elevated levels of sperm competition were associated with more rapid phenotypic divergence in sperm size (i.e., midpiece, flagellum, and total sperm length), suggesting that postcopulatory sexual selection accelerates the

evolution of sperm morphology in this group. These findings demonstrate that postcopulatory sexual selection can influence both the direction (e.g., selection for longer/shorter sperm) and speed of sperm evolution in a group of internally fertilizing vertebrates. Moreover, our results highlight the potential for sperm morphological traits to play a role in avian speciation, and we suggest that studies linking intra- and interspecific variation in sperm phenotype to fertilization success under conspecific and heterospecific scenarios will help elucidate the evolutionary processes underlying sperm evolution and mechanisms of postcopulatory prezygotic reproductive isolation between species.

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.qb2c7.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Data on sperm morphology (i.e., length of sperm components, total sperm length), combined testes mass (CTM), and body mass for the 114 species used in this study.
Table S2. Regression of sperm morphology on relative testes mass (i.e., testes mass and body mass as covariates in the model), controlling for phylogeny via PGLS.
Table S3. Test of bias in parameter estimates under the BM linear, OU null, and OU linear models.
Table S4. Estimates of robustness of model parameter estimates.
Table S5. Type I error for null models without the effect of sperm competition and the associated threshold ΔAICc value required to maintain a Type I $\leq$ 0.05.
Figure S1. Maximum clade credibility tree illustrating the evolutionary relationships of the 114 passerine species included in this study.