Biological Journal of the Linnean Society, 2025, **146**(**2**), blaf103
https://doi.org/10.1093/biolinnean/blaf103

OXFORD

Original Article

Weak sperm differentiation in Darwin's finches

Jan T. Lifjeld[1,*], Eduardo Garcia-del-Rey[2], Gaute Grønstøl[1], Carlos A. Valle[3], Erica H. Leder[1,4,5]

[1]Natural History Museum, University of Oslo, PO Box 1172 Blindern, Oslo, 0318, Norway
[2]Macaronesian Institute of Field Ornithology, Santa Cruz, Tenerife, 38001, Spain
[3]Colegio de Ciencias Biológicas y Ambientales, Galápagos Science Center GSC, Universidad San Francisco de Quito USFQ, Quito, 170901, Ecuador
[4]Department of Marine Sciences, University of Gothenburg, Strömstad, 45296, Sweden
[5]Department of Biology, University of Turku, Turku, 20520, Finland

[*]Corresponding author. Natural History Museum, University of Oslo, PO Box 1172 Blindern, Oslo, 0318, Norway. E-mail: j.t.lifjeld@nhm.uio.no

ABSTRACT

Spermatozoa may provide insights into the evolutionary history, reproductive isolation, and mating systems of species. Here we combine sperm and genomic data to conduct the first comparative analysis of sperm differentiation among Darwin's finches, an iconic adaptive radiation with considerable gene flow across species borders. All eight study species had the typical form of songbird spermatozoa, but shorter than most other species in the Thraupidae family. There was no detectable differentiation in sperm length among four ground finch species (*Geospiza*) and two tree finch species (*Camarhynchus*). In both genera, autosomal single nucleotide polymorphisms (SNPs) revealed signatures of genetic admixture. The grey warbler-finch *Certhidea fusca* and the vegetarian finch *Platyspiza crassirostris* had significantly shorter sperm than the sister genera *Geospiza* and *Camarhynchus* from which they diverged about 0.90 and 0.43 Mya, respectively. The largest intergeneric divergences in sperm length were of the same magnitude as divergences observed within the speciation continuum in other songbirds over similar time spans. Relatively high among-male variation in sperm length indicates a moderate-to-low level of extrapair paternity and a divergence rate in sperm length that is lower than in more promiscuous songbirds. We conclude that sperm size evolution is too slow to drive prezygotic isolation in this radiation.

Keywords: adaptive radiation; extrapair paternity; Galápagos Islands; promiscuity; reproductive isolation; speciation; spermatozoa; Thraupidae

INTRODUCTION

Spermatozoa are highly differentiated across the animal kingdom and adapted to locate and fertilize conspecific ova (Birkhead *et al.* 2009). They may therefore play a role in speciation through prezygotic isolation (Howard 1999). Internal fertilizers have on average longer sperm than external fertilizers (Kahrl *et al.* 2021), and female promiscuity seems to drive sperm elongation in a wide range of animal taxa with internal fertilization (Gomendio and Roldan 2008), including mammals (Tourmente *et al.* 2011) and passerine birds (Briskie *et al.* 1997, Kleven *et al.* 2009, Lüpold *et al.* 2009, Lifjeld *et al.* 2010). The Passerides songbirds cover the entire range of sperm lengths known for birds, i.e. from 30 to 290 μm (Fitzpatrick *et al.* 2022), and long sperm have evolved independently multiple times in this group from an ancestral state of short sperm (Omotoriogun *et al.* 2020). Moreover, female promiscuity is negatively associated with intrapopulation variance in sperm length in songbirds (Calhim *et al.* 2007, Kleven *et al.* 2008, Lifjeld *et al.* 2010, 2019), as well as in other taxa (Fitzpatrick and Baer 2011, Varea-Sánchez *et al.* 2014, Rowley *et al.* 2019). This is generally interpreted as an effect of promiscuity-mediated stabilizing selection (Lüpold and Pitnick 2018). The reduced sperm size variance implies less overlap between divergent allopatric populations, which may lead to rapid prezygotic isolation in promiscuous species (Lifjeld *et al.* 2024, Ottenburghs 2024). Thus, spermatozoa, in combination with the mating system, can play an important role in speciation.

Birds are quite well studied regarding phenotypic variation, phylogeny and systematics, ecology, and behaviour, but there are still major gaps in our knowledge about their spermatozoa. In the most comprehensive sperm morphology database published to date, i.e. SpermTree (Fitzpatrick *et al.* 2022), there is morphometric information from around 560 bird species. This number amounts to only 5% of known species of Aves (Gill *et al.* 2024). There is considerable potential for better insights to the role of spermatozoa in many aspects of the biology of birds.

Received 23 June 2025; revised 8 August 2025; accepted 30 August 2025
© The Author(s) 2025. Published by Oxford University Press on behalf of The Linnean Society of London.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

Here, we present the first study of sperm morphology in Darwin's finches, an iconic adaptive radiation, and a textbook example of allopatric speciation (Grant 1986, Newton 2003, Coyne and Orr 2004, Price 2008). The radiation encompasses 18 currently recognized species (Gill *et al.* 2024) across the Galápagos archipelago and Cocos Island that have evolved rapidly from a single common ancestor during the past million years (Lamichhaney *et al.* 2015). Species are mainly diversified in beak shape and body size, and to a lesser extent in plumage coloration (Lack 1947, Grant and Grant 2008). They are taxonomically grouped into four genera in the Galápagos Islands: *Certhidea* (two warbler-finches), *Platyspiza* (one species, the vegetarian finch), *Camarhynchus* (five tree finches) and *Geospiza* (nine ground finches), plus *Pinaroloxias inornata* (Cocos finch) on Cocos Island. Genomic studies have estimated a time-calibrated phylogeny for all species (Lamichhaney *et al.* 2015), but also verified extensive introgressive hybridization among tree finches (Kleindorfer *et al.* 2014, Peters *et al.* 2017, Kleindorfer and Dudaniec 2020) and ground finches (Farrington *et al.* 2014, Lamichhaney *et al.* 2015, Grant and Grant 2019, Rubin *et al.* 2022, Enbody *et al.* 2023). There is even a case of hybrid speciation (Lamichhaney *et al.* 2017). These new insights have led to an amplified theory of how the group has radiated by a combination of allopatric divergence and sympatric resource competition, natural selection, and introgressive hybridization (Grant and Grant 2024b). Nonetheless, because species borders are clearly porous and blurred in this group, the taxonomic delineation of species remains a controversial issue (Zink 2009, McKay and Zink 2015, Cadena *et al.* 2017, Zink and Vázquez-Miranda 2019, Cadena and Zapata 2021).

Our study includes eight species from all four genera occurring in the Galápagos islands, but is restricted to two islands, i.e. San Cristóbal and Santa Cruz. Our primary aim was to describe the sperm morphology and differentiation of these populations in a phylogenetic context, and in relation to their mating system. Because several of our study populations have not been incorporated in previous phylogenetic analyses, and gene flow makes the recognition of discrete gene pools uncertain, we performed our own analyses of population genetic structure and phylogeny based on genome-wide single nucleotide polymorphisms (SNPs).

Darwin's finches have a socially monogamous mating system with biparental care (Grant and Grant 2008). Extrapair paternity has been documented in several molecular paternity studies of *Geospiza scandens*, *Geospiza magnirostris*, and *Geospiza fortis* on the small island of Daphne Major (Grant and Grant 2024a). They indicate a moderate to low frequency of extrapair paternity in the range of 6–10% extrapair young (EPY) for *G. scandens and G. magnirostris* (Petren *et al.* 1999, Grant and Grant 2011, 2024a) and 14–17% for *G. fortis* (Grant and Grant 2011, 2019). These frequencies are lower than the level for passerine species in general, i.e. 19% (Brouwer and Griffith 2019, Valcu *et al.* 2021). Here we use the coefficient of among-male variation in sperm length as a proxy for the frequency of EPY (Lifjeld *et al.* 2010, 2019). Because female promiscuity is a potential driver of sperm length differentiation in incipient species of passerine birds (Lifjeld *et al.* 2023, 2024), but also an evolutionary labile trait with much variation within species (Valcu *et al.* 2021), it is important to assess the two traits within the same set of populations. Ultimately, we wanted to explore the potential for rapid sperm length divergence as a mechanism of postcopulatory prezygotic isolation (Lifjeld *et al.* 2023, 2024, Ottenburghs 2024) that might complement the current understanding of premating isolation in this adaptive radiation.

MATERIAL AND METHODS

Study area and study species

Field work took place during 18–22 March 2023 near Jardín de Opuntias, San Cristóbal, and during 24–29 March 2023 around the Charles Darwin Research Station, Puerto Ayora, Santa Cruz (Fig. 1). We targeted territorial, breeding males, and used playback of conspecific song to elicit an aggressive response to lure them into mist-nets. We measured wing chord (flattened and straightened right wing) with a wing ruler to the nearest 0.5 mm, and beak length (from skull), height (at the nostrils), and width (perpendicular to the same position as the height measurement), using a caliper to the nearest 0.1 mm. All measurements were taken by one person only. We collected blood samples (20–80 µl) by brachial venipuncture and preserved them in 1 ml 96% ethanol. Sperm samples (0.5–2 µl) were collected by cloacal massage, diluted in 10 µl of phosphate-buffered saline, and then preserved in 1 ml 5% formaldehyde solution (= 12.5% formalin).

Our study included a total of 106 sampled male individuals (13 females and one suspected male hybrid were excluded from the data set). Sex was determined on plumage characters and/or the shape of the cloacal protuberance. Species identification was based on a combination of body size, beak dimensions and plumage. Male ground finches (*Geospiza*) have variable amount of black body plumage, whereas male tree finches (*Camarhynchus*) and the vegetarian finch have variable amounts of black on the head only. Within both groups, species can best be separated by body size and beak dimensions. We used a Principal Components Analysis (PCA) to examine the clustering of species and verify species identification based on the measurements of beak length, beak height, beak width, and wing length. Figure 1 gives the sample numbers per species and island. Five males had no detectable sperm, so the total number of sperm samples was 101.

Analysis of sperm traits

Sperm samples were prepared for light microscopy using the protocol described in Cramer *et al.* (2021) and Grønstøl *et al.* (2023). Briefly, 15 µl of fixed ejaculate was applied to a microscope slide, air-dried, and rinsed with distilled water. Spermatozoa were photographed at 320× in a Leica DM6000B microscope with a digital camera (Leica Flexacam C1) and 10 haphazardly chosen, morphologically normal cells were measured to the nearest pixel (0.14 µm) using Leica Application Suite software (LAS X v3.7.2). We measured the length of the sperm head (acrosome + nucleus), midpiece (containing the fused mitochondria wrapped helically around most of the flagellum), and tail (i.e. the midpiece-free end of the flagellum). The sum of the three measurements gives the total sperm length, and the sum of the midpiece and tail equals the flagellum length. We calculated the mean value for each segment based on the 10 sperm cells per male. We also calculated the coefficient of variation of sperm length within males (CV_{wm}) and among males (CV_{am}) using their mean values with the correction factor of $(1 + 1/4N)$ for variable sample sizes, since CV is

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

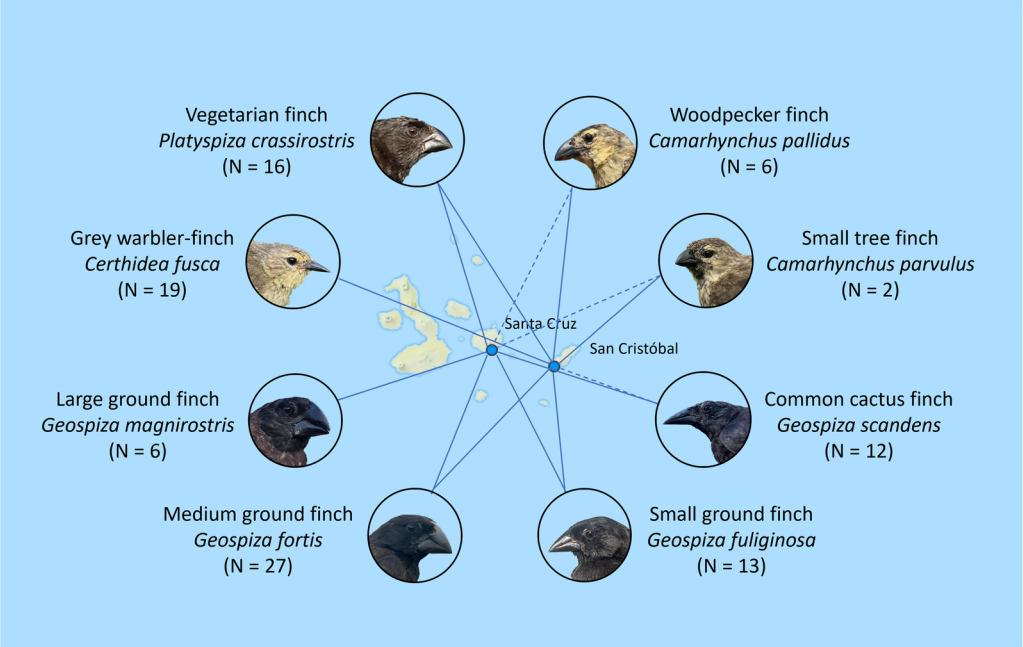

Figure 1. The eight species of Darwin's finches on Santa Cruz and San Cristóbal in the Galápagos Islands included in the study. The blue dots indicate the sampling locations, and the blue lines indicate the presence of the species on each island; the dashed line indicates that no sperm samples were collected. N denotes the total number of sperm samples.

generally underestimated at low sample sizes (Sokal and Rohlf 1981). We used the sperm length CV_{am} to estimate the frequency of EPY, using the conversion formula in Lifjeld *et al.* (2019). All sperm measurements were carried out by one person only. Pairwise divergences were calculated on log-transformed raw data and expressed in Hedges' *g*, which calculates the difference between means in units of the pooled standard deviation (Hedges 1981). Divergence rates were expressed in units of generations, where estimated divergence times (see below) were divided by species-specific generation lengths (in years) taken from the database of Bird *et al.* (2020).

For the comparison of sperm lengths in Darwin's finches with other species in the Thraupidae family, we compiled a data set from the literature, the SpermTree database (Fitzpatrick *et al.* 2022) and the Avian Sperm Collection database at the Natural History Museum Oslo (Lifjeld 2019). For a more detailed view of the structure of the sperm head, i.e. the size of the acrosome and the nucleus, we made scanning electron micrographs of one sperm sample from three species: *C. fusca*, *P. crassirostris*, and *G. fortis*. Sample preparation and imaging procedure followed the protocol outlined in Støstad *et al.* (2018).

Genome assembly and SNP calling

We used a whole-genome sequencing approach to identify SNPs and examine the genetic structure of the study populations and to construct a dated phylogeny for the recognized genetic clusters. We sequenced the whole genomes of 50 of our sampled specimens and downloaded sequences from an additional 14 individuals from San Cristóbal (Rubin *et al.* 2022) from the Sequence Read Archive of NCBI (https://www.ncbi.nlm.nih.gov/sra).

DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol. DNA library preparation and sequencing (150 bp, paired end) on an Illumina Novaseq SP flowcell were performed by the Norwegian Sequencing Centre at Oslo University Hospital. The sequencing output yielded between 36 and 290 million read pairs per individual, while the downloaded genomes had between 36 and 178 million read pairs. Since most samples had between 10× and 30× coverage, we reduced the number of reads for samples with very high sequencing output (> 170 million fragments) to have a similar read depth as the other samples by taking a random sample of the reads using seqkit v.2.5.1 (Shen *et al.* 2016).

Adapter removal and quality trimming were performed with *cutadapt* v.3.5 with a quality threshold of 20 and a minimum length threshold of 80 (Martin 2011). Reads were mapped to a high quality *Camarhynchus parvulus* reference genome (Rubin *et al.* 2022) using BWA v.0.7.18 (Li and Durbin 2010) and converted to unsorted binary files using SAMtools v.1.21 (Li *et al.* 2009, Danecek *et al.* 2021). Duplicates were removed using *fixmate*, *sort*, and *markdup* from SAMtools v.1.21.

The *mpileup* function implemented in BCFtools v.1.18.1 (Danecek *et al.* 2021) was used for SNP calling with a threshold of 20 for minimum mapping quality and the *call* function with the consensus caller option (-m) was used to call the genotypes. Small contigs (< 1000 bases) were omitted from the genome for SNP calling. We also excluded the Z chromosome to avoid any potential biases in SNP heterozygosity since five of the 14 downloaded genomes from NCBI were female and thus have just one Z chromosome. SNPs occurring in repeat regions (soft-masked regions) were removed. Genotypes were further filtered by retaining only loci where the SNP was more than eight bases away from an indel, the locus was a bi-allelic SNP, the locus quality was 30 or greater, the coverage depth was between 500 and 1600 over all individuals, the SNP had a depth of eight reads or greater, and a genotype

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

quality greater than 20 for an individual. Three missing individuals were allowed per SNP, and a minor allele frequency of 0.02. Both BCFtools v.1.17 and VCFtools v.0.1.16 (Danecek *et al.* 2011, 2021) were used for filtering. This filtering retained 1 231 339 SNPs scored in all individuals. These SNPs were further pruned by keeping only one SNP (the first SNP) every 2000 bases, leaving 261 754 SNPs.

Population structure, ancestry, and divergence time estimation

We performed a phylogenetic PCA of the 261 754 SNPs using the program *smartpca* in the *smartsnp* package (https://christianhuber.github.io/smartsnp/). This is an ordination method that reduces the complexity of variation in a huge number of SNP markers into a few axes that best describe the total variation among and within populations. The 64 individuals represented 12 island populations, i.e. four species from both islands and four species from one island. We used the same data set to estimate the most likely number of ancestral gene pools being the source for the current population structure, using the software ADMIXTURE (Alexander *et al.* 2009). For each individual, the method calculates an admixture proportion, which is the estimated genetic contribution from each of the ancestral populations.

In order to estimate the divergence time of the ancestry groups, we performed a Bayesian phylogenetic analysis on the SNP data using SNAPP v.1.6.1 (Bryant *et al.* 2012), an add-on package for the program BEAST2 v.2.7.3 (Bouckaert *et al.* 2019). We used two individuals from each of five ancestral groups identified by the ADMIXTURE analysis to reduce the computational load. We also employed stricter filtering to ensure high quality SNPs since the number of SNPs was also reduced. In this case, we increased the individual minimum read depth to 10 and the genotype quality to 30 and allowed no missing data. Only the first SNP every 2000 bases was retained.

SNAPP calculates the probability of a species tree by mathematically integrating over all possible gene trees from the individual SNP markers. Branch lengths can be converted to a time scale by specifying a time constraint on the deepest divergence event in the tree, following Stange *et al.* (2018). As a calibration point, we used a divergence time of 0.901 Mya for the split between *C. fusca* and the rest of Darwin's finches (Lamichhaney *et al.* 2015) and defined it through a lognormal prior distribution with a mean of 0.901 Mya and a standard deviation of 0.063.

The script prep_snapp.rb of Stange *et al.* (2018) was used to prepare the XML format input file for SNAPP with further random sampling to keep only 50 000 SNPs of the 411 678 SNPs present in this set of individuals. The Markov-chain Monte Carlo (MCMC) process was run in three replicates with a chain length of 100 million and results were stored every 25 000 iterations. We assumed that the population sizes were not equal and thus allowed variable population sizes in the model for divergence by using the parameters. We did so by modifying the XML file and adding SNAPP's 'GammaMover' and 'RateMixer' operators to allow changes in each branch's population size over the course of the MCMC.

The SNAPP log files were checked in Tracer v.1.7.2 (Rambaut *et al.* 2014), ensuring that the effective samples sizes of all model parameters were > 200. The log and tree files from the three replicate SNAPP runs were combined in LogCombiner, a utility program within BEAST2 (Bouckaert *et al.* 2019). Node ages (mean height) and credible intervals [95% highest posterior density (HPD)] were estimated together with the maximum clade credibility tree in TreeAnnotator v.1.7.4 (Drummond *et al.* 2012) and visualized in FigTree v.1.4.4 (Rambaut 2017).

RESULTS

Morphological differentiation among species

Because congeneric species of Darwin's finches are mostly differentiated in body size and beak dimensions, we used a combination of these traits for species identification and confirmation. Genera are more easily distinguished on plumage characters, in addition to body size and beak shape. The results of the PCA of the four structural variables are visualized in Figure 2 for the two most explanatory axes. The first axis (PC1) explained almost 90% of the variation from the small-bodied and thin-beaked *C. fusca* to the large and thick-beaked *G. magnirostris* (One-way ANOVA: $F_{8,97} = 258.8$, $P < .001$). The second axis (PC2) described another 6% in relative beak length, from the pointed-beaked *G. scandens* to the blunt-beaked *P. crassirostris* (One-way ANOVA: $F_{8,97} = 106.7$, $P < .001$). Note that the two island populations of *G. fortis* are shown separately, because birds on San Cristóbal were significantly larger than those on Santa Cruz (PC1; Tukey HSD test: $P = .047$).

Sperm size differentiation

Sperm length data for the eight species of Darwin's finches are summarized in Table 1. Total sperm length averages varied between 116–124 μm, which is in the medium size range for songbirds (Omotoriogun *et al.* 2020). *Certhidea fusca* and *P. crassirostris* had shorter sperm than the *Camarhynchus* and *Geospiza* species. The difference was caused by a shorter flagellum (with a shorter midpiece) while head lengths were similar. All species had an elongated midpiece wrapped helically around 80–90% of the length of the flagellum. There was no significant variation in sperm dimensions among the four *Geospiza* species (One-way ANOVAs: all $P > .20$). Samples sizes of the two *Camarhynchus* species were too small for any meaningful statistical comparison ($N = 2$ and $N = 6$), but they were within the same size range as *Geospiza*. When species within *Geospiza* and *Camarhynchus* were lumped together (Table 1), there were no significant differences between the two genera in any of the sperm traits.

SEM micrographs of sperm heads of three Darwin's finches and two other tanager species revealed very similar head structure with a longer acrosome than nucleus and with a helical membrane along the acrosome (Fig. 3). An acrosome-to-nucleus length ratio above 1 and the acrosomal helix, together with the elongated midpiece, follow the typical spermatozoan 'Bauplan' for songbirds (Jamieson 2007, Støstad *et al.* 2018, Omotoriogun *et al.* 2020).

The total sperm lengths of Darwin's finches were relatively short compared to other species in the Thraupidae family, but similar to their close relatives in the Coerebinae subfamily (Fig. 4). Notably, species in other Thraupidae genera show much larger divergences in sperm length than the Darwin's finch radiation, for example in *Thraupis* and *Sporophila* (Fig. 4).

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

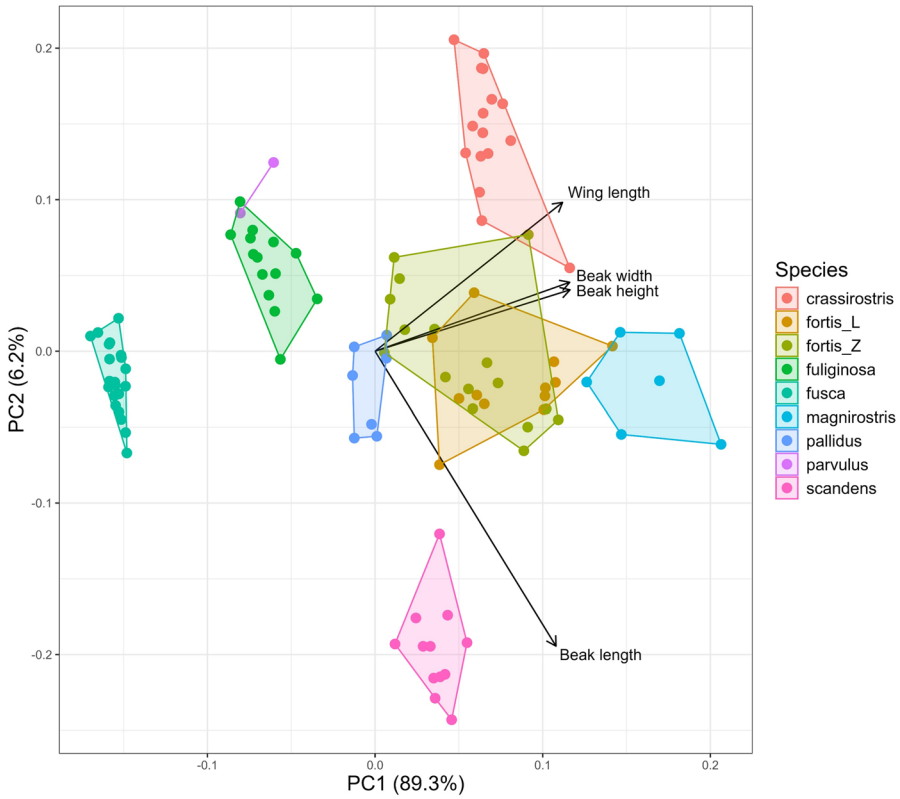

Figure 2. Principal Component Analysis of beak dimensions and body size in Darwin's finches. PC1 explains most of the variation (89.3%) in body size and beak size, whereas PC2 (6.2%) reflects beak shape (from pointed to blunt). Arrowed lines indicate the eigenvectors of the four morphological variables and polygons outline the extreme values within each population. Note that *Geospiza fortis* is shown separately for the two islands (L = San Cristóbal, Z = Santa Cruz) because there was a significant difference between them in PC1 (Tukey's HSD test: $P = .047$).

The among-male coefficient of variation in total sperm length of Darwin's finches with a sample size above eight varied in the range of 2.0–3.0%, which corresponded to an estimated frequency of 8.1–22.5% EPY (Table 1). Overall, these estimates suggested that the EPY frequency varied in the range of 10–15% in the study populations.

Genome-wide SNPs: diversity and ancestry groups

Given the diffuse species borders and horizontal gene flow among Darwin's finches, particularly within *Camarhynchus* and *Geospiza*, their differentiation in spermatozoa (or lack thereof) should be interpreted in relation to their genetic distances. We therefore analysed the population genetic structure among the eight study species, as well as between conspecifics from both islands.

After mapping the Illumina reads to the *C. parvulus* reference genome, filtering and SNP calling, we ended up with a dataset of about 260 000 autosomal SNPs from a total of 64 individuals. A phylogenetic PCA revealed a clear separation of genera (Fig. 5A, B). The first axis (PC1) separated *C. fusca* from all other species. PC2 separated *P. crassirostris* from the *Camarhynchus* and *Geospiza* species, whereas PC3 split *Camarhynchus* from *Geospiza*. The fourth axis (PC4) spaced out the four *Geospiza* species with some between-island overlap for *scandens* and *fortis*. Interestingly, the two *Camarhynchus* species clustered together on all four PCA axes and separated first on PC5 (not illustrated).

We also ran a population structure analysis using the ADMIXTURE software to identify the most likely number of ancestral groups that can best describe the current SNP variation in the data set. The outputs revealed lowest cross-validation errors for K = 5 (Fig. 5D), which meant that the best model assigned the 64 sampled individuals to five ancestral groups. The estimated contribution of each ancestral group to each sample is illustrated by colours for K = 3–6 in Figure 5C. For K = 5, the five ancestral groups matched closely with the four genera, while the four *Geospiza* species were admixed from two ancestral groups. *Geospiza scandens* and *magnirostris* were more purely assigned to each of the two ancestral groups, whereas *fuliginosa* and *fortis* were more admixed. This pattern was consistent with the intermediate position of *fuliginosa* and *fortis* on PC4 in the PCA analysis (Fig. 5B). Moreover, the two *Camarhynchus* species were assigned to the same ancestral group, which agrees with their high similarity in the PCA clusters (Fig. 5A, B).

A dated phylogeny

In order to estimate the divergence times among the five ancestral groups, we ran a Bayesian phylogenetic analysis using SNAPP (Bryant *et al.* 2012) with the basal split between *C. fusca* and the rest of Darwin's finches as a calibration point of 0.901 Mya (Lamichhaney *et al.* 2015). The resulting maximum clade credibility tree is visualized in Figure 6A. The topology was largely congruent with Lamichhaney *et al.* (2015), except for the relationships within *Geospiza* (see below), but divergence times were younger than theirs. Our analysis estimated a split between *P. crassirostris* and the main lineage at about 0.431 Mya (95% HPD

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

Table 1. Length of sperm components for the eight species and four genera of Darwin's finches (mean ± SD). All length measurements are given in μm, coefficients of variation within-male (CV_{wm}) and among-male (CV_{am}), and predicted extrapair young (EPY) in percent. – indicates sample size too small for estimation. Predicted EPY percentages were calculated from the CV_{am} of total sperm length (cf. Lifjeld *et al.* 2019)

Taxa	Total sperm length (TSL)	Head length	Midpiece length	Flagellum length	CV_{wm} of TSL	CV_{am} of TSL	Predicted EPY %
***Certhidea fusca* (*N* = 19)**	116.36 ± 3.49	14.78 ± 0.45	82.16 ± 6.39	101.58 ± 3.50	2.02 ± 0.75	3.04	8.1
***Platyspiza crassirostris* (*N* = 16)**	116.69 ± 2.58	14.41 ± 0.53	84.61 ± 1.86	102.28 ± 2.32	2.14 ± 0.73	2.25	17.3
***Camarhynchus pallidus* (*N* = 6)**	120.86 ± 1.50	14.55 ± 0.28	94.81 ± 3.05	106.31 ± 1.29	2.30 ± 0.67	–	–
***Camarhynchus parvulus* (*N* = 2)**	123.79 ± 0.06	14.12 ± 0.32	98.47 ± 2.68	109.67 ± 0.26	1.81 ± 0.31	–	–
***Geospiza scandens* (*N* = 12)**	123.73 ± 2.43	14.50 ± 0.31	93.72 ± 1.69	109.23 ± 2.39	1.76 ± 0.56	2.00	22.5
***Geospiza fuliginosa.* (*N* = 13)**	121.71 ± 3.18	14.42 ± 0.54	93.17 ± 2.26	107.30 ± 2.83	1.62 ± 0.54	2.66	11.4
***Geospiza fortis* (*N* = 27)**	123.39 ± 3.39	14.57 ± 0.46	94.10 ± 3.13	108.82 ± 3.27	1.86 ± 0.51	2.78	10.2
***Geospiza magnirostris* (*N* = 6)**	121.61 ± 4.28	14.71 ± 0.52	93.69 ± 2.46	106.91 ± 4.29	1.85 ± 0.39	–	–
Merged congeneric species:							
***Camarhynchus* two species (*N* = 8)**	121.60 ± 1.86	14.44 ± 0.34	95.72 ± 3.25	107.15 ± 1.90	2.18 ± 0.62	–	–
***Geospiza* four species (*N* = 58)**	122.90 ± 3.30	14.54 ± 0.45	93.77 ± 2.60	108.37 ± 3.17	1.78 ± 0.51	2.70	11.0

interval: 0.373–0.488), and a split between the *Camarhynchus* and *Geospiza* clades about 0.160 Mya (0.136–0.186). The corresponding estimates by Lamichhaney *et al.* (2015) were 0.546 ± 0.074 and 0.412 ± 0.097 Mya, respectively. The divergence time for the two ancestral groups of *Geospiza* was estimated to 0.069 Mya (0.043–0.095), but this is probably an underestimate due to recent gene flow between the two lineages. Different patterns of gene flow may also explain why Lamichhaney *et al.* (2015) found that *scandens* were more closely related to *magnirostris*, and *fuliginosa* to *fortis*, in contrast to our findings of *scandens* and *fuliginosa*, and *fortis* and *magnirostris*, being more related. Finally, a traitgram (Fig. 6B) illustrates how the mean sperm lengths may have differentiated with time in this radiation.

The rate of sperm length divergence

With the estimates of divergence times among the distinct genetic groups in the radiation and the magnitude of divergences in sperm length among them, it is possible to compare the rates of sperm length divergence with those of other passerine groups for which similar data exist. We extracted such data from Lifjeld *et al.* (2024) within the same time frame as the Darwin's finch radiation, i.e. the past million years or < 500 000 generations. The pairwise divergences in sperm length, quantified in Hedges' *g*, among the four Darwin's finch genera were largely at the same scale as other songbird divergences (Hedges' *g* in the range of 0–2, i.e. mean values differed within 0–2 standard deviations) for similar divergence times and for similar level of extrapair paternity (Fig. 7). However, the divergences were larger (Hedges' $g > 2$) for a few songbird species with higher rates of extrapair paternity, viz. the polygynandrous alpine accentor *Prunella collaris*, two highly promiscuous *Ammospiza* sparrows and two subspecies of the Eurasian bluethroat *Luscinia svecica* (Fig. 7). This pattern shows that sperm length in Darwin's finch radiation has differentiated at a similar rate as incipient songbird species with intermediate to low frequency of extrapair paternity, but at a lower rate than species with more female promiscuity. The rate of sperm length divergence in songbirds is mostly influenced by the trait's variance, as more female promiscuity reduces the variance, and small variances reduce overlap between trait distributions (Lifjeld *et al.* 2024). As an illustrative example, Figure 8 compares the sperm length distributions of *P. crassirostris* and the combined *Geospiza* group with those of a Spanish and a Moroccan population of *Prunella collaris* reported in Lifjeld *et al.* (2023). The two pairs have similar sperm lengths and a similar gap between mean values, but different trait variances presumably driven by different levels of female promiscuity. The higher variance in Darwin's finches translates into a higher overlap between sperm length distributions (31.0%) than that of the two alpine accentor populations (12.4%). The alpine accentor populations have also diverged over a shorter time span (82 000 years) than Darwin's finches (431 000 years).

DISCUSSION

We have shown here that Darwin's finches exhibit the typical sperm morphology of Passerides songbirds, with a helical acrosome that is longer than the nucleus, an extended midpiece coiled around the flagellum, and an intermediate total length (Jamieson 2007, Omotoriogun *et al.* 2020). Moreover, the eight study species were rather weakly differentiated in their sperm lengths compared to many other clades of closely related songbird species (Rowe *et al.* 2015, Omotoriogun *et al.* 2020) and even conspecific populations with shorter divergence times (Lifjeld *et al.* 2024). This is a relevant observation for understanding speciation in songbirds because spermatozoa may function as a speciation phenotype in some radiations, but not in others. In the following, we discuss the most likely factors contributing to the observed pattern of

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

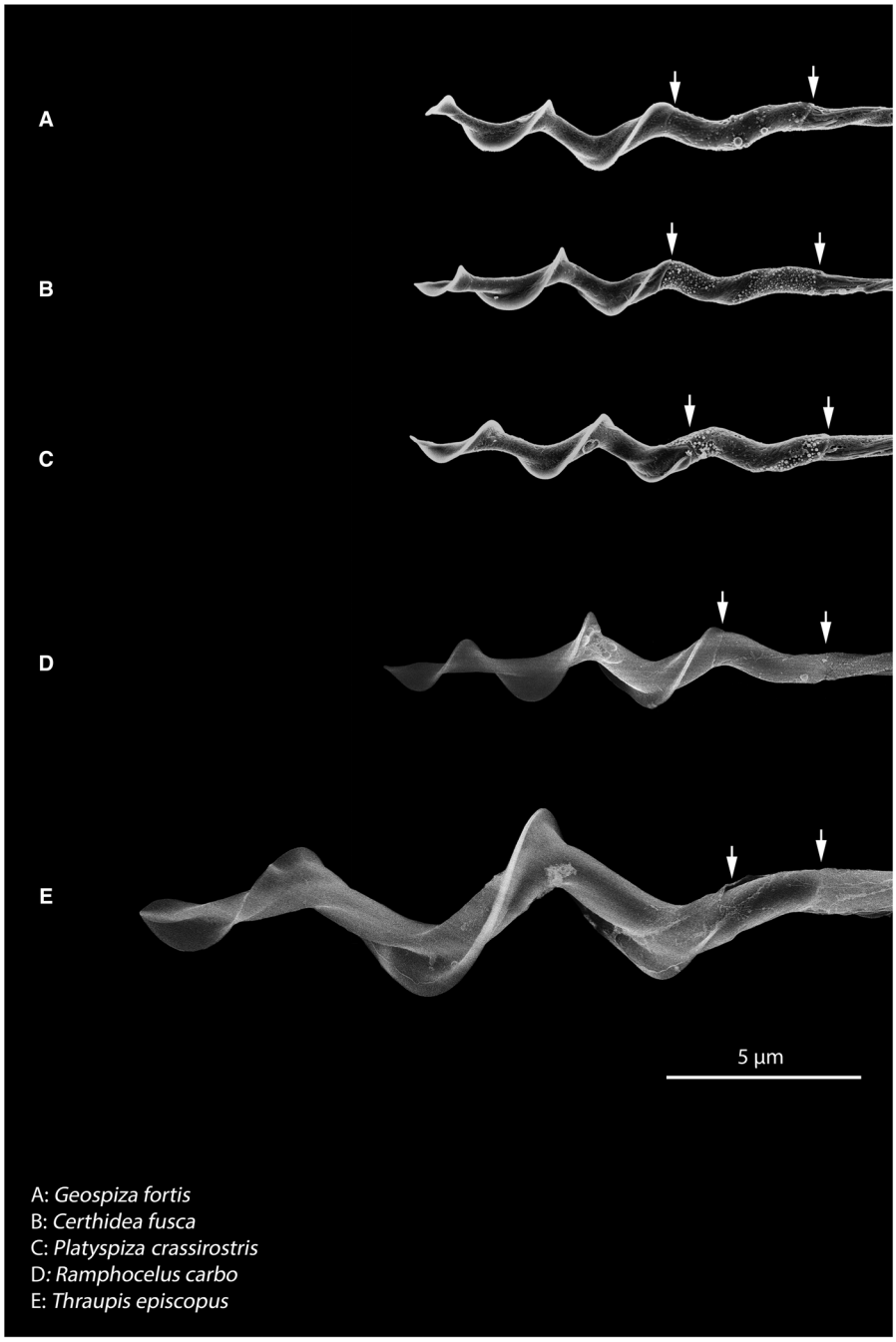

Figure 3. Scanning electron micrographs of sperm heads of three Darwin's finches and two other tanager species shown at the same scale. Arrows indicate the transition between the acrosome and the nucleus, and between the nucleus and the flagellum with the coiled midpiece. In all species the acrosome is longer than the nucleus.

weak sperm differentiation, including the role of genes, selection, and time, and thus why spermatozoa seem to play a minor role as a reproductive barrier in Darwin's finches.

Sperm and beak phenotypes

Darwin's finches are a young adaptive radiation that has diversified into 18 species from a single common ancestor in less than a million years (Lamichhaney *et al.* 2015). This high diversification rate can hardly be explained by the regular allopatry-then-sympatry model of speciation (Grant and Grant 2024b), because it usually takes a million years or more of isolation in allopatry for reproductive isolation to evolve (Newton 2003, Coyne and Orr 2004, Price 2008), and especially so at lower latitudes (Weir and Schluter 2007). Instead, the current understanding of this radiation is that species are at an early stage of the speciation process where full reproductive isolation has not been reached. Species integrity is still maintained by a few genomic regions under strong natural selection that are not eroded by gene flow or recombination. To the contrary, gene flow through introgressive hybridization fuels genetic variation at these loci and ensures a potential for rapid adaptive responses to changes in selection pressures (Grant and Grant 2024b). Rubin *et al.* (2022) found that the phenotypic differentiation in beak shape and size, as

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

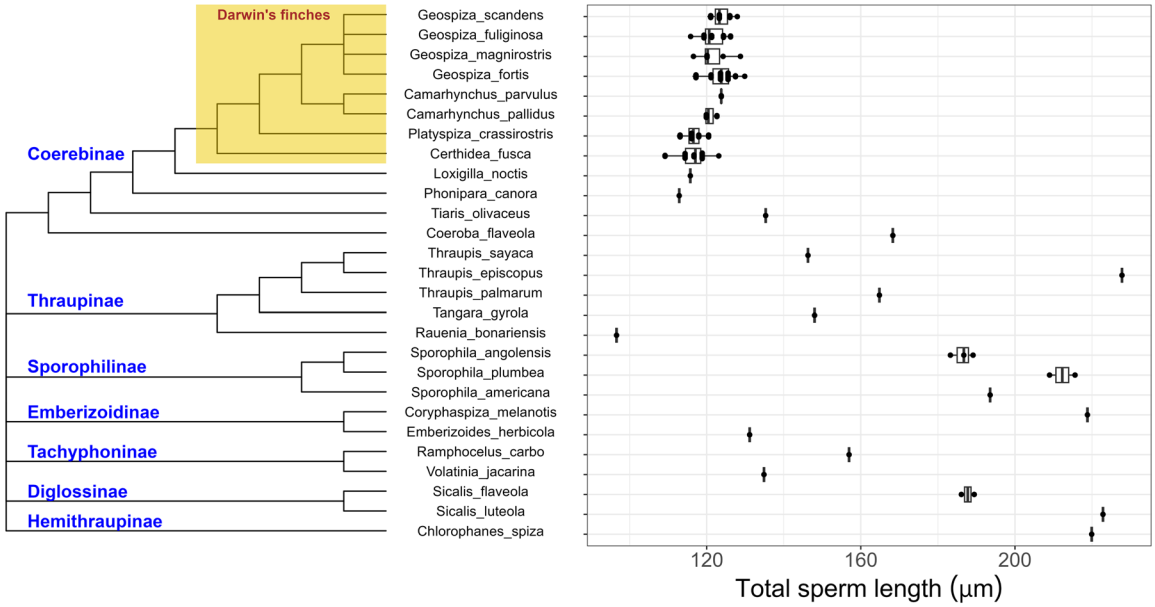

Figure 4. Sperm length variation in the tanager family (Thraupidae). The left panel illustrates the phylogenetic relationships (no branch lengths) among species within each subfamily following the topology in Burns *et al.* (2014). Subfamilies (blue font) are shown as a polytomy following Burns *et al.* (2016). The right panel shows species-level sperm lengths as a box plot where the box indicates the interquartile range with the median as a vertical bar, and horizontal bars connect minimum and maximum values. For species without a box, there is only one data point.

well as body size, among three *Geospiza* species were encoded by a set of 28 genomic regions located on the larger autosomes. Enbody *et al.* (2023) identified six loci that explained 45% of the variation in beak size in *G. fortis*. It is the combination of alleles at these genetic modules that determines the phenotypes that define the species.

Grant and Grant (2024b) coined this speciation model as a 'competition-selection-hybridization' process. The differentiation in beak size and shape clearly reflects adaptations to different dietary sources (Lack 1947, Grant and Grant 2008), and these phenotypes can rapidly respond to shifts in selection pressures (Gibbs and Grant 1987). The theory also assumes that these adaptive peaks may be shifted by competition among species. We observed a trait shift between the two island populations of *G. fortis* in the first component (PC1) of the PCA of beak dimensions and body size (Fig. 2), with significantly larger individuals on San Cristóbal than on Santa Cruz. This shift might be explained by a size reduction on Santa Cruz due to competition from the larger *G. magnirostris* on the island. Alternatively, *G. fortis* on San Cristóbal may have shifted towards a larger size following the extinction of *G. magnirostris* on the island (and on Floreana) in a few decades after Charles Darwin's visit to the Galapagos in 1835 (Grant and Grant 2021). Another possibility is that there is a within-island bimodality, which has previously been documented for the Santa Cruz population (Hendry *et al.* 2006, 2009), and a sampling bias in our data. Our restricted data set does not allow any further interpretation of this observation.

We found that sperm length variation in the radiation was not associated with the differentiation in beak phenotypes. Instead, sperm lengths clustered in two size groups that diverged early in the evolutionary history of the radiation, i.e. between the *Certhidea/Platyspiza* lineages and the *Camarhynchus/Geospiza* lineages (Fig. 6B). There was no clear differentiation among species within either of them. It seems therefore reasonable to assume that genes affecting sperm length are not associated or linked with the genetic modules that affect the species-determining phenotypes. They must be located elsewhere in the genome and are therefore more exposed to the homogenizing forces of past or ongoing gene flow.

Sperm length and sperm competition

Compared to the rapid differentiation in beak size and shape, the evolution of sperm length is clearly slower. A basic difference between the two types of traits is their selective environments. While the beak is under natural selection for adaptation to the dietary environment, sperm size is under sexual selection for performance, i.e. fertilization success, inside the female reproductive tract. Spermatozoa are adapted to locate, penetrate, and fertilize ova in that environment, but sperm traits may also be shaped by competition from sperm of other males in the race for fertilization. Sperm competition results from female promiscuity through extrapair copulations in socially monogamous birds. The risk and strength of sperm competition is regarded an important selective force that shapes sperm length in songbirds in two ways. First, species with elevated rates of extrapair paternity have generally evolved longer sperm (Briskie *et al.* 1997, Kleven *et al.* 2009). The sperm lengths of Darwin's finches lie at the shorter end of the spectrum for the Thraupidae family, but not very different from their closest relatives outside the Galápagos. Thus, there is no indication that sperm lengths in Darwin's finches have moved much away from their ancestors. Other members of the Thraupidae family have evolved longer sperm, as shown in Figure 4. Long sperm has evolved independently multiple times among Passerides songbirds and particularly among families in the Passeroidea superfamily (Omotoriogun *et al.* 2020), which includes the Thraupidae. In another family of the Passeroidea, the Fringillidae, sperm length varies widely among species and is positively correlated with relative testis size (Immler and Birkhead 2007),

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

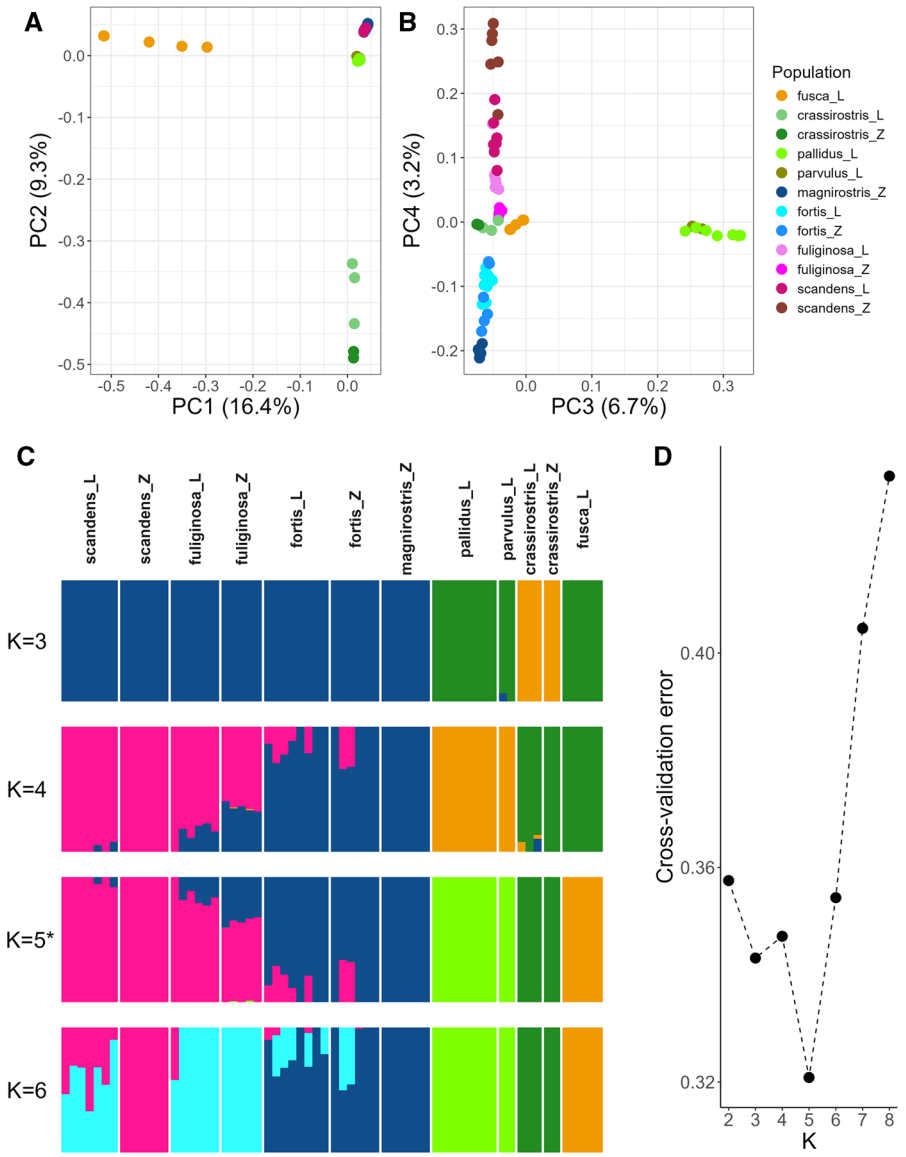

Figure 5. Population structure of Darwin's finches based on 261 754 autosomal SNPs among 64 individuals from 12 island populations. Island is indicated after the species names (L= San Cristóbal, Z = Santa Cruz). A Principal Component Analysis (panels A and B) shows the clustering of species and island along PC1 and PC2 (A), and PC3 and PC4 (B). The results of an ADMIXTURE analysis are shown in (C) for K = 3–6, where K = 5* has the lowest cross-validation errors (D).

another common proxy for female promiscuity and sperm competition.

The other signature of female promiscuity is a reduced variation in sperm lengths among individual males (Immler *et al.* 2008, Kleven *et al.* 2008, Lifjeld *et al.* 2010, 2019). In fact, the coefficient of among-male variation (CV_{am}) in sperm length explains around 75% of the variation in EPY rates among songbird populations (Lifjeld *et al.* 2019). A general interpretation is that female promiscuity increases the strength of stabilization selection around an optimum size that matches the female environment (Calhim *et al.* 2007, Cramer *et al.* 2023, Lifjeld *et al.* 2023, 2024). The sperm length CV estimates for Darwin's finches indicate a moderate-to-low rate of EPY in line with the previous paternity estimates for two *Geospiza* species on Daphne Major (Grant and Grant 2011, 2019). Thus, the large among-male variation in sperm length suggests that the socially monogamous mating system of Darwin's finches is characterized by low levels of female promiscuity in the contemporary populations, and the short sperm lengths overall indicate no promiscuity-driven selection for longer sperm over evolutionary time scales.

Sperm and speciation

The rate of sperm evolution in Darwin's finches seems slow when we compare their divergences to other radiations. For example, species within the genera *Thraupis*, *Sporophila*, and *Sicalis* span a wider range in sperm lengths than the intergeneric differences among Darwin's finches (Fig. 4). *Thraupis episcopus* has the longest sperm known in the family (228 μm) and considerably longer than its close relative *Thraupis sayaca* (146 μm). According to Cueva *et al.* (2022), the two species diverged about 0.93 Mya which is comparable to the age of the Darwin's finch radiation.

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

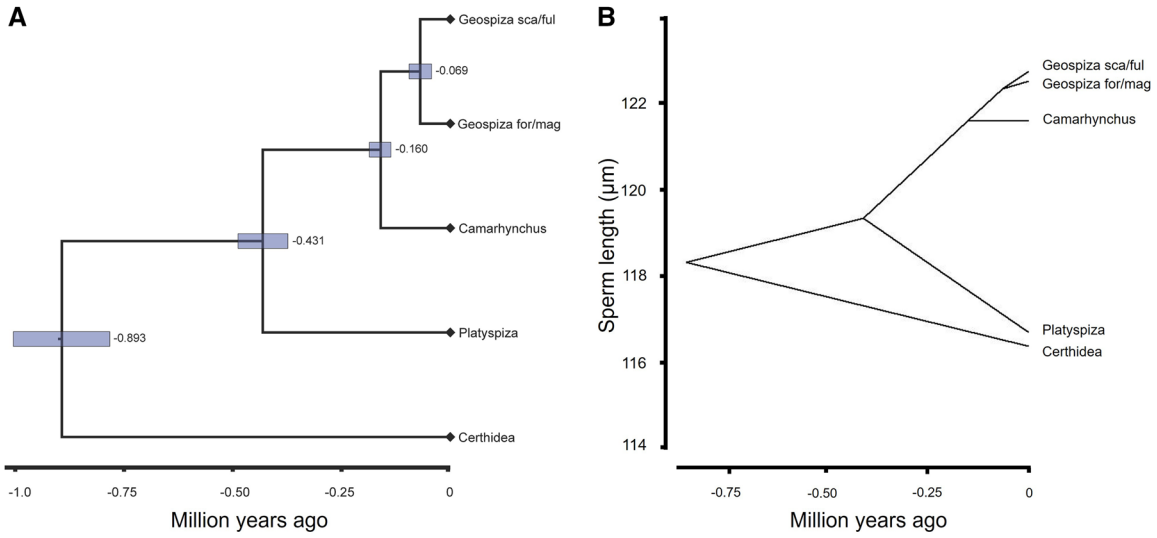

Figure 6. A, a time-calibrated species tree for Darwin's finches based on 50 000 autosomal SNPs and a constraint of 0.901 Mya divergence time for the basal split between *Certhidea fusca* and the main lineage (Lamichhaney *et al.* 2015). Node ages are given with the 95% highest probability density interval indicated in blue bars. B, a traitgram depicting the differentiation of mean sperm length through time for the five genetic clusters of Darwin's finches.

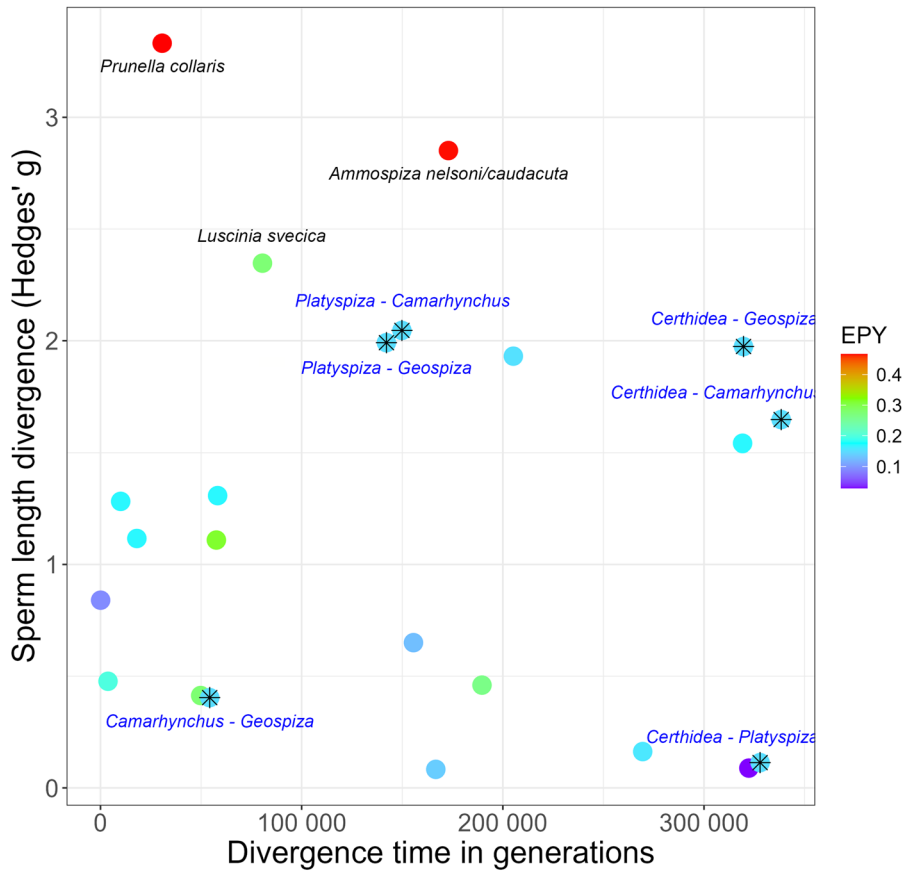

Figure 7. Sperm length divergence in relation to divergence time for various songbird populations. Genus pairs of Darwin's finches are indicated with starred points and blue font; the other points represent populations pairs taken from Lifjeld *et al.* (2024) with a divergence time less than 500 000 generations. The three largest sperm length divergences in high-promiscuity species are annotated in black font. The colour scale refers to the proportion of extrapair young (EPY: see Lifjeld *et al.* 2024 for details). For Darwin's finches, the frequency of EPY was set to 0.10.

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

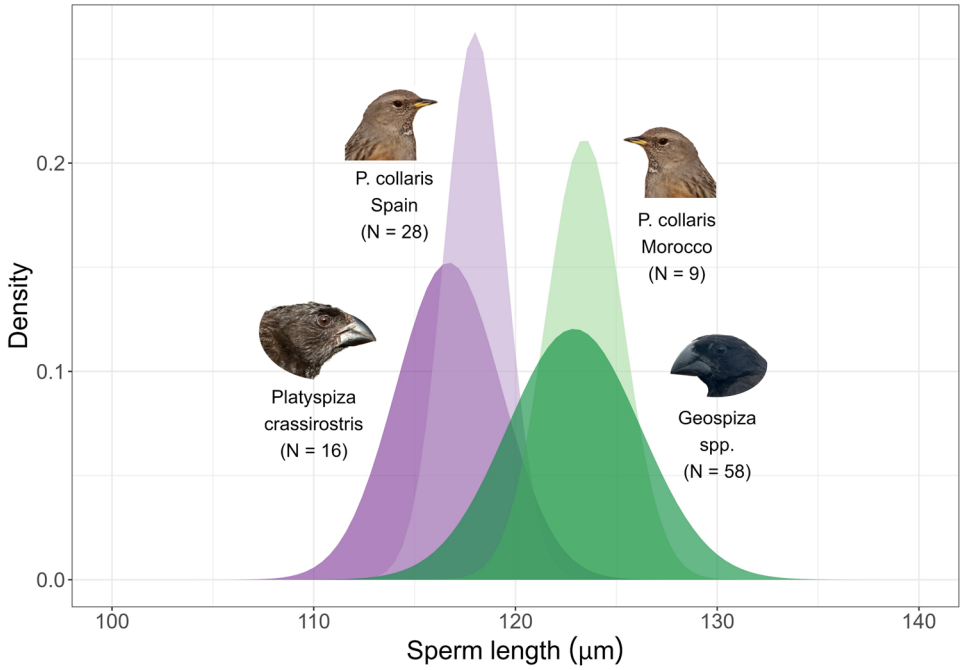

Figure 8. Comparison of sperm length divergence between two Darwin's finch groups (the vegetarian finch *Platyspiza crassirostris* and ground finches *Geospiza* spp.) and two populations of the polygynandrous alpine accentor *Prunella collaris* (data from Lifjeld *et al.* 2023). The curves are normal distributions drawn from the mean and the standard deviation. The estimated divergence time is 431 000 years for the Darwin's finch populations and 82 000 years for the alpine accentor populations. Images of *Prunella collaris* by Thore Koppetsch.

Female promiscuity is an important driver of sperm length divergence (Rowe *et al.* 2015) and seems to have a stronger effect than divergence time on sperm length divergence in songbirds (Lifjeld *et al.* 2024). The hypothesis that rapid sperm length divergence can promote prezygotic isolation in promiscuous lineages assumes that sperm length is more influential in causing assortative fertilization than other traits driving assortative mating. In Darwin's finches, phenotypic traits evolve much faster than sperm traits, and they function as mating traits through assortative pair formation (Grant and Grant 2024b). Sperm lengths seem too undifferentiated and sperm competition too weak for assortative fertilization on sperm length to evolve. Hybridization and introgression are also fairly common among *Camarhynchus* species (Kleindorfer *et al.* 2014, Kleindorfer and Dudaniec 2020) and *Geospiza* species (Grant and Grant 2024a, b) that are undifferentiated in sperm size. There are only a few reported cases of hybrids involving *C. fusca* and apparently none with *P. crassirostris* (McCarthy 2006), which both have shorter sperm than *Camarhynchus* and *Geospiza*. Here, heterospecific sperm might have reduced fertility or competitiveness, but the frequency of such intergeneric mating is presumably very low.

The Darwin's finch radiation, and particularly that of the *Camarhynchus* and *Geospiza* species, will probably continue to evolve under strong natural selection pressures for ecologically adaptive traits and horizontal gene flow that will counteract any disruptive selection on sperm phenotypes. A scenario that might alter the selection dynamics in this system is an event that would favour more female promiscuity, for example stronger pathogen-mediated selection for immune-compatible offspring (Johnsen *et al.* 2000, Fossøy *et al.* 2008, Lindsay *et al.* 2019) that in effect could strengthen selection on sperm phenotypes and accelerate divergence through reduced trait variance (Lifjeld *et al.* 2024, Ottenburghs 2024).

CONCLUSION

Our study of eight Darwin's finches has revealed a clear differentiation in genotypes and somatic phenotypes, but a much weaker differentiation in sperm phenotypes. We attribute the low sperm differentiation to the rather young age of the radiation combined with a monogamous mating system with moderate to low levels of extrapair paternity. We conclude that sperm diverge too slowly to cause reproductive isolation in this system.

ACKNOWLEDGEMENTS

We thank Lars Erik Johannessen for curating the sperm databases, Audun Schrøder-Nielsen and Jarl Andreas Anmarkrud for DNA extractions and sample submission to the Norwegian Sequencing Centre and the NCBI SRA, and members of the Sex and Evolution Research Group at NHM Oslo for comments to the manuscript. Samples were collected under permits issued by Ministerio del Ambiente de Ecuador (permit number MAATE-DBI-CM-2022-0270) and the Galápagos National Park (permit number 034-2023 DPNG). The samples were exported to Norway under permit number 021-2023-EXP-CM-FAU-DBI/MAATE issued by Ministerio del Ambiente de Ecuador. We are grateful to the Dirección de Biodiversidad del Ministerio del Ambiente, Agua y Transición Ecológica de Ecuador MAATE, the Galapagos National Park Directorate, and the Ecuadorian authorities for the permits. We also thank the Charles Darwin Research Station for allowing us to catch and sample birds on their land.

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

AUTHOR CONTRIBUTIONS

J.T.L. conceived of, designed, and acquired funding for the study. J.T.L. and C.A.V. acquired permits. J.T.L., E.G.d.R., C.A.V., and E.H.L. collected the field data. J.T.L., G.G., and E.H.L. performed the formal analyses and created visualizations. J.T.L. and E.H.L. wrote the original draft of the manuscript, which all authors reviewed and edited.

CONFLICT OF INTEREST

None declared.

FUNDING

The study was funded by The Research Council of Norway (grant number 301592 to J.T.L.). The computations were performed on resources provided by Sigma2—the national infrastructure for high-performance computing and data storage in Norway.

DATA AVAILABILITY

The paired-end sequencing reads are available on NCBI Sequence Read Archive (SRA) under the BioProject ID PRJNA1261677. Data and R scripts used in the analyses are deposited in the Dryad digital repository (https://doi.org/10.5061/dryad.3ffbg79w8).

REFERENCES

Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* 2009;**19**:1655–64. https://doi.org/10.1101/gr.094052.109

Bird JP, Martin R, Akçakaya HR *et al.* Generation lengths of the world's birds and their implications for extinction risk. *Conservation Biology: The Journal of the Society for Conservation Biology* 2020;**34**:1252–61. https://doi.org/10.1111/cobi.13486

Birkhead TR, Hosken DJ, Pitnick S. *Sperm Biology: an Evolutionary Perspective*. Oxford: Elsevier, 2009.

Bouckaert R, Vaughan TG, Barido-Sottani J *et al.* BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 2019;**15**:e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Briskie JV, Montgomerie R, Birkhead TR. The evolution of sperm size in birds. *Evolution; International Journal of Organic Evolution* 1997;**51**:937–45. https://doi.org/10.2307/2411167

Brouwer L, Griffith SC. Extra-pair paternity in birds. *Molecular Ecology* 2019;**28**:4864–82. https://doi.org/10.1111/mec.15259

Bryant D, Bouckaert R, Felsenstein J *et al.* Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution* 2012;**29**:1917–32. https://doi.org/10.1093/molbev/mss086

Burns KJ, Shultz AJ, Title PO *et al.* Phylogenetics and diversification of tanagers (Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Molecular Phylogenetics and Evolution* 2014;**75**:41–77. https://dx.doi.org/10.1016/j.ympev.2014.02.006

Burns KJ, Unitt P, Mason NA. A genus-level classification of the family Thraupidae (Class Aves: Order Passeriformes). *Zootaxa* 2016;**4088**:329–54. https://doi.org/10.11646/ZOOTAXA.4088.3.2

Cadena CD, Zapata F. The genomic revolution and species delimitation in birds (and other organisms): why phenotypes should not be overlooked. *Ornithology* 2021;**138**:138. https://doi.org/10.1093/ornithology/ukaa069

Cadena CD, Zapata F, Jiménez I. Issues and perspectives in species delimitation using phenotypic data: Atlantean evolution in Darwin's finches. *Systematic Biology* 2017;**67**:181–94. https://doi.org/10.1093/sysbio/syx071

Calhim S, Immler S, Birkhead TR. Postcopulatory sexual selection is associated with reduced variation in sperm morphology. *PLoS ONE* 2007;**2**:e413. https://doi.org/10.1371/journal.pone.0000413

Coyne JA, Orr HA. *Speciation*. Sunderland, MA: Sinauer, 2004.

Cramer ERA, Grønstøl G, Maxwell L *et al.* Sperm length divergence as a potential prezygotic barrier in a passerine hybrid zone. *Ecology and Evolution* 2021;**11**:9489–97. https://doi.org/10.1002/ece3.7768

Cramer ERA, Yilma ZB, Lifjeld JT. Selection on sperm size in response to promiscuity and variation in female sperm storage organs. *Journal of Evolutionary Biology* 2023;**36**:131–43. https://doi.org/10.1111/jeb.14120

Cueva D, Bravo GA, Silveira LF. Systematics of *Thraupis* (Aves, Passeriformes) reveals an extensive hybrid zone between *T. episcopus* (Blue-gray Tanager) and *T. sayaca* (Sayaca Tanager). *PLoS ONE* 2022;**17**:e0270892. https://doi.org/10.1371/journal.pone.0270892

Danecek P, Auton A, Abecasis G; 1000 Genomes Project Analysis Group*et al.* The variant call format and VCFtools. *Bioinformatics* 2011;**27**:2156–8. https://doi.org/10.1093/bioinformatics/btr330

Danecek P, Bonfield JK, Liddle J *et al.* Twelve years of SAMtools and BCFtools. *GigaScience* 2021;**10**:10. https://doi.org/10.1093/gigascience/giab008

Drummond AJ, Suchard MA, Xie D *et al.* Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 2012;**29**:1969–73. https://doi.org/10.1093/molbev/mss075

Enbody ED, Sendell-Price AT, Sprehn CG *et al.* Community-wide genome sequencing reveals 30 years of Darwin's finch evolution. *Science* 2023;**381**:eadf6218. https://doi.org/10.1126/science.adf6218

Farrington HL, Lawson LP, Clark CM *et al.* The evolutionary history of Darwin's finches: speciation, gene flow, and introgression in a fragmented landscape. *Evolution; International Journal of Organic Evolution* 2014;**68**:2932–44. https://doi.org/10.1111/evo.12484

Fitzpatrick JL, Baer B. Polyandry reduces sperm length variation in social insects. *Evolution; International Journal of Organic Evolution* 2011;**65**:3006–12. https://doi.org/10.1111/j.1558-5646.2011.01343.x

Fitzpatrick JL, Kahrl AF, Snook RR. SpermTree, a species-level database of sperm morphology spanning the animal tree of life. *Scientific Data* 2022;**9**:30. https://doi.org/10.1038/s41597-022-01131-w

Fossøy F, Johnsen A, Lifjeld JT. Multiple genetic benefits of female promiscuity in a socially monogamous passerine. *Evolution; International Journal of Organic Evolution* 2008;**62**:145–56. https://doi.org/10.1111/j.1558-5646.2007.00284.x

Gibbs HL, Grant PR. Oscillating selection on Darwin's finches. *Nature* 1987;**327**:511–3. https://doi.org/10.1038/327511a0

Gill F, Donsker D, Rasmussen P. IOC World Bird List (v14.1), 2024. https://www.worldbirdnames.org/new/ioc-lists/

Gomendio M, Roldan ES. Implications of diversity in sperm size and function for sperm competition and fertility. *The International Journal of Developmental Biology* 2008;**52**:439–47. https://doi.org/10.1387/ijdb.082595mg

Grant PR. *Ecology and Evolution of Darwin's Finches*. Princeton, NJ: Princeton University Press, 1986.

Grant PR, Grant BR. *How and Why Species Multiply. The Radiation of Darwin's Finches*. Princeton, NJ: Princeton University Press, 2008.

Grant PR, Grant BR. Causes of lifetime fitness of Darwin's finches in a fluctuating environment. *Proceedings of the National Academy of Sciences* 2011;**108**:674–9. https://doi.org/10.1073/pnas.1018080108

Grant PR, Grant BR. Adult sex ratio influences mate choice in Darwin's finches. *Proceedings of the National Academy of Sciences* 2019;**116**:12373–82. https://doi.org/10.1073/pnas.1903838116

Grant PR, Grant BR. Morphological ghosts of introgression in Darwin's finch populations. *Proceedings of the National Academy of Sciences* 2021;**118**:e2107434118. https://doi.org/10.1073/pnas.2107434118

Grant PR, Grant BR. *40 Years of Evolution. Darwin's Finches on Daphne Major Island*, 2nd edn. Princeton, NJ: Princeton University Press, 2024a.

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

Grant PR, Grant BR. From microcosm to macrocosm: adaptive radiation of Darwin's finches. *Evolutionary Journal of the Linnean Society* 2024b;**3**:3. https://doi.org/10.1093/evolinnean/kzae006

Grønstøl G, Danielsen M, Cramer ERA *et al.* Effects of fixatives and storage duration on avian sperm morphology. *Journal of Ornithology* 2023;**164**:171–81. https://doi.org/10.1007/s10336-022-02015-x

Hedges LV. Distribution theory for Glass's estimator of effect size and related estimators. *Journal of Educational Statistics* 1981;**6**:107–28. https://doi.org/10.3102/10769986006002107

Hendry AP, Grant PR, Rosemary Grant B *et al.* Possible human impacts on adaptive radiation: beak size bimodality in Darwin's finches. *Proceedings of the Royal Society B: Biological Sciences* 2006;**273**:1887–94. https://doi.org/10.1098/rspb.2006.3534

Hendry AP, Huber SK, De León LF *et al.* Disruptive selection in a bimodal population of Darwin's finches. *Proceedings of the Royal Society B: Biological Sciences* 2009;**276**:753–9. https://doi.org/10.1098/rspb.2008.1321

Howard DJ. Conspecific sperm and pollen precedence and speciation. *Annual Review of Ecology and Systematics* 1999;**30**:109–32. https://doi.org/10.1146/annurev.ecolsys.30.1.109

Immler S, Birkhead TR. Sperm competition and sperm midpiece size: no consistent pattern in passerine birds. *Proceedings of the Royal Society B: Biological Sciences* 2007;**274**:561–8.

Immler S, Calhim S, Birkhead TR. Increased postcopulatory sexual selection reduces the intramale variation in sperm design. *Evolution* 2008;**62**:1538–43. https://doi.org/10.1111/j.1558-5646.2008.00393.x

Jamieson BGM. Avian spermatozoa: structure and phylogeny. In: Jamieson BGM (eds), *Reproductive Biology and Phylogeny of Birds Part A*. Enfield, NH: Science Publishers Inc., 2007, 349–511.

Johnsen A, Andersen V, Sunding C *et al.* Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* 2000;**406**:296–9. https://doi.org/10.1038/35018556

Kahrl AF, Snook RR, Fitzpatrick JL. Fertilization mode drives sperm length evolution across the animal tree of life. *Nature Ecology & Evolution* 2021;**5**:1153–64. https://doi.org/10.1038/s41559-021-01488-y

Kleindorfer S, Dudaniec RY. Hybridization fluctuates with rainfall in Darwin's tree finches. *Biological Journal of the Linnean Society* 2020;**130**:79–88. https://doi.org/10.1093/biolinnean/blaa029

Kleindorfer S, O'Connor JA, Dudaniec RY *et al.* Species collapse via hybridization in Darwin's tree finches. *The American Naturalist* 2014;**183**:325–41. https://doi.org/10.1086/674899

Kleven O, Fossøy F, Laskemoen T *et al.* Comparative evidence for the evolution of sperm swimming speed by sperm competition and female sperm storage duration in passerine birds. *Evolution; International Journal of Organic Evolution* 2009;**63**:2466–73. https://doi.org/10.1111/j.1558-5646.2009.00725.x

Kleven O, Laskemoen T, Fossøy F *et al.* Intraspecific variation in sperm length is negatively related to sperm competition in passerine birds. *Evolution; International Journal of Organic Evolution* 2008;**62**:494–9. https://doi.org/10.1111/j.1558-5646.2007.00287.x

Lack D. *Darwin's Finches*. Cambridge: Cambridge University Press, 1947.

Lamichhaney S, Berglund J, Almen MS *et al.* Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature* 2015;**518**:371–5. https://doi.org/10.1038/nature14181

Lamichhaney S, Han F, Webster MT *et al.* Rapid hybrid speciation in Darwin's finches. *Science* 2017;**359**:224–8. https://doi.org/10.1126/science.aao4593

Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* 2010;**26**:589–95. https://doi.org/10.1093/bioinformatics/btp698

Li H, Handsaker B, Wysoker A; 1000 Genome Project Data Processing Subgroup*et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009;**25**:2078–9. https://doi.org/10.1093/bioinformatics/btp352

Lifjeld JT. The avian sperm collection in the Natural History Museum, University of Oslo. *Alauda* 2019;**87**:93–101.

Lifjeld JT, Cramer ERA, Leder EH *et al.* Sperm as a speciation phenotype in promiscuous songbirds. *Evolution; International Journal of Organic Evolution* 2024;**79**:134–43. https://doi.org/10.1093/evolut/qpae154

Lifjeld JT, de Gabriel Hernando M, Fuertes Marcos B *et al.* Rapid sperm length divergence in a polygynandrous passerine: a mechanism of cryptic speciation?*Evolution; International Journal of Organic Evolution* 2023;**77**:2352–64. https://doi.org/10.1093/evolut/qpad146

Lifjeld JT, Gohli J, Albrecht T *et al.* Evolution of female promiscuity in Passerides songbirds. *BMC Evolutionary Biology* 2019;**19**:169. https://doi.org/10.1186/s12862-019-1493-1

Lifjeld JT, Laskemoen T, Kleven O *et al.* Sperm length variation as a predictor of extrapair paternity in passerine birds. *PLoS ONE* 2010;**5**:e13456. https://doi.org/10.1371/journal.pone.0013456

Lindsay WR, Andersson S, Bererhi B *et al.* Endless forms of sexual selection. *PeerJ* 2019;**7**:e7988. https://doi.org/10.7717/peerj.7988

Lüpold S, Linz G, Birkhead T. Sperm design and variation in the New World blackbirds (Icteridae). *Behavioral Ecology and Sociobiology* 2009;**63**:899–909. https://doi.org/10.1007/s00265-009-0733-6

Lüpold S, Pitnick S. Sperm form and function: what do we know about the role of sexual selection?*Reproduction* 2018;**155**:R229-R243. https://doi.org/10.1530/rep-17-0536

Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 2011;**17**:10. https://doi.org/10.14806/ej.17.1.200

McCarthy EM. *Handbook of Avian Hybrids of the World*. Oxford: Oxford University Press, 2006.

McKay BD, Zink RM. Sisyphean evolution in Darwin's finches. *Biological Reviews of the Cambridge Philosophical Society* 2015;**90**:689–98. https://doi.org/10.1111/brv.12127

Newton I. *The Speciation and Biogeography of Birds*. London: Academic Press, 2003.

Omotoriogun TC, Albrecht T, Gohli J *et al.* Sperm length variation among Afrotropical songbirds reflects phylogeny rather than adaptations to the tropical environment. *Zoology* 2020;**140**:125770. https://doi.org/10.1016/j.zool.2020.125770

Ottenburghs J. Digest: Stabilizing selection drives sperm length divergence in promiscuous passerines. *Evolution; International Journal of Organic Evolution* 2024;**79**:324–5. https://doi.org/10.1093/evolut/qpae176

Peters KJ, Myers SA, Dudaniec RY *et al.* Females drive asymmetrical introgression from rare to common species in Darwin's tree finches. *Journal of Evolutionary Biology* 2017;**30**:1940–52. https://doi.org/10.1111/jeb.13167

Petren K, Grant BR, Grant PR. Low extrapair paternity in the cactus finch (*Geospiza scandens*). *The Auk* 1999;**116**:252–6.

Price T. *Speciation in Birds*. Greenwood Village, CO: Roberts & Co, 2008.

Rambaut A. FigTree, version 1.4. 3, a graphical viewer of phylogenetic trees *Computer program distributed by the author*, 2017. http://treebioe-dacuk/software/figtree/2017

Rambaut A, Suchard MA, Xie D, Drummond AJ. Tracer v.1.6, 2014. http://beast.bio.ed.ac.uk/Tracer.

Rowe M, Albrecht T, Cramer ERA *et al.* Postcopulatory sexual selection is associated with accelerated evolution of sperm morphology. *Evolution; International Journal of Organic Evolution* 2015;**69**:1044–52. https://doi.org/10.1111/evo.12620

Rowley A, Locatello L, Kahrl A *et al.* Sexual selection and the evolution of sperm morphology in sharks. *Journal of Evolutionary Biology* 2019;**32**:1027–35. https://doi.org/10.1111/jeb.13501

Rubin C-J, Enbody ED, Dobreva MP *et al.* Rapid adaptive radiation of Darwin's finches depends on ancestral genetic modules. *Science Advances* 2022;**8**:eabm5982. https://doi.org/10.1126/sciadv.abm5982

Shen W, Le S, Li Y, Hu F. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLoS ONE* 2016;**11**:e0163962. https://doi.org/10.1371/journal.pone.0163962

Sokal RR, Rohlf FJ. *Biometry*. San Francisco, CA: W. H. Freeman & Co, 1981.

Stange M, Sánchez-Villagra MR, Salzburger W *et al.* Bayesian divergence-time estimation with genome-wide single-nucleotide

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025

polymorphism data of sea catfishes (Ariidae) supports Miocene closure of the Panamanian Isthmus. *Systematic Biology* 2018;**67**:681–99. https://doi.org/10.1093/sysbio/syy006

Støstad HN, Johnsen A, Lifjeld JT *et al.* Sperm head morphology is associated with sperm swimming speed: a comparative study of songbirds using electron microscopy. *Evolution* 2018;**72**:1918–32. https://doi.org/10.1111/evo.13555

Tourmente M, Gomendio M, Roldan ERS. Sperm competition and the evolution of sperm design in mammals. *BMC Evolutionary Biology* 2011;**11**:12. https://doi.org/10.1186/1471-2148-11-12

Valcu C-M, Valcu M, Kempenaers B. The macroecology of extra-pair paternity in birds. *Molecular Ecology* 2021;**30**:4884–98. https://doi.org/10.1111/mec.16081

Varea-Sánchez M, Gómez Montoto L, Tourmente M, Roldan ERS. Post-copulatory sexual selection results in spermatozoa with more uniform head and flagellum sizes in rodents. *PLoS ONE* 2014;**9**:e108148. https://doi.org/10.1371/journal.pone.0108148

Weir JT, Schluter D. The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science* 2007;**315**:1574–6. https://doi.org/10.1126/science.1135590

Zink RM. Darwin's finches: multiply and subtract. *BioScience* 2009;**59**:86–7.

Zink RM, Vázquez-Miranda H. Species limits and phylogenomic relationships of Darwin's finches remain unresolved: potential consequences of a volatile ecological setting. *Systematic Biology* 2019;**68**:347–57. https://doi.org/10.1093/sysbio/syy073

Downloaded from https://academic.oup.com/biolinnean/article/146/2/blaf103/8287203 by Turku University user on 21 November 2025