

# Sperm length variation among Afrotropical songbirds reflects phylogeny rather than adaptations to the tropical environment



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## ABSTRACT

Sperm cells vary tremendously in size and shape across the animal kingdom. In songbirds (Aves: Passeri), sperm have a characteristic helical form but vary considerably in size. Most of our knowledge about sperm morphology in this group stems from studies of species in the Northern temperate zone, while little is known about the numerous species in the tropics. Here we examined sperm size in 125 Afrotropical songbird species with emphasis on the length of the major structural components (head, midpiece, flagellum), and total sperm length measured using light microscopy. Mean total sperm length varied from 51  $\mu\text{m}$  to 212  $\mu\text{m}$  across species. Those belonging to the Corvoidea superfamily had relatively short sperm with a small midpiece, while those of the three major Passeridan superfamilies Passeroidea, Muscicapoidae and Sylvioidea showed large interspecific variation in total sperm length and associated variation in midpiece length. These patterns are consistent with previous findings for temperate species in the same major clades. A comparative analysis with songbird species from the Northern temperate zone ( $N = 139$ ) showed large overlap in sperm length ranges although certain temperate families (e.g. Parulidae, Emberizidae) typically have long sperm and certain Afrotropical families (e.g. Cisticolidae, Estrildidae) have relatively short sperm. Afrotropical and temperate species belonging to the same families showed no consistent contrasts in sperm length. Sperm length variation among Afrotropical and Northern temperate songbirds exhibits a strong phylogenetic signal with little or no evidence for any directional latitudinal effect among closely related taxa.

## 1. Introduction

Sperm show extraordinary morphological diversity across the animal kingdom (Pitnick et al., 2009). For example, sperm length varies in insects from  $\sim 7 \mu\text{m}$  in a parasitoid wasp (Uzbekov et al., 2017) to  $\sim 58,290 \mu\text{m}$  in a fruit fly (Pitnick et al., 1995). The evolutionary forces underlying this diversification are not well understood although it is widely acknowledged that sperm competition, i.e. the competition

among males for fertilizations (Parker, 1970), must play an important role (Pitnick et al., 2009; Simmons and Fitzpatrick, 2012). Changes in sperm length are also associated with changes in the architecture of the female reproductive tract (Briskie et al., 1997; Miller and Pitnick, 2002; Higginson et al., 2012). The theory of anisogamy generally emphasizes the selective advantage for males to produce tiny gametes in vast numbers (e.g., Parker, 1982; Lessells et al., 2009). Overall, there is good evidence for sperm competition across the animal kingdom and that

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males enhance their success in sperm competition through an increase in sperm numbers, i.e. the raffle principle (Parker, 1982, Pizzari and Parker, 2009). However, it does not automatically follow that sperm competition selects for smaller sperm. Empirical evidence suggests that the effect of sperm competition on sperm size evolution can be rather complex (Snook, 2005) and comparative analyses of many animal taxa have in fact revealed a positive association between sperm competition and sperm length (Fitzpatrick and Lüpold, 2014). There is also experimental evidence in insects that sperm competition selects for an increase in sperm length (Godwin et al., 2017). In passerine birds, sperm competition accelerates the rate of sperm length evolution, but changes in sperm length can go either way (Rowe et al., 2015). This means that sperm competition may select for both sperm number and larger or smaller sperm, and that the trade-off between numbers and size may vary considerably among taxa (Immler et al., 2011).

We report on a study of sperm length variation among Afrotropical songbirds (Aves: Passeriformes: Passeri). Across all songbird species, regardless of geographic location, sperm length varies in the range of 40–290  $\mu\text{m}$ , which is a modest size range compared to insects, but still much wider than in other groups of birds (Jamieson, 2007). Most knowledge of variation in sperm traits across the global avifauna originates from studies of birds in the Northern temperate zone with little known from the tropics. This might imply a biased picture as ecological requirements and adaptation may affect trait evolution differently in the two regions, especially the evolution of various reproductive traits and mating systems (Stutchbury and Morton, 2008), and hence the evolution of sperm. Life-history traits show some differences between tropical and temperate birds in adult mortality (Peach et al., 2001), reproductive rates and clutch size (Ricklefs and Wikelski, 2002), pace of life (Wiersma et al., 2007) and parental investment (Ghalambor and Martin, 2001; Jetz et al., 2008; McNamara et al., 2008). There is also correlative evidence showing associations between various life-history and socioecological traits and proxies for sperm competition in birds (reviewed in Lifjeld et al., 2019), from which one might predict that tropical species in general should have lower levels of sperm competition than those in the temperate zones (Stutchbury and Morton, 2001). However, the available evidence for a contrast in sperm competition is far from conclusive (Macedo et al., 2008; Albrecht et al., 2013; Lifjeld et al., 2019).

There are some comparative studies on sperm lengths in African birds (Omotoriogun et al., 2016a, b) including those that list sperm length data for African species (Immler et al., 2011, Albrecht et al., 2013), but a more comprehensive analysis of variation in sperm length in Afrotropical birds relative to Northern temperate birds is lacking. Here we present the first comparative analysis of this kind based on 125 Afrotropical species, and combined this with data on 139 temperate species of songbird to investigate whether they follow the general patterns known for temperate species of the same clade or whether there are specific patterns to Afrotropical species.

## 2. Materials and methods

### 2.1. Field work

Our study samples were collected from birds in Nigeria and Cameroon, specifically at Amurum Forest Reserve, Jos (09° 53' N, 08° 59' E); Yankari Game Reserve, Bauchi (09° 50' N, 10° 30' E); Omo Forest Reserve, Ogun (06° 51' N, 4° 30' E), Okomu National Park, Benin (06° 15' N, 05° 09' E); and IITA Forest, Ibadan (07° 30' N, 03° 55' E); Mt Cameroon (primary lowland to montane forest; 04° 15' N, 09° 09' E), Big Babanki, and Laide Farm Bamenda-Banso Highlands (forest-farm-land mosaics; 06° 05' N, 10° 28' E). Birds were captured using mist-nets (assisted with song-playback), and breeding males were sampled i.e., February to October in 2010–2015 in Nigeria, and November to June in 2008–2014 in Cameroon. Sperm samples (~ 0.5–3  $\mu\text{l}$ ) were collected with microcapillary tubes from the cloacal protuberance of male birds

after a gentle massage (Wolfson, 1952; Kleven et al., 2008; Kucera and Heidinger, 2018) and diluted in a small volume (~20  $\mu\text{l}$ ) of Phosphate-buffered saline (PBS) solution before fixed in ~300  $\mu\text{l}$  of 5% formaldehyde solution for later slide preparation. Sampled birds were fitted with uniquely numbered aluminium bands (from SAFRING) to avoid resampling, and immediately released into site of capture after sampling.

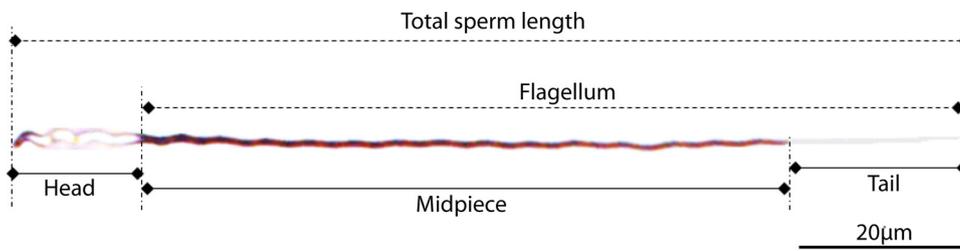
### 2.2. Species coverage

Altogether, we analysed sperm data from 2937 males of 125 species from 28 taxonomic families from Africa. They all belong to four superfamilies of the Passeri clade (songbirds): Corvoidea (8 species from 4 families), Passeroidea (52 species from 7 families), Muscicapoidea (15 species from 2 families), and Sylvioidea (50 species from 15 families). The Corvoidea belongs to the infraorder Corvidae, whereas the three other superfamilies belong to the Passerides (*sensu* Cracraft, 2014). For the comparison with species of the Northern temperate zone, we compiled a data set from the Avian Sperm Collection Database at the Natural History Museum, University of Oslo (NHMO) (Lifjeld, 2019). This data set contained 139 species from 34 families, all belonging to the same two infraorders Corvidae and Passerides as the Afrotropical species. The two data sets, with sperm length measurements averaged for species and the raw data from individual specimens, are available as supplementary material (Additional file 2). The data set of sperm measurements with metadata can also be downloaded from GBIF.org (Omotoriogun et al., 2020).

### 2.3. Species phylogeny

The phylogenetic trees of songbird species used for analyses were pruned from a larger tree (Marki, 2018) which was obtained using a supermatrix approach (Sanderson et al., 1998). DNA sequences of species were downloaded from GenBank; where sequence data was unavailable from focal species a substitute sequences from close relatives was used instead (*sensu* Price et al., 2014). The sequences originate from nuclear intron 2 of the myoglobin gene (Myo2), introns 6 and 7 of the ornithine decarboxylase (ODC) gene, intron 11 of the glyceraldehyde-3-phosphodehydrogenase (GAPDH) gene, and the two mitochondrial genes cytochrome b (cytb) and NADH dehydrogenase subunit 2 (ND2). A matrix of the GenBank accession numbers is available as supplementary material (Additional file 4).

Sequences alignment was performed using the Muscle algorithm (Edgar, 2004) in SeaView v4.5.4 (Gouy et al., 2009); ambiguous regions contained in the resulting alignments were removed using GBlocks (Castresana, 2000). The data matrix of all five genes (3240 bp) was concatenated and analysed in RAxML v8.2.4. The best fitting model of nucleotide evolution was determined by the Bayesian Information Criterion (BIC) in jModelTest 2 (Darrriba et al., 2012) and applied as follows: GTR + I +  $\Gamma$  model for cytb, ND2, and ODC; GTR +  $\Gamma$  for Myo2, and SYM + I +  $\Gamma$  for GAPDH partitions. We applied relaxed uncorrelated log-normal distribution for the molecular clock models that was unlinked across all five gene partitions (Drummond et al., 2006). A Yule speciation process was used for the tree prior, with rate heterogeneity, base frequencies, and substitution rates were unlinked across the five gene partitions. In BEAST, Markov Chain Monte Carlo chains was ran for 100 million generations, sampling every 10 000 generations. Convergence diagnostics based on Effective Sample Size was assessed in Tracer v1.6 (Rambaut et al., 2014) discarding 10% as burn-in. Results were summarized as posterior distribution as a maximum clade credibility tree (with mean node heights) using TreeAnnotator v1.8.3 (Drummond et al., 2012). A nexus file for this tree, containing all 264 species included in this study, is available as a supplementary material (Additional file 1).



**Fig. 1.** The sperm component measurements. The length of the three components, head, midpiece and tail, were measured separately, the length of the flagellum was calculated as the sum of midpiece and tail, and total sperm length was calculated as the sum of head and flagellum. The sperm image is from a Blue-billed Malimbe *Malimbe nitens*.

#### 2.4. Measurement of sperm morphology

We prepared microscope glass slides from a small aliquot (~15 µl) of the formaldehyde fixed sperm samples by spreading a drop of the fixed sperm sample on a clean microscope slide and let it air-dry for 24 hours. Slides were then rinsed with distilled water and allowed to air-dry. Using a digital camera (DFC420, Leica Microsystems, Heerbrugg, Switzerland) mounted onto a digital light microscope (DM6000 B, Leica Microsystems), high-resolution digital images of individual spermatozoon were captured at microscope magnifications (160× or 320×). Leica application suite version 2.6.0 R1 was applied to measure ( $\pm 0.1 \mu\text{m}$ ) the length of sperm head, midpiece and tail (i.e. the section of the flagellum not entwined by the midpiece) following Laskemoen et al. (2007). We calculated sperm total length (sum of head, and midpiece, and tail length) and flagellum length (sum of midpiece and tail length). The measured sperm components are illustrated in Fig. 1. Repeated measurements of the same 15 sperm cells from a single individual showed high repeatability (head:  $r = 0.87$ ,  $F_{14,15} = 14.75$ ,  $P < 0.001$ ; midpiece:  $r = 0.81$ ,  $F_{14,15} = 9.76$ ,  $P < 0.001$ , tail:  $r = 0.83$ ,  $F_{14,15} = 10.94$ ,  $P < 0.001$ ), as calculated according to Lessells and Boag (1987). Measurements of sperm head, midpiece, flagellum and total length for individual males were based on the mean of 10 spermatozoa measured per male as a standard (mean of 9.8, minimum of 3 sperm measured in samples with few sperm).

#### 2.5. Statistics and comparative analysis

Analyses were performed in R version 3.1.2 (R Development Core Team, 2017). Prior to analysis, we log-transformed data for all sperm traits. We reconstructed ancestral character states of sperm total length using the “phytools” (Revell, 2013) and the “ape” package (Paradis and Schliep, 2018) in R. Character states were estimated at internal nodes using maximum likelihood with “fastAnc” and “contMap” functions, both in “phytools” (Revell, 2013).

We tested for the presence of phylogenetic signal in sperm traits using Pagel’s  $\lambda$  (Pagel, 1999) and Blomberg’s  $K$  (Blomberg et al., 2003) in “phytools” (Revell, 2013). These measures of phylogenetic signal are not identical,  $\lambda$  measures the strength of phenotypic-genotypic covariance assuming Brownian motion ( $\lambda = 1$  equals Brownian motion) whereas  $K$  reflects the partitioning of trait variance among and within clades: with high  $K$  values implying more variance among clades and low  $K$  values meaning more variance among the terminal branches. For Pagel’s  $\lambda$ , log-likelihood ratio tests were used to determine if the estimated maximum likelihood values of  $\lambda$  differed from 0 (no phylogenetic signal) or 1 (total dependence on phylogeny); whereas randomization test were used to determine whether traits exhibited a significant phylogenetic signal ( $K > 0$ ) in Blomberg’s  $K$ .

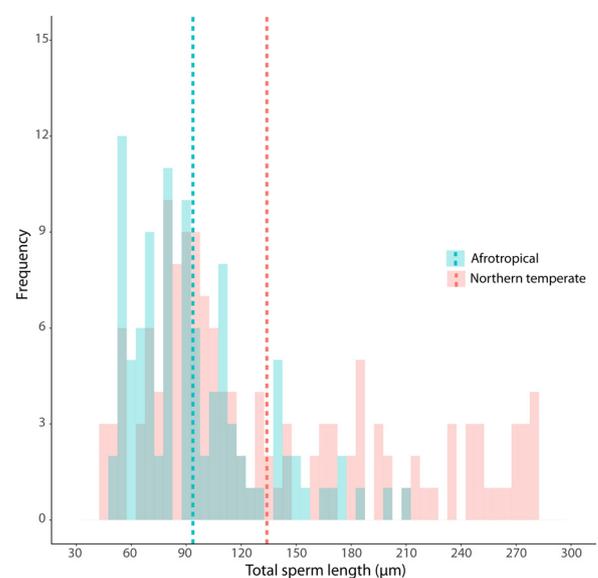
We performed phylogenetic generalized least squares (PGLS) regressions to test for differences in sperm traits (as response variable) between Afrotropical and Northern temperate (region as predictor variable). The PGLS approach accounts for the statistical non-independence of data points as a result of common ancestry of species (Pagel, 1999; Freckleton et al., 2002) and allows the estimation (via maximum likelihood) of the phylogenetic scaling parameter lambda ( $\lambda$ ) as above. We tested the likelihood ratio of  $\lambda$  value against  $\lambda = 1$  and

$\lambda = 0$ . We also restricted analysis to family levels with species representation from both regions, and tested the effect of region on total sperm length, and length of sperm components. PGLS regressions were performed using the package “caper” (Orme, 2013).

### 3. Results and Discussion

For Afrotropical songbirds, sperm lengths ranged from 51 µm in the Singing Cisticola *Cisticola cantans* to 212 µm in the Streaky-headed Seedeater *Crithagra gularis*. Among the temperate species, sperm lengths ranged from 43 µm (White-throated Dipper *Cinclus cinclus*) to 280 µm (Indigo Bunting *Passerina cyanea*, Eastern Towhee *Pipilo erythrophthalmus* and Chipping Sparrow *Spizella passerina*). A histogram covering all species of the two regions illustrates the more restricted range of sperm lengths for Afrotropical species, and that sperm on average across all species are shorter for Afrotropical than for Northern temperate species (Fig. 2).

When the Afrotropical species were broken down by the four superfamilies (see example images in Fig. 3), sperm were relatively short in Corvoidea (54 – 83 µm) compared to the more diverse sperm lengths in the three Passerides superfamilies, i.e. Passeroidea (53 – 212 µm), Muscicapoidea (81 – 184 µm) and Sylvioidea (51 – 148 µm). These patterns of sperm length variation among African songbirds largely match the patterns seen among the major clades, i.e. above the family level, in the Passeri (Jamieson, 2007); longer sperm (> 100 µm) are typically found within the Passerides songbirds, while Corvids songbirds have shorter sperm, similar to the sister group of the Passeri, the Tyranni (Jamieson, 2007). It suggests that longer sperm have evolved frequently within each of the superfamilies of the Passerides, from an



**Fig. 2.** Histogram of total sperm lengths of songbird species from the Afrotropical and Northern temperate zones, the dash lines representing the mean of sperm length (Afrotropical, mean = 93.6 µm, N = 125; Northern temperate, mean = 134.0 µm, N = 139).



**Fig. 3.** Examples of sperm length variation across four superfamilies of Afrotropical songbirds illustrated by digital images scaled to the same magnification (the 40 µm scalebar indicated). The 27 species are: Muscicapoidae: M1-*Turdus pelios*, M2-*Cossypha albicapillus*, M3-*Cossypha niveicapilla*, M4-*Neocossyphus poensis*, M5-*Melaenornis pallidus*, M6-*Stiphornis erythrothorax*. Passeroidea: P1-*Vidua macroura*, P2-*Emberiza affinis*, P3-*Emberiza tahapisi*, P4-*Ploceus cucullatus*, P5-*Ploceus heuglini*, P6-*Euplectes macroura*, P7-*Cryptospiza reichenovii*, P8- *Cinnyris venustus*, P9- *Cinnyris cupreus*. Sylvioidea: S1-*Cisticola guinea*, S2-*Cercotrichas harlaubi*, S3- *Sylvietta brachyura*, S4- *Eremomela pusilla*, S5-*Prinia subflava*, S6-*Psalidoprocne obscura*, S7-*Phyllastrephus albigularis*, S8-*Pycnonotus barbatus*, S9-*Nicator chloris*. Corvoidea: C1-*Platysteira cyanea*, C2-*Terpsiphone rufiventer*, C3-*Dyaphorophya blissetti*.

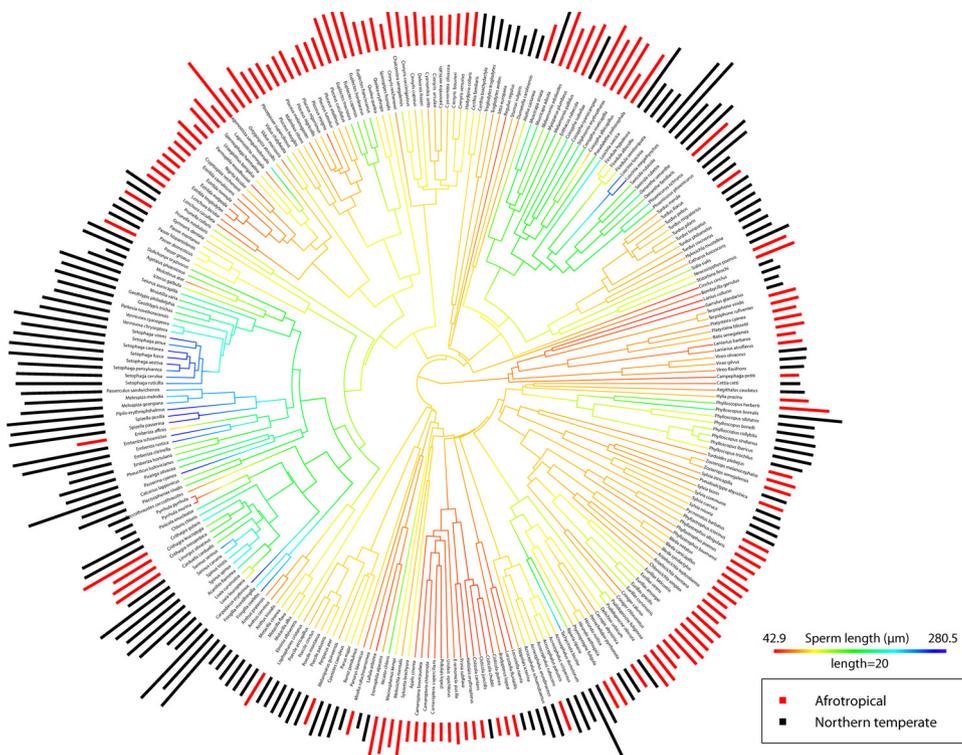
ancestral state of short sperm.

When the sperm lengths are mapped onto the phylogeny (Fig. 4), it becomes evident that certain clades stand out with some distinct patterns in sperm lengths. For example, the Fringillidae and Muscicapidae have longer sperm whereas the Cisticolidae have consistently shorter sperm than all the other families. Within the Ploceidae, species of the genus *Euplectes* have consistently longer sperm than those of their sister genus *Ploceus* (Fig. 4). There are, however, also some large intrageneric contrasts in sperm lengths among Afrotropical species, such as *Crithagra gularis* (Fringillidae) and *Lagonosticta sanguinodorsalis* (Estrildidae) having rather long sperm compared to their congeners.

Another major contrast between Corvids and Passerides sperm is the length of the midpiece. It is short in Corvids and long in Passerides, except for the atypical Passerides sperm in some *Pyrrhula* finches (Lifjeld et al., 2013). The long midpiece is strongly correlated with the flagellum length in Passerides, and covers up to almost 90% of total sperm length in the species with the longest sperm (Lifjeld, 2019). The length of the sperm head is not much differentiated across the Passeriformes. In the present data set, only members of the Muscicapidae

family stand out as having a longer sperm head than the rest. Figures visualizing the length of the different sperm components mapped onto the phylogeny (as in Fig. 4) can be found in the supplementary material (Additional file 3).

There was no effect of region (Afrotropical versus Northern temperate zone) on overall sperm length or the length of sperm components when controlling for phylogeny (Table 1). The skewed distributions apparent in Fig. 2 must therefore be largely due to family level differences in sperm length and uneven family representation from the two regions. Some African families (e.g. Cisticolidae, Estrildidae) typically have short sperm while some Northern temperate families (e.g. Emberizidae, Parulidae) have rather long sperm. There was also a strong phylogenetic signal in sperm length components across Afrotropical and Northern temperate birds as revealed by Pagel's  $\lambda$  ( $\lambda \geq 0.884$ ,  $P < 0.0001$ ) and Blomberg's  $K$  ( $K \geq 0.893$ ,  $P = 0.001$ ) (Table 1). Midpiece, flagellum and total sperm length tended to be more similar among related species than expected under Brownian motion but with certain marked exceptions (e.g. *Crithagra gularis* and *Lagonosticta sanguinodorsalis*). Most of the variation in sperm length can therefore be



**Fig. 4.** Variations in total sperm length ( $\mu\text{m}$ ) in Afrotropical ( $N = 125$ ) and Northern temperate ( $N = 139$ ) songbird mapped on their phylogeny. Sperm length is visualized in colour along the branches and nodes in the phylogeny in a gradient from red (short sperm) to blue (longer sperm). Branch lengths are given in million years (myr). The barplots on the periphery of the phylogeny show sperm lengths for species of the two regions separated by colour of the bars (Afrotropical = red, Northern temperate = black).

attributed to differences among the deeper nodes in the phylogeny, e.g. the family level.

When we restricted the analysis to four larger families (Fringillidae, Hirundinidae, Muscicapidae, and Turdidae) with multiple species represented in both regions, there was still no significant effect of region on sperm length or sperm components (Fig. 5, Table 2). The only exception was head length in the Turdidae for the separate family analysis, but this could be an artefact of multiple tests. Again, there were strong phylogenetic signals in total sperm length and the length of the different sperm components (Table 2).

We therefore conclude that the large variation in sperm length observed among songbirds has a strong phylogenetic component and that much of the differentiation occurred early in the evolutionary history of the clade. There are marked differences in sperm length between taxonomic families, which explains the tendency for Afrotropical species to have shorter sperm than Northern temperate species. We find no evidence that current selection pressures related to the tropical environment and associated adaptations in ecology and life history, can explain any differences in sperm length among closely related species. Nonetheless, we must emphasize that species coverage was not exhaustive for this study, as sperm morphology remains unknown for the majority of Afrotropical passerines.

**Table 1**

Phylogenetic least squares (PGLS) analysis of differences in sperm components between Afrotropical ( $N = 125$ ) and temperate ( $N = 139$ ) species, with the component of interest as a response variable and climate zone as explanatory variable. The model including the maximum-likelihood of lambda ( $\lambda$ ) value was compared against the models assuming  $\lambda = 1$  and  $\lambda = 0$ , and superscripts following the  $\lambda$  values indicate probability (P) of likelihood-ratio of sperm trait (first position: against  $\lambda = 0$ ; second position: against  $\lambda = 1$ ). The table also gives two estimates of the phylogenetic signal in the sperm components as expressed by Pagel's  $\lambda$  (with P based on a likelihood ratio test) and Blomberg's K (with P based on randomisation test of the null hypothesis of  $K = 0$ ).

Sperm trait	PGLS				Pagel's $\lambda$		Blomberg's K	
	Estimate ( $\pm$ SE)	t	P	$\lambda$	$\lambda$	P	K	P
Head length	$-0.05 \pm 0.31$	-0.172	0.864	$1 < 0.001, 1.00$	1.0044	< 0.0001	0.8926	< 0.001
Midpiece length	$-0.05 \pm 0.09$	-0.505	0.614	$1 < 0.001, 1.00$	1.0155	< 0.0001	1.6142*	< 0.001
Flagellum length	$-0.06 \pm 0.05$	-1.118	0.265	$1 < 0.001, 1.00$	1.0132	< 0.0001	1.5085	< 0.001
Total sperm length	$-0.05 \pm 0.05$	-1.089	0.277	$1 < 0.001, 1.00$	1.0129	< 0.0001	1.5175*	< 0.001

\* K also significantly higher than 1 (P < 0.05).

#### 4. Authors' contribution

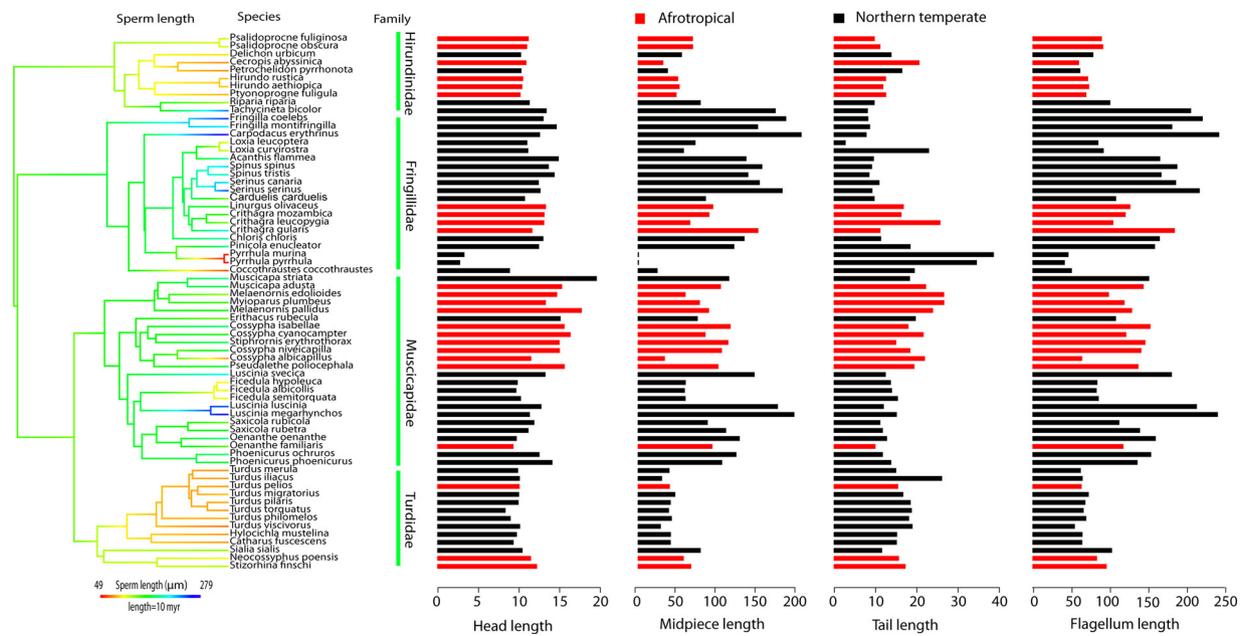
Conceived and designed the study: TCO, JTL; data collection: all authors; data analyses: TCO; data management and interpretation: TCO, JTL, LEJ, drafted the manuscript: TCO; All authors read, commented and approved the final manuscript.

#### 5. Availability of data and materials

The datasets for this article are included within the article and its additional supporting files. The dataset of sperm measurements is also published on GBIF.org (Omotoriogun et al., 2020). Sperm samples are deposited in the Avian Sperm Collection at Natural History Museum, Oslo (Lifjeld, 2019), which can be accessed online at the museum's website (<https://www.nhm.uio.no/>).

#### 6. Consent for publication

Not applicable



**Fig. 5.** Variation in sperm total lengths and components in the Fringillidae, Muscipidae, Turdidae and Hirundinidae with species represented in both Afrotropical and Northern temperate regions with barplot differentiated by colours (Afrotropical = red; Northern temperate = black) for the head, midpiece and flagellum for the two regions. The phylogeny illustrates variation of sperm total length for the families with a scale bar of colour ranging from red to blue; the scale bar also indicates branch lengths in million years (myr).

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**Declaration of Competing Interest**

The authors declare that they have no competing interests.

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**Table 2**

Phylogenetic least squares (PGLS) analysis of regional differences in sperm components of songbird species (N = 67) from four families with representation from both the Afrotropical and the Northern temperate region. The model including the maximum-likelihood of lambda ( $\lambda$ ) value was compared against the models assuming  $\lambda = 1$  and  $\lambda = 0$ , and superscripts following the  $\lambda$  values indicate probability (P) of likelihood-ratio of sperm component (first position: against  $\lambda = 0$ ; second position: against  $\lambda = 1$ ). The table also gives two estimate of the phylogenetic signal in the sperm components as expressed by Pagel's  $\lambda$  (with P based on a likelihood ratio test) and Blomberg's K (with P based on a randomisation test).

Family	Sperm trait	PGLS					Pagel's Lambda		Blomberg's K	
		Estimate ( ± SE)	t	P	$\lambda$	$\lambda$	P	K	P	
Fringillidae (N = 20)	Head length	0.09 ± 0.23	0.438	0.666	0.996 < 0.0001, 0878	1.0216	< 0.0001	1.1941	0.006	
	Midpiece length	-0.08 ± 0.67	-0.122	0.904	1 < 0.0001, 1	1.0307	< 0.0001	0.9448	0.003	
	Flagellum length	-0.09 ± 0.29	-0.307	0.762	1 0.0005, 1.00	1.0287	0.0005	0.9066	0.002	
	Total sperm length	-0.07 ± 0.27	-0.261	0.797	1 0.0005, 1.00	1.0284	0.0005	0.9218	0.001	
Muscipidae (N = 24)	Head length	-0.05 ± 0.08	-0.621	0.541	1 0.0005, 1	1.0952	< 0.0001	1.4670	0.001	
	Midpiece length	-0.19 ± 0.17	-1.093	0.284	1 0.015, 1	1.0969	0.0001	1.1146	0.001	
	Flagellum length	-0.13 ± 0.13	-1.002	0.327	1 0.0054, 1	1.0972	0.0002	1.0860	0.001	
	Total sperm length	-0.12 ± 0.12	-1.044	0.308	1 0.0038, 1	1.0971	0.0002	1.0848	0.001	
Turdidae (N = 13)	Head length	0.15 ± 0.05	3.043	0.011	0 1, 0.0085	0.7044	0.0748	0.9254	0.052	
	Midpiece length	0.04 ± 0.14	0.304	0.767	1 0.035, 1	1.1699	0.0010	1.9568	0.003	
	Flagellum length	-0.01 ± 0.08	-0.121	0.906	1 0.010, 1	1.1688	0.0006	2.0087	0.005	
	Total sperm length	0.00 ± 0.07	0.011	0.992	1 0.010, 1	1.1386	0.0008	2.0747	0.006	
Hirundinidae (N = 10)	Head length	-0.01 ± 0.05	-0.280	0.787	1 0.059, 1	1.0680	0.0118	1.1467	0.004	
	Midpiece length	-0.31 ± 0.26	-1.195	0.266	1 0.013, 1	1.0781	0.0007	1.1867	0.001	
	Flagellum length	-0.22 ± 0.21	-1.051	0.324	1 0.026, 1	1.0784	0.0006	1.1543	0.002	
	Total sperm length	-0.19 ± 0.19	-1.015	0.340	1 0.029, 1	1.0785	0.0005	1.1568	0.001	

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.zool.2020.125770>.

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