



Reddish male swallows have short sperm

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Abstract

Sexual selection favors the evolution of pre-copulatory sexual traits such as ornamentation and post-copulatory sexual traits such as long sperm, but the interrelationships of the two types of sexual traits remain unclear. Here, using Japanese barn swallows, *Hirundo rustica gutturalis*, and an interspecific analysis of the family Hirundinidae, we examined sperm length in relation to pheomelanin-based plumage coloration. As predicted by the fact that pheomelanogenesis consumes antioxidants, which would be detrimental to develop long sperm, reddish coloration (but not other ornaments) were negatively related to sperm length both within and across species, suggesting an evolutionary tradeoff between these traits.

Keywords Pheomelanin-based pigmentation · Plumage ornaments · Sperm size · Phylogenetic comparative approach · Tail feather length

Sexual selection favors the evolution of pre-copulatory sexual traits that enhance mating success via mate choice and intrasexual contest (e.g., conspicuous ornaments; reviewed in Andersson 1994). Likewise, sexual selection favors the evolution of post-copulatory sexual traits, enhancing fertilization success via sperm choice/competition (e.g., long sperm; Bennison et al. 2015; reviewed in Birkhead and Møller 1998). The two evolutionary processes might not be independent if pre- and post-copulatory sexual traits are interrelated: positive relationship facilitates and negative relationship constrains the evolution of sexual traits. Therefore, it is important to know whether and how the two types of sexual traits are interrelated.

Concerning this point, two alternative hypotheses are proposed with particular attention to oxidative stress due to our understanding that sperm quality is susceptible to oxidative stress (reviewed in Friesen et al. 2020). First, the redox-based

“phenotype-linked fertility hypothesis” predicts a positive relationship between pre- and post-copulatory sexual traits based on the assumption that individuals with enough supply of resources (antioxidants) invest more in both pre- and post-copulatory sexual traits (Sheldon 1994; Blount et al. 2001). Second, “sperm competition theory” predicts a negative relationship between them, because resources (e.g., antioxidants) invested in pre-copulatory sexual traits is unavailable for post-copulatory sexual traits, or vice versa (Parker 1998; Tazzyman et al. 2009). Although empirical studies of animals, particularly those with carotenoid-based coloration (i.e., a sexual trait that uses dietary antioxidants), sometimes found positive (e.g., Helfenstein et al. 2010) and negative relationships (e.g., Rowe et al. 2010), no clear pattern was observed across studies (reviewed in Mautz et al. 2013).

Pheomelanin is a reddish-brown pigment widespread among birds and mammals (McGraw 2006). In contrast to carotenoids, (pheo)melanin pigments are synthesized in animals, and are long thought to be mostly independent of individual physiological state or resource availability (e.g., McGraw 2008; Roulin 2016). However, pheomelanogenesis consumes glutathione, an important antioxidant, and thus pheomelanin production trades off with anti-oxidative defence (e.g., Ito and Wakamatsu 2008; Galván and Alonso-Alvarez 2009; Arai et al. 2017). Therefore, as is the case for carotenoid-based coloration, pheomelanin-based coloration would affect sperm quality, because glutathione is potentially linked to sperm quality as an antioxidant (Friesen

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et al. 2020), and because consumption of an antioxidant (glutathione) affects other antioxidants as well (Blount et al. 2001).

The barn swallow, *Hirundo rustica*, is a model species for pre- and post-copulatory sexual selection (Møller 1994; Turner 2006). They have several sexually selected ornaments, including pheomelanin-based reddish throat coloration (reviewed in Romano et al. 2017). Extrapair paternity is generally high (e.g., ca. 20% of young in the European subspecies, *H. r. rustica*) but varies markedly across populations, and sperm morphology tracks the frequency of extrapair paternity in each population (i.e., small and variable sperm in a population with low extrapair paternity; Laskemoen et al. 2013; Hasegawa et al. 2019). Although sperm quality is found to be related to some traits in this species (e.g., Møller et al. 2008 for covariation between sperm swimming performance and male tarsus length), the relationship between pheomelanin-based coloration and sperm quality has not been reported.

Here, we examined sperm length in relation to pheomelanin plumage coloration in Joetsu population of the Asian barn swallow (*H. r. gutturalis*). They have reddish throat patches that are twice as large as those of European ones (Hasegawa et al. 2010a). In this low-density population (ca. 20 m between the nearest breeding males), extrapair paternity is virtually absent (<3% of young; Hasegawa et al. 2010b), and hence small and variable sperm length is observed (Hasegawa et al. 2019). This population provides a unique opportunity to examine how intense pre-copulatory, but not post-copulatory, sexual selection affects sperm quality and its relationship with pre-copulatory sexual traits (see Hasegawa 2018 for a review of sexual selection in Japanese populations). We predicted a negative relationship between sperm length and throat coloration, because spermiogenesis, including the sperm elongation process, is easily affected by oxidative stress (e.g., see Ricketts et al. 2011 for in vivo and in vitro experiments in *Drosophila*: note that this is also important in vertebrate; Henkel 2011), and because pheomelanin-based reddish throat coloration is shown to be negatively related to oxidative balance in Japanese barn swallows even during the breeding period (e.g., Arai et al. 2017, 2018). We also tested the interspecific patterns using the family Hirundinidae.

Methods

Study site

The current study was conducted during the early breeding season (1 April–30 June) in 2017 and 2018 in a residential area of the Joetsu City, Niigata Prefecture, Japan (37°07'N,

138°15'E). We inspected nests every third day to record breeding events.

Capture and measurement

Adults were captured in sweep nets while roosting at night. Each bird was provided with a numbered aluminum ring and a unique combination of half-sized colored rings. The sex of each individual was determined by the tail shape and by the presence (female) or absence (male) of an incubation patch (Turner 2006). We obtained ejaculate samples by gently massaging the males' cloacal protuberance and measured sperm sizes afterwards (Hasegawa et al. 2019). Detailed measurements are given in Supplementary material 1.

We measured wing length, tarsus length, keel length, body mass, and four measures of plumage ornaments, i.e., outermost tail feather length, the size of white-tail spots, throat patch size, and throat coloration at capture (see Hasegawa et al. 2010a for details). Detailed information on measuring coloration is given in Supplementary material 2.

Statistics

We standardized all measurements to zero mean and unit variance in each study year to exclude possible year effects before analysis (see Table S1). Body condition index was calculated as residual body mass on keel length after standardizing each variable in each year (linear model: coefficient \pm SE = 0.52 ± 0.21 , $F_{1,17} = 6.44$, $P = 0.02$). We also conducted phylogenetic comparative analysis using the family Hirundinidae (see Supplementary material 3 for details). All data analyses (e.g., Pearson's product-moment correlation, multiple linear model) were performed using the R statistical package (ver. 4.0.0; R Core Team 2020).

Results

Sperm length was not significantly correlated with male ornaments (Pearson's product-moment correlation coefficient, $|r| < 0.38$, $n = 19$, $P > 0.10$) except for throat coloration value: Males with redder throats had shorter sperm ($r = 0.50$, $n = 19$, $P = 0.029$; Fig. 1). Each component of sperm, i.e., sperm head length, midpiece length, and tail length was not significantly correlated with male ornaments (i.e., outermost tail feather length, the size of white-tail spots, throat patch size, and throat coloration: $|r| < 0.45$, $n = 19$, $P > 0.05$). Qualitatively similar results were found when we used multiple linear models to exclude the possible confounding effects of other ornaments, although the sample size was limited (Table 1).

Wing length, tarsus length, keel length, body mass, and body condition were not significantly correlated with sperm

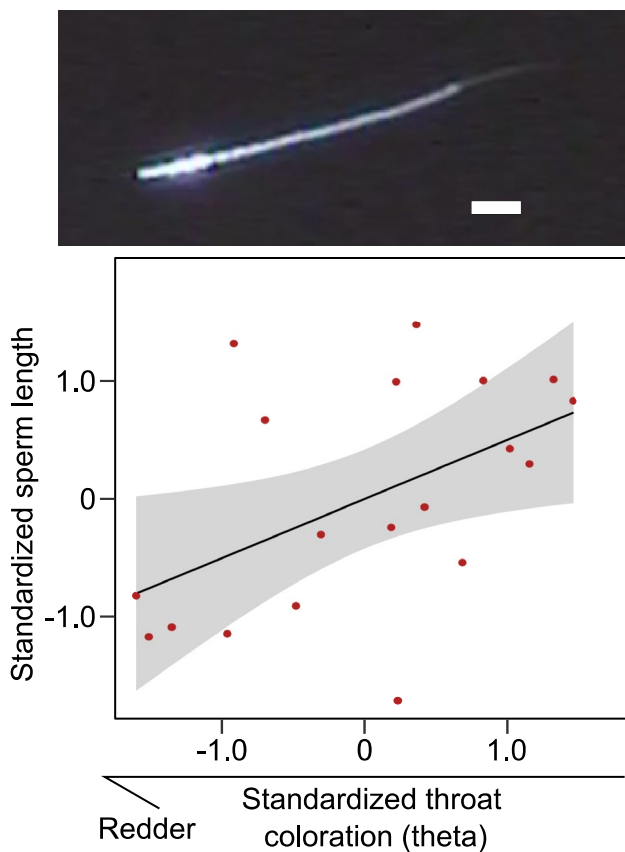


Fig. 1 Relationship between throat coloration and sperm length in Joetsu population of the barn swallow. Line (and shade) indicates a simple regression line (and 95% confidence interval). A photograph of sperm (captured by a phase-contrast microscope) is shown above the figure, in which sperm head, midpiece, and tail correspond to bright spiral piece, thick central piece, and thin dark tail, respectively. The white bar in the photograph indicates 10 μm

length ($|r| < 0.31$, $P > 0.19$), and its components ($|r| < 0.44$, $P > 0.06$). Neither body mass nor body condition index was significantly related to male ornaments in the current data set ($|r| < 0.29$, $n = 19$, $P = 0.22$).

When using a phylogenetic comparative analysis while controlling for the extrapair mating opportunity (see Supplementary material 3), we found that species with reddish plumage had significantly shorter sperm than species without reddish plumage (Fig. 2; Table 2).

Discussion

We found a negative relationship between sperm length and pheomelanin reddish plumage coloration both within and across species, as predicted by sperm competition theory (see Introduction). Confounding effects of other ornamental traits and its correlates (e.g., age) are unlikely, because we found no detectable correlations between sperm sizes and

Table 1 Multiple linear models explaining sperm sizes in relation to male ornaments in the Japanese barn swallows (each: $n = 19$)

Variables	Coefficient \pm SE	<i>t</i>	<i>P</i>
(a) sperm length			
Outermost tail feather length	-0.34 ± 0.20	-1.67	0.12
The size of white-tail spots	0.03 ± 0.21	0.15	0.88
Throat patch area	0.35 ± 0.20	1.77	0.10
Throat coloration	0.47 ± 0.20	2.36	0.033
(b) head length			
Outermost tail feather length	-0.24 ± 0.25	-0.97	0.35
The size of white-tail spots	-0.33 ± 0.25	-1.31	0.21
Throat patch area	-0.10 ± 0.24	-0.43	0.68
Throat coloration	0.08 ± 0.24	0.33	0.75
(c) midpiece length			
Outermost tail feather length	-0.35 ± 0.21	-1.69	0.11
The size of white-tail spots	0.43 ± 0.21	2.03	0.06
Throat patch area	0.32 ± 0.20	1.61	0.13
Throat coloration	0.35 ± 0.20	1.76	0.10
(d) tail length			
Outermost tail feather length	0.15 ± 0.25	0.62	0.54
The size of white-tail spots	-0.47 ± 0.25	-1.90	0.08
Throat patch area	0.11 ± 0.24	0.44	0.67
Throat coloration	0.01 ± 0.24	0.03	0.98

All variables were standardized to zero mean and unit variance in each study year before analysis

Max VIF (i.e., variance inflation factor) = 1.14, indicating that multicollinearity might have few effects on the estimates

Bold indicates a significant variable

other ornaments including a highly age-dependent trait (i.e., outermost tail feather length; e.g., Hasegawa et al. 2010a). To our knowledge, the current study is the first study demonstrating a negative relationship between sperm size and pheomelanin-based coloration in any animal species and clades.

A simple explanation for the observed pattern is that pre-copulatory sexual selection favors the evolution of pheomelanin-rich throat patches at the expense of sperm size by consuming glutathione, an antioxidant. Because extrapair paternity is virtually absent in the study population, post-copulatory sexual selection for long sperm would be negligible (see Introduction), possibly contributing to uncovering the tradeoff (due to the limited confounding effects of extrapair mating; also see Table 2 for the interspecific pattern controlling for the extrapair mating opportunity). In addition to such a direct tradeoff, selection for more pheomelanin (i.e., selection for glutathione consumption for pigmentation) might indirectly favor short sperm, because long sperm is more likely to be affected by oxidative stress (e.g., Hermosell et al. 2013). Another explanation that less-colorful males invest more in sperm competition (i.e., have longer sperm) is unlikely due to the lack of extrapair paternity (see

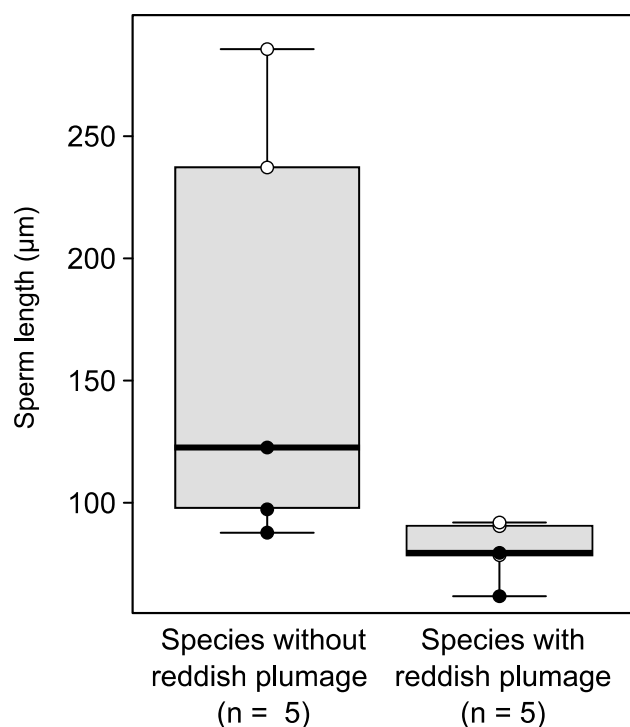


Fig. 2 Boxplots of sperm length for species with and without reddish plumage in the family Hirundinidae. The horizontal bar in each boxplot indicates the median, and the box shows the first and third quartiles of data. The whiskers range from the lowest to the highest data points within $1.5 \times$ interquartile range of the lower and upper quartiles, respectively. Black and white circles indicate species with biparental incubation and female-only incubation, respectively

Table 2 Multivariable phylogenetic generalized least square (PGLS) model for sperm length in the family Hirundinidae ($n = 10$)

Predictor	Coefficient \pm SE	95% CI
Incubation type (female-only vs biparental)	0.0037 ± 0.0009	$0.0015, 0.0059$
Reddish plumage coloration (absence vs presence)	0.0048 ± 0.0014	$0.0016, 0.0081$
Model-averaged lambda (λ) = 1.00		

Model-averaged coefficients, SEs, and 95% confidence intervals (CI) are shown

Significant test results (i.e., 95% CI does not contain zero) are indicated in bold

Inverse sperm length was used as a dependent variable to fit normal distribution (and thus sign of the coefficient was reversed from the raw values)

Note that when including tail fork depth instead of reddish plumage coloration, tail fork depth was far from significant (i.e., 95% CI largely overlapped with zero). This was also the case when we used $\log(\text{wing length})$ as a measure of body size

above; note that the rare paternity loss was found in males with small white-tail spots rather than males with the colorful throat; Hasegawa 2018).

The current correlational study, however, could not exclude all confounding factors. Because colorful males hold high-quality territories in this population (Hasegawa et al. 2014), intense agonistic interaction for such preferable territories might increase energy expenditure and reduce male physiological conditions as well (though colorful males often have better body conditions than drab males: e.g., Arai et al. 2018). Likewise, via intraspecific interactions, pheomelanin coloration feeds back their physiological state (including oxidative balance) in barn swallows (e.g., Safran et al. 2008; Vitousek et al. 2013), and thus behavioral interaction would reinforce the relationship between oxidative balance and plumage coloration (Arai et al. 2018). These phenotypic plasticities alone cannot explain the observed interspecific pattern, which suggests an evolutionary trade-off between the two kinds of sexual traits, constraining the evolution of each sexual trait. The causality behind the relationship, however, remains to be clarified, possibly with experimental manipulation of oxidative status.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10164-021-00726-x>.

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Author's contribution MH did most field survey, performed statistical analysis, and wrote most of the manuscript, EA and MN assisted field survey, provided technical support and improved the manuscript.

Data availability The datasets were uploaded to osf.io (<https://doi.org/10.17605/osf.io/b5cs7>).

Declarations

Conflict of interest We have no conflict of interest.

Ethical approval The permits for animal capture and handling were provided by Niigata Prefecture in Japan (#1 and #24 for 2017 and 2018, respectively), following the Wildlife Protection and Hunting Management Law.

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