

Morphology-Function Relationships and Repeatability in the Sperm of *Passer* Sparrows

Emily R.A. Cramer,^{1*} Terje Laskemoen,¹ Even Stensrud,¹ Melissa Rowe,¹ Fredrik Haas,^{2,3} Jan T. Lifjeld,¹ Glenn-Peter Sætre,² and Arild Johnsen¹

¹Natural History Museum, University of Oslo, Blindern, 0318 Oslo, Norway

²Department of Biosciences, Center for Ecological and Evolutionary Synthesis, University of Oslo, Blindern, 0316 Oslo, Norway

³Department of Biology, Ecology Building, Lund University, 223 62 Lund, Sweden

ABSTRACT Sperm performance is likely to be an important determinant of male reproductive success, especially when females copulate with multiple males. Understanding sperm performance is therefore crucial to fully understand the evolution of male reproductive strategies. In this study, we examined the repeatability of sperm morphology and motility measures over three breeding seasons, and we studied relationships between sperm morphology and function. We conducted this study in wild-derived captive house sparrows (*Passer domesticus*) and Spanish sparrows (*P. hispaniolensis*). Results for the two species were similar. As predicted from results in other passerine species, total sperm length was highly repeatable across ejaculates, and repeatability for the length of other components was moderate. The repeatability of sperm swimming speed across ejaculates was lower, but statistically significant, suggesting that sperm velocity may be a relatively dynamic trait. Surprisingly, swimming speed did not correlate with the relative length of the midpiece, and it correlated negatively with the relative length of the flagellum and with total sperm length. This pattern is the opposite of what theory predicts and differs from what has been found in house sparrows before. Also contrary to previous work, we found no evidence that total sperm length correlates with sperm longevity. These results therefore highlight the need for a better understanding of relationships between sperm morphology and function in passerine birds. *J. Morphol.* 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: sperm morphology; sperm velocity; form-function relationships

INTRODUCTION

In many species, females copulate with more than one male in a single reproductive cycle (e.g., Simmons, 2001; Griffith et al., 2002), which generates the opportunity for female choice of sperm traits (Eberhard, 1996) and for sperm from rival males to compete (Parker, 1970). Sperm characteristics can therefore play an important evolutionary role if they confer an advantage in male competition or female choice contexts. As predicted under this theoretical framework, sperm characteristics vary across species according to the level of multi-

ple mating. In passerine birds, species with higher levels of multiple mating have longer sperm (Kleven et al., 2009; Lüpold et al., 2009a, 2009b; but see Immler and Birkhead, 2007), less variability in sperm length among males (Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010), faster-swimming sperm (Kleven et al., 2009; but see Lüpold et al., 2009a), and a higher proportion of motile, morphologically normal sperm (Rowe and Pruett-Jones, 2011), compared to species with lower levels of multiple mating. With this evidence of strong selection on sperm morphology and function across species, it is necessary to investigate within-species processes and to improve our understanding of basic sperm biology.

Sperm swimming speed is thought to be important in passerines, because sperm speed influences

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*Correspondence to: Emily R.A. Cramer, Natural History Museum, Post Box 1172, Blindern, 0318 Oslo, Norway.
E-mail: becky.cramer@nhm.uio.no

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fertilization success in a wide range of animals (reviewed in Simmons and Fitzpatrick, 2012). It did not, however, correlate with reproductive success in tree swallows (*Tachycineta bicolor*), in the only study on passerines to date (Laskemoen et al., 2010). In turn, it is widely hypothesized that sperm morphology is an important factor affecting sperm swimming speed. Physical models predict that longer relative flagellum lengths should increase sperm swimming speed (Humphries et al., 2008). This prediction has been supported in some studies on passerines (interspecific study: Lüpold et al., 2009a; intraspecific studies: Mossman et al., 2009; Helfenstein et al., 2010; Immler et al., 2010). Other studies on passerines, however, find no support for a correlation between relative flagellum length and sperm swimming speed (interspecific studies: Kleven et al., 2009; Lüpold et al., 2009b; Rowe et al., 2013; intraspecific studies: Immler et al., 2010; Laskemoen et al., 2010) or find support for correlations between other measures of morphology and swimming speed (e.g., total sperm length, Lüpold et al., 2009a, but see Lifjeld et al., 2012; relative mid-piece length, Laskemoen et al., 2010). The precise relationship between sperm form and function therefore appears to vary across species in passerines, and no general pattern is yet known.

Sperm morphology may also be important in and of itself. In several invertebrates, sperm size affects their ability to displace other males' sperm within the female reproductive tract (reviewed in Snook, 2005), or it affects interaction with the female sperm storage organs (Pattarini et al., 2006; Lüpold et al., 2012b). Less is known in passerines, but, across passerine species, sperm length evolves in response to changes in the length of females' sperm storage tubules (Briskie et al., 1997). Moreover, males with sperm with relatively long flagella were found to have higher success at maintaining paternity within their own nests in superb fairy-wrens (*Malurus cyaneus*), although they had lower success at gaining fertilizations with females paired to other males (Calhim et al., 2011). However, in house wrens (*Troglodytes aedon*), sperm morphology was not found to relate to reproductive success (Cramer et al., 2013a).

Given the complex and sometimes contradictory patterns documented to date, it is particularly important that we have a firm understanding of basic sperm biology in passerines. In this study, we examined the sperm of house sparrows (*Passer domesticus*) and Spanish sparrows (*P. hispaniolensis*). We tested repeatability in sperm morphology and movement among ejaculates collected over three breeding seasons, predicting, based on work in other species (e.g., Lüpold et al., 2012a; Cramer et al., 2013b; Laskemoen et al., 2013b) that repeatability would be high for the length of sperm morphological components and swimming speed. We

also tested the relationship between sperm morphology and sperm swimming speed, predicting that longer sperm or sperm with a higher flagellum: head ratio should swim faster (Humphries et al., 2008). Finally, we tested whether sperm morphology predicts sperm longevity; we predicted that shorter sperm would continue swimming longer than longer sperm (Helfenstein et al., 2010; Lifjeld et al., 2012).

MATERIALS AND METHODS

Study Animals

We used a total of 27 Spanish sparrows, 28 house sparrows, and two hybrids of the two species, all of which were kept in aviaries at the University of Oslo, Norway. Most samples came from males housed with conspecific females, but on two occasions, we also sampled males housed with only females of the other species. Details on the aviaries are given in Cramer et al. (2014). Most individuals were wild-caught in 2010 [house sparrows in Oslo, Norway (59.934N, 10.723E) and Spanish sparrows in Badajoz, Spain (38.649N, 7.215W)], but all available captive-born individuals (three house sparrows and two hybrids) were also sampled. Ethical permission was issued to FH (Norwegian Animal Research Authority—FOTS ID 2394), and we followed legal requirements of the countries in which the research was conducted.

Sampling Methods

We collected sperm on six different sample events across three breeding seasons (two per season; dates and details on sample sizes are given in Supporting Information Table S1). Only a subset of individuals was sampled in most events, and in one event, only house sparrows were sampled. Each male was sampled only once per event. Several of these sampling events involved experiments unrelated to this article (Cramer et al., 2014, and unpublished data), and here we used only data from the control treatments. Details of the procedures used to record sperm swimming behavior differed slightly, according to the design of the different experiments; precise differences are shown in Supporting Information Table S1 and Figure S1. Because of these differences, we statistically control for sampling event in our analyses and do not interpret changes in mean velocity and proportion of motile sperm (PM) across sampling events.

In all events, we collected ejaculates via cloacal massage and gently mixed the ejaculate into 50–400 μ L of a prewarmed neutral medium (see Supporting Information Table S1), with the volume of medium adjusted according to the estimated volume of sperm collected. Our goal was to obtain a final sperm concentration appropriate for sperm motility analysis. Diluted concentrations varied from 11.6 ± 0.9 to 17.3 ± 1.5 million cells per mL, as estimated from cell counts from video analysis (see below). Sperm samples were filmed immediately on prewarmed Leja slides (two or four chamber; see Supporting Information Table S1, Fig. S1) mounted on a MiniTherm stage heater set to 40°C. Recordings were taken at 400 X total magnification on a microscope (Olympus CX41, Olympus, Japan) with a mounted video camera (HDR-HC1E, PAL, Sony, Japan; or Legria HF S200, Canon, Japan). Each ejaculate was filmed in 2–12 different locations within the slide chamber, with locations being distant enough that it is unlikely that individual sperm were filmed twice. For event 6, we noted the time delay between the start of filming and the beginning of each filming location, for testing longevity effects. Excess diluted sperm was mixed with 300 μ L 5% formaldehyde and stored at room temperature for later morphological analyses.

Videos were analyzed using computer-assisted sperm analysis (Hamilton-Thorne CEROS), with quality control settings

TABLE 1. Descriptive statistics on sperm morphology (TSL: total sperm length, in μm ; F:H: the ratio of the lengths of the flagellum to the head) and motility (PM: proportion of motile cells; VCL: curvilinear velocity, in $\mu\text{m/s}$) and for house sparrows, Spanish sparrows, and hybrid males, across six sampling events

		Mean \pm SE (N males)					
	Species	Event 1	Event 2	Event 3	Event 4	Event 5	Event 6
F:H	House	5.78 \pm 0.10 (6)	5.41 \pm 0.07 (14)	5.88 \pm 0.08 (12)	5.91 \pm 0.07 (7)	6.27 \pm 0.22 (4)	6.19 \pm 0.09 (13)
	Spanish	5.86 \pm 0.10 (6)	5.49 \pm 0.05 (16)	—	6.02 \pm 0.07 (10)	6.42 \pm 0.14 (6)	6.46 \pm 0.07 (17)
	Hybrid	—	—	—	—	5.78 \pm 0.13 (2)	5.72 \pm 0.17 (2)
TSL	House	100.59 \pm 1.07 (6)	100.08 \pm 0.98 (14)	100.56 \pm 1.01 (12)	100.52 \pm 1.00 (7)	99.13 \pm 2.29 (4)	99.50 \pm 0.94 (13)
	Spanish	100.36 \pm 1.21 (6)	99.98 \pm 0.59 (16)	—	101.48 \pm 1.14 (10)	100.13 \pm 1.69 (6)	100.65 \pm 0.68 (17)
	Hybrid	—	—	—	—	92.89 \pm 0.60 (2)	94.34 \pm 0.05 (2)
VCL	House	101.45 \pm 3.22 (6)	101.69 \pm 3.63 (12)	128.62 \pm 3.00 (11)	107.28 \pm 3.45 (6)	125.22 \pm 3.50 (5)	122.73 \pm 1.52 (22)
	Spanish	100.40 \pm 5.09 (5)	98.51 \pm 1.79 (11)	—	112.41 \pm 1.33 (7)	124.46 \pm 3.94 (6)	111.44 \pm 2.63 (21)
	Hybrid	—	—	—	—	135.81 \pm 10.27 (2)	125.24 \pm 4.63 (2)
PM	House	0.84 \pm 0.05 (6)	0.79 \pm 0.03 (13)	0.79 \pm 0.03 (12)	0.54 \pm 0.11 (7)	0.70 \pm 0.04 (5)	0.58 \pm 0.04 (23)
	Spanish	0.77 \pm 0.06 (6)	0.76 \pm 0.05 (12)	—	0.58 \pm 0.08 (10)	0.72 \pm 0.05 (6)	0.48 \pm 0.04 (23)
	Hybrid	—	—	—	—	0.80 \pm 0.09 (2)	0.31 \pm 0.01 (2)

following Cramer et al. (2014). That is, moving tracks with elongation scores >50 were considered nonsperm contaminants and were deleted from the dataset. Tracks with a straight-line velocity (VSL) $< 25 \mu\text{m/s}$ or a smoothed velocity (VAP) $< 30 \mu\text{m/s}$ were moving due to drift and were considered static cells. The proportion of motile cells was calculated as the number of motile cells divided by the total number of cells detected. Only videos with at least 20 cells (static and motile) were included in analyses of the proportion of motile cells. Motile tracks with straightness < 80 , linearity < 35 , with fewer than 10 detection points, with gaps in the detection series, or with large single motions between CEROS detections were considered to be poorly tracked motile cells. These tracks were therefore not included in calculating average swimming speed (estimated as mean curvilinear velocity, VCL). Only videos with at least 20 motile tracks that passed these criteria were included for analyses on VCL, so that our final sample size for velocity data was 128 ejaculates. For most analyses, we calculated the proportion of motile cells and the mean velocity from all 2–12 recording locations for each video; for longevity analysis, we calculated a separate mean for each recording location.

We measured sperm morphology of 115 of the ejaculates that we filmed, following the procedure of Laskemoen et al. (2007). Approximately 15- μL fixed sperm was streaked onto a glass slide, allowed to dry overnight, and rinsed gently with distilled water. After allowing the slide to dry again, we photographed sperm and measured the lengths of the head, midpiece, and exposed flagellum for 10 sperm cells using a camera (Leica DFC420, Leica Microsystems, Heerbrugg, Switzerland) connected to a digital light microscope (Leica DM6000B). We calculated sperm total length as the sum of the three components, and flagellum length as the sum of the midpiece and exposed flagellum. We also calculated the ratio of the lengths of the flagellum: head and of midpiece: total sperm length for each sperm cell, as well as the coefficient of variation in total sperm length within an ejaculate. For statistical analyses, we used the mean of the component lengths or ratios across the 10 measured cells. Within a sampling event, all males were measured by a single observer, but different observers measured different events. We control for this variation statistically by including sampling event as a factor in the models, and we do not interpret changes in mean sperm morphology across sampling events.

Statistical Analyses

We compared mean values for morphology and motility between house and Spanish sparrows by constructing linear mixed models (LMM) including male identity as a random effect, with species and sampling event as factors and an inter-

action between the latter two variables. If the interaction term was not significant (using a cutoff of $P = 0.05$, following Zuur et al., 2009, page 125), we removed it from the model.

We estimated repeatability across different ejaculates for each sperm trait by calculating the percent of variance explained by a random effect of male identity in LMMs with the sperm trait as the response variable. We tested the significance of this random effect using a likelihood ratio test, following Nakagawa and Schielzeth (2010), and we constructed models using restricted maximum likelihood estimation, following Zuur et al. (2009). This approach allowed us to control for the fixed factors of sampling event and species. Only males sampled in at least two events (including hybrids and captive-bred pure males) were included in these analyses.

To test the relationship between velocity and morphology, we constructed separate LMMs for each sperm morphology measure, with mean VCL as the response variable, a morphological trait as the predictor of interest, sampling event and male species as factors, and a random effect of male identity. We used the mean values for sperm morphology for each male within each sampling event. We initially included a three-way interaction between species, event, and the morphological measure, as well as the pair-wise interactions of these variables, to assess whether morphology-speed correlations differed between species or among events. Nonsignificant ($P > 0.05$) interactions were removed from the model in a backwards step-wise process (Zuur et al., 2009). Following the same procedure, we tested whether the proportion of motile cells related to the mean swimming speed. Because of substantial variation between sampling events in mean values for some parameters (Table 1), we centered values around the mean for the sampling event before testing for morphology-function correlations (Schielzeth, 2010).

We also tested for morphology-longevity relationships, within sampling event 6 only, since that was the event when we filmed for the longest time (see Supporting Information). In separate models for each sperm morphology measure, we tested for interactions between morphology, the time of filming of that location, and species, in predicting the proportion of motile cells or mean VCL in each filming location. We initially tested a three-way interaction between time, morphology, and species, as well as including the constituent pairwise interactions and main effects; nonsignificant interaction terms were removed from the model as above. Male identity was included as a random effect. Models including random slopes (with respect to filming time). Including temporal autocorrelation structures typically did not substantially improve model fit as assessed by the Akaike Information Criterion, and did not qualitatively affect results, except where noted.

TABLE 2. Repeatability estimates for sperm morphology and motility in house and Spanish sparrows, estimated following Nakagawa and Schielzeth (2010) while controlling for species and sample event

Sperm trait tested	Repeatability	Likelihood ratio (<i>P</i> -value)	<i>N</i> males (<i>N</i> samples)
Total sperm length	0.85	89.90 (0.0001)	37 (107)
Flagellum: head ratio	0.61	30.05 (0.0001)	37 (107)
Midpiece: total sperm length ratio	0.56	23.92 (0.0001)	37 (107)
Head length	0.15	1.44 (0.23)	37 (107)
Midpiece length	0.55	30.37 (0.0001)	37 (107)
Flagellum length	0.85	89.23 (0.0001)	37 (107)
Within-ejaculate variability in total length	<0.01	<0.01 (>0.99)	37 (107)
Velocity	0.24	4.45 (0.04)	37 (96)
Proportion of motile cells	<0.01	<0.01 (>0.99)	39 (108)

In tests of species-level differences, we did not include hybrids, as too few individuals were available for robust testing. For tests that initially included interactions between sample event and species, we excluded sampling event 3, because only house sparrows were sampled in that event. We assessed model assumptions (normality and heterogeneity of variance of residuals) by eye, following the recommendation of Zuur et al. (2009). Statistics were conducted in R v 3.0.3 using the package nlme (Pinheiro and Bates, 2013).

RESULTS

Species Level Differences

Across all sample events, Spanish sparrow sperm had a slightly higher flagellum: head ratio than house sparrows (parameter estimate \pm SE 0.16 ± 0.08 ; $F_{1,40} = 4.16$, $P = 0.048$), and Spanish sparrow sperm swam approximately 5.14 ± 2.10 $\mu\text{m/s}$ more slowly than those of house sparrows ($F_{1,53} = 5.99$, $P = 0.02$; Table 1). Neither total sperm length ($F_{1,40} = 0.45$, $P = 0.51$) nor proportion of motile sperm ($F_{1,54} = 1.64$, $P = 0.2$) differed between species. Mean swimming speed ($F_{4,42} = 22.11$, $P < 0.0001$), proportion of motile cells ($F_{4,51} = 11.83$, $P < 0.0001$), and flagellum: head ratios ($F_{4,53} = 80.34$, $P < 0.0001$) differed across sampling events, though total sperm length did not ($F_{4,53} = 1.29$, $P = 0.29$). As stated above, differences across events are likely due in part to different recording protocols for sperm swimming parameters, and different measurers for sperm morphology.

We provide descriptive information on hybrid individuals (Table 1), but caution that direct comparison with the older, wild-caught, pure-bred birds may be inappropriate, as there were suggestions of age effects in some variables (though we were unable to effectively test age effects, as we only sampled three captive-hatched pure-species males; analyses not shown). We also provide descriptive information on sampling event 3, which was not included in models where a species by event interaction term was included.

Repeatability

After controlling for differences due to measurement events, total sperm length and flagellum

length were highly repeatable (Table 2). Flagellum: head ratio, midpiece: total sperm length ratio, and midpiece length were moderately repeatable, and swimming speed was repeatable at a lower, but significant, level (Table 2). Head length had a low and nonsignificant repeatability. Within-male variability in total sperm length and the proportion of motile sperm had repeatability values approaching 0 (Table 2).

Structure-Function Relationships

Relationships between morphological traits and swimming speed were highly consistent between species and across sampling events; interaction terms between morphological traits, sampling event, and species were always highly nonsignificant ($P > 0.2$) and dropped from the models. Sperm swimming speed was significantly negatively correlated with the flagellum: head ratio (Fig. 1), flagellum length, midpiece length, and total sperm length (Table 3). Head length and the midpiece: total sperm length ratio were not significantly related to swimming speed (Table 3).

The relationship between mean sperm swimming speed and the proportion of motile cells in the ejaculate differed across sampling event and species (three-way interaction term, $F_{4,28} = 3.88$, $P = 0.01$). Estimated relationships between swimming speed and the proportion of motile cells for each species and event varied dramatically, with no consistent pattern with respect to species or event (Supporting Information Table S3).

The proportion of motile cells and sperm swimming speed decreased over time within event 6 recordings (which lasted 70–98 s). We found no evidence that morphology affected the rate of decline in the proportion of motile cells ($P > 0.60$ for interactions between morphology and time in reduced models). There was weak evidence that, in Spanish sparrows, swimming speed declined faster in ejaculates with longer midpieces and higher midpiece: total sperm length ratios (see Supporting Information for more information). However, these patterns were driven by a single time point for a single male and became

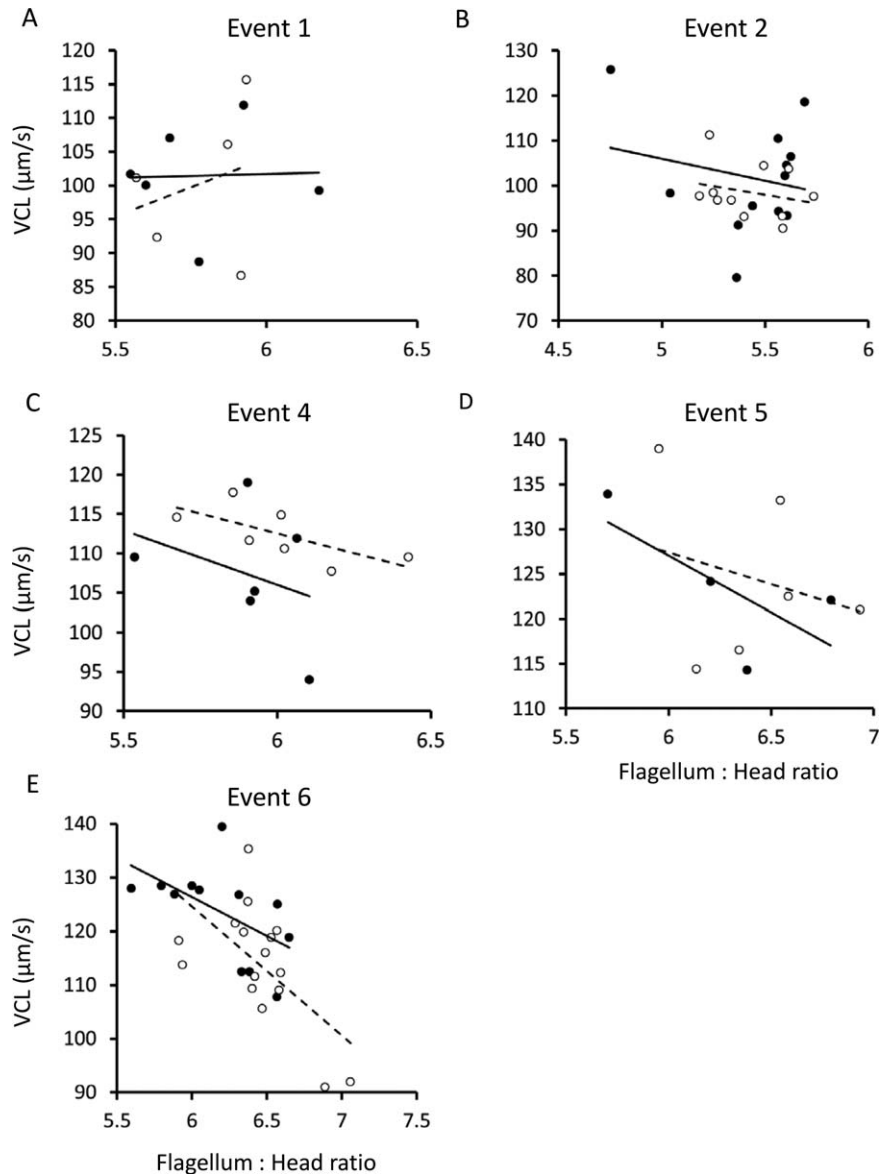


Fig. 1. The relationship between sperm curvilinear velocity (VCL) and the ratio of the lengths of the flagellum and head, across five sampling events (panels A–E corresponding to sampling events 1–2 and 4–6; sample event 3 only included house sparrows and so was not included in these tests). House sparrows are indicated in black dots and solid lines, while Spanish sparrows are indicated in white dots and dotted lines. Here, simple correlations within each species and event are calculated separately, for visualization purposes. See main text for statistical details.

nonsignificant when a temporal autocorrelation structure or random slope term was introduced to the model. This pattern therefore does not appear to be robust. Other morphological traits did not affect the rate of change in swimming speed over time, as the interaction between time and morphology was not significant (interaction terms $P > 0.20$ for all other morphological variables in reduced models).

DISCUSSION

Species Level Differences

Sperm traits and morphology-function relationships were quite similar between house and Spanish

sparrows (see also Cramer et al., 2014). Total sperm length did not differ between species, and the difference in the flagellum: head ratio is slight compared to variation observed among passerines. That is, the difference in flagellum: head ratio between house and Spanish sparrows is about 0.16, while differences of up to 1.14 have previously been documented among species of the genus *Passer* (Immler et al., 2011), and differences greater than 10 occur among oscine passerine species (Lüpold et al., 2009a). Moreover, differences between populations of the same species can exceed that described here (difference of up to 1.1 between populations of red-winged black-bird *Agelaius phoeniceus*, Lüpold et al., 2011, and

TABLE 3. Estimated relationship of sperm swimming speed (VCL) to sperm morphological traits

Predictor variable	Parameter estimate \pm SE	F-test statistic and P-value
Flagellum: head ratio	-13.37 ± 3.75	$F_{1,39} = 12.72$, $P = 0.001$
Midpiece: total sperm length	36.25 ± 68.55	$F_{1,39} = 0.28$, $P = 0.60$
Flagellum length	-1.08 ± 0.33	$F_{1,39} = 11.10$, $P = 0.002$
Head length	3.37 ± 2.91	$F_{1,39} = 1.35$, $P = 0.25$
Midpiece length	-1.75 ± 0.56	$F_{1,39} = 9.94$, $P = 0.003$
Total sperm length	-1.03 ± 0.33	$F_{1,39} = 9.94$, $P = 0.003$

Statistical models included sample event and species as fixed factors; the relationship between speed and morphology did not differ among events or species. Sample sizes were 40 house sparrow ejaculates from 18 males and 45 Spanish sparrow ejaculates from 23 males.

bluethroat *Luscinia svecica*, Hogner et al., 2013; up to 0.7 in barn swallows *Hirundo rustica*, Laskemoen et al., 2013a; and up to 0.3 in coal tits *Periparus ater*, Schmoll and Kleven, 2011). The difference in sperm swimming speed is also relatively minor compared to the variation across passerine species (e.g., values for passerines range from about 80 to 160 $\mu\text{m/s}$, Kleven et al., 2009, while the estimated difference between house and Spanish sparrows is 5 $\mu\text{m/s}$). House and Spanish sparrows diverged approximately 3.4 million years ago according to molecular clock estimates based on mitochondrial DNA (Allende et al., 2001; Elgvin et al., 2011). This should be sufficient time for sperm traits to change, judging from a study of another passerine, the bluethroat, where sperm morphology has changed dramatically among subspecies that diverged less than 350,000 years ago (Hogner et al., 2013). While house sparrows have moderate levels of extra-pair paternity (Wetton and Parkin, 1991; Cordero et al., 1999; Griffith et al., 1999; Veiga and Boto, 2000; Whitekiller et al., 2000; Stewart et al., 2006; Edly-Wright et al., 2007), and Spanish sparrows likely do as well (Calhim and Birkhead, 2007; Cramer et al., 2014), it is plausible that selection on sperm has been largely stabilizing in these two species over evolutionary time, preventing sperm traits from diverging.

While we had too small of a sample size for robust statistical testing, we found no evidence that first generation male hybrids had reduced sperm performance, which contrasts to substantially reduced ovarian development in female hybrids from this captive population (Eroukhanoff et al., submitted; also c.f. reduced sperm performance in hybrids from *Ficedula flycatchers*; Alund et al., 2013).

Repeatability

As found in previous studies in other species, total sperm length is highly repeatable across dif-

ferent ejaculates by the same male, and repeatability was moderate for most other sperm morphology components. Repeatable sperm morphology therefore appears to be a robust pattern in passerine birds (Lüpold et al., 2012a; Cramer et al., 2013b; Laskemoen et al., 2013b). Repeatability in swimming speed is low in house and Spanish sparrows, and the proportion of motile cells is not repeatable; this result contrasts with findings in barn swallows, where these traits are highly repeatable (Laskemoen et al., 2013b) but matches work in mallard ducks (*Anas platyrhynchos*; Denk et al., 2005). Both sperm morphology (Immler et al., 2010) and sperm mobility (Pizzari et al., 2007) can change in response to changes in social status in birds, and such phenotypic plasticity would be expected to reduce repeatability among ejaculates. Relatively low repeatability in sperm velocity in sparrows may therefore indicate that it is a relatively dynamic trait in these species.

Using captive populations could potentially bias the results toward higher repeatability, if the captive conditions are relatively constant compared to typical conditions of free-living birds. On the other hand, using different recording procedures and having different people measure sperm morphology could decrease repeatability, despite statistical control for these factors. Our values for repeatability of morphology are comparable to those found in wild populations of other species (Lüpold et al., 2012a; Cramer et al., 2013b; Laskemoen et al., 2013b;), and domestication and captivity seem to have minimal, if any, effects on sperm biology in zebra finches (*Taeniopygia guttata*, Immler et al., 2012).

While values describing mean sperm characteristics were moderately to highly repeatable across sample events, values describing variation in sperm characteristics (i.e., variability in sperm total length, proportion of motile sperm) were not repeatable, as also reported for variability in total length in house wrens (Cramer et al., 2013b); though not for the proportion of motile cells in barn swallows (Laskemoen et al., 2013b). The difference in repeatability for means compared to repeatability of proportional values and variance may be partly explained by statistical factors: it may be necessary to have a higher sample size to obtain a robust estimate of the latter measures. Alternatively, variation in motility and morphology across ejaculates of a single male may be heightened by factors such as time since the last copulation, which may have less influence on mean values.

Morphology-Function Relationships

Fluid dynamic modeling predicts that the flagellum: head ratio should correlate positively with sperm swimming speed (Humphries et al., 2008),

as has been found in a number of studies in passerines (Lüpold et al., 2009a; Mossman et al., 2009; Immler et al., 2010), including one on house sparrows (Helfenstein et al., 2010). In contrast, we found strong evidence for a negative relationship between flagellum: head ratio and velocity, and this relationship was robust to variation in the video recording protocols used across sampling events. Together with several other studies in passerines that find no relationships between velocity and the flagellum: head ratio (Kleven et al., 2009; Lüpold et al., 2009b; Immler et al., 2010; Laskemoen et al., 2010), we suggest that the fluid dynamic models are either too simplified or are not consistently relevant for sperm that swim as passerine sperm do. That is, the models are based on mammal-like sperm that swim via whip-like motions of the flagellum, while passerine sperm spiral through the medium by rotating around their longitudinal axis (Vernon and Woolley, 1999). This difference may make it difficult to apply general models of sperm motion to passerines. In short, we suggest that associations between sperm morphology and function may be driven by unmeasured, latent variables that correlate with both morphology and function, rather than being due to a direct, physical effect of morphology on function, which would be expected to be more consistent across studies.

We found that the flagellum: head ratio correlated negatively with swimming speed, and that total sperm length did not correlate with longevity. In a Swiss house sparrow population, the flagellum: head ratio correlated positively with speed, and total sperm length correlated negatively with longevity (Helfenstein et al., 2010). One methodological factor that could help explain this difference in results is that we sampled a large number of birds on a single day per event, rather than spreading sampling over several days to weeks. As sperm morphology and velocity change over time in at least some species (Lüpold et al., 2012a; Cramer et al., 2013b; but see Laskemoen et al., 2013b), using data from a broad and continuous span of dates could introduce noise or bias into the analysis of relationships among sperm traits. Alternatively, there may be true variation across populations of house sparrows in how morphology relates to swimming parameters, though at this time it is unclear what could cause such geographic variation.

In summary, we find that house sparrows and Spanish sparrows have similar sperm morphology and motility parameters, and that the relationships between morphology and function are similar for the two species. In contrast to this consistency between species, some of our results differ from previous findings on house sparrows, suggesting that there could be substantial within-species variation, perhaps linked to differences

among populations. The low repeatability of sperm swimming speed in this study, combined with evidence for phenotypic plasticity in swimming speed from other work, suggests that this trait is highly dynamic, while morphological traits are more stable. Moreover, we have found the opposite correlation between sperm form and function from what theory predicts, and many other studies also do not find the predicted correlation. This degree of variation in the relationship between sperm morphology and velocity would be unexpected if the relationship was actually driven by the physics of sperm motion, as has been thought. We suggest that the theoretical framework for testing the relationship between sperm swimming speed and morphology needs to be revised for passerine birds.

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