CHAPTER 1

Global variation in birdsong complexity is best explained by environmental variability

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Avian song is one of the most prominent traits in the study of animal sexual signalling[1]. Because of its potential importance for intrasexual competition and mate choice, song has been considered the acoustic "peacock's tail"[2–5]. However, the idea that sexual selection has been the driving force behind variation in song complexity across avian species remains controversial[6–8]. Drivers of broad-scale patterns in birdsong complexity are generally poorly understood, calling for a global synthesis. Using estimates of song complexity for ~5,000 species of passerines (Order: Passeriformes), we here show that, both in Oscines, a clade with learned songs, and Suboscines with innate songs, complex songs are typical for assemblages characterized by low sexual dimorphism but highly colourful males, large proportion of migratory species, and in habitat generalists. However, most associations emerged as a result of spatially non-random distribution of passerine lineages across assemblages and disappeared in a phylogenetically informed cross-species analyses. After controlling for common ancestry in cross-species models, we found that song complexity in Oscines, but not Suboscines, was positively correlated only with habitat generalism. Our results suggest that, at least in song-learning Oscines, sexual selection may be partly involved in the evolution of complex songs, possibly via environmentally-driven processes leading to intensified selection on sexual signals of quality, or co-evolution with enhanced cognitive abilities associated with living in variable environments.

In many vertebrate and invertebrate groups, conspicuous vocal performances are used during intra-sexual, competitive interactions and in the context of attracting potential mates[9]. Among the most obvious examples are the complex male songs of the largest clade of extant birds, the passerines (Order: Passeriformes). Song complexity varies widely among species, from single notes of many Suboscines to the large repertoires consisting of thousands of different elements, in mockingbirds and nightingales[7,10,11]. Sexual selection has been proposed as the major driver explaining the interspecific variation of complex avian song[2–5]. Previous studies have yielded conflicting results as to the role of sexual selection in the evolution of avian song complexity probably owing to the (1) the lack of a robust and widely applicable measure of song complexity and (2) the use of a restricted number and different sets of species in analyses.

Here, we use birdsong recordings from large, citizen-science databases and quantify song complexity for 4,939 species of passerines (~83% of extant passerine species[12]). To do this we derived an easy to measure proxy for avian song complexity which allows for an effective and meaningful comparisons across various passerine clades and with previous studies. We counted the number of different element types within a 50-element sequence of the song of each individual. This simple measure of song complexity is positively correlated with previously used song complexity metrics, such as the syllable repertoire size (r = 0.73), number of syllables per song (r = 0.62) and song repertoire size (r = 0.51) (Supplementary Table 1). Moreover, it reflects the relative volume of the high vocal centre (HVC), region in the avian brain involved in song production and vocal learning ($P < 0.001$, $R^2 = 0.21$) (Supplementary Table 2).

Overall, song complexity varied markedly across passerine species and world regions (Fig. 1, 2), yet showing a strong phylogenetic signal (Pagel's $\lambda = 0.75$). Several passerine lineages were characterized by either very simple or very complex songs. Oscines, members of the song-learning passerine clade, produce more complex songs than Suboscines, a clade with innate songs (Supplementary Fig. S1). Interestingly, most Oscines still produce relatively simple songs (Fig. 1), indicating that vocal learning is a necessary, but not sufficient condition for the evolution of complex song[19].

We evaluated a set of life-history, social and environmental indices of sexual selection as predictors of avian song complexity. First, we estimated the effects of sexual dimorphism and dichromatism, polygyny, territoriality, and social bonds duration on song complexity[6,7,13,14]. We also had a closer look on the long proposed relationship between song complexity and male plumage colouration[15–17]. Passerines rely on multiple signalling modalities and ornamental types, and show remarkable variation in both acoustic[3,4] and visual[18] ornaments. Evolution of multiple ornaments could undergo different trajectories: (1) joint evolution to increase the efficiency of communication[16], (2) evolutionary trade-offs due to signal production costliness and maintenance[17] or (3) independent evolution when communicating different information to receivers[15]. Furthermore, we estimated effects of environmental indices, such as environmental variability, migratory behaviour and species ecological generalism[19–23]. Our analyses were conducted on assemblage level as well as by a phylogenetically-informed cross-species approach. Because of the fundamental difference in the mechanism of acquiring song in Oscines (song-learning clade) and Suboscines (clade with innate songs) passerines, and a clear difference in the levels of song complexity and its variation in these two groups, we performed further analyses separately for each clade.

Spatial multivariate analyses across Oscine and Suboscines assemblages (112.5 × 112.5 km grid cells) revealed that complex songs characterize avian assemblages also characterized by a set of certain life-history, social and environmental traits indicative of strong intensity of sexual selection (Fig. 3). However, phylogenetic simulations revealed that all these effects fall within the distribution of simulated effects, indicating that observed patterns were driven by the geography of speciation (Fig. 3) or possibly lineage sorting[24]. Consistent with these findings, multivariate phylogenetically informed cross-species analysis revealed no effect of all life-history and social and most of environmental indices of the strength of sexual selection on song complexity, neither in Oscines nor in Suboscines (Tables 1, 2). The finding of no association between male plumage colouration and song complexity in either Oscines or Suboscines in a cross-species analyses indicate that two these types of signalling modalities evolved largely independently across passerines, and may transfer different information to conspecifics and reflect different selective pressures[15,25].

In the Oscines, only habitat generalism remained a significant predictor of song complexity in a multivariate cross-species model controlling for the co-variation between predictors. This indicates that species that are found breeding in more biomes produce more complex songs than habitat specialists. However, the proportion of variation explained by this predictor was rather low (<2%, Table 1). Oscines tolerating heterogeneous environments are, in general, both widespread and locally abundant[26] which may, in turn, lead to stronger competition for mates[21]. Alternatively, species living in variable environment may face intense competition for mates and resources[27] which, in turn, may lead to increased elaboration of signals of mate quality[19,22]. It is possible that life in heterogeneous environment produces a direct positive selection on song elaboration or a co-opted selection on song elaboration and cognitive skills and innovation abilities required for living in variable evironments[19,22,28]. In the Suboscines, we found no association between any of the environmental predictors and song complexity in cross-species model (Table 2).

Our analysis of the largest data set on avian song complexity available to date revealed a clear global biogeographic structure in this trait for which several life-history, social and environmental factors are important predictors across passerine assemblages. However, results accounting for phylogeny indicate that it is the environment occupied by the species which to some extent predicts global patterns of song complexity, but only in Oscines, a song-learning passerine clade. The overall effect size is low, however, and much of the variation in avian song complexity is either explained by phylogeny or remains unexplained. The exact processes underlying the environment complexity−song complexity pattern remains to be investigated. Our song complexity metric only represents one of several song dimensions. Detail studies on intraspecific variation in song found that other aspects of birdsong such as performance, consistency or the presence of particular structures may provide other targets of sexual selection[2]. The situation may also be complicated by the multidirectionality of effects of selection pressures acting on song complexity or their interactions[7]. A major challenge lies now in a deeper understanding of mechanisms generating acoustic phenotype at the level of individual species which may provide important insights into the ecology and evolution of this behaviour over large scales and the ongoing debate over the functions of elaborated signals.

1. Catchpole, C. K. & Slater, P. J. B. *Bird song: biological themes and variations*. (Cambridge University Press, 2008).

2. Nowicki, S. & Searcy, W. A. Song function and the evolution of female preferences: why birds sing, why brains matter. *Ann. N. Y. Acad. Sci.* **1016**, 704–723 (2004).

3. Catchpole, C. K. Bird song, sexual selection and female choice. *Trends Ecol. Evol.* **2**, 94–97 (1987).

4. Searcy, W. A. & Andersson, M. Sexual selection and the evolution of song. *Annu. Rev. Ecol. Syst.* **17**, 507–533 (1986).

5. Gil, D. & Gahr, M. The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol. Evol.* **17**, 133–141 (2002).

6. Cooney, C. R., MacGregor, H. E. A., Seddon, N. & Tobias, J. A. Multi-modal signal evolution in birds: re-examining a standard proxy for sexual selection. *Proc. R. Soc. B Biol. Sci.* **285**, 20181557 (2018).

7. Snyder, K. T. & Creanza, N. Polygyny is linked to accelerated birdsong evolution but not to larger song repertoires. *Nat. Commun.* **10**, 884 (2019).

8. Najar, N. & Benedict, L. The relationship between latitude, migration and the evolution of bird song complexity. *Ibis* **161**, 1–12 (2019).

9. Simmons, A. & Fay, R. R. *Acoustic communication*. (Springer, 2002).

10. Beecher, M. D. & Brenowitz, E. A. Functional aspects of song learning in songbirds. *Trends Ecol. Evol.* **20**, 143–149 (2005).

11. Weir, J. T. & Wheatcroft, D. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proc. R. Soc. B Biol. Sci.* **278**, 1713–1720 (2011).

12. Dale, J., Dey, C. J., Delhey, K., Kempenaers, B. & Valcu, M. The effects of life history and sexual selection on male and female plumage colouration. *Nature* **527**, 367–370 (2015).

13. Mahler, B. & Gil, D. The evolution of song in the *Phylloscopus* leaf warblers (Aves: Sylviidae): a tale of sexual selection, habitat adaptation, and morphological constraints. *Adv. Study Behav.* **40**, 35–66 (2009).

14. Shutler, D. & Weatherhead, P. J. Targets of sexual selection: song and plumage of wood warblers. *Evolution* **44**, 1967–1977 (1990).

15. Mason, N. A., Shultz, A. J. & Burns, K. J. Elaborate visual and acoustic signals evolve independently in a large, phenotypically diverse radiation of songbirds. *Proc. R. Soc. B Biol. Sci.* **281**, 20140967 (2014).

16. Ligon, R. A. *et al.* Evolution of correlated complexity in the radically different courtship signals of birds-of-paradise. *PLoS Biol.* **16**, e2006962 (2018).

17. Badyaev, A. V., Hill, G. E. & Weckworth, B. V. Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. *Evolution* **56**, 412–419 (2002).

18. Valcu, M., Dale, J., Griesser, M., Nakagawa, S. & Kempenaers, B. Global gradients of avian longevity support the classic evolutionary theory of ageing. *Ecography* **37**, 930–938 (2014).

19. Medina, I. & Francis, C. D. Environmental variability and acoustic signals: a multi-level approach in songbirds. *Biol. Lett.* **8**, 928–931 (2012).

20. Gomes, A. C. R., Funghi, C., Soma, M., Sorenson, M. D. & Cardoso, G. C. Multimodal signalling in estrildid finches: song, dance and colour are associated with different ecological and life-history traits. *J. Evol. Biol.* **30**, 1336–1346 (2017).

21. Tobias, J. A. & Seddon, N. Sexual selection and ecological generalism are correlated in antbirds. *J. Evol. Biol.* **22**, 623–636 (2009).

22. Botero, C. A., Boogert, N. J., Vehrencamp, S. L. & Lovette, I. J. Climatic patterns predict the elaboration of song displays in mockingbirds. *Curr. Biol.* **19**, 1151–1155 (2009).

23. Byers, B. E. Migration and song elaboration in wood-warblers (*Geothlypis*). *Auk* **132**, 167–179 (2015).

24. Warren, D. L., Cardillo, M., Rosauer, D. F. & Bolnick, D. I. Mistaking geography for biology: inferring processes from species distributions. *Trends Ecol. Evol.* **29**, 572–580 (2014).

25. Drury, J. P. *et al.* Contrasting impacts of competition on ecological and social trait evolution in songbirds. *PLOS Biol.* **16**, e2003563 (2018).

26. Gaston, K. J. *et al.* Abundance-occupancy relationships. *J. Appl. Ecol.* **37**, 39–59 (2000).

27. Botero, C. A. & Rubenstein, D. R. Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. *PLoS One* **7**, e32311 (2012).

28. Sayol, F. *et al.* Environmental variation and the evolution of large brains in birds. *Nat. Commun.* **7**, 13971 (2016).

29. Symonds, M. R. E. & Moussalli, A. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav. Ecol. Sociobiol.* **65**, 13–21 (2011).

30. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. *Nature* **491**, 444–448 (2012).

Table 1. Results of multivariate phylogenetically-informed cross-species model assessing correlation between song complexity and life-history, social and environmental indices of sexual selection across 1,861 species of Oscines. The number of scored elements was included as fixed effect in the model. We accounted for phylogenetic uncertainty by running models on 100 phylogenies and combining model coefficients using a model averaging approach[29]. We report also conditional (R^2_{cond}; variance explained by the fixed and random effects) and marginal (R^2_{marg}; variance explained by the fixed effects only) proportion of variance explained by model.

Term	Estimate	Lower	Upper	Std. Error	t	*P*	*P* (2.5%)	*P* (97.5%)
Number of elements	0.363	0.328	0.398	0.018	20.300	<0.001	<0.001	<0.001
Sexual size dimorphism	0.016	-0.028	0.060	0.023	0.702	0.490	0.412	0.734
Sexual dichromatism	-0.005	-0.057	0.046	0.026	-0.201	0.831	0.758	0.979
Male ornamentation	0.028	-0.025	0.081	0.027	1.040	0.304	0.258	0.435
Social polygyny	-0.019	-0.069	0.031	0.025	-0.756	0.454	0.374	0.618
Sociality	-0.006	-0.061	0.049	0.028	-0.212	0.811	0.718	0.983
Territoriality	-0.027	-0.077	0.024	0.026	-1.040	0.306	0.249	0.486
Precipitation seasonality	0.041	0.001	0.082	0.021	2.020	0.047	0.028	0.091
Temperature seasonality	0.026	-0.034	0.087	0.031	0.847	0.402	0.349	0.569
Migration	0.051	-0.007	0.110	0.030	1.720	0.089	0.071	0.144
Generalism	0.071	0.032	0.110	0.020	3.590	<0.001	<0.001	<0.001
Pagel's λ	0.752	0.737	0.791					
R^2_{cond}	0.575	0.572	0.582					
R^2_{marg}	0.221	0.218	0.228					

Table 2. Results of multivariate phylogenetically-informed cross-species model assessing correlation between song complexity and life-history, social and environmental indices of sexual selection across 277 species of Suboscines. The number of scored elements was included as fixed effect in the model. We accounted for phylogenetic uncertainty by running models on 100 phylogenies and combining model coefficients using a model averaging approach[29]. We report also conditional (R^2_{cond}; variance explained by the fixed and random effects) and marginal (R^2_{marg}; variance explained by the fixed effects only) proportion of variance explained by model.

Term	Estimate	Lower	Upper	Std. Error	t	*P*	*P* (2.5%)	*P* (97.5%)
Number of elements	0.290	0.159	0.42	0.067	4.36	<0.001	<0.001	<0.001
Sexual size dimorphism	-0.003	-0.112	0.107	0.056	-0.048	0.935	0.907	0.995
Sexual dichromatism	-0.021	-0.193	0.151	0.088	-0.238	0.813	0.757	0.963
Male ornamentation	-0.050	-0.210	0.111	0.082	-0.607	0.545	0.511	0.624
Social polygyny	-0.241	-0.481	-0.001	0.122	-1.970	0.050	0.046	0.065
Sociality	-0.064	-0.286	0.158	0.113	-0.564	0.574	0.55	0.649
Territoriality	-0.147	-0.330	0.035	0.093	-1.580	0.115	0.106	0.151
Precipitation seasonality	0.063	-0.039	0.164	0.052	1.210	0.227	0.211	0.266
Temperature seasonality	0.017	-0.154	0.187	0.087	0.191	0.849	0.816	0.938
Migration	0.118	-0.059	0.295	0.090	1.310	0.192	0.174	0.247
Generalism	0.036	-0.085	0.157	0.062	0.587	0.558	0.537	0.610
Pagel's λ	0.581	0.555	0.656					
R^2full	0.504	0.499	0.519					
R^2fixef	0.111	0.108	0.121					

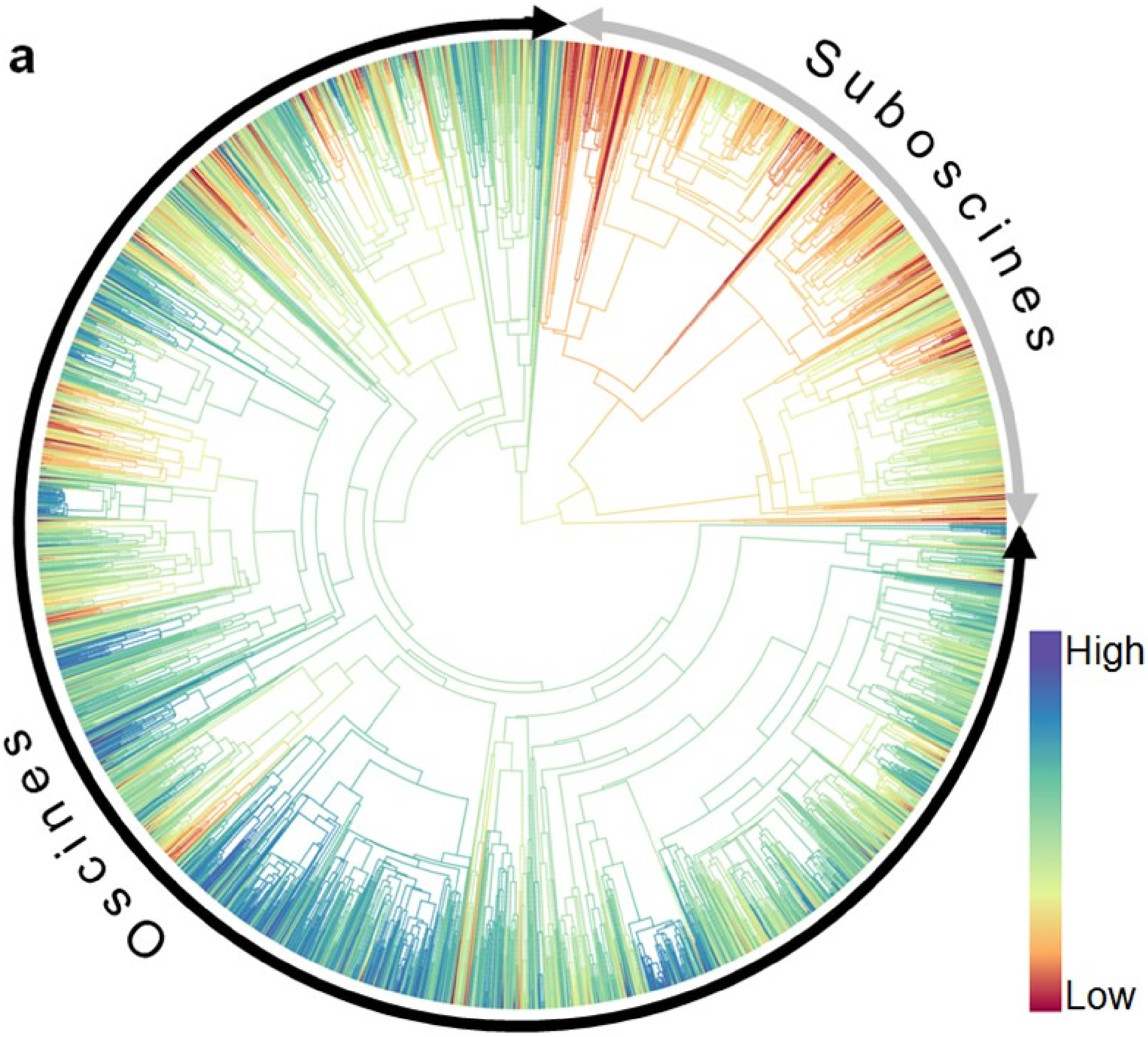

Figure 1. | Evolutionary history of song complexity in passerines. A reconstruction of the evolutionary history of song complexity across 4,939 species of passerines. Song complexity (log10-transformed) was mapped as a continuous character on a maximum credibility tree which was constructed using 100 trees extracted from Bird Tree project[30]. Internal nodes are coloured according to ancestral states estimated by maximum likelihood methods. For reconstruction we used only species with 50 scored elements.

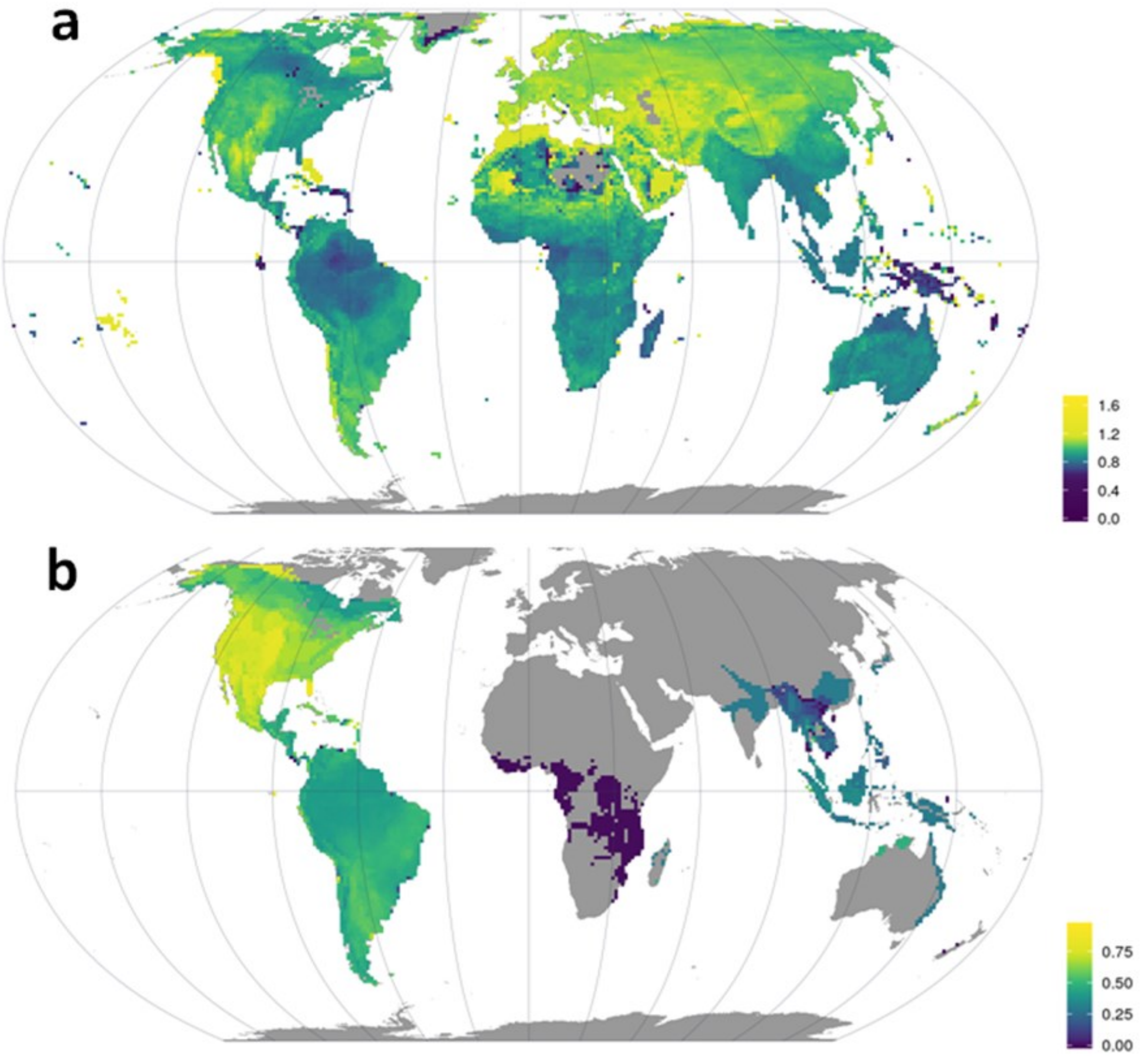

Figure 2 | Geographic variation in song complexity across passerines. Mapped song complexity (log10-transformed) for **a,** Oscines (*N* = 10,940 grid cells), and **b,** Suboscines (*N* = 3,968 grid cells) across grid cell assemblages of 112.5 × 112.5 km. Only cells with more than five species were included. Maps illustrating the geographic distribution of predictor variables are included in Supplementary Figures S3−S4.

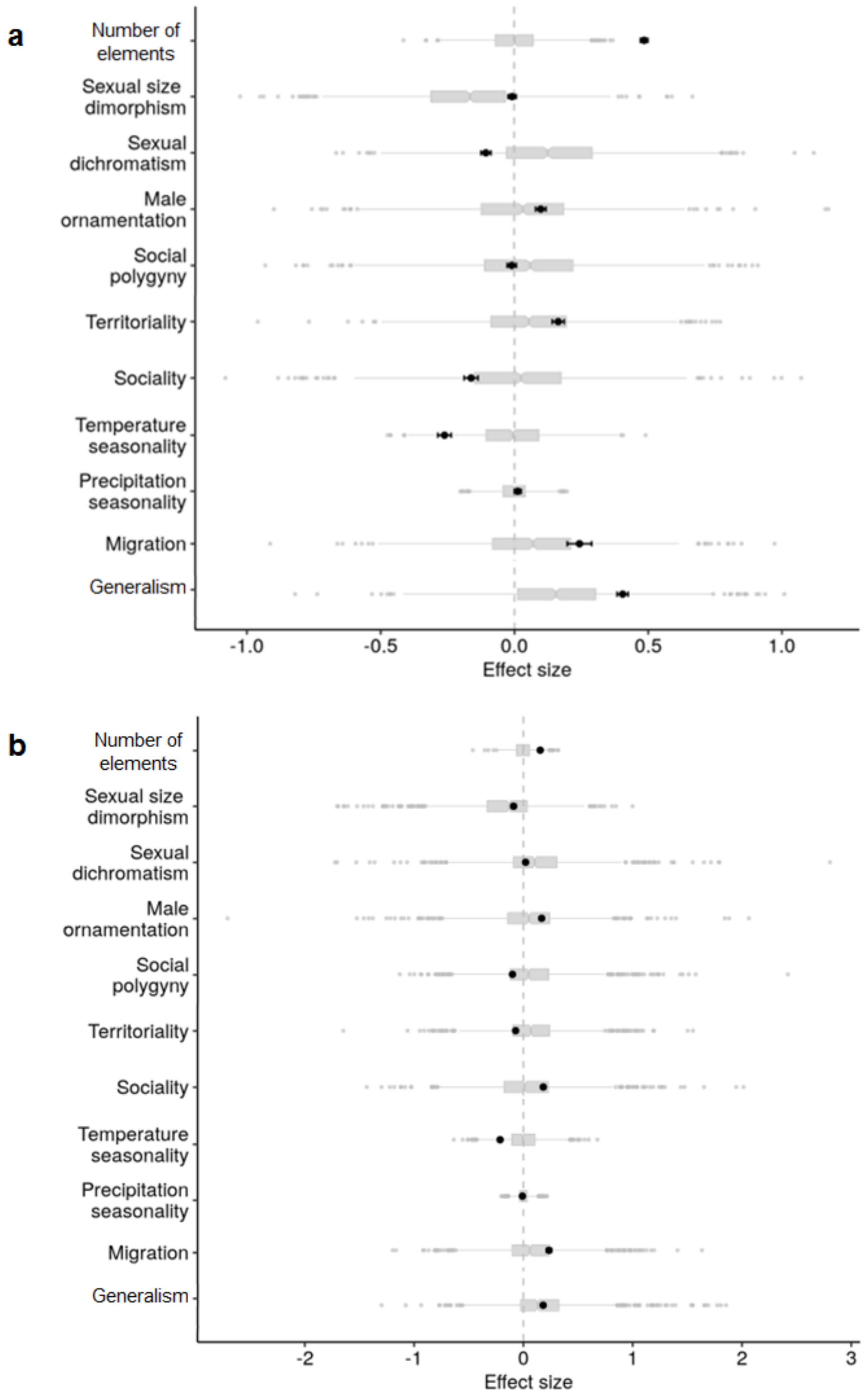

Figure 3 | Predictors of song complexity across passerine assemblages. Linear mixed models examined the effects of life-history, social and environmental variables on song complexity across assemblages (112.5 × 112.5 km) of **a,** Oscines, and **b,** Suboscines (black symbols). Grey symbols show 95% confidence intervals of results obtained from a sample of 1000 phylogenetic simulations. Models included zoogeographic realm as random intercept.

METHODS

The song complexity dataset.

Data collection. We collected song complexity data from recordings available at two large citizen-science databases of bird vocalization: xeno-canto (www.xeno-canto.org) and Macaulay Library (The Cornell Lab of Ornithology; www.macaulaylibrary.org). We defined song, as primarily long-range acoustic signal used in courtship and territory defence and stereotyped in some way. We primarily focused on recordings of adult male birds. Although females of many particularly tropical species also sing[31,32], we argue that this should not have significant effect on patterns observed in our data because (1) females often sing in duets and female solo song is rarer[33–35]; hence, most of recordings with solo song are expected to cover male vocal performance; (2) species where female sing represent generally < 20% of all species in most (even tropical) assemblages[32], (3) males and females of species where both sexes sing regularly often produce similarly complex songs[36–40].

Based on availability of recordings for species, one of us (PM) collected 1–5 recordings for each species. For most of species we included only recordings spatially separated by ≥ 1 km. For small range species or species with poor recording coverage we collected also recordings with did not met these criteria but had to differ altitude by ≥ 100 metres and/or were recorded in different seasons.

For each recording (individual), the same person (PM) counted the first 50 elements and determined the number of different element types (i.e. element repertoire size) as a measure of song complexity. This method provides a simplified approach to quickly assess song complexity across species and is useful particularly in species where it is difficult to define what is one song (e.g. in continuous singers). Because song complexity could be affected by the number of scored elements, for each species we also computed a maximum number of scored elements (up to 50). We defined an element as the smallest continuous structure on a sonogram, separated from other continuous structures by at least 10 milliseconds[15]. Elements were inspected visually and different element types were defined based on element morphology, frequency, and length. In some species, particularly Suboscines, song consisted of morphologically similar elements that were repeated many times but gradually changed in frequency. Following ref.[11], we scored this recording as showing two element types. We estimated species-specific song complexity as the upper quantile (0.9) of the number of element types of all recordings. In a result, we determined a species position on high–low song complexity continuum; species whose songs had many different element types were considered to have complex songs while those with one or few element types have simple songs.

We measured song complexity using spectrograms with a FFT length of 256 Hz and Hamming window with 50% overlap in the software Avisoft SASLab Lite, version 5.2.09 (Avisoft Bioacoustics; www.avisoft.com). Recordings for different species were analysed in random order and data collector did not have knowledge on species ID during whole process of song complexity data collection. In the final set of measurements entering main analyses we included song complexity scores for 4,939 species of passerines (18,061 recordings; mean ± SD = 3.7 ± 1.6 recordings per species, median = 4).

Predictor variables.

Sexual size dimorphism. We obtained data on body size estimated as wing length from ref.[12] and calculated sexual size dimorphism (SSD) as log10(male wing length) − log10(female wing length). Positive values indicate species with larger males than females, suggesting more intense sexual selection[41].

Male plumage colouration and sexual dichromatism. For male plumage colouration, we used the scores provided in ref.[12], which correlates with other indicators of sexual selection[19]. These scores are based on RGB values of six plumage patches (nape, crown, forehead, throat, upper breast, and lower breast) extracted from digital image processing software on scanned handbook illustrations and correlate with measures derived from spectrophotometry[12,42]. Low score values correspond to "female-like" plumage, while high values are assigned to elaborated, "male-like" plumage. We defined sexual dichromatism as the male plumage score minus the female score.

Social polygyny. Social polygyny data were retrieved from ref.[12]. Polygyny was scored on a four-point scale: 0 = strict social monogamy, 1 = monogamy with infrequent instances of polygyny observed (< 5% of males), 2 = mostly social monogamy with regular occurrences of facultative social polygyny (5–20% of males), and 3 = obligate resource defence polygyny (> 20% of males) or lek polygyny. A few passerines species are polygynandrous (e.g. the dunnock *Prunella modularis*). These species were pooled with the monogamous species because we reasoned that sexual selection would be more similar in each sex in polygynandrous species compared with polygynous species.

Territoriality and social bonds. We extracted data on territoriality and on the stability of social bonds from ref.[32]. Territoriality was scored on a three-point scale: 0 = non-territorial, 1 = seasonally or weakly territorial, and 2 = year-round territorial. Social bond stability was also scored on a three-point scale: 0 = solitary, 1 = having short-term pair/group bonds, and 2 = having long-term pair/group bonds.

Breeding ranges. We obtained extant species breeding ranges from ref.[43] by subsetting the database to "taxonomic order = Passeriformes, season = 1 (resident) and season = 2 (breeding Season)" and removing "presence = 5 (extinct before 1500) and origin = 3 (species introduced outside of their historical distribution range through either direct or indirect human activity)".

Environmental seasonality. Based on the breeding range of each species, we used data on temperature and precipitation seasonality from the CHELSA (Climatologies at high resolution for the earth's land surface areas) climatological data (period: 1979–2013) with high spatial resolution (30 arc sec)[44]. Seasonality was estimated as within-year variation in temperature and precipitation[45]. First, we prepared seasonality raster maps by re-scaling the original rasters from ref.[44] to 10-km resolution and reprojecting them to the Equal Earth projection[46] with GDAL 2.4.1.[47]. We aggregated variables into three-month averages (three months convey a typical avian breeding period[45]) and computed the standard deviation between the four three-month periods per year and over the entire 35 year period. Species-specific environmental seasonality was then computed by overlaying each species breeding range with the seasonality raster map, extracting all raster cell values under the range and compute mean raster cell values. Both variables (temperature and precipitation) were log10-transformed (variable + 1) before analysis.

Migration. We retrieved data on migration from ref.[12]. Migration was scored on a three-point scale: 0 = resident (breeding and non-breeding ranges are identical), 1 = partial migration (some overlap between breeding and non-breeding ranges), 2 = complete migration (no overlap between breeding and non-breeding ranges).

Habitat generalism. We estimated ecological generalism as the number of different biome types within the distribution range of each species, using the data on distribution of terrestrial biomes from ref.[48] (https://www.worldwildlife.org/publications/terrestrial-ecoregions-of-the-world; classification into 14 biomes). The breeding range of each species was intersected with the biome polygons and the intersection polygon was extracted. Habitat generalism was calculated as the total number of distinct biomes remained in the intersection polygon.

Phylogenetic data. We extracted a species-level phylogeny from the online tool available at http://birdtree.org[30] (the Hackett backbone). To assess the uncertainty associated with phylogenies, we randomly sampled 100 phylogenetic trees from the phylogenetic database. For visualization purposes we used a maximum credibility tree (MCC) reconstructed from these 100 trees using the function maxCladeCred in the *phangorn* package[49].

Statistical analyses. All statistical analyses were performed using R v. 3.5.3[50].

Validation and reproducibility. We used Pearson's r to validate our scores of song complexity against previously published data on an important avian song complexity metric, including syllable (N = 120 species) and song repertoire size (N = 206 species), and number of unique syllables per song (N = 174 species), from ref.[7] (Supplementary Table S1). We explored the biological relevance of our metric by investigating the association between song complexity and the relative volume of a major song control centre in the passerine brain, the high vocal centre (HVC; N = 56 species) assembled primarily from ref.[51]. This association was explored by multivariate phylogenetic generalized least- squares model where HVC was controlled for brain and body size of passerine species (both collected from ref.[51]) by including them as fixed effects to the same model (Supplementary Table S2). For validation we used only species with 50 scored elements. We found that our metric positively correlates with syllable repertoire size and other important song complexity metrics, hence, it is suitable for the exploration of large-scale variation in song complexity because it can be effectively quantified and interpreted across global diversity of passerines. As proposed by ref.[7], metrics deriving song complexity from number of different elements and syllables are more resistant to the variable song structure and to definitions of basic song unit than other metrics such as the song repertoire size and the number of unique syllables per song. Furthermore, we validated the reproducibility of our approach by asking six volunteers (all but one were untrained and never worked in the field of bioacoustics) to score 25 sonograms (19 for Oscines, six for Suboscines; each from different species and family) for the number of element types among selected recording frame using abovementioned definition and approach. Volunteers had no knowledge about the identity of the recorded species or about the aim of the study. Repeatability of our scores of song complexity (log10-transformed) was estimated by the rpt function in the rptR package[52]. We performed a significance test against a null distribution and estimated the 95% confidence interval of repeatability with 1000 bootstrap samples. Model was fitted using Gaussian family of distribution by the glmer function in the lme4 package[53]. Our song complexity metrics exhibited high repeatability between scorers (R = 0.96) (Supplementary Fig. S2).

Assemblage analyses. Global mapping was performed using rangeMapper v. 0.3.6[54]. Analyses were performed on digitized breeding range distributions of passerine species using a grid with a cell size of 112.5 × 112.5 km (~1° scale) using an Equal Earth projection[46]. Only cells with more than five species were included, obtaining altogether 10,940 grid cells/assemblages for Oscines and 3,968 for Suboscines. For each grid cell, we calculated a mean value of song complexity and life-history, social and environmental traits (Supplementary Fig. S3−4). Then, we built multivariable model with song complexity as dependant variable and life-history, social and environmental factors as predictors. To correct for the unequal sampling across species, we also included number of scored elements as fixed predictor in analysis. All variables were standardized by centring and dividing by one standard deviation prior to analyses[55]. To account for different evolutionary history across world regions, we included terrestrial zoogeographic realms as a random intercept in analysis (11 realms according ref.[56]). Predictor variables were modelled with a random slope, allowing them to have a different slope in each realm. We accounted for spatial autocorrelation using a spatial covariate[57] by extracting response residuals of the non-spatial model[58]. We computed a spatial auto-covariate with function autocov_dist in spdep package[59] using a symmetric neighbourhood matrix[57], a neighbourhood distance of 120 km and an inverse squared weighting scheme. The visual inspection of the residuals shows that this method is effective in dealing with spatial autocorrelation. We found no major departure from the assumptions of normality and heterogeneity of variance in model residuals. We calculated variance explained by the fixed and random effects (conditional R^2) and by the fixed effects only (marginal R^2) using MuMIn package[60]. Assemblage models were carried out with the lme4 package[61].

The empirical patterns might be solely driven by phylogenetic clustering[24]. Hence, we generated multivariable dataset simulations from a multivariable Brownian motion model for the co-evolution of song complexity with the species life-history, social and environmental traits using function fit_bm_model in castor package[62]. We computed diffusivity matrices of the model for each of the 100 phylogenetic trees and averaged them to obtain one final diffusivity matrix. We also simulated 1000 datasets using function simulate_bm_model in the same package[62]. We imported each of the simulated datasets to the rangeMapper project and generated maps for each simulated variable[54]. We constructed the spatial covariate for each iteration. We fitted multivariable linear mixed-effect models for each of the simulated life history, social and environmental traits. We calculated a 95% inference interval based on the effect sizes as 2.5th–97.5th percentiles range and compared the empirical estimates from the real data with the simulated data. When the empirical estimate fall within the inference interval, we assumed that the reported effect can be caused solely by phylogenetic relatedness.

Cross-species analyses. We tested for the presence of phylogenetic signal in song complexity using Pagel's λ[63] using function phylosig in phytools package[64]. We tested differences in song complexity between Oscines and Suboscines using a two-way ANOVA (Type-III sums of squares) using function Anova in car package[65]. In these analyses, we used only species with 50 scored elements. Phylogenetically related taxa tend to share more similar characteristics than distantly related species. We hence fitted phylogenetic generalized least-squares (PGLS) regressions using Pagel's lambda (λ) model[63] to explore associations between song complexity and predictor variables while accounting for statistical non-independence between species due to shared evolutionary history. We built multivariable models, one for Oscines and one for Suboscines, with song complexity as dependant variable and life-history, social and environmental factors as predictors. To correct for the unequal sampling across species, we also included number of scored elements as fixed predictor in analysis. We checked for multicollinearity between predictors, revealing low intercorrelation between most of predictors (Supplementary Fig. S5). All variables were standardized by centring and dividing by one standard deviation prior to analyses[55]. We accounted for phylogenetic uncertainty by running models on 100 phylogenies and combining model coefficients using a model averaging approach[29]. The proportion of variance (conditional and marginal R^2) explained by the phylogenetic linear models was computed according to ref.[66] using rr2 package[67]. Model residuals showed no major departures from the assumptions of normality and heterogeneity of variance. Confidence intervals (95% CI) of fixed effects are based on 1000 bootstrap replicates and were calculated using function glht implemented in the multcomp package[68] while controlling for multiple testing. Cross-species analyses were performed using phylolm package[69].

31. Odom, K. J., Hall, M. L., Riebel, K., Omland, K. E. & Langmore, N. E. Female song

is widespread and ancestral in songbirds. *Nat. Commun.* **5**, 3379 (2014).

32. Tobias, J. A. *et al.* Territoriality, social bonds, and the evolution of communal signaling in birds. *Front. Ecol. Evol.* **4**, 74 (2016).

33. Hall, M. L. A review of hypotheses for the functions of avian duetting. *Behav. Ecol. Sociobiol.* **55**, 415–430 (2004).

34. Mann, N. I., Marshall-Ball, L. & Slater, P. J. B. The complex song duet of the plain wren. *Condor* **105**, 672–682 (2003).

35. Mikula, P. *et al.* Female solo song and duetting are associated with different territoriality in songbirds. *Behav. Ecol.* **31**, 322–329 (2020).

36. Langmore, N. E. Functions of duet and solo songs of female birds. *Trends Ecol. Evol.* **13**, 136–140 (1998).

37. Price, J. J. Evolution and life-history correlates of female song in the New World blackbirds. *Behav. Ecol.* **20**, 967–977 (2009).

38. Hall, M. L. A review of vocal duetting in birds. *Adv. Study Behav.* **40**, 67–121 (2009).

39. Pilowsky, J. A. & Rubenstein, D. R. Social context and the lack of sexual dimorphism in song in an avian cooperative breeder. *Anim. Behav.* **85**, 709–714 (2013).

40. Mann, N. I., Dingess, K. A., Barker, K. F., Graves, J. A. & Slater, P. J. B. A comparative study of song form and duetting in neotropical *Thryothorus* wrens. *Behaviour* **146**, 1–43 (2009).

41. Fairbairn, D. J., Blanckenhorn, W. U. & Székely, T. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. (Oxford University Press, 2007).

42. Delhey, K., Dale, J., Valcu, M. & Kempenaers, B. Reconciling ecogeographical rules: rainfall and temperature predict global colour variation in the largest bird radiation. *Ecol. Lett.* **22**, 726–736 (2019).

43. BirdLife International and NatureServe. Bird species distribution maps of the world. http://datazone.birdlife.org/ (2018).

44. Karger, D. N. *et al.* Climatologies at high resolution for the earth's land surface areas.

Sci. Data **4**, 170122 (2017).

45. Jetz, W. & Rubenstein, D. R. Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Curr. Biol.* **21**, 72–78 (2011).

46. Šavrič, B., Patterson, T. & Jenny, B. The Equal Earth map projection. *Int. J. Geogr. Inf. Sci.* **33**, 454–465 (2019).

47. GDAL/OGR contributors. GDAL/OGR Geospatial Data Abstraction Software Library. *Open Source Geospatial Found.* (2019).

48. Olson, D. M. *et al.* Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* **51**, 933–938 (2001).

49. Schliep, K. P. phangorn: phylogenetic analysis in R. *Bioinformatics* **27**, 592–593 (2011).

50. R Development Core Team. *A Language and Environment for Statistical Computing*. (R Foundation for Statistical Computing, 2019).

51. Moore, J. M., Székely, T., Büki, J. & Devoogd, T. J. Motor pathway convergence predicts syllable repertoire size in oscine birds. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 16440–16445 (2011).

52. Stoffel, M. A., Nakagawa, S. & Schielzeth, H. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* vol. 8 1639–1644 (2017).

53. Bates, D., Maechler, M., Bolker, B. & Walker, S. lme4: Linear mixed-effects models using Eigen and S4. *R Packag. version* **1**, 1–23 (2014).

54. Valcu, M., Dale, J. & Kempenaers, B. rangeMapper: a platform for the study of macroecology of life-history traits. *Glob. Ecol. Biogeogr.* **21**, 945–951 (2012).

55. Schielzeth, H. Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evol.* **1**, 103–113 (2010).

56. Holt, B. G. *et al.* An update of Wallace's zoogeographic regions of the world. *Science* **339**, 74–78 (2013).

57. Bardos, D. C., Guillera-Arroita, G. & Wintle, B. A. Valid auto-models for spatially autocorrelated occupancy and abundance data. *Methods Ecol. Evol.* **6**, 1137–1149 (2015).

58. Crase, B., Liedloff, A. C. & Wintle, B. A. A new method for dealing with residual spatial autocorrelation in species distribution models. *Ecography* **35**, 879–888 (2012).

59. Bivand, R. S., Pebesma, E. & Gómez-Rubio, V. *Applied Spatial Data Analysis with R*. (Springer, 2013).

60. Nakagawa, S. & Schielzeth, H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142 (2013).

61. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).

62. Louca, S. & Doebeli, M. Efficient comparative phylogenetics on large trees. *Bioinformatics* **34**, 1053–1055 (2018).

63. Pagel, M. Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884 (1999).

64. Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).

65. Fox, J., Weisberg, S., Bates, D. & Fox, M. J. Package "car". http://cran.r-project.org/web/packages/car/car.pdf (2012).

66. Ives, A. R. R^2s for correlated data: phylogenetic models, LMMs, and GLMMs. *Syst. Biol.* **68**, 234–251 (2019).

67. Ives, A. R. & Li, D. rr2: an R package to calculate R^2s for regression models. *J. Open Source Softw.* **3**, 1028 (2018).

68. Hothorn, T. *et al.* Package 'multcomp'. https://cran.r-project.org/web/packages/multcomp/index.html (2019).

69. Tung Ho, L. si & Ané, C. A linear-time algorithm for gaussian and non-gaussian trait evolution models. *Syst. Biol.* **63**, 397–408 (2014).

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Author contributions T.A. and P.M. conceived the study. P.M. collected song complexity data. All authors contributed to development of the methods. M.V. and P.M. analysed the data. P.M. prepared the Supplementary Information. P.M. under supervision of T.A. wrote the paper with input from all authors.

Song metric	*N*	Pearson's r	95% CI	*P*
Syllable repertoire size	120	0.73	0.64–0.81	<0.001
Syllables per song	174	0.62	0.52–0.70	<0.001
Song repertoire size	206	0.51	0.40–0.61	<0.001

Table S1. Validation of song complexity metric used in a present study against other widely used metrics. Association between log10-transformed scores of song complexity used in a present study and metrics used as proxies of song complexity in previous studies. We used only species with 50 scored elements. We report Pearson's correlation coefficient (Pearson's r), 95% confidence interval (95% CI), associated *P* values and sample sizes (*N*; number of species).

Variable	**β ± SE**	**t**	***P***	**λ**	**R^2_{adj}**
Intercept	2.337 ± 0.959	2.437	0.018	$0.535^{0.386;0.076}$	0.207
HVC	0.733 ± 0.182	4.033	<0.001		
Brain size	-0.366 ± 0.575	-0.637	0.527		
Body size	-0.048 ± 0.453	-0.105	0.917		

Table S2. Neural correlates of song complexity in passerines. Multivariate phylogenetic generalized least-squares model[1] examining association between song complexity and relative size of high vocal centre (HVC) across 56 passerine species. We used only species with 50 scored elements. Model was build using the pgls function in the caper package[2] and Pagel's lambda (λ). HVC was controlled for brain and body size of passerine species by including body size to the same model. To control for phylogenetic relatedness of species, we used a single maximum credibility tree which was constructed using 100 trees extracted from Bird Tree project[3] and maxCladeCred function in the phangorn package[4]. Superscripts following the λ values refer to *P* values of likelihood-ratio tests against models with $\lambda = 0$ and 1, respectively. R^2_{adj} is adjusted coefficient of determination.

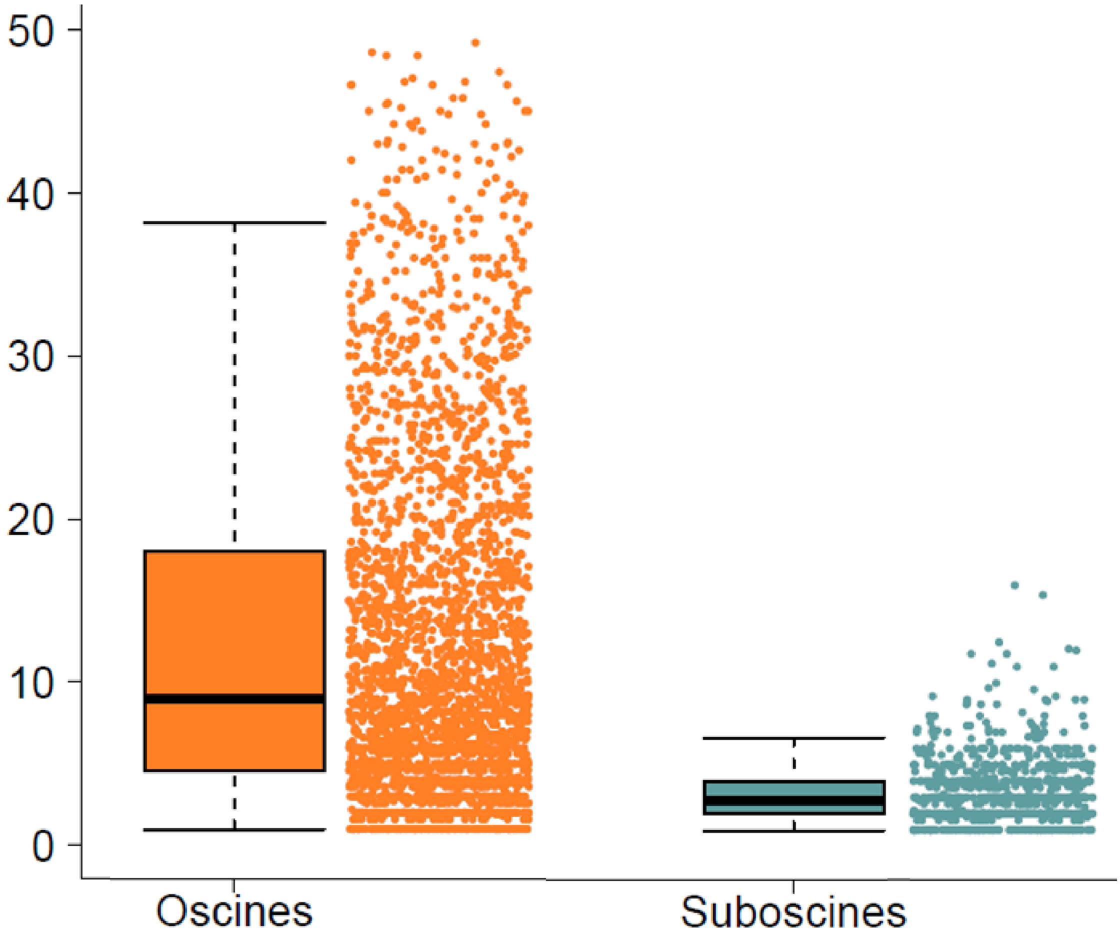

Figure S1. Distribution of song complexity in passerines with different song learning ability. Oscines (learned songs; $N = 2{,}782$ species) produce more complex song than Suboscines (innate songs; $N = 865$ species) (two-way Anova, $F_{1,3645} = 1127.4$, $P < 0.001$). We used only species with 50 scored elements. Box plots show the median (thick horizontal line), upper (75%) and lower (25%) quartiles (top and bottom of the box) and 1.5 times the inter-quartile range (whiskers). Scatter plots show the raw values.

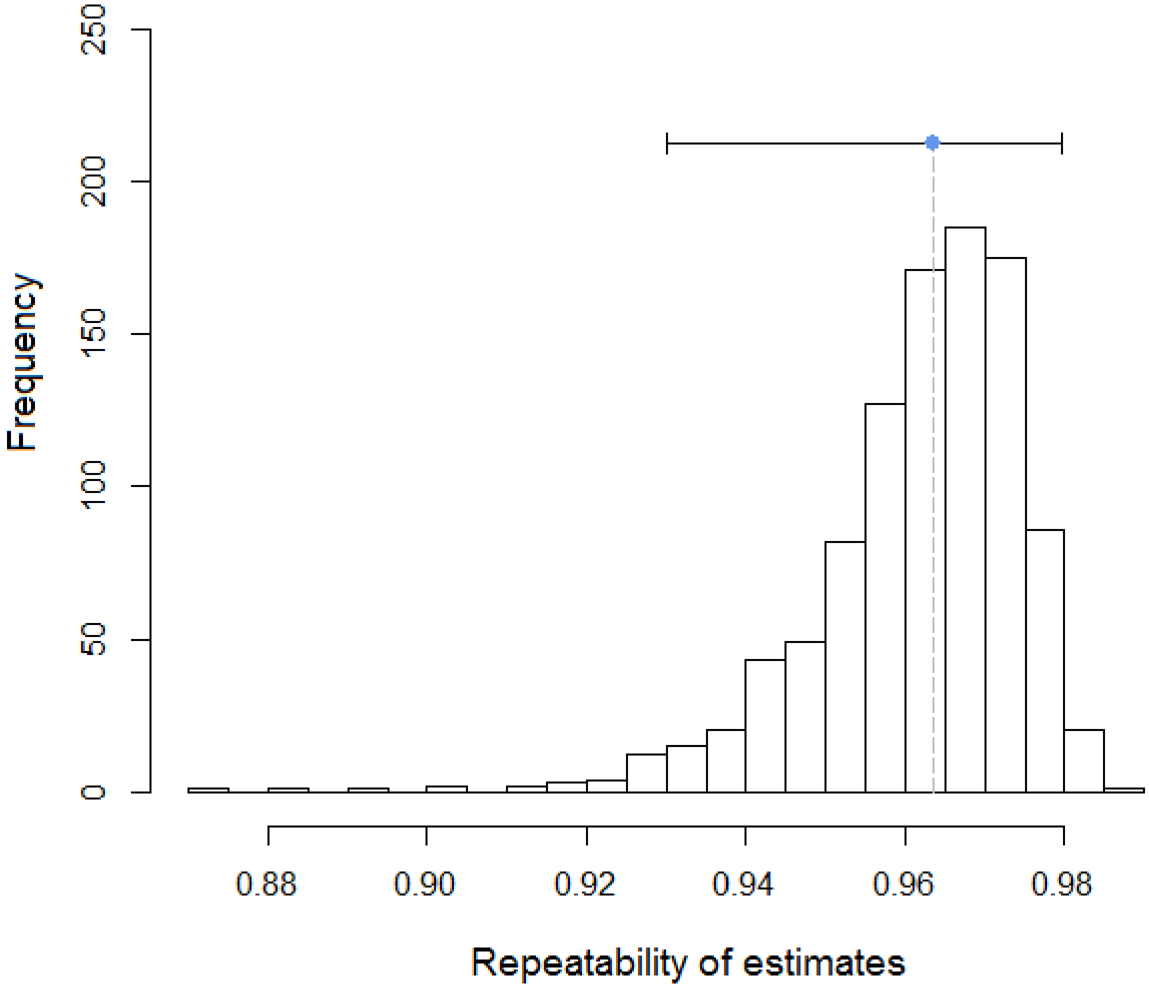

Figure S2. Repeatability of song complexity estimates. Repeatability of our scores of song complexity (log10-transformed) was estimated by the rpt function in the rptR package[5]. We performed a significance test against a null distribution and estimated the 95% confidence interval of repeatability with 1000 bootstrap samples. Model was fitted using Gaussian family of distribution by the glmer function in the lme4 package[6].

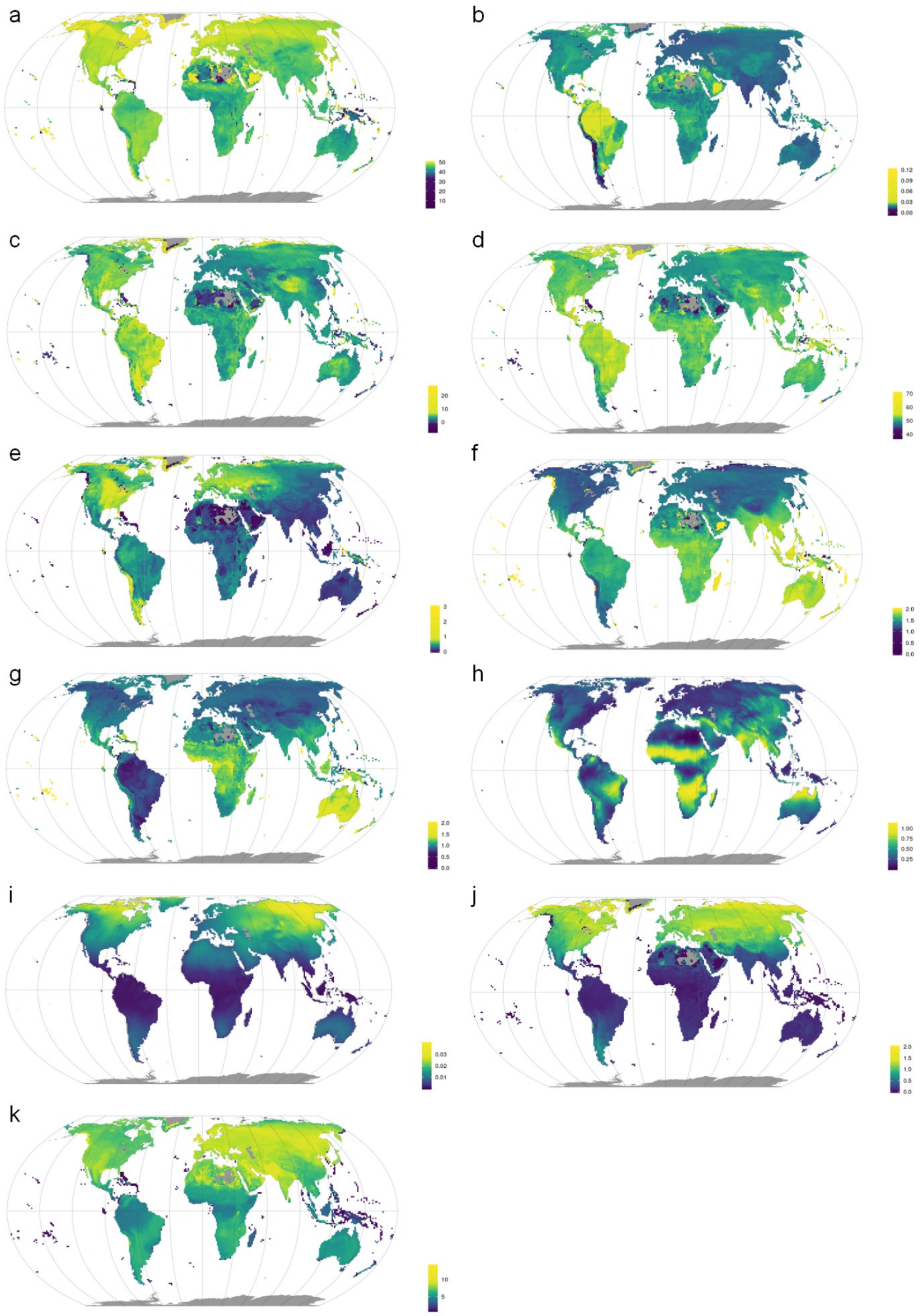

Figure S3. Maps illustrating the geographic distribution of predictors of song complexity for Oscines. a, number of scored elements, **b,** sexual size dimorphism, **c,** sexual dichromatism, **d,** male plumage colouration, **e,** polygyny, **f,** social bonds, **g,** territoriality, **h,** precipitation seasonality, **i,** temperature seasonality, **j,** migration and **k,** habitat generalism.

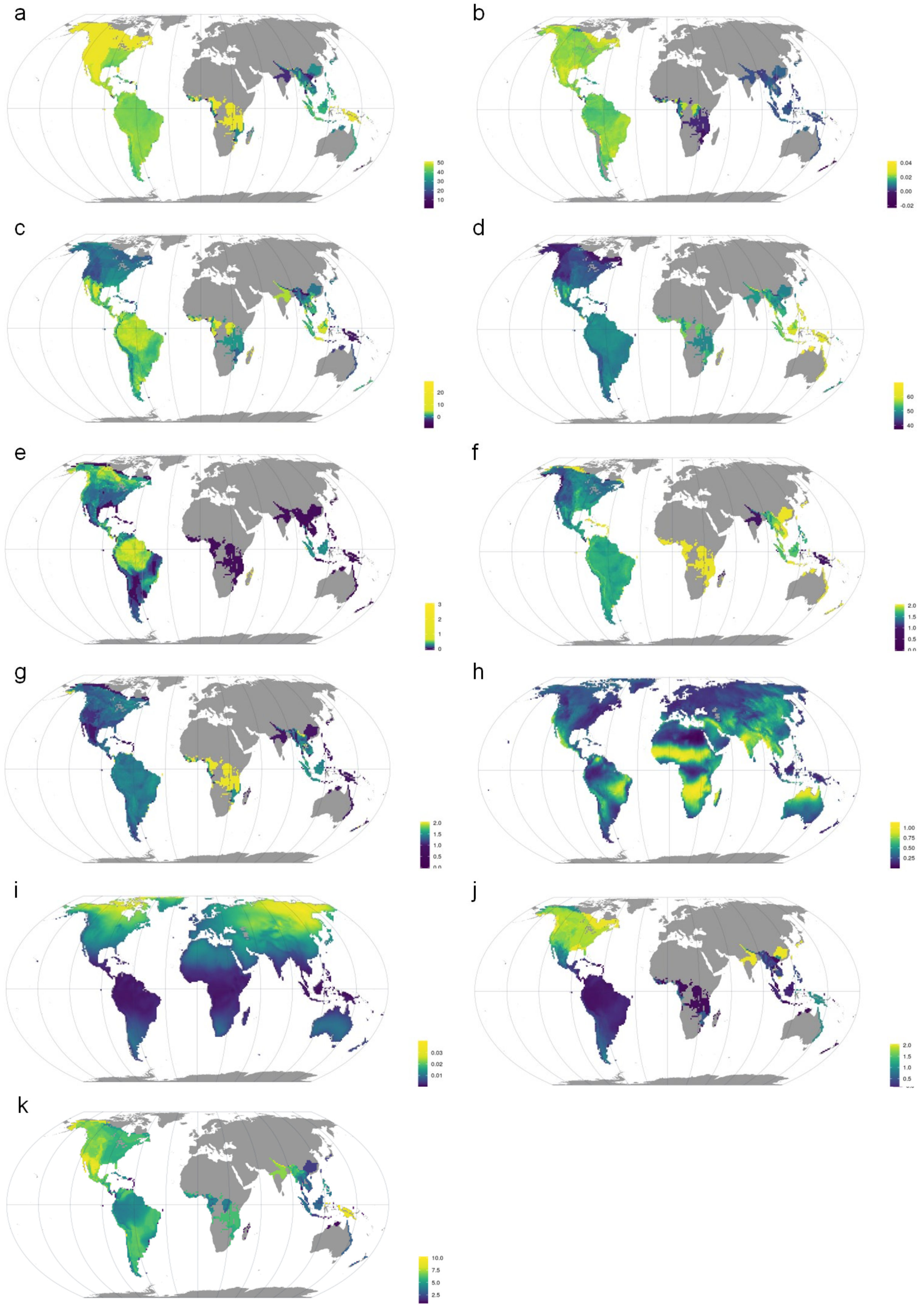

Figure S4. Maps illustrating the geographic distribution of predictors of song complexity for Suboscines. **a,** number of scored elements, **b,** sexual size dimorphism, **c,** sexual dichromatism, **d,** male plumage colouration, **e,** polygyny, **f,** social bonds, **g,** territoriality, **h,** precipitation seasonality, **i,** temperature seasonality, **j,** migration and **k,** habitat generalism.

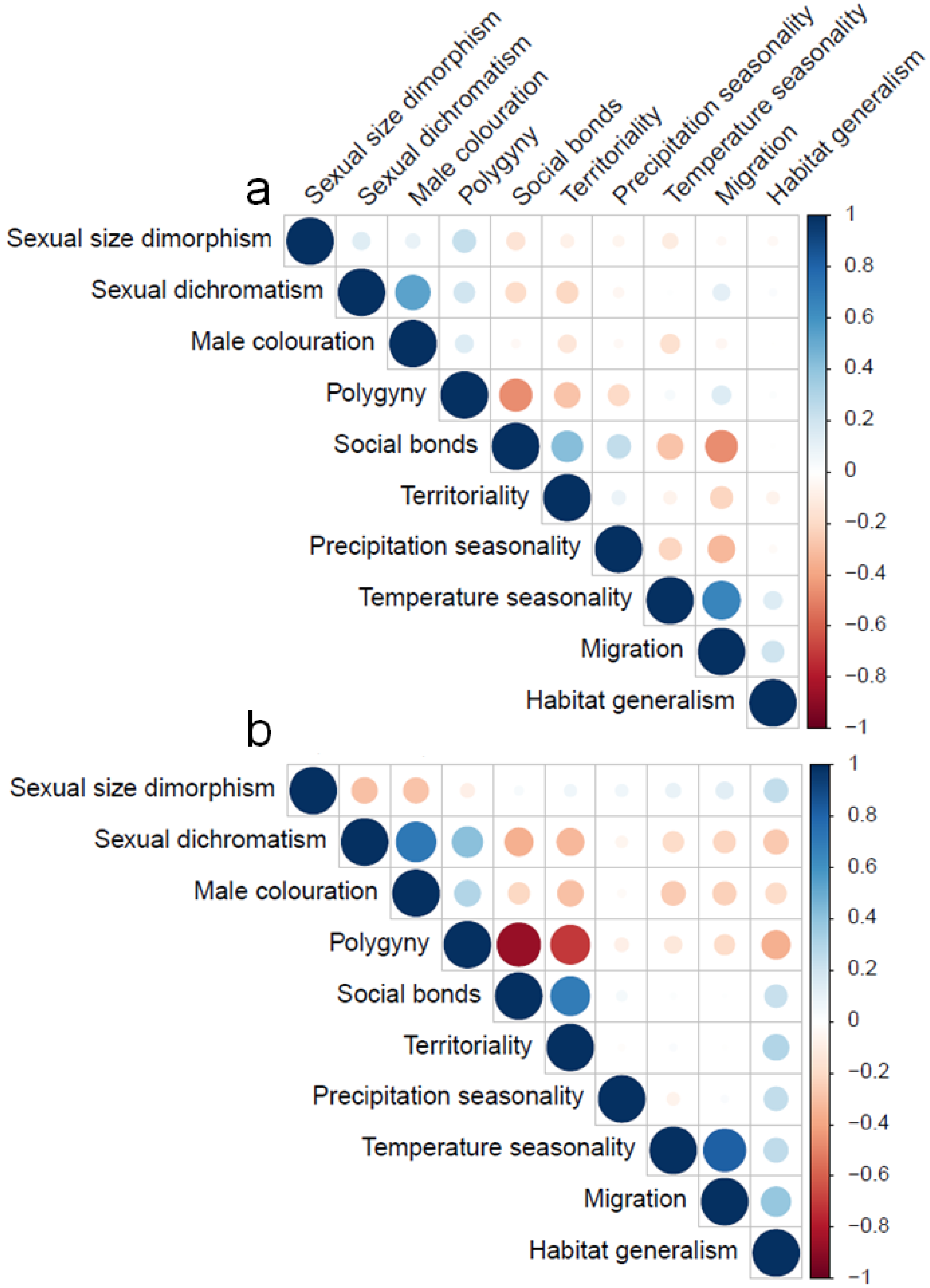

Figure S5. Correlation matrices for indices of sexual selection. Figure depicts strength of correlation between life-history, social and environmental indices of sexual selection for **a,** Oscines (*N* = 1,861) and **b,** Suboscines (*N* = 277) species. Correlation coefficients were estimated using Spearman's rank correlation approach.

1. Freckleton, R. P., Harvey, P. H. & Pagel, M. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**, 712–726 (2002).

2. Orme, D., Freckleton, R., Thomas, G. & Petzoldt, T. The caper package: comparative analysis of phylogenetics and evolution in R. http://caper.r-forge.r-project.org (2012) doi:http://caper.r-forge.r-project.org.

3. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. *Nature* **491**, 444–448 (2012).

4. Schliep, K. P. phangorn: phylogenetic analysis in R. *Bioinformatics* **27**, 592–593 (2011).

5. Stoffel, M. A., Nakagawa, S. & Schielzeth, H. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* vol. 8 1639–1644 (2017).

6. Bates, D., Maechler, M., Bolker, B. & Walker, S. lme4: Linear mixed-effects models using Eigen and S4. *R Packag. version* **1**, 1–23 (2014).

CHAPTER 2

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SSE SOCIETY for the STUDY of EVOLUTION

Song complexity—no correlation between standard deviation of frequency and traditionally used song complexity metrics in passerines: A comment on Pearse et al. (2018)

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Recently, Pearse et al. explored the macroecology of passerine song using a large citizen science database of bird songs and machine learning techniques. They used standard deviation of frequency (SDF) as a surrogate for song complexity, finding only weak support for correlation between SDF and life-history traits such as monogamy and sexual dimorphism. Their finding that song complexity increases toward more productive environments and warmer areas seemingly contradicts several previous multitaxonomic studies. By comparing SDF scores with traditionally used song complexity metrics (syllable repertoire size and the number of syllable types per song), we found no evidence of any correlation. This may help to explain the discrepancy between their findings and findings of previous studies. While we agree that simple metrics that can be quantified and compared between multiple, highly variable species are crucial for progress in large-scale analysis of birdsong complexity, the biological relevance of SDF remains unclear and more research is needed to clarify its relevance for further studies of birdsong complexity.

KEY WORDS: birdsong, song complexity, syllable repertoire size.

In a recent paper, Pearse et al. (2018) used a large citizen science dataset, Xeno-canto (www.xeno-canto.org), and machine learning techniques to characterise avian song characteristics, including song complexity. While they used several metrics to capture this parameter in passerines, all were intercorrelated; hence, they used a single metric as the main surrogate for song complexity, defined as SD of frequency (hereafter SDF). Then, using phylogenetically informed comparative and assemblage-based analyses, they evaluated the effects of biotic and abiotic factors on interspecific song complexity variation in 578 passerine species. Pearse et al. (2018) found only weak support for correlation between life-history traits such as monogamy and sexual dimorphism and the evolution of song complexity. Surprisingly, using comparative and assemblage-based analyses, they found a significant positive link between climate factors such as net primary productivity (NPP) and NPP and temperature, respectively, and song complexity, i.e., that birds with more complex songs occupied more productive (and presumably more complex) environments and warmer areas than species with less elaborate songs. This seems to contradict results of several previous multitaxonomic studies showing that birdsong complexity increases at higher latitudes, i.e., toward less productive (and less complex) environments and more variable/seasonal environments (e.g., Botero et al. 2009; Weir and Wheatcroft 2011; Medina and Francis 2012). While we appreciate the novel approach used to analyze such a large birdsong database, as well as the attempt to find simple metrics for quantifying birdsong complexity across large spatial and taxonomic scales, we believe a likely reason for the discrepancy

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Table 1. Results of a set of univariate phylogenetically-informed regression analyses (PGLS) evaluating the association between (a) log-transformed scores on total syllable repertoire size in 45 species published by Moore et al. (2011) and Garamszegi et al. (2005) and (b) syllable diversity in 37 species published by Weir and Wheatcroft (2011) as the dependent variable and various song complexity metrics reported by Pearse et al. (2018) as predictors. Superscripts following the λ values refer to significance (P values) of likelihood-ratio tests against models with $\lambda = 0$ (no phylogenetic association in traits) or 1 (complete phylogenetic association in traits), respectively. The adjusted coefficient of determination – r^2_{adj}.

Song complexity metrics	β ± SE	*t*	P	λ	r^2_{adj}
(a) Syllable repertoire size					
Standard deviation of frequency	0.730 ± 0.729	1.002	0.322	$0.554^{0.012;\ 0.097}$	<0.001
Inter-quartile range of frequency	0.606 ± 0.441	1.373	0.177	$0.589^{0.008;\ 0.078}$	0.020
Renyi entropy	2.941 ± 4.065	0.724	0.473	$0.502^{0.016;\ 0.079}$	0.012
Shannon entropy	−2.740 ± 1.631	−1.680	0.100	$0.505^{0.012;\ 0.154}$	0.040
Temporal entropy	3.549 ± 9.578	0.371	0.713	$0.528^{0.016;\ 0.153}$	−0.020
Spectrotemporal entropy	2.941 ± 4.065	0.724	0.473	$0.502^{0.016;\ 0.079}$	−0.011
Total entropy	−2.179 ± 1.531	−1.423	0.162	$0.511^{0.012;\ 0.147}$	0.023
(b) Syllable diversity					
Standard deviation of frequency	−0.063 ± 0.348	−0.182	0.857	$0.809^{<0.001;\ 0.009}$	−0.028
Inter-quartile range of frequency	0.111 ± 0.171	0.649	0.521	$0.830^{<0.001;\ 0.017}$	−0.016
Renyi entropy	−0.562 ± 2.401	−0.234	0.816	$0.805^{<0.001;\ 0.008}$	−0.027
Shannon entropy	−0.562 ± 2.401	−0.234	0.816	$0.805^{<0.001;\ 0.008}$	−0.027
Temporal entropy	−2.459 ± 2.774	−0.887	0.381	$0.860^{<0.001;\ 0.053}$	−0.006
Spectrotemporal entropy	−0.448 ± 0.955	−0.469	0.642	$0.795^{<0.001;\ 0.007}$	−0.022
Total entropy	−0.927 ± 0.889	−1.043	0.304	$0.822^{<0.001;\ 0.016}$	0.002

between the results of previously published works and those presented by Pearse et al. (2018) has been missed.

Bird species differ markedly in their vocal expression and song characteristics (Catchpole and Slater 2008), making it difficult to find approaches and metrics that effectively quantify temporal and structural organization of birdsong across wider taxonomic scales. An important characteristic of avian song is its complexity. This is widely estimated on an interspecific scale as (a) repertoire size (i.e., the number of unique elements, syllables, or song types in the repertoire) and/or song versatility (e.g., Garamszegi et al. 2005; Botero et al. 2009; Moore et al. 2011; Soma and Garamszegi 2011), or (b) within-song traits (e.g., based on the average number of syllables and syllable types per song per species; Mahler and Gil 2009; Weir and Wheatcroft 2011). These metrics have been shown to have good biological relevance; serving, for example, as an honest signal of mate quality (summarized in Catchpole and Slater 2008). Pearse et al. (2018) attempted to use simple automatically derived metrics, estimated as SDF, as a substitute for these birdsong complexity metrics. Surprisingly, Pearse et al. (2018) did not validate their novel song complexity scores against previously reported data on song complexity derived from single-species repertoire size estimates.

Initially, we extracted data on song complexity for 39 passerine species with well-known syllable repertoire sizes, primarily from Moore et al. (2011), with six other species added from Garamszegi et al. (2005) (hereafter referred to as syllable repertoire size). Subsequently, we extracted data on within-song complexity expressed as PC scores highly correlated with the number of syllable types per song for 37 species from Weir and Wheatcroft (2011) (hereafter referred to as syllable diversity). Each of the 45 and 37 species were included in the publication of Pearse et al. (2018). When multiple song complexity estimates were found for a species, we used mean values corrected for sample size. The relationships between known syllable repertoire size and syllable diversity (dependent variables) and various song complexity metrics provided by Pearse et al. (2018) (predictors) were then investigated using a phylogenetic generalized least squares (PGLS) approach and the "pgls" function in the *caper* package (Orme 2013) to account for the non-independence of data owing to shared ancestry of species. The PGLS uses maximum likelihood to simultaneously optimize the phylogenetic signal (Pagel's λ) in the residuals. The consensus phylogenetic tree of songbirds was built from 1000 randomly generated trees based on a Hackett backbone using the phylogenetic tool available at http://birdtree.org/ (Jetz et al. 2012). The maximum clade credibility tree was generated using "maxCladeCred" function in *phangorn* package (Supplementary Material 1, 2) (Schliep 2018). All statistical analyses were performed in R v. 3.4.3 (R Core Team 2017).

The PGLS revealed no relationship between the log-transformed scores on total syllable repertoire size and song

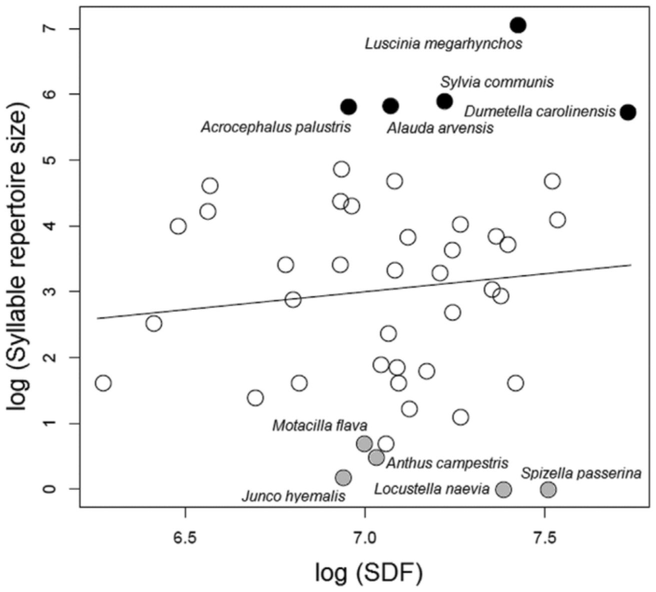

Figure 1. Relationship between log-transformed scores on total syllable repertoire size published by Moore et al. (2011) and Garamszegi et al. (2005) and scores on song complexity (standard deviation of frequency; SDF) reported by Pearse et al. (2018). The five species with highest reported syllable repertoire size according to Moore et al. (2011) and Garamszegi et al. (2005) are highlighted by black dots, while the five species with the lowest reported syllable repertoire size are highlighted by grey dots. The line is the linear regression line not controlled for phylogeny. *N* = 45 species.

complexity metrics reported by Pearse et al. (2018) (Table 1a). The lack of relationship between SDF and total syllable repertoire size is highlighted by a species-to-species comparison of song complexity scores. When comparing the common nightingale *Luscinia megarhynchos* (which has an extremely large repertoire of >1100 syllable types; Hultsch 1980) and the common grasshopper warbler *Locustella naevia* (which has a single syllable repertoire; Wahlström 1966), SDF-based song complexity treated them as producing songs of similar complexity. Some other species with large repertoire sizes had even lower scores than species with the lowest syllable repertoire sizes (Fig. 1). Similarly, we found no association between within-song complexity estimated as syllable diversity and log-transformed scores of song complexity metrics reported by Pearse et al. (2018) (Table 1b).

Our findings show that, counterintuitively, having more unique syllables does not necessarily increase song SDF. We further demonstrate that the novel SDF metric of Pearse et al. (2018) is unassociated with species-specific repertoire size and syllable diversity, two important song complexity metrics widely used in previous ecological and evolutionary studies (e.g., Garamszegi et al. 2005; Botero et al. 2009; Moore et al. 2011; Weir and Wheatcroft 2011; Medina and Francis 2012). We agree that simple metrics that can be quantified and compared between multiple, highly variable species are crucial for progress in large-scale analysis of birdsong complexity. However, although SDF may capture some component of birdsong complexity, its biological relevance remains unclear and more research is needed to clarify its relevance for further studies of birdsong complexity.

AUTHOR CONTRIBUTIONS

PM and TA analysed the data, all co-authors contributed to the writing of the comment.

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DATA ARCHIVING

Data can be accessed at Dryad (https://doi.org/10.5061/dryad.kg0mr83).

LITERATURE CITED

Botero, C. A., N. J. Boogert, S. L. Vehrencamp, and I. J. Lovette. 2009. Climatic patterns predict the elaboration of song displays in mockingbirds. Curr. Biol. 19:1151–1155.

Catchpole, C. K., and P. J. B. Slater. 2008. Bird song: biological themes and variations. Cambridge Univ. Press, Cambridge.

Garamszegi, L. Z., T. J. Balsby, B. D. Bell, M. Borowiec, B. E. Byers, T. Draganoiu, M. Eens, W. Forstmeier, P. Galeotti, D. Gil, L. Gorissen, P. Hansen, H. M. Lampe, and S. Leitner. 2005. Estimating the complexity of bird song by using capture-recapture approaches from community ecology. Behav. Ecol. Sociobiol. 57:305–317.

Hultsch, H. 1980. Beziehungen zwischen Struktur, zeitlicher Variabilität und sozialem Einsatz im Gesang der Nachtigall (*Luscinia megarhynchos*). PhD dissertation. Freie Universität, Berlin.

Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. Nature 491: 444–448.

Mahler, B., and D. Gil. 2009. The evolution of song in the *Phylloscopus* leaf warblers (Aves: Sylviidae): a tale of sexual selection, habitat adaptation, and morphological constraints. Adv. Stud. Behav. 40:35–66.

Medina, I., and C. D. Francis. 2012. Environmental variability and acoustic signals: a multi-level approach in songbirds. Biol. Lett. 8: 928–931.

Moore, J. M., T. Székely, J. Büki, and T. J. DeVoogd. 2011. Motor pathway convergence predicts syllable repertoire size in oscine birds. Proc. Natl. Acad. Sci. USA 108:16440–16445.

Orme, D. 2013. The caper package: comparative analysis of phylogenetics and evolution in R. R package version 0.5.2. http://cran.r-project.org/package=caper.

Pearse, W. D., I. Morales-Castilla, L. S. James, M. Farrell, F. Boivin, T. J. Davies. 2018. Global macroevolution and macroecology of passerine song. Evolution 72:944–960.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

Schliep, K. P. 2018. Package ‘phangorn’: phylogenetic reconstruction and analysis. R package version 2.4.0. https://cran.r-project.org/web/packages/phangorn/.

Soma, M., and L. Z. Garamszegi. 2011. Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. Behav. Ecol. 22:363–371.

Wahlström, S. 1966. En akustisk jämförelse mellan sången hos tre olika Locustellaarter. Vår Fågelvärld 25:161–166.

Weir, J. T., and D. Wheatcroft. 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. Proc. R. Soc. B Biol. Sci. 278:1713–1720.

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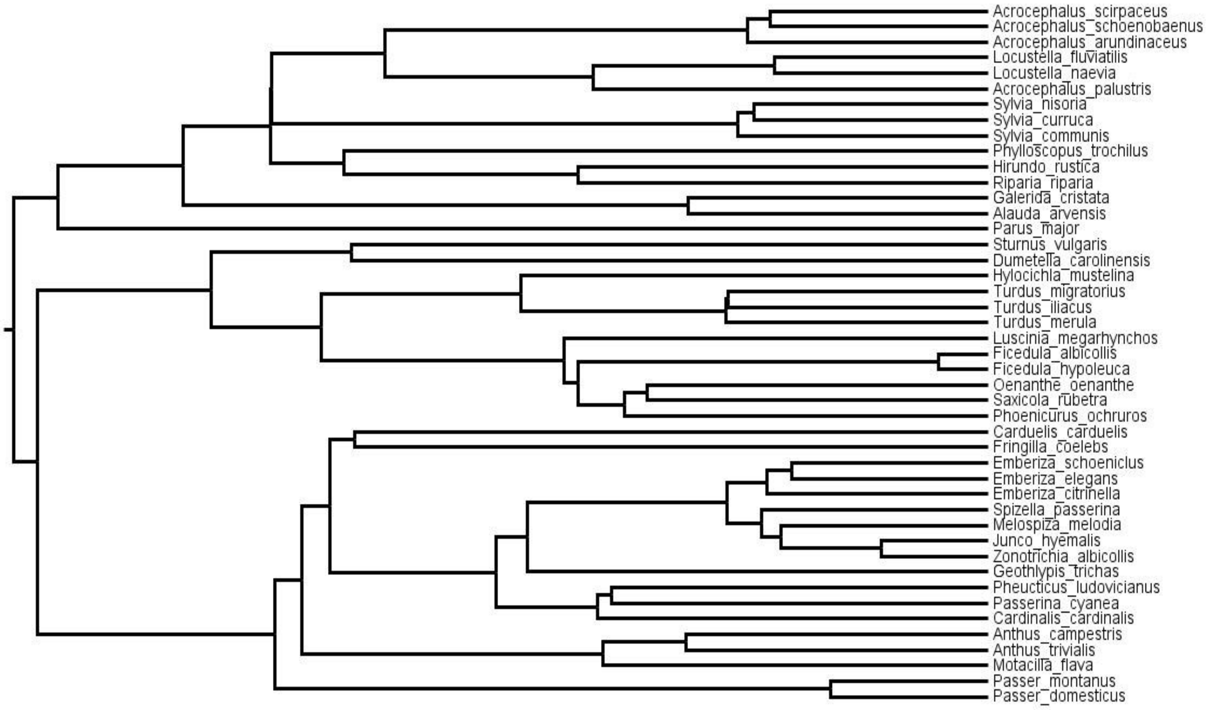
Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Material

Supplementary Information

Supplementary Material 1. Maximum clade credibility tree for the phylogenetic relationships between the 45 songbird species involved in the study of relationship between syllable repertoire size and song complexity metrics reported by Pearse et al. (2018). The phylogenetic tree was built from 1000 randomly generated trees based on a Hackett backbone topology using the phylogenetic tool available at http://birdtree.org/ (Jetz et al. 2012). The maximum clade credibility tree was generated using "maxCladeCred" function in *phangorn* package (Schliep 2011).



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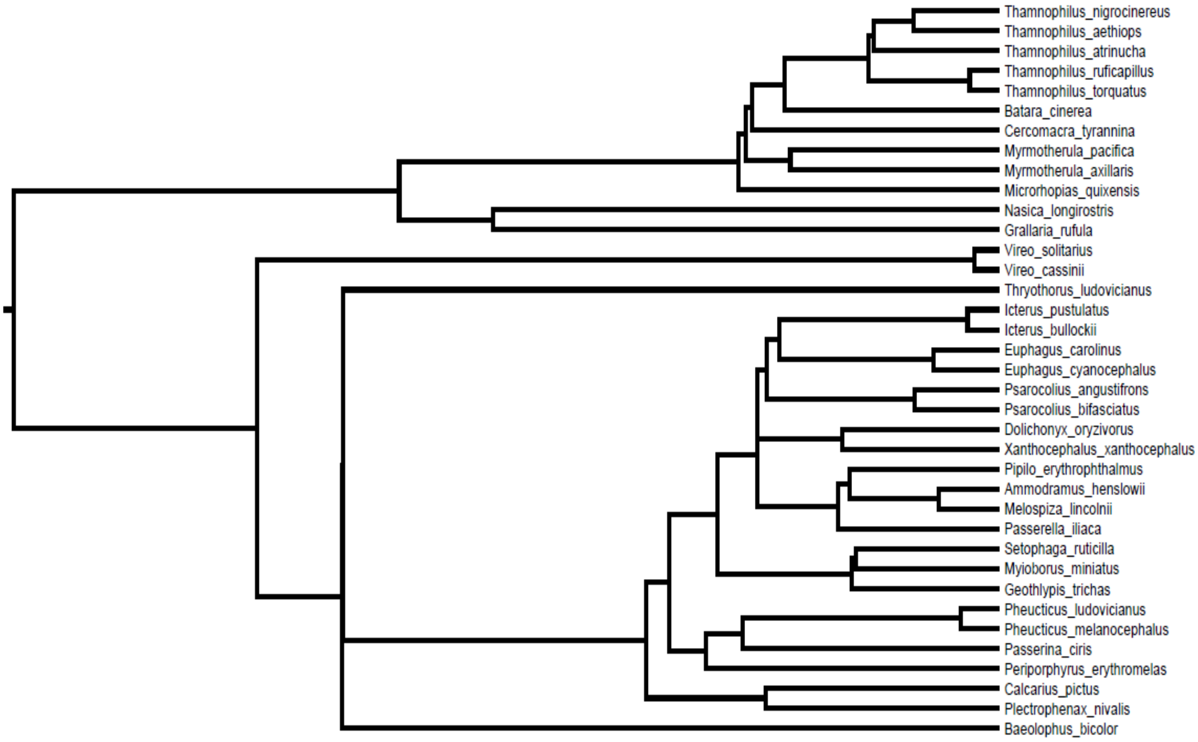
Supplementary Material 2. Maximum clade credibility tree for the phylogenetic relationships between the 37 songbird species involved in the study of relationship between syllable diversity and song complexity metrics reported by Pearse et al. (2018). The phylogenetic tree was built from 1000 randomly generated trees based on a Hackett backbone topology using the phylogenetic tool available at http://birdtree.org/ (Jetz et al. 2012). The maximum clade credibility tree was generated using "maxCladeCred" function in *phangorn* package (Schliep 2011).



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References

Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. *Nature* **491**:444–448.

Pearse, W. D., I. Morales-Castilla, L. S. James, M. Farrell, F. Boivin, T. J. Davies. 2018. Global macroevolution and macroecology of passerine song. *Evolution* **72**:944–960.

Schliep, K. P. 2018. Package ‘phangorn’: phylogenetic reconstruction and analysis. R package version 2.4.0. https://cran.r-project.org/web/packages/phangorn/

CHAPTER 3

A global analysis of song frequency in passerines provides no support for the acoustic adaptation hypothesis but suggests a role for sexual selection

Running title: Evolutionary ecology of song frequency

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Statement of authorship: TA and PM conceived and designed the study with input from all authors. TA and BK coordinated the study. PM collected the song data. MV performed the statistical analyses with input from WF. MB made the figures with help from MV and PM. TP and HB provided bioacoustic expertise. PM drafted the first version of the manuscript. TA, BK and PM revised and finalized the manuscript with input from all authors.

Data availability statement: The data used in this study were collected from publicly available databases. If the manuscript will be accepted, the data supporting the results and computer code used to generate the results will be archived at Github, and the data DOI will be included at the end of the article.

Abstract

Many animals use acoustic signals for communication, implying that the properties of these signals can be under strong selection. The acoustic adaptation hypothesis predicts that species living in dense habitats emit lower-frequency sounds than those in open areas, because low-frequency sounds generally propagate further in denser vegetation. Signal frequency may also be under sexual selection, because it correlates with body size and lower-frequency sounds are perceived as more intimidating. Here, we evaluate these hypotheses by analysing variation in peak song frequency across 5,085 passerine species (Passeriformes). A phylogenetically-informed analysis revealed that song frequency decreases with increasing body mass and with male-biased sexual size dimorphism. However, we found no support for the predicted relationship between frequency and habitat. Our results suggest that the global variation in passerine song frequency is mostly driven by natural and sexual selection causing evolutionary shifts in body size rather than by habitat-related selection on sound propagation.

Keywords: acoustic adaptation hypothesis, allometry, animal communication, bird song, macroecology, morphological constraints, sexual selection

INTRODUCTION

Acoustic signalling is widespread among animals (Bradbury & Vehrencamp 1998; Gerhardt & Huber 2002; Catchpole & Slater 2008). Successful transmission and reception of acoustic signals between conspecifics are essential in diverse contexts, including predation avoidance (alerting others to a threat), territory defence, mate attraction, and synchronization of breeding activities (Bradbury & Vehrencamp 1998; Catchpole & Slater 2008). One of the fundamental characteristics of acoustic signals is the frequency of the sound, because it strongly affects signal propagation through the environment (Morton 1975; Wiley & Richards 1982; Padgham 2004). Low frequency sounds are generally less attenuated during transmission than high frequency sounds (Wiley & Richards 1982; Padgham 2004). Nevertheless, the frequency of acoustic signals is tremendously diverse across the animal kingdom (Gerhardt 1994; Fitch 2006; Gillooly & Ophir 2010; Pijanowski *et al.* 2011) and several hypotheses have been proposed to explain this diversity. Here, we focus on the three most compelling ones: (1) the acoustic adaptation hypothesis, (2) the morphological constraint hypothesis, and (3) the sexual selection hypothesis.

Since the 1970s, it has been postulated that the frequency of acoustic signals could reflect an adaptation to maximize the effectiveness of sound transmission in specific habitats (Morton 1975). This is known as the acoustic adaptation hypothesis (Boncoraglio & Saino 2007; Ey & Fischer 2009). Sounds transmitted through the natural environment are subject to degradation, for example due to environmental absorption, reverberation and scattering. The degree of this degradation depends both on the sound structure and on the physical characteristics of the environment (Wiley & Richards 1982; Brumm & Naguib 2009). Specifically, because of frequency-dependent attenuation, low-frequency sounds transmit generally further than high-frequency sounds. However, the slope of the frequency dependence is steeper in dense, forested habitats because of the high degree of sound absorption and scattering from foliage. Hence, high-frequency signals are attenuated more strongly in closed than in open habitats (Morton 1975; Marten & Marler 1977; Wiley & Richards 1978). Therefore, species living in forested habitats are expected to produce vocalizations of lower frequencies than those living in open habitats (Ey & Fischer 2009). Despite this strong theoretical underpinning, empirical evidence for the acoustic adaptation hypothesis is equivocal (Morton 1975; Wiley 1991; Buskirk 1997; Bertelli & Tubaro 2002; Blumstein & Turner 2005; Ey & Fischer 2009). For instance, a meta-analysis by Boncoraglio & Saino (2007) showed that song frequency in birds tends to be lower in closed compared with open habitats, but the effect size was small. A review by Ey & Fischer (2009) concluded that habitat-related adjustments of frequency parameters of acoustic signals of birds, anurans and mammals are not as widespread as previously thought.

The morphological constraint hypothesis simply posits that body size sets a limit on the frequency of the sound an animal can produce. Morphological constraints generally seem to play a pervasive role in the evolution of animal acoustic communication (Ryan & Brenowitz 1985; Bradbury & Vehrencamp 1998; Fitch & Hauser 2002). A negative relationship between body size and frequency of acoustic signals, i.e. larger species tend to produce lower frequency sounds than smaller species, seems to be a general rule in animal bioacoustics and has been documented across various groups, including insects, fishes, amphibians, reptiles, birds, and mammals (Wallschläger 1980; McClatchie *et al.* 1996; Fitch & Hauser 2002; Gillooly & Ophir 2010; Pearse *et al.* 2018). In birds, it has been suggested that the frequency of vocalizations negatively scales with body size, simply because body size influences the morphology and functional aspects of the vocal apparatus, such as the size of vibratory structures (Bertelli & Tubaro 2002; Suthers & Zollinger 2008; Seneviratne *et al.* 2012; Gonzalez-Voyer *et al.* 2013; Tietze *et al.* 2015). However, body size alone does not explain the entire variation in song frequency across animals. Departures from the negative allometric relationship between frequency of acoustic signals and body size may reflect (a) differences in evolutionary history that caused variation in syrinx or vocal tract morphology (phylogenetic constraints) and (b) differences in costs or benefits of producing low-frequency sounds. Thus, variation in frequency may inform about current or past selection on acoustic signals (Searcy & Nowicki 2005; Ophir *et al.* 2010; Wagner *et al.* 2012).

This brings us to the hypothesis that the frequency of acoustic signals may be sexually selected, acting as an indicator of an individual’s size, dominance or fighting ability. In various taxa, the frequency of male vocalizations indeed seems to indicate individual body size and can influence territory establishment (or other forms of male−male competition), attractiveness (female choice) and ultimately an individual’s reproductive success (Morton 1977; Fitch & Hauser 2002; Apicella *et al.* 2007; Hardouin *et al.* 2007; Mager *et al.* 2007; Vannoni & McElligott 2008; Forstmeier *et al.* 2009; Brumm & Goymann 2017). For instance, the frequency of advertising vocalizations negatively correlates with body size in males of common toads *Bufo bufo* and during the mating period smaller males were less often attacked by larger males when natural croaks of the small males were experimentally replaced by deep croaks (Davies & Halliday 1978). Similarly, heavier individuals of scops owl *Otus scops* produced lower-frequency hoots and territorial males responded less intensely to hoots simulating heavier intruders (Hardouin *et al.* 2007). Thus, if low-frequency sounds are advantageous during agonistic interactions between males and as a means of dominance status signalling (Davies & Halliday 1978; Wagner 1989; Briefer *et al.* 2010; Bro-Jørgensen & Beeston 2015), we predict correlated evolution of male vocal frequency and indices of the intensity of sexual selection such as male-biased sexual size dimorphism (Trivers 1972; Fairbairn 1997).

Here, we use a large data set of 5,085 passerine species (Order: Passeriformes), representing 85% of all passerines and 50% of all avian taxa (Jetz *et al.* 2012), to explore interspecific variation in peak frequency of male song. Applying a phylogenetically-informed cross-species analysis, we evaluate the association between song frequency and habitat density, body size (expressed as body mass), and the intensity of sexual selection (expressed as sexual size dimorphism). Based on the hypotheses outlined above, we test the one-tailed predictions that lower-frequency songs are associated with (1) more closed (forested) habitats, (2) larger body size and (3) stronger male-biased sexual size dimorphism.

Passerines are an excellent study system for evaluating sources of interspecific variation in signal frequency. First, their song represents a textbook example of a long-range acoustic signal that plays an important role in mate attraction and territory defence (Catchpole 1987; Catchpole & Slater 2008). Second, passerines are globally distributed, show a more than 300-fold difference in body mass, vary in sexual selection pressures and mating systems, and occupy a wide range of habitats (del Hoyo *et al.* 2018). Although song (or call) frequency has been widely studied in birds, previous comparative studies often evaluated the effects of body size, sexual selection, and habitat effects separately and without accounting for phylogeny (reviewed by Ey & Fischer 2009). Moreover, previous studies were restricted to a few species only (Ey & Fischer 2009).

MATERIALS AND METHODS

Data on peak song frequency

We collected song recordings primarily from xeno-canto (https://www.xeno-canto.org), a citizen science repository of bird vocalizations. When access to recordings of endangered or vulnerable species was restricted, we directly contacted the authors. For species with missing recordings on xeno-canto, we used recordings from the Macaulay Library (The Cornell Lab of Ornithology, https://www.macaulaylibrary.org/). We focused exclusively on the song, ignoring other types of vocalizations (e.g. calls). Song is commonly defined as a long-range vocalization that is used mainly in mate attraction and territory defence. The definition of the song may, however, vary across sources or authorities, and functions of particular vocalizations are still poorly known for several passerine species. Therefore, we used the classification of vocalizations as provided on the platform storing the recordings. Although some recordings might be misclassified, we primarily focused on high-quality recordings (scored as quality “A” or “B” in xeno-canto, or rated four or more stars in Macaulay Library), usually collected by skilled observers with in-depth knowledge of particular bird species’ vocalizations. Both repositories also provide a space for discussion and correction of misclassified recordings by community members, increasing the reliability of the available information.

We collected 1−5 (median = 4, mean ± SD = 3.7 ± 1.5) recordings of adult male song for each species (total of 18,789 recordings from 5,085 species). We did not use recordings of female and juvenile song. However, recordings often lacked information on sex, age, or the number of singing individuals. Although most of such recordings presumably documented adult male song, females of many species sing, either solo, in duets (coordinated joint singing of a mated pair) or in a chorus (three and more singing individuals) (Odom *et al.* 2014; Tobias *et al.* 2016; Mikula *et al.* 2020). A few recording annotations mentioned “duet” or “chorus” and in some cases we could disentangle parts produced by different individuals. We then measured song frequency for the individual producing the more complex song, i.e. containing more elements and syllable types (presumably a male). For a few species, we were not able to separate the song of multiple individuals. In these cases, we assumed that the recording was representative of the song of the males of the species. Although this procedure might have introduced some error, we do not expect systematic bias in species-specific frequency values. We assigned geographic coordinates to all song recordings as reported by the person who made the recording. In widely distributed species, recordings were typically separated by tens to thousands of kilometres. However, in species with smaller ranges, we used recordings made at least 1 km apart to reduce the possibility that two or more analysed recordings contained song of the same individual. In several species (all island or mountain endemics or poorly sampled species) this was not possible. In these cases, we a priori maximized the altitudinal and temporal separation of recordings, by only selecting recordings that differed in altitude by at least 100 metres or were collected in different years.

After downloading, all recordings were converted to *.wav* format with an online converter (www.online-audio-converter.com) at a sampling rate of 44.1 kHz. We characterized song frequency by a single parameter, namely peak frequency (i.e. the frequency at maximum amplitude), using the Raven Pro 1.4 software (Cornell Lab of Ornithology, Ithaca, NY, USA, www.ravensoundsoftware.com). We then calculated the median value for each species. Peak frequency is central to our hypotheses because: (1) unlike minimum and maximum frequencies, it is crucial for signal transmission (Brumm & Naguib 2009), (2) it may differ between habitats (see meta-analysis in Boncoraglio & Saino 2007), and (3) it is a key trait in other studies investigating the effect of morphological constraints and sexual selection on acoustic communication (Gillooly & Ophir 2010; Greig *et al.* 2013; Mason & Burns 2015; Thiagavel *et al.* 2017). First, we measured peak song frequency based on a fast Fourier transform length of 256 points (Hann window), resulting in a frequency resolution of 172 Hz. In a second step, we re-measured peak song frequency for species with median peak frequency < 1.2 kHz (n = 90 species), using a higher frequency resolution of 21.5 Hz (fast Fourier transform length of 2,048 points) to capture the lower end of the range in peak song frequency more accurately. To ensure consistency, all recordings were downloaded and analysed by a single person (PM).

Predictor variables

Body size and sexual size dimorphism

As a proxy of species-specific body size, we used mean body mass (in grams; pooling sexed and unsexed individuals from Dunning 2008; n = 4,602 species) or male body mass (from Dunning 2008; n = 984 species). To estimate sexual size dimorphism we used data on male and female body mass (from Dunning 2008; n = 984 species) or wing length (in millimetres; from Dale *et al.* 2007; n = 2,463 species). We then calculated sexual size dimorphism either as log(male body mass) − log(female body mass) or as log(male wing length) − log(female wing length). Positive values indicate species where males are larger than females, i.e. male-biased sexual size dimorphism. Sexual size dimorphism is associated with other indices of the intensity of sexual selection, such as the mating system (polygyny versus monogamy) or testis size (Dunn *et al.* 2001).

Habitat density

As a proxy for habitat density, we used tree cover data from Collection 2 of the Copernicus Global Land Cover project (Buchhorn *et al.* 2020). For each geographic location of a song recording, we extracted the percentage of tree cover in a 100 × 100 metres quadrant using the *exactextractr* package (v.0.2.1) in R (Baston 2020). Species-specific tree cover was then estimated as the mean of all conspecific recordings.

We also extracted data on habitat type for each species based on descriptions in del Hoyo *et al.* (2018). We assigned each species to the most prevalent habitat type on a three-point scale: (1) closed (covering species living in densely vegetated habitat types such as forest, woodland and mangrove), (2) mixed (covering generalist species and species inhabiting ecotones), and (3) open (covering species inhabiting grassland, steppe, desert and semi-desert, savannah, bushland, rocky habitats and seashores).

Statistical analyses

All statistical analyses were performed using R v. 4.0.0 (R Development Core Team 2019).

Data visualization

To help interpret the investigated relationships, we assessed whether peak song frequency evolved within diverged groups of passerines by plotting the evolutionary tree of song frequency, as well as of the predictors (Fig. S1). We mapped these variables on a maximum credibility tree reconstructed from 100 trees using the function maxCladeCred in the *phangorn* package (v. 2.5.5) (Schliep 2011). Character states at internal nodes were mapped using a maximum-likelihood approach implemented in the contMap function (Revell 2013) from the *phytools* package (Revell 2012). To illustrate the geographic distribution of peak song frequency, we used the breeding range distribution of all passerines (obtained from BirdLife International and NatureServe 2018) to visualize mean peak song frequency values across passerine assemblages with grid cells of 112.5 × 112.5 km (~1° scale) (Valcu *et al.* 2012).

General modelling procedures

All comparative analyses were performed using the *phylolm* package (v. 2.6) (Tung Ho & Ané 2014). To control for non-independence due to common ancestry (Paradis 2011), we used phylogenetic generalized least-squares (PGLS) regressions with Pagel's lambda (λ) transformation of a correlation structure (Pagel 1999). This method explicitly models how the covariance between species declines as they become more distantly related. If $\lambda = 1$, modelled traits co-vary in direct proportion to shared evolutionary history, whereas $\lambda = 0$ indicates phylogenetic independence of traits (Freckleton *et al.* 2002). We randomly sampled 100 phylogenetic trees (Hackett backbone) from those available at http://birdtree.org (Jetz *et al.* 2012), which included all species in our data set. We ran all models using these 100 phylogenies to account for uncertainties associated with different tree topologies and combined model coefficients by model averaging (Symonds & Moussalli 2011). For each model, we also calculated the proportion of variance explained (R^2) according to Ives (2019) using the *rr2* package (Ives & Li 2018), including the conditional R^2 (the variance explained by fixed and random effects) and the marginal R^2 (the variance explained by the fixed effects only), and report these as mean values from 100 models each based on a different phylogenetic tree. Model residuals revealed no major violation of the assumptions of normality and homogeneity of variance. Peak song frequency and body mass were log-transformed before analysis. Peak song frequency and all predictors were also mean-centred and divided by their standard deviation (Schielzeth 2010).

Sex-specific body mass and wing length data were only available for 984 and 2,463 species, respectively. Hence, we estimated the missing values with the phylogenetic imputation method in the *Rphylopars* package (v 0.2.12) (Goolsby *et al.* 2017), using Pagel's lambda model of trait evolution. We did this separately for each of the 100 phylogenetic trees, such that each tree was associated with specific imputed values. This method performs well in predicting missing species' data (Penone *et al.* 2014) and imputed data increase the statistical power of analysis (Nakagawa & Freckleton 2008). Importantly, the bias in imputed data sets tends to be lower than the bias in data sets with missing data omitted, particularly when values for many species are missing (Penone *et al.* 2014). To minimize concerns that imputed data may affect our conclusions, we validated the robustness of our findings by performing all analyses also on the subset of species for which we have data on body mass and sexual size dimorphism.

Model specification

We specified two types of models. First, we ran a set of univariate models with peak song frequency as the dependent variable and with either body mass (species or male), sexual size dimorphism (based on wing length or body mass) or habitat density (tree cover or habitat type) as predictor. Second, we ran multivariate models, which included different sets of predictors. The first models included combinations of species body mass, wing-based sexual size dimorphism and tree cover (or habitat type), the second models included combinations of male body mass and body mass-based sexual size dimorphism as predictors. Note that the results from univariate and multivariate models, from analyses based on imputed or raw data, from analyses with species- or male-specific body mass, as well as from analyses based on tree cover or habitat type were qualitatively almost identical (Fig. S2 and Table S1). Hence, in the main text we report only findings from multivariate model containing species-specific body mass, wing-based sexual size dimorphism and tree cover with imputed missing data for body mass and sexual size dimorphism.

RESULTS

Species-specific median peak song frequency ranged from 215 Hz to 10,659 Hz (*n* = 5,085 species), but most passerine species emitted songs of intermediate frequencies (mean ± SD = 4,030 ± 1,626 Hz; median = 3,790 Hz; Fig. 1a). Median peak song frequency shows a strong evolutionary signal with a coefficient $\lambda \approx 0.87$ (see also Table S1). Nevertheless, low and high peak song frequencies occur within phylogenetically distinct groups (Fig. 1a).

Passerines sang at low frequencies predominantly in large parts of Australia, in tropical rainforests of the Neotropical, Afrotropical, and Papua New Guinea regions, and possibly in the Sahara where data coverage was sparse (Fig. 1b). Conversely, high-frequency songs characterize passerine communities in the northern parts of the Nearctic and Palearctic regions, in large mountain ranges such as the Andes and Himalayas, in southern parts of the Neotropical region, and in belts of grassland and savannah in Africa (Fig. 1b).

Body mass was the strongest predictor of global variation in peak song frequency (Fig. 2a and Fig. S2), explaining 11−16% of the variation (59−67% together with phylogeny; Table S1). As predicted from the morphological constraint hypothesis, heavier species sang at lower frequencies (Fig. 2a and Fig. S2); this pattern was observed for all but two families (*n* = 52 families with more than 15 species; Fig. 2b and Fig. S3).

Peak song frequency was also significantly associated with sexual size dimorphism (either measured in wing length or in body mass), although the effect size was substantially smaller, explaining 1−3% of the variation (Fig. 2a and Fig. S2; Table S1). As predicted based on the sexual selection hypothesis, species with a stronger male-biased sexual size dimorphism (i.e. a higher intensity of sexual selection) sang with lower frequencies, even after controlling for body mass *per se* (Fig. 2a and Fig. S2; Table S1). This effect of decreasing frequency with increasing dimorphism was seen in 67% of families (35 out of 52 families with more than 15 species) while in the remaining families the trend was in the opposite direction (Fig. 2b and S3). Note that in this analysis data on body mass were not sex-specific. Hence, adding sexual size dimorphism might improve model fit, simply because our measure of body mass and sexual size dimorphism together better reflect male size than species-specific mass alone. However, sexual dimorphism in body mass remained influential even when limiting the analysis to a subset of 984 species for which data on male body mass were available (Fig. S2).

Peak song frequency of passerines was weakly, but significantly associated with tree cover or habitat type (Fig. 2a and Fig. S2; Table S1); however, the effect explained only around 0.2% of the variation and was opposite to that predicted from the acoustic adaptation hypothesis: species living in open habitats had lower (not higher) peak song frequencies than those living in more dense, forested habitats (Fig. 2a and Fig. S2; Table S1). Moreover, this effect was observed in only 24 out of 52 families (46%) with more than 15 species (with the random expectation being 50% of the families; Fig. 2b and S3). This unexpected relationship was close to zero and not statistically significant in multivariate models that used the original, non-imputed values of body mass and sexual size dimorphism (based either on wing length or body mass; Fig. S2; Table S1).

DISCUSSION

Our data revealed remarkable variation in peak song frequency among the world's passerine birds. Our analyses show that most of the interspecific diversity in peak song frequency can be explained by evolutionary history and by body mass, with an additional effect of sexual size dimorphism as a proxy of the intensity of sexual selection. In contrast, our study does not support the acoustic adaptation hypothesis. Opposite to the prediction, we found at best a weakly positive association between habitat density and peak song frequency. Our results thus indicate that the evolution of peak song frequency in passerines is primarily controlled by morphological constraints, as expected from basic physical principles. We further show that peak song frequency may be shaped by sexual selection, but not by habitat-driven selection to maximize song transmission.

We found that after controlling for phylogeny 11−16% of interspecific variation in peak song frequency of passerines is explained by variation in body mass (Table S1). However, phylogeny also explains some of the variation in body mass (Fig. S1) and in a simple linear regression body mass explains ~27% of the variance in peak song frequency. Together, body mass and phylogeny explained almost 70% of the variation in peak song frequency (Table S1). Our results confirm that body size (estimated as body mass in our study) imposes a strong morphological limit on the production of vocalizations of certain frequencies, presumably through a strong correlation with the length of the vocal tract and the size of the labia in the syrinx (Podos 2001; Suthers & Zollinger 2008; Rodríguez *et al.* 2015). The morphological constraint hypothesis can thus be seen as a kind of “null model” (also see Pearse *et al.* 2018) and it is the remaining variation in peak song frequency that needs explanation.

After accounting for body mass, peak song frequency was lower in species where males were larger than females, i.e. in species with – presumably − stronger sexual selection on males. This result is robust to different ways of analysis (Table S1) and supports the hypothesis that sexual selection has shaped the evolution of song frequency (Greig *et al.* 2013; Hall *et al.* 2013; Geberzahn & Aubin 2014; Linhart & Fuchs 2015; Pearse *et al.* 2018). Our comparative study provides evidence that sexual selection led to low-frequency song performance in many families of passerines, presumably in those where song frequency is indicative of the competitive ability of individuals during male–male interactions (Christie *et al.* 2004; Seddon *et al.* 2004; Price *et al.* 2006). Notably, the songs that departed the most in peak frequency from the expected association with body mass − those of three related species from the Cotingidae family (the Amazonian umbrellabird *Cephalopterus ornatus*, the long-wattled umbrellabird *C. penduliger*, and the red-ruffed fruitcrow *Pyroderus scutatus*) – were also those that had the lowest peak frequencies documented for any passerine in our data set (< 260 Hz); their peak frequencies are so low that they partly overlap with the fundamental speech frequencies of humans (100−300 Hz), who are, however, more than 100 times heavier (Baken 1987). The umbrellabirds and their close relatives show high male-biased sexual size dimorphism (compared to other passerines) and a lekking mating system where males display together on traditional "exploded" leks and presumably do not provide parental care (del Hoyo *et al.* 2018). In species that produce substantially lower-frequency songs than predicted from the negative frequency−size relationship, sexual selection may have led to the development of a specific vocal apparatus to produce these sounds (Riede *et al.* 2016), such as the unique pendulous oesophageal vocal sacs that are used as a resonator in umbrellabirds (Sick 1954, see also Riede *et al.* 2015 for a non-passerine example). Although selection for low-frequency sounds may in some cases cause a corresponding change in body size (Fitch 1999), it seems more likely that natural (Woodward *et al.* 2005; Ricklefs 2010) and sexual (Björklund 1990) selection on body size underlies most evolutionary shifts in the song frequency of passerines, with an additional effect of sexual selection on the vocal apparatus.

Despite the theoretical basis and some empirical evidence for a negative association between song frequency and habitat density (Morton 1975; Badyaev & Leaf 1997; Buskirk 1997; Bertelli & Tubaro 2002; Blumstein & Turner 2005; Boncoraglio & Saino 2007), our comparative study provides clear evidence against the acoustic adaptation hypothesis. Peak song frequency across the world's passerines was, if anything, weakly positively instead of negatively correlated with habitat density. Thus, forest-inhabiting species produced sounds that were higher or similar in peak frequency than those of species living in open areas. While other unmeasured biotic and abiotic characteristics of the environment, including consistent background noise produced by wind, rain, insects or other birds, may drive the evolution of peak song frequencies (reviewed in Brumm & Zollinger 2013), we provide solid evidence that habitat density – as used and widely evaluated in bioacoustic studies – had at best a negligible effect on peak song frequency of passerines. Of course, this does not exclude singing-associated behavioural adaptations of birds that improve signal transmission, such as microhabitat selection during perch-singing or display flights (Menezes & Santos 2020). It is noteworthy that at the intraspecific level, birds can adjust their song frequency to local conditions, but these shifts are relatively minor compared to the interspecific variation in frequency we documented in this study (Slabbekoorn & Peet 2003; Slabbekoorn & den Boer-Visser 2006; Nemeth & Brumm 2010; Brumm & Zollinger 2013).

In conclusion, using data of most passerine species and half of the global avian diversity, our study provides three insights into the evolution of acoustic signals. (1) A strong allometric relationship between body size and peak song frequency imposes a clear limit on the evolution of song frequency. (2) Sexual selection seems to cause departures from this allometric relationship, leading to lower-frequency signals than predicted by body size. Further research into the mechanism (e.g. selection on the structure of the vocal apparatus) is of interest. (3) There is no evidence that species in more dense, forested habitats produce songs of lower frequencies. Our study thus challenges the idea that habitat-dependent selection to maximize sound propagation influences the evolution of signal frequency in songbirds. Future work should focus on the link between song frequency, behaviour during vocal performance (e.g. aerial displays), and habitat properties that influence sound transmission and degradation. In general, our study calls for large-scale empirical studies on acoustic signal frequency in other animal groups as independent replication studies.

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References

Apicella, C.L., Feinberg, D.R. & Marlowe, F.W. (2007). Voice pitch predicts reproductive success in male hunter-gatherers. *Biol. Lett.*, 3, 682–684.

Badyaev, A. V. & Leaf, E.S. (1997). Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* warblers. *Auk*, 114, 40–46.

Baken, R.J. (1987). *Clinical Measurement of Speech and Voice*. Taylor and Francis Ltd., London.

Baston, D. (2020). *exactextractr: Fast extraction from raster datasets using polygons*. *R Packag. version 0.2.1.* Available at: https://cran.r-project.org/package=exactextractr. Last accessed .

Bertelli, S. & Tubaro, P.L. (2002). Body mass and habitat correlates of song structure in a primitive group of birds. *Biol. J. Linn. Soc.*, 77, 423–430.

BirdLife International and NatureServe. (2018). *Bird species distribution maps of the world*. Available at: http://datazone.birdlife.org/.

Björklund, M. (1990). A phylogenetic interpretation of sexual dimorphism in body size and ornament in relation to mating system in birds. *J. Evol. Biol.*, 3, 171–183.

Blumstein, D.T. & Turner, A.C. (2005). Can the acoustic adaptation hypothesis predict the structure of Australian birdsong? *Acta Ethol.*, 8, 35–44.

Boncoraglio, G. & Saino, N. (2007). Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Funct. Ecol.*, 21, 134–142.

Bradbury, J.W. & Vehrencamp, S.L. (1998). *Principles of animal communication*. Sinauer Associates, Sunderland, MA.

Briefer, E., Vannoni, E. & McElligott, A.G. (2010). Quality prevails over identity in the sexually selected vocalisations of an ageing mammal. *BMC Biol.*, 8, 1–15.

Bro-Jørgensen, J. & Beeston, J. (2015). Multimodal signalling in an antelope: Fluctuating facemasks andknee-clicks reveal the social status of eland bulls. *Anim. Behav.*, 102, 231–239.

Brumm, H. & Goymann, W. (2017). On the natural history of duetting in White-browed Coucals: sex- and body-size-dependent differences in a collective vocal display. *J. Ornithol.*, 158, 669–678.

Brumm, H. & Naguib, M. (2009). Environmental acoustics and the evolution of bird song. *Adv. Study Behav.*, 40, 1–33.

Brumm, H. & Zollinger, S.A. (2013). Avian Vocal Production in Noise. In: *Animal Communication and Noise* (ed. Brumm, H.). Springer, Berlin, Heidelberg, pp. 187–227.

Buchhorn, M., Lesiv, M., Tsendbazar, N.-E., Herold, M., Bertels, L. & Smets, B. (2020). Copernicus Global Land Cover Layers−Collection 2. *Remote Sens.*, 12, 1044.

Buskirk, J. V. (1997). Independent evolution of song structure and note structure in American wood warblers. *Proc. R. Soc. B Biol. Sci.*, 264, 755–761.

Catchpole, C.K. (1987). Bird song, sexual selection and female choice. *Trends Ecol. Evol.*, 2, 94–97.

Catchpole, C.K. & Slater, P.J.B. (2008). *Bird song: biological themes and variations*. Cambridge University Press, Cambridge, UK.

Christie, P.J., Mennill, D.J. & Ratcliffe, L.M. (2004). Pitch shifts and song structure indicate male quality in the dawn chorus of black-capped chickadees. *Behav. Ecol. Sociobiol.*, 55, 341–348.

Dale, J., Dunn, P.O., Figuerola, J., Lislevand, T., Székely, T. & Whittingham, L.A. (2007). Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. *Proc. R. Soc. B Biol. Sci.*, 274, 2971–2979.

Davies, N.B. & Halliday, T.R. (1978). Deep croaks and fighting assessment in toads *Bufo bufo*. *Nature*, 274, 683–685.

Dunn, P.O., Whittingham, L.A. & Pitcher, T.E. (2001). Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution*, 55, 161–175.

Dunning, J.B. (2008). *CRC Handbook of Avian Body Masses*. CRC Press, Boca Raton.

Ey, E. & Fischer, J. (2009). The "acoustic adaptation hypothesis" – a review of the evidence from birds, anurans and mammals. *Bioacoustics*, 19, 21–48.

Fairbairn, D.J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.*, 28, 659–687.

Fitch, T.W. (2006). Production of vocalizations in mammals. *Vis. Commun.*, 3, 115–121.

Fitch, W.T. (1999). Acoustic exaggeration of size in birds via tracheal elongation: comparative and theoretical analyses. *J. Zool.*, 248, 31–48.

Fitch, W.T. & Hauser, M.D. (2002). Unpacking "honesty": vertebrate vocal production and the evolution of acoustic signals. In: *Acoustic communication.* (eds. Simmons, A.M., Fay, R.R. & Popper, A.N.). Springer, New York, pp. 65–137.

Forstmeier, W., Burger, C., Temnow, K. & Derégnaucourt, S. (2009). The genetic basis of zebra finch vocalizations. *Evolution*, 63, 2114–2130.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.*, 160, 712–726.

Geberzahn, N. & Aubin, T. (2014). How a songbird with a continuous singing style modulates its song when territorially challenged. *Behav. Ecol. Sociobiol.*, 68, 1–12.

Gerhardt, H.C. (1994). The evolution of vocalization in frogs and toads. *Annu. Rev. Ecol. Syst.*, 25, 293–324.

Gerhardt, H.C. & Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. 2nd ed. The University of Chicago Press, Chicago.

Gillooly, J.F. & Ophir, A.G. (2010). The energetic basis of acoustic communication. *Proc. R. Soc. B Biol. Sci.*, 277, 1325–1331.

Gonzalez-Voyer, A., den Tex, R.-J., Castelló, A. & Leonard, J.A. (2013). Evolution of acoustic and visual signals in Asian barbets. *J. Evol. Biol.*, 26, 647–659.

Goolsby, E.W., Bruggeman, J. & Ané, C. (2017). Rphylopars: fast multivariate phylogenetic comparative methods for missing data and within-species variation. *Methods Ecol. Evol.*, 8, 22–27.

Greig, E.I., Price, J.J. & Pruett-Jones, S. (2013). Song evolution in Maluridae: influences of natural and sexual selection on acoustic structure. *Emu*, 113, 270–281.

Hall, M.L., Kingma, S.A. & Peters, A. (2013). Male songbird indicates body size with low-pitched advertising songs. *PLoS One*, 8, e56717.

Hardouin, L.A., Reby, D., Bavoux, C., Burneleau, G. & Bretagnolle, V. (2007). Communication of male quality in owl hoots. *Am. Nat.*, 169, 552–562.

del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (2018). *Handbook of the birds of the world Alive*. Available at: http://www.hbw.com/.

Ives, A.R. (2019). R^2s for correlated data: phylogenetic models, LMMs, and GLMMs. *Syst. Biol.*, 68, 234–251.

Ives, A.R. & Li, D. (2018). rr2: an R package to calculate R^2s for regression models. *J. Open Source Softw.*, 3, 1028.

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K. & Mooers, A.O. (2012). The global diversity of birds in space and time. *Nature*, 491, 444–448.

Linhart, P. & Fuchs, R. (2015). Song pitch indicates body size and correlates with males' response to playback in a songbird. *Anim. Behav.*, 103, 91–98.

Mager, J.N., Walcott, C. & Piper, W.H. (2007). Male common loons, *Gavia immer*, communicate body mass and condition through dominant frequencies of territorial yodels. *Anim. Behav.*, 73, 683–690.

Marten, K. & Marler, P. (1977). Sound transmission and its significance for animal vocalization. I. Temperate habitats. *Behav. Ecol. Sociobiol.*, 2, 271–290.

Mason, N.A. & Burns, K.J. (2015). The effect of habitat and body size on the evolution of vocal displays in Thraupidae (tanagers), the largest family of songbirds. *Biol. J. Linn. Soc.*, 114, 538–551.

McClatchie, S., Alsop, J. & Coombs, R.F. (1996). A re-evaluation of relationships between fish size, acoustic frequency, and target strength. *ICES J. Mar. Sci.*, 53, 780–791.

Menezes, J.C.T. & Santos, E.S.A. (2020). Habitat structure drives the evolution of aerial displays in birds. *J. Anim. Ecol.*, 89, 482– 493.

Mikula, P., Tószögyová, A., Hořák, D., Petrusková, T., Storch, D. & Albrecht, T. (2020). Female solo song and duetting are associated with different territoriality in songbirds.

Behav. Ecol., 31, 322–329.

Morton, E.S. (1975). Ecological sources of selection on avian sounds. *Am. Nat.*, 109, 17–34.

Morton, E.S. (1977). On the occurrence and significance of motivation structural rules in some bird and mammal sounds. *Am. Nat.*, 111, 855– 869.

Nakagawa, S. & Freckleton, R.P. (2008). Missing inaction: the dangers of ignoring missing data. *Trends Ecol. Evol.*

Nemeth, E. & Brumm, H. (2010). Birds and anthropogenic noise: are urban songs adaptive? *Am. Nat.*, 176, 465–475.

Odom, K.J., Hall, M.L., Riebel, K., Omland, K.E. & Langmore, N.E. (2014). Female song is widespread and ancestral in songbirds. *Nat. Commun.*, 5, 3379.

Ophir, A.G., Schrader, S.B. & Gillooly, J.F. (2010). Energetic cost of calling: general constraints and species-specific differences. *J. Evol. Biol.*, 23, 1564–1569.

Padgham, M. (2004). Reverberation and frequency attenuation in forests – implications for acoustic communication in animals. *J. Acoust. Soc. Am.*, 115, 402–410.

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884.

Paradis, E. (2011). *Analysis of Phylogenetics and Evolution with R*. Springer, Berlin.

Pearse, W.D., Morales-Castilla, I., James, L.S., Farrell, M., Boivin, F. & Davies, T.J. (2018). Global macroevolution and macroecology of passerine song. *Evolution*, 72, 944–960.

Penone, C., Davidson, A.D., Shoemaker, K.T., Di Marco, M., Rondinini, C., Brooks, T.M., *et al.* (2014). Imputation of missing data in life-history trait datasets: which approach performs the best? *Methods Ecol. Evol.*, 5, 961–970.

Pijanowski, B.C., Villanueva-Rivera, L.J., Dumyahn, S.L., Farina, A., Krause, B.L., Napoletano, B.M., *et al.* (2011). Soundscape ecology: The science of sound in the landscape. *Bioscience*, 61, 203–216.

Podos, J. (2001). Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature*, 409, 185–188.

Price, J.J., Earnshaw, S.M. & Webster, M.S. (2006). Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Anim. Behav.*, 71, 799–807.

R Development Core Team. (2019). *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Revell, L.J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.*, 3, 217–223.

Revell, L.J. (2013). Two new graphical methods for mapping trait evolution on phylogenies. *Methods Ecol. Evol.*, 4, 754–759.

Ricklefs, R.E. (2010). Insights from comparative analyses of aging in birds and mammals. *Aging Cell*, 9, 273–284.

Riede, T., Eliason, C.M., Miller, E.H., Goller, F. & Clarke, J.A. (2016). Coos, booms, and hoots: The evolution of closed-mouth vocal behavior in birds. *Evolution*, 70, 1734–1746.

Riede, T., Forstmeier, W., Kempenaers, B. & Goller, F. (2015). The functional morphology of male courtship displays in the Pectoral Sandpiper (*Calidris melanotos*). *Auk*, 132, 65–77.

Rodríguez, R.L., Araya-Salas, M., Gray, D.A., Reichert, M.S., Symes, L.B., Wilkins, M.R., *et al.* (2015). How acoustic signals scale with individual body size: common trends across diverse taxa. *Behav. Ecol.*, 26, 168–177.

Ryan, M.J. & Brenowitz, E.A. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *Am. Nat.*, 126, 87–100.

Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evol.*, 1, 103–113.

Schliep, K.P. (2011). phangorn: phylogenetic analysis in R. *Bioinformatics*, 27, 592–593.

Searcy, W.A. & Nowicki, S. (2005). *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton University Press, Princeton.

Seddon, N., Amos, W., Mulder, R.A. & Tobias, J.A. (2004). Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. *Proc. R. Soc. B Biol. Sci.*, 271, 1823–1829.

Seneviratne, S.S., Jones, I.L. & Carr, S.M. (2012). Patterns of vocal divergence in a group of non-oscine birds (auklets; Alcidae, Charadriiformes). *Evol. Ecol. Res.*, 14, 95–112.

Sick, H. (1954). Zur Biologie des amazonischen Schirmvogels, *Cephalopterus ornatus*. *J. für Ornithol.*, 95, 233–244.

Slabbekoorn, H. & den Boer-Visser, A. (2006). Cities change the songs of birds. *Curr. Biol.*, 16, 2326–2331.

Slabbekoorn, H. & Peet, M. (2003). Birds sing at a higher pitch in urban noise. *Nature*, 424, 267.

Suthers, R.A. & Zollinger, S.A. (2008). From brain to song: the vocal organ and vocal tract. In: *Neuroscience of Birdsong* (eds. Zeigler, P.H. & Marler, P.). Cambridge University Press, Cambridge, pp. 78–98.

Symonds, M.R.E. & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav. Ecol. Sociobiol.*, 65, 13–21.

Thiagavel, J., Santana, S.E. & Ratcliffe, J.M. (2017). Body size predicts echolocation call peak frequency better than gape height in vespertilionid bats. *Sci. Rep.*, 7, 1–6.

Tietze, D.T., Martens, J., Fischer, B.S., Sun, Y.-H., Klussmann-Kolb, A. & Päckert, M. (2015). Evolution of leaf warbler songs (Aves: Phylloscopidae). *Ecol. Evol.*, 5, 781–798.

Tobias, J.A., Sheard, C., Seddon, N., Meade, A., Cotton, A.J. & Nakagawa, S. (2016). Territoriality, social bonds, and the evolution of communal signaling in birds. *Front. Ecol. Evol.*, 4, 74.

Trivers, R.L. (1972). Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871–1971* (ed. Campbell, B.). Aldine, Chicago, pp. 136–179.

Tung Ho, L. si & Ané, C. (2014). A linear-time algorithm for gaussian and non-gaussian trait evolution models. *Syst. Biol.*, 63, 397–408.

Valcu, M., Dale, J. & Kempenaers, B. (2012). rangeMapper: a platform for the study of macroecology of life-history traits. *Glob. Ecol. Biogeogr.*, 21, 945–951.

Vannoni, E. & McElligott, A.G. (2008). Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. *PLoS One*, 3, e3113.

Venables, W.N. & Ripley, B.D. (2002). *Modern Applied Statistics with S. Fourth Edition*. Springer, New York.

Wagner, W.E. (1989). Fighting, assessment, and frequency alteration in Blanchard's cricket frog. *Behav. Ecol. Sociobiol.*, 25, 429–436.

Wagner, W.E., Beckers, O.M., Tolle, A.E. & Basolo, A.L. (2012). Tradeoffs limit the evolution of male traits that are attractive to females. *Proc. R. Soc. B Biol. Sci.*, 279, 2899–2906.

Wallschläger, D. (1980). Correlation of song frequency and body weight in passerine birds. *Experientia*, 36, 412.

Wiley, H.R. & Richards, D.G. (1982). Adaptations for acoustic communication in birds: sound transmission and signal detection. In: *Acoustic Communication in Birds* (eds. Kroodsma, D.E. & Miller, E.H.). Academic Press, New York, pp. 131–181.

Wiley, R.H. (1991). Associations of song properties with habitats for territorial oscine birds of eastern North America. *Am. Nat.*, 138, 973–993.

Wiley, R.H. & Richards, D.G. (1978). Physical constraints on acoustic communication in the atmosphere: Implications for the evolution of animal vocalizations. *Behav. Ecol. Sociobiol.*, 3, 69–94.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A., *et al.* (2005). Body size in ecological networks. *Trends Ecol. Evol.*, 20, 402–409.

Figure legends

Figure 1. Distribution of peak song frequency across passerines. (a) Distribution across a maximum credibility phylogenetic tree (based on 100 trees sampled from http://birdtree.org) with colour scale reflecting variation (Kernel densities) in species median values (*n* = 5,085 species). Highlighted are 10 major groups of passerines with their representative species, scaled according to size, except for the downscaled representatives of the Tyrannida (should be ~20% larger) and the basal Oscines (should be three times larger); starting with Acanthisittia and going counterclockwise, the pictures depict *Xenicus gilviventris* (10 cm body size), *Smithornis sharpei* (17 cm), *Cephalopterus penduliger* (41 cm; example of low-frequency singer: https://www.xeno-canto.org/75792), *Campylorhamphus trochilirostris* (25 cm), *Menura novaehollandiae* (103 cm), *Paradisaea raggiana* (34 cm), *Eupetes macrocerus* (29 cm), *Cisticola chiniana* (14 cm), *Turdus migratorius* (25 cm) and *Setophaga tigrina* (13 cm; example of high-frequency singer: https://www.xeno-canto.org/182791). Illustrations reproduced by permission of Lynx Edicions. (b) Geographical distribution in peak song frequency across species assemblages (based on the species' breeding range) defined for 112.5 × 112.5 km (~1° scale) areas. Colour scale reflects variation (Kernel densities) in assembly mean peak song frequency (*n* = 10,856 points; for clearer illustration of differences, outliers were assigned a single value causing the "bumps" on both ends of the distribution).

Figure 2. Associations between peak song frequency and body mass, sexual size dimorphism (in wing length) and tree cover across passerines (*n* = 5,085 species). (a) Standardized effect sizes (dots) with their 95% confidence intervals (horizontal lines) based on a multivariate analysis with imputed missing data for body mass and sexual size dimorphism (see Material and Methods and Table S1 for details). Values represent averages from 100 multivariate models, each using a different phylogenetic tree. (b) Relationship between peak song frequency and each of the three explanatory variables. Each dot represents the median peak song frequency of a given species. Lines show the results of univariate robust linear regressions for each of the 52 families with more than 15 species. Positive slopes are indicated in dark blue, negative slopes in yellow. Note the log-scale for peak song frequency and body mass and that for clearer visualisation two lower and ten higher sexual size dimorphism points are not displayed. Robust regressions were fitted to the data with imputed missing values using the rlm function from the *MASS* package (Venables & Ripley 2002). For results of univariate models and those using the original, non-imputed data only, see Fig. S2 and S3, and Table S1.

Figure 1

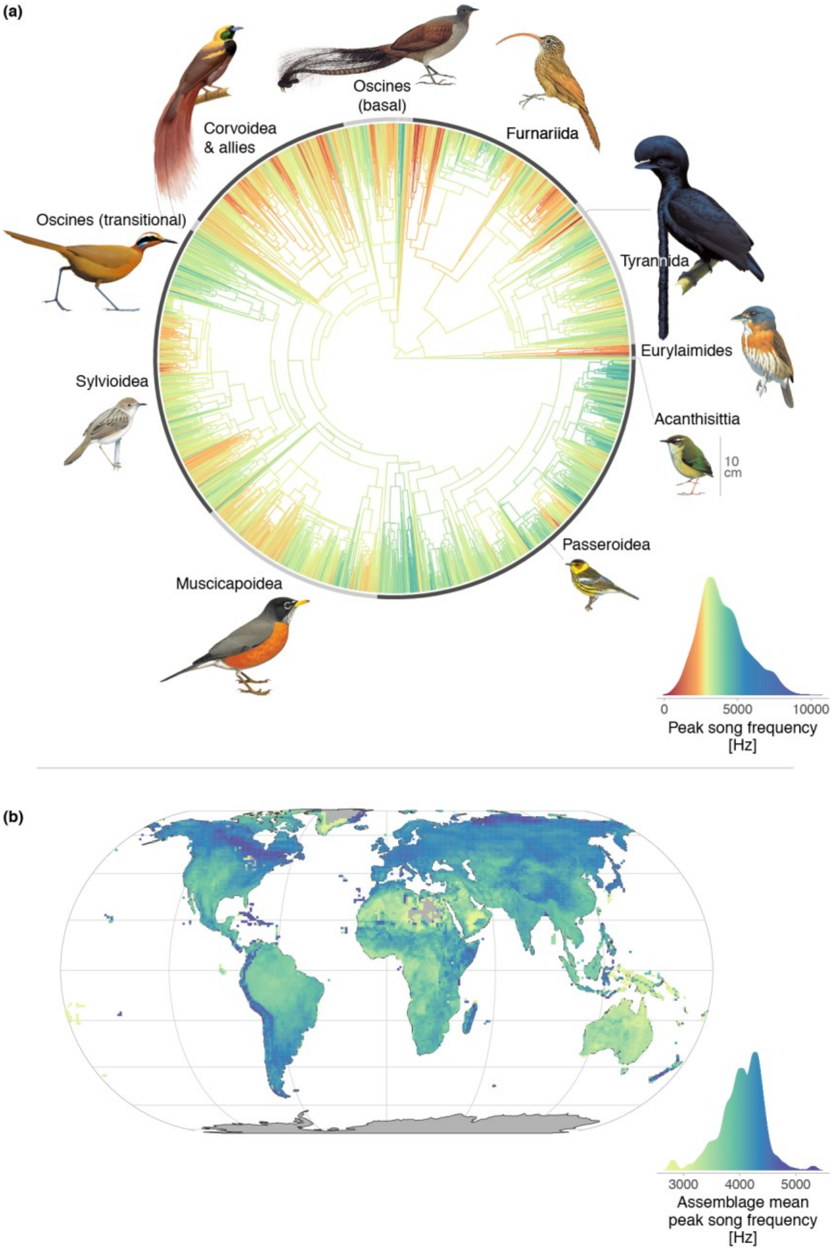
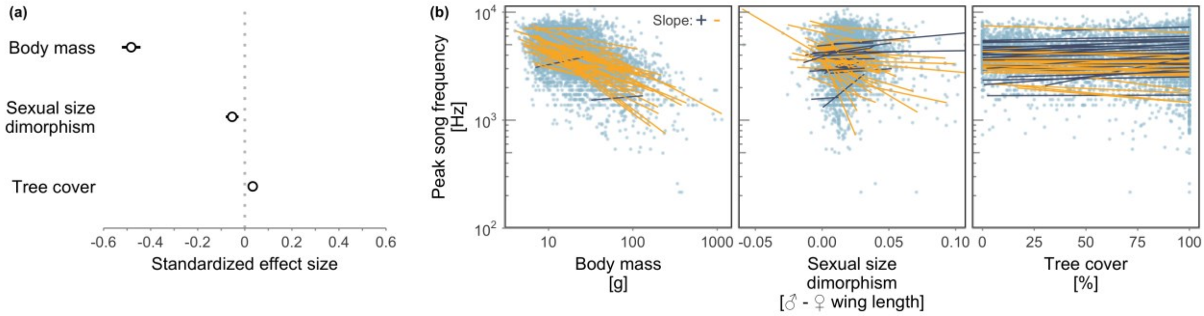

(a)
Oscines (basal)
Corvoidea & allies
Furnariida
Oscines (transitional)
Tyrannida
Eurylaimides
Sylvioidea
Acanthisittia
10 cm
Passeroidea
Muscicapoidea
0
5000
10000
Peak song frequency [Hz]
(b)
3000
4000
5000
Assemblage mean peak song frequency [Hz]

Figure 2


(a)
Body mass
Sexual size dimorphism
Tree cover
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
Standardized effect size
(b)
Peak song frequency [Hz]
10^4
10^3
10^2
Slope: + –
10
100
1000
Body mass [g]
-0.05
0.00
0.05
0.10
Sexual size dimorphism [♂ - ♀ wing length]
0
25
50
75
100
Tree cover [%]

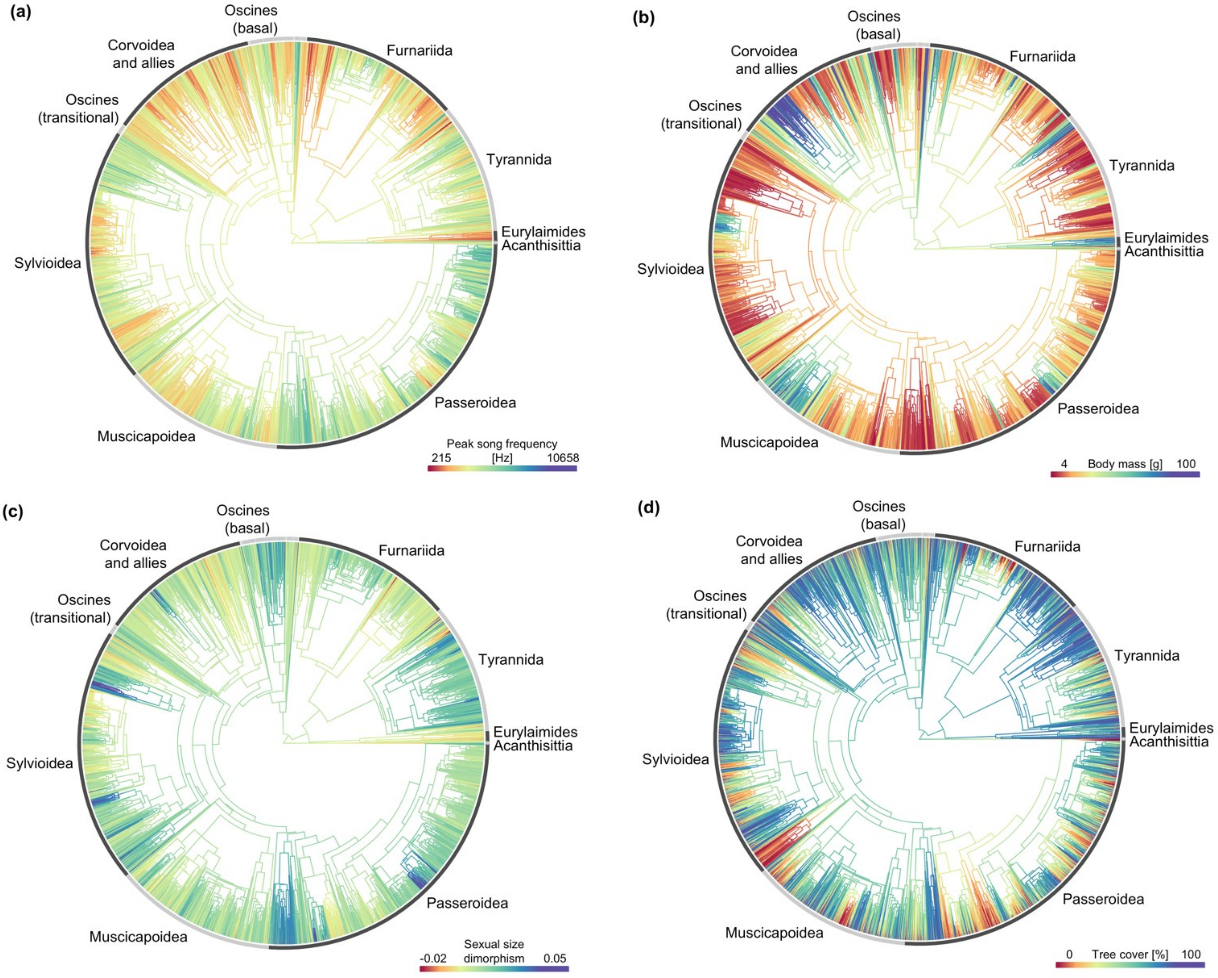


Figure S1. Distribution of peak song frequency, body mass, sexual size dimorphism (in wing length) and percentage of tree cover across passerines (*n* = 5,085 species), using maximum credibility phylogenetic tree (based on 100 trees sampled from http://birdtree.org). Missing values for body mass (*n* = 483 species) and sexual size dimorphism (*n* = 2,622 species) were imputed (see Materials and Methods). Note that for clearer illustration of differences, outliers in (b) and (c) were assigned to a single cut off value (i.e. one indicated in the colour scale)

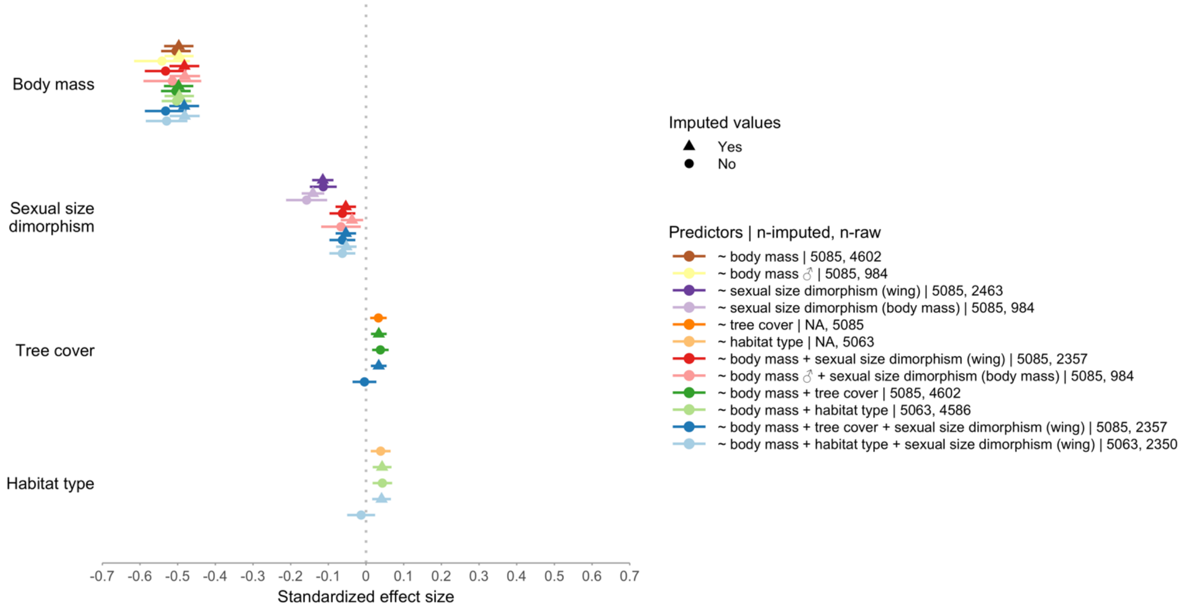


Figure S2. Association between peak song frequency and body mass (species or ♂), sexual size dimorphism (in wing length or body mass) and tree cover or habitat type across passerines. Triangle and dots depict standardized effect sizes, horizontal lines their 95% confidence intervals based on univariate and multivariate cross-species analysis with imputed missing data for body mass or sexual size dimorphism (triangles) or with original, non-imputed data only (dots; see Material and Methods). The values represent model averaged estimates from 100 models, each using a different phylogenetic tree. Full statistical results are reported in Table S1. Note, the dark blue triangle estimates are those reported in Fig. 2a in the main test.

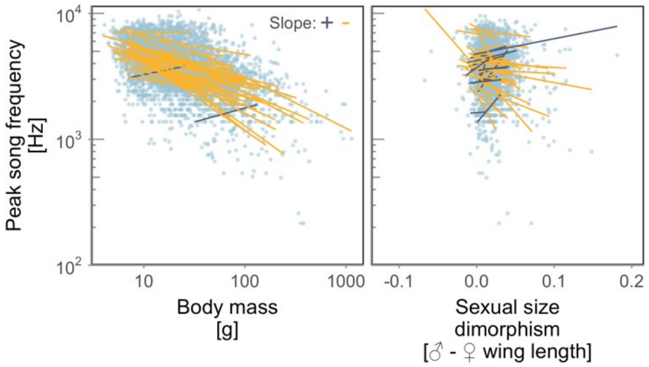


Figure S3. Relationship between peak song frequency and body mass (*n* = 4,602 species) and sexual size dimorphism (in wing length; *n* = 2,463 species) across passerines. Each dot represents the median peak song frequency of a given species. Lines show the relationship based on univariate robust linear regressions for each family with more than 15 species (52 families in case of body mass and 51 families in a case of sexual size dimorphism). Positive slopes are indicated in the dark blue, negative slopes in yellow. Note the log-scale for peak song frequency and body mass. Robust regressions were fitted to the original, non-imputed data using the rlm function from *MASS* package (Venables & Ripley 2002).

Table S1. Results from comparative analyses examining the effects of body mass, sexual size dimorphism and habitat density on peak song frequency across passerines.

Model	Imputed	*n*	Term	Est.	SE	Lower est.	Upper est.	t-value	P	P (2.5%)	P (97.5%)
Univariate											
f ~ BM	Yes	5,085	Intercept	-0.020	0.390	-0.784	0.744				
			BM	-0.497	0.020	-0.536	-0.458	-25.004	<0.0001	<0.0001	<0.0001
			Pagel's λ	0.840		0.833	0.858				
			R^2_{cond}	0.669		0.667	0.675				
			R^2_{marg}	0.105		0.103	0.111				
	No	4,602	Intercept	-0.029	0.394	-0.801	0.743				
			BM	-0.504	0.020	-0.544	-0.465	-25.070	<0.0001	<0.0001	<0.0001
			Pagel's λ	0.846		0.841	0.863				
			R^2_{cond}	0.667		0.664	0.673				
			R^2_{marg}	0.117		0.115	0.123				
f ~ BM♂	Yes	5,085	Intercept	-0.024	0.388	-0.785	0.737				
			BM	-0.496	0.020	-0.535	-0.458	-25.284	<0.0001	<0.0001	<0.0001
			Pagel's λ	0.838		0.832	0.857				
			R^2_{cond}	0.670		0.667	0.676				
			R^2_{marg}	0.107		0.105	0.113				
	No	984	Intercept	-0.130	0.321	-0.760	0.499				
			BM	-0.542	0.038	-0.616	-0.468	-14.344	<0.0001	<0.0001	<0.0001
			Pagel's λ	0.705		0.688	0.747				
			R^2_{cond}	0.590		0.587	0.601				
			R^2_{marg}	0.155		0.153	0.164				
f ~ SSD$_{WL}$	Yes	5,085	Intercept	-0.323	0.440	-1.186	0.540				
			SSD	-0.115	0.014	-0.143	-0.086	-7.951	<0.0001	<0.0001	<0.0001
			Pagel's λ	0.870		0.865	0.884				
			R^2_{cond}	0.635		0.632	0.642				
			R^2_{marg}	0.012		0.012	0.014				
	No	2,463	Intercept	-0.304	0.427	-1.140	0.532				
			SSD	-0.113	0.018	-0.149	-0.078	-6.243	<0.0001	<0.0001	<0.0001
			Pagel's λ	0.859		0.852	0.877				
			R^2_{cond}	0.605		0.602	0.615				
			R^2_{marg}	0.016		0.015	0.019				
f ~ SSD$_{BM}$	Yes	5,085	Intercept	-0.279	0.436	-1.134	0.575	-9.208	<0.0001	<0.0001	<0.0001
			SSD	-0.141	0.015	-0.171	-0.111				
			Pagel's λ	0.867		0.862	0.881				
			R^2_{cond}	0.636		0.633	0.643				
			R^2_{marg}	0.016		0.016	0.019				
	No	984	Intercept	-0.296	0.393	-1.066	0.474	-5.666	<0.0001	<0.0001	<0.0001
			SSD	-0.158	0.028	-0.212	-0.103				
			Pagel's λ	0.789		0.775	0.817				
			R^2_{cond}	0.530		0.526	0.542				
			R^2_{marg}	0.030		0.028	0.035				
f ~ TC	No	5,085	Intercept	-0.283	0.447	-1.160	0.594				
			TC	0.033	0.011	0.011	0.055	2.940	0.0048	0.0016	0.0165
			Pagel's λ	0.875		0.869	0.888				
			R^2_{cond}	0.631		0.628	0.638				
			R^2_{marg}	0.002		0.001	0.003				
f ~ HT	No	5,063	Intercept	-0.277	0.448	-1.154	0.600				
			HT	0.039	0.013	0.013	0.065	2.891	0.0054	0.002	0.02
			Pagel's λ	0.875		0.869	0.888				
			R^2_{cond}	0.631		0.628	0.638				
			R^2_{marg}	0.002		0.001	0.002				
Multivariate											
f ~ BM + SSD$_{WL}$	Yes	5,085	Intercept	-0.048	0.388	-0.809	0.713				
			BM	-0.482	0.020	-0.522	-0.443	-23.856	<0.0001	<0.0001	<0.0001
			SSD	-0.054	0.014	-0.081	-0.027	-3.873	0.0002	<0.0001	0.0009
			Pagel's λ	0.838		0.832	0.857				
			R^2_{cond}	0.670		0.668	0.676				
			R^2_{marg}	0.108		0.106	0.113				
	No	2,357	Intercept	-0.065	0.366	-0.783	0.652				
			BM	-0.532	0.028	-0.587	-0.477	-18.892	<0.0001	<0.0001	<0.0001
			SSD	-0.063	0.018	-0.097	-0.028	-3.573	0.0006	0.0002	0.0023
			Pagel's λ	0.814		0.806	0.842				
			R^2_{cond}	0.649		0.646	0.657				
			R^2_{marg}	0.138		0.136	0.145				

Table S1 continued

Model	Imputed	*n*	Term	Est.	SE	Lower est.	Upper est.	t-value	P	P (2.5%)	P (97.5%)
$f \sim BM$♂ $+ SSD_{BM}$	Yes	5,085	Intercept	-0.032	0.387	-0.789	0.726				
			BM	-0.481	0.021	-0.521	-0.441	-23.385	<0.0001	<0.0001	<0.0001
			SSD	-0.037	0.015	-0.067	-0.008	-2.463	0.0159	0.0090	0.0368
			Pagel's λ	0.837		0.830	0.855				
			R^2_{cond}	0.670		0.668	0.676				
			R^2_{marg}	0.108		0.106	0.114				
	No	984	Intercept	-0.146	0.317	-0.768	0.476				
			BM	-0.514	0.039	-0.591	-0.437	-13.136	<0.0001	<0.0001	<0.0001
			SSD	-0.067	0.027	-0.119	-0.014	-2.482	0.0138	0.0106	0.0225
			Pagel's λ	0.698		0.681	0.741				
			R^2_{cond}	0.592		0.589	0.603				
			R^2_{marg}	0.160		0.157	0.168				
$f \sim BM + TC$	Yes	5,085	Intercept	-0.025	0.391	-0.790	0.741				
			BM	-0.497	0.020	-0.536	-0.459	-25.030	<0.0001	<0.0001	<0.0001
			TC	0.034	0.011	0.013	0.055	3.186	0.0021	0.0008	0.0064
			Pagel's λ	0.841		0.835	0.859				
			R^2_{cond}	0.670		0.667	0.676				
			R^2_{marg}	0.107		0.105	0.113				
	No	4,602	Intercept	-0.035	0.395	-0.810	0.740				
			BM	-0.505	0.020	-0.544	-0.465	-25.089	<0.0001	<0.0001	<0.0001
			TC	0.038	0.011	0.016	0.060	3.411	0.0009	0.0003	0.0031
			Pagel's λ	0.848		0.843	0.865				
			R^2_{cond}	0.667		0.665	0.674				
			R^2_{marg}	0.119		0.117	0.126				
$f \sim BM + HT$	Yes	5,063	Intercept	-0.023	0.392	-0.790	0.745				
			BM	-0.496	0.020	-0.535	-0.457	-24.913	<0.0001	<0.0001	<0.0001
			HT	0.043	0.013	0.018	0.068	3.362	0.0012	0.0004	0.004
			Pagel's λ	0.842		0.836	0.861				
			R^2_{cond}	0.670		0.667	0.676				
			R^2_{marg}	0.107		0.104	0.113				
	No	4,586	Intercept	-0,033	0,396	-0,809	0,742				
			BM	-0,503	0,020	-0,542	-0,463	-24.984	<0.0001	<0.0001	<0.0001
			HT	0,043	0,013	0,018	0,069	3.284	0.0015	0.0006	0.0047
			Pagel's λ	0,849		0,843	0,866				
			R^2_{cond}	0,668		0,665	0,675				
			R^2_{marg}	0,118		0,116	0,125				
$f \sim BM + SSD_{WL} + TC$	Yes	5,085	Intercept	-0.053	0.389	-0.816	0.709				
			BM	-0.483	0.020	-0.522	-0.443	-23.884	<0.0001	<0.0001	<0.0001
			SSD	-0.054	0.014	-0.081	-0.026	-3.859	0.0002	<0.0001	0.001
			TC	0.034	0.011	0.013	0.055	3.170	0.0022	0.0009	0.0067
			Pagel's λ	0.840		0.834	0.858				
			R^2_{cond}	0.671		0.668	0.677				
			R^2_{marg}	0.110		0.107	0.115				
	No	2,357	Intercept	-0.064	0.366	-0.781	0.654				
			BM	-0.532	0.028	-0.587	-0.477	-18.882	<0.0001	<0.0001	<0.0001
			SSD	-0.063	0.018	-0.097	-0.028	-3.576	0.0006	0.0002	0.0022
			TC	-0.004	0.016	-0.036	0.027	-0.258	0.783	0.6914	0.9863
			Pagel's λ	0.813		0.806	0.842				
			R^2_{cond}	0.649		0.646	0.657				
			R^2_{marg}	0.138		0.136	0.145				
$f \sim BM + SSD_{WL} + HT$	Yes	5,063	Intercept	-0.051	0.390	-0.815	0.714				
			BM	-0.481	0.020	-0.521	-0.442	-23.786	<0.0001	<0.0001	<0.0001
			SSD	-0.052	0.014	-0.080	-0.025	-3.767	0.0003	<0.0001	0.0013
			HT	0.041	0.013	0.016	0.066	3.258	0.0016	0.0005	0.0055
			Pagel's λ	0.841		0.835	0.860				
			R^2_{cond}	0.671		0.668	0.677				
			R^2_{marg}	0.109		0.107	0.115				
	No	2,350	Intercept	-0.060	0.366	-0.778	0.658				
			BM	-0.529	0.028	-0.585	-0.474	-18.799	<0.0001	<0.0001	<0.0001
			SSD	-0.063	0.018	-0.097	-0.028	-3.574	0.0006	0.0002	0.0021
			HT	-0.013	0.019	-0.05	0.024	-0.687	0.5011	0.4095	0.7631
			Pagel's λ	0.814		0.806	0.842				
			R^2_{cond}	0.649		0.646	0.657				
			R^2_{marg}	0.138		0.136	0.144				

Shown are standardized effect sizes, with their SEs, 95% confidence intervals and test statistics based on univariate and multivariate cross-species analyses. Values represent averages from 100 multivariate models, each using a different phylogenetic tree. For each model we also report whether we used imputed or original values (for body mass and sexual size dimorphism), sample size, lambda values and conditional and marginal R^2. The abbreviations represent: *f* = Peak song frequency (log-transformed), BM = Body mass (log transformed), BM♂ = Male body mass (log transformed), SSD_{WL} = Sexual size dimorphism (log-transformed wing length of male − log-transformed wing length of female), SSD_{BM} = Sexual size dimorphism (log-transformed body mass of male – log-transformed body mass of female), TC = Tree cover (%) and HT =

habitat type (0 = open, 1 = mixed, 2 = closed; used as continuous variable). All variables, including peak song frequency, were z-transformed (mean-centred and divided by standard deviation).

References

Venables, W.N. & Ripley, B.D. (2002). *Modern Applied Statistics with S. Fourth Edition*. Springer, New York.

CHAPTER 4

Behavioral
Ecology

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Original Article

Female solo song and duetting are associated with different territoriality in songbirds

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Recently, there has been an increased effort to unravel selective factors behind female song evolution in songbirds. Female birds which produce songs may sing either solo or in duets; although the 2 vocal performances likely evolved through different selection forces and mechanisms, the majority of large-scale studies to date have focused only on duetting or female song in general (pooling female solo song and duetting into a single category). Hence, here we estimate the effect of behavioral life-history traits (territoriality, social bonds, and cooperative breeding) and environmental productivity on the occurrence of female solo song and duetting separately in songbirds of South Africa and Lesotho. The focal region is characterized by subtropical/tropical climate, clear spatial environmental productivity gradient, and detailed knowledge on avian species distribution and behavioral life-history traits. Phylogenetically informed comparative analyses revealed that species where females produce only solo songs exhibited higher levels of territoriality than species with nonsinging females (in an univariable model) but, simultaneously, lower levels than duetting species. Although both species with female solo song and duetting establish mainly long-term social bonds, the former defend their territories seasonally while the latter exhibit mainly year-round territoriality. Cooperative breeding and environmental productivity were not associated with the distribution of female solo song and duetting in any model. Our results indicate that when exploring female song ecology and evolution, female solo song and duetting are likely to be distinct song categories associated with different levels of territoriality.

Lay Summary: Female birds produce songs either solo or in duets. Although large-scale relationships between duetting and social or environmental factors have been studied intensively, such associations remain largely unexplored for female solo song. We found that species with female solo song are less territorial than duetting species and both groups are simultaneously more territorial than species where female song is absent. Hence, female solo song and duetting may represent distinct song categories associated with different levels of territoriality.

Key words: **behavioral life-history traits, macroecology, normalized difference vegetation index, phylogenetic comparative analyses.**

INTRODUCTION

The complex song of songbirds (Passeriformes: Passeri) has long been attributed to males only, with the assumption that it evolved through sexual selection via male–male competition and female choice (Darwin 1871; Catchpole 1987). Present opinion is that female song is ancestral to songbirds and that it is phylogenetically widespread and occurs in species inhabiting a range of climatic zones (Garamszegi et al. 2007; Odom et al. 2014). In general, females may sing either independently of males, that is, emitting solo songs, or in duets, that is, coordinated with the mate. For species in which both sexes sing, the prevailing view is that females sing for similar reasons as males (but the distribution of functions of female song could to be more variable than in males) (Langmore 1998; Hall 2004). In addition to intrapair contact (de Silva et al. 2004) and coordination of breeding activities (Gilbert and Carroll 1999), female singing may also be associated with mate attraction, mate guarding, and defense of territories (Langmore 1998; Hall 2004, 2009; Cain and Langmore 2015; Krieg and Getty 2016; Tobias et al. 2016). Although female solo song and duetting can

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share some similar functions, the 2 types of performances are not necessarily equivalent (Langmore 1998; Hall 2009; Odom et al. 2015; Tobias et al. 2016). Moreover, although duets are rare in temperate zone birds, female solo song occurs more often there than duetting (Garamszegi et al. 2007; Tobias et al. 2016). Differences in function and geographical distribution between these 2 types of vocal performance indicate that they likely evolved through different selection forces and mechanisms (Odom et al. 2015).

Previous studies have shown an association between the distribution of female song in general (involving both duetting and female solo song) and behavioral life-history traits such as the long-term territoriality (Robinson 1948; Benedict 2008), occurrence of social monogamy (Price 2009; Odom et al. 2015; but see Benedict 2008), absence of migration (Price 2009; Logue and Hall 2014; Odom et al. 2015), and convergent sex-roles (Slater and Mann 2004). A global synthesis on social and environmental factors behind the evolution of duetting revealed that duetting was, out of many factors evaluated, most strongly linked to year-round territoriality and stable social bonds (Tobias et al. 2016). Nevertheless, it is difficult to make a deeper insight into female solo song evolution from these studies, because results in female song studies might be largely driven by the inclusion of duetting species. Evolutionary and ecological factors driving the evolution of female solo song remain largely unexplored and large-scale studies on female solo song are, according to the best of our best knowledge, still missing. For instance, it is unclear which selective factors favor the evolution of female solo song and duetting with respect to each other.

Global variation in avian behavioral traits, as, for instance, territoriality (Maher and Lott 2000), breeding systems (Jetz and Rubenstein 2011), and migration (Alerstam et al. 2003), is largely determined by environmental conditions including those presumably associated with the distribution of female song evolution. Species with females that sing are predicted to inhabit regions with high-resource availability and little seasonal change. Such birds are expected to reside and breed over the prolonged season (Ricklefs 1969; Price et al. 2009) and experience increased pressure from both intra- and interspecific intruders (Ballance et al. 1997; Justino et al. 2012). In fact, predictable environments rich in food resources seem to favor birds exhibiting more sedentary lifestyles and longer-term partnerships, thus driving the evolution of singing in female birds (Logue and Hall 2014; Odom et al. 2015; Tobias et al. 2016). Indeed, species with females that sing have been found to be particularly common among highly productive tropical regions characterized by low levels of environmental seasonality (Morton 1996; Slater and Mann 2004; Price et al. 2009; Odom et al. 2014, 2015; Tobias et al. 2016).

In this study, we employed phylogenetic comparative analyses to explore interspecific variability in female solo song and duetting in association with important intrinsic (behavioral) and extrinsic (environmental productivity across species distribution range) traits in songbirds to determine whether both vocal performances are associated with the same or different selective factors. First, we explored variability in duetting, because a substantial body of theoretical and empirical work regarding female song ecology and evolution focuses on duetting, and predict that females that sing in duets belong mainly to species with year-round territoriality, establishing long-term social bonds as well as inhabiting more productive areas (Tobias et al. 2016). However, female solo song seems to be relatively more common than duetting in temperate zone species (Garamszegi et al. 2007; Odom et al. 2015) which exhibit different behavioral traits and face more variable environment; hence, we predict associations of female solo song with decreased level of territoriality, shorter-term social bonds, and lower environmental productivity compared with duetting species. We tested these hypotheses on a sample of songbirds breeding in sub-Saharan Africa that is rarely studied in regard to avian female song. We specifically focused on South Africa and Lesotho, a region occupied by more than 350 songbird species of 39 families (Lepage 2018). The knowledge on species distribution, life-histories, and environmental conditions is exceptionally detailed there (see METHODS), making the region very well suited for our research purposes. From a global perspective, South African songbirds typically exhibit slow ("tropical") pace of life (Jetz et al. 2008), but on regional scale these traits tend to vary across species, and species in the region significantly differ in production of female song (Hockey et al. 2005). Furthermore, although the climate is generally subtropical to tropical, key environmental conditions in the region show a clear longitudinal trend (driven by a longitudinal precipitation gradient), from dry and unproductive desert in the west to moist and productive woodland in the east (Hořák et al. 2015).

METHODS

Data collection

Species distribution data

Comprehensive data on the distribution of songbird species across South Africa and Lesotho were obtained from the Southern African Bird Atlas Project (SABAP) (Harrison et al. 1997), which surveyed all birds living in this region between 1987 and 1992. Each set of songbird species recorded within a fine ~25 × 25 km grid cell (0.25° spatial resolution) was considered as a local assemblage (the region covered by 1,858 grid cells/assemblages in total).

Female song data collection

Song was defined as primary long-range complex acoustic vocalization used by birds mainly during the breeding season. In accordance with this definition, information on the presence or absence of female song in South African songbirds was primarily obtained from data compiled for global assessments, with further relevant data taken from other literature sources (see below for further details). Species producing only simple call-like vocalization (e.g., some corvids) were omitted from the analyses because the lack of song in both sexes might be because of different selection pressures than in species with male song only. Similarly, species for which relevant information on vocalization was lacking were omitted from further analyses.

Duetting was defined as coordinated or alternated long-range vocalization that is performed communally by two bird individuals (usually the members of a mated pair) (Tobias et al. 2016). This working definition harbors duets as generally understood, but excludes simple vocalizations such as different types of calls. Following suggestions by Logue and Hall (2014) and Tobias et al. (2016), duetting species were pooled together with chorusing species (involving 3 or more individuals), because both types of signalizations are similar in structure and function (Logue and Hall 2014). It is thus often difficult to distinguish between them (e.g., many duetting species were observed chorusing and vice versa) and, moreover, duets and choruses are thought to have evolved under similar selection pressures (e.g., Seddon and Tobias 2003; Tobias et al. 2016).

Because information that females of particular species sing only solo songs is rarely explicitly stated in literature, we have to extract such information by focusing on indirect evidence. Firstly, we prepared a female song dataset where we combined data on presence/

absence of female song in general provided by Webb et al. (2016), then Hockey et al. (2005), which directly focus on birds of South Africa and Lesotho, and finally continuously updated online edition of "Handbook of the Birds of the World Alive" (del Hoyo et al. 2015). In general, female song was scored as present when females were reported as singing or where species accounts stated that song is persistent in both sexes. Female song was scored as absent when production of song was reported by males only, or when female produced only simple calls. Finally, scores on female solo song distribution among species were obtained by combination of data included in female song and duetting data set. We considered that a given species only has a female solo song if female song was scored as present for that species in the female song data set and simultaneously the exhaustive update on duetting distribution across world birds by Tobias et al. (2016) stated that females of this species do not sing in duets or choruses.

After excluding temperate zone migrants (13 species) and species lacking information on all traits (nine species), of the 278 songbird species for which we had breeding distributional data, we were able to obtain information on presence or absence of duetting for 269 species (out of these 52 species produce duets) and presence or absence of female song for 163 species (out of these 30 species produce female solo song).

Behavioral life-history traits

We collected data on several key behavioral life-history traits hypothesized to play a role in the evolution of song production in female songbirds, including the level of territoriality, stability of social bonds, and cooperative breeding (Logue and Hall 2014; Najar and Benedict 2015; Odom et al. 2015; Tobias et al. 2016). Information on these traits for species breeding in South Africa and Lesotho were obtained from recent large-data compilations by Tobias et al. (2016) (territoriality and social bond type) and Dale et al. (2015) (cooperative breeding). Tobias et al. (2016) also scored data according to the level of quality (i.e., uncertainty of classification assignment). The majority of data on territoriality and social bond for birds of South Africa were of high quality; we thus decided to omit quality scores from the analysis. Because definitions and categories in some traits change from source to source, we provide definitions as used in data-source studies.

Territoriality. Species were scored following definitions provided by Tobias et al (2016) as follows: 1) nonterritorial that do not defend territories, or defend only very small areas around nest sites, or species where males defend song or display posts only; 2) seasonally or weakly territorial, having home ranges that largely overlap, or that usually join mixed flocks with poorly defined spatial ranges; and 3) year-round territorial that defend territories all year (for further details see Tobias et al. 2016).

Social bonds. Species were scored as 1) solitary if they do not form pairs, or form them only for a short time during the courtship period, 2) having short-term pair/group bonds if they establish only seasonal partnership and change it in subsequent breeding attempts (low partner fidelity and >50% divorce rate per year), and 3) having long-term pair/group bonds if pair/group members establish year-round partnership or their seasonal pair/group lasts toward subsequent breeding attempts (high partner fidelity and <50% divorce rate per year) (for further details see Tobias et al. 2016).

Cooperative breeding. Species were classified in the following way: 1) noncooperative; species with no more than 2 adult birds caring for the offspring, and 2) cooperative; species with more than 2 birds taking care for the offspring (for further details see Dale et al. 2015).

Environmental productivity

We obtained the "normalized difference vegetation index" (hereafter NDVI), an estimate of environmental productivity based on the spectral properties of vegetation, for each grid cell from the data set provided by the U.S. Geological Survey (http://iridl.ldeo.columbia.edu/SOURCES/.USGS/.ADDS). Maximum NDVI values (hereafter $\mathrm{NDVI}_{\mathrm{max}}$), represented by the average NDVI value of the month with the highest NDVI (available for 1981–2008), were chosen as a proxy of environmental productivity. As the NDVI describes the "greenness" of vegetation and is closely correlated with rainfall, total green biomass, and net primary productivity (Goward and Dye 1987; Chong et al. 1993; Paruelo et al. 1997; Schmidt and Karnieli 2002), it is expected to reflect food availability to songbirds. This assumption is supported by the findings of Lassau and Hochuli (2008) and Lafage et al. (2014, who found a positive correlation between the amount of food available (e.g., arthropod abundance) in the breeding season and NDVI. For subsequent statistical analyses concerning individual species, we calculated the average values of $\mathrm{NDVI}_{\mathrm{max}}$ across all cells occupied by given species. We used $\mathrm{NDVI}_{\mathrm{max}}$ because we expected that the analyzed traits could play an important role during a breeding period, typically a period of the year when birds can potentially obtain the maximum amount of food resources from the environment.

Phylogenetic tree construction

A phylogenetic tree of South African songbirds was constructed using the avian phylogenetic tool available at http://birdtree.org/ (Jetz et al. 2012). The maximum credibility tree was built from 1000 randomly generated trees based on a Hackett backbone (Hackett et al. 2008). Moreover, for subsequent analyses, we also used 100 randomly selected phylogenetic trees. The maximum clade credibility tree was then determined using the TreeAnnotator tool v. 1.8.2 in the BEAST software package v. 1.8.2 (Drummond and Rambaut 2007).

Statistical analyses

As phylogenetically related taxa have a higher probability of sharing characteristics from a common ancestor, phylogenetic relatedness of species could affect presence of duetting and female solo song. Therefore, we modeled associations between variables, including nonindependence in species data, using phylogenetic generalized linear mixed-effect models (PGLMM) (Ives and Helmus 2011). We produced models containing all behavioral life-history traits and environmental productivity as explanatory variables, and with duetting and female solo song as response variables. Duetting, female solo song, and cooperative breeding were used as binary (presence/absence) variables, whereas environmental productivity was used as a continuous variable. We decided to use territoriality and social bonds as continuous rather than categorical variables in the analyses because there is a gradient in the levels of these variables, which means that the cutoff criteria for their categorization into distinct categories were somewhat arbitrary. Moreover, models with continuous variables are less sensitive to available sample size and the symmetry of the observations for each category. It should be noted, however, that inclusion of territoriality and social bonds as categorical explanatory variables did not change the overall conclusions (see below).

We performed four comparisons contrasting: 1) duetting species and nonduetting species (with either nonsinging or solo singing females), 2) duetting species and species with nonsinging females, 3) species with females producing solo song and duetting species, and 4) species with females producing solo song and species with nonsinging females. First, we conducted a multivariable model and subsequently univariable models for each explanatory variable separately (for univariable models, see Supplementary Table S1). We used species-level phylogeny; to enhance the informative value of results, we used 2 ways how to deal with phylogenetic uncertainty. In one case, we performed a model with single maximum credibility phylogenetic tree, and in the second case, we used 100 randomly selected phylogenetic trees and combined the outputs as the average values of the posterior outcomes (Supplementary Table S2). These 2 approaches were applied for both multivariable and univariable models, respectively, and showed the same results; hence, in the main text, we report only models using single maximum credibility phylogenetic tree. However, because every species was represented by only one sample in our data set, the random effect of the species could potentially be confounded with the residuals (Hadfield 2010a). To avoid this obstacle and test the robustness of these results, we also performed the same models with different parameterization using family-level phylogeny (Supplementary Tables S3–S5); importantly, both approaches gave identical results.

We performed PGLMM by Bayesian inference using the Markov chain Monte Carlo technique (MCMCglmm; Hadfield 2010b). This approach allowed us to control for phylogenetic co-variation among species by including phylogenetic distance into the model as a random variable (Hadfield and Nakagawa 2010). We used the inverse-gamma prior for random effects and an uninformative prior for the residual variance in all MCMCglmms. The choice of priors was determined by the character of our data, sometimes containing a small number of samples for some variable categories. All models were run for 1,000,000 iterations with elimination of the first 20,000 iterations as a burn-in period and thinning to every 100th iteration. All binary variables were centered by subtracting their mean and the numeric variables were centered and standardized on the same scale (range 1) to improve interpretability. For models with a maximum credibility phylogenetic tree, we used the Gelman–Rubin statistic to check convergence of multiple MCMC chains runs in parallel. This compares within-chain variance with between-chain variance and calculates the potential scale reduction factor (Gelman and Rubin 1992). The iteration chains mixed well, exhibiting no observable autocorrelation. The Gelman–Rubin statistic threshold for models with a maximum credibility phylogenetic tree was < 1.05 in all models. Posterior fixed effect distribution was examined for overlap with zero (as the significance test), using 95% highest posterior density as a credible interval. We also calculated phylogenetic signal as an estimate of the proportion of variance in duetting and female solo song after conditioning on explanatory variables explained by the effect of phylogenetic relatedness. All data were processed and statistically analyzed using R 3.3.1 (R Development Team 2013).

RESULTS

We found that in the first 2 comparisons, that is, duetting species versus nonduetting species, and duetting species versus species with nonsinging females, the strongest predictors of duetting distribution were the level of territoriality and length of social bonds. However, it was not possible to test the effect of social bonds in these contrasts as it was not possible to estimate the posteriors for this parameter. This was because all species singing in duets fall into one category of social bonds (long-term). This, however, indicates that the association of duetting song with the long-term social bonds is indeed strong. Univariable models lead to the same results (Figure 1, Table 1, see also Supplementary Tables S1 and S2). Species where females sing in duets maintain longer-term territoriality and social bonds when compared with relatively shorter-term territoriality and social bonds in species where females produce no duets and no female song, respectively. However, when contrasting species with females singing solo songs and duetting species, the only statistically significant association in both multivariable and univariable models was with the level of territoriality such that the level of territoriality increases toward duetting species (Figure 1, Table 1, see also Supplementary Tables S1 and S2). Finally, when contrasting species with female solo song and species with nonsinging females, no significant predictor was identified in multivariable models. However, univariable models revealed association with the level of territoriality; species wherein females produce solo songs exhibited higher level of territoriality than species with no female song (Figure 1, Table 1, see also Supplementary Tables S1 and S2). In all contrasts, cooperative breeding and the environmental variable, NDVI_{max}, did not explain the presence of any singing characteristic.

To test robustness of these results, we also prepared models where territoriality and social bonds were coded as categorical variables (results are summarized in Supplementary Tables S6 and S7). Duetting species exhibited significantly more often year-round territoriality than seasonal or weak territoriality when compared with nonduetting species and species with no female song. Furthermore, species with solo singing females were significantly more often seasonally territorial when compared with mainly year-round territorial duetting species but exhibited lower proportion of weak territoriality than species with no female song.

The above results are nicely supported by spatial geographical patterns. There was a clear spatial pattern in duetting distribution across the study region (Figure 2a, b), where regions with a high proportion of duetting songbirds strongly overlapped spatially with regions with high proportion of year-round territorial species (Supplementary Figure S1). At the same time, the association between the proportion of duetting and environment (NDVI_{max}) was less clear; however, higher proportion of duetting species was partially recorded in humid and highly productive areas (Supplementary Figure S1), including savannahs, woodlands, and forests in eastern and north-eastern South Africa, as well as areas along the Indian Ocean coastline. A low proportion (occasionally approaching zero) of duetting species was detected in central, higher elevated grasslands, and in Succulent Karoo, Nama Karoo, and Kalahari and Namib Deserts in western South Africa. When contrasting species with the presence of female solo song against species with the presence of duetting, the proportion of female solo song was highest in central parts of South Africa (Figure 2c), copying geographical trends in the distribution of seasonal territoriality; these areas were also characterized by less productive environments (Supplementary Figure S1). Finally, when contrasting species with the presence of female solo song against species without female song, areas with the high proportion of species with female solo song geographically coincide with areas with the high proportion of year-round territorial species, similarly to the first 2 previous contrasts (duetting vs. non-duetting and duetting vs. no female song; Figure 2d, Supplementary Figure S1).

DISCUSSION

We found high variation among South African songbirds with regard to the presence of female singing. It is worth noting that

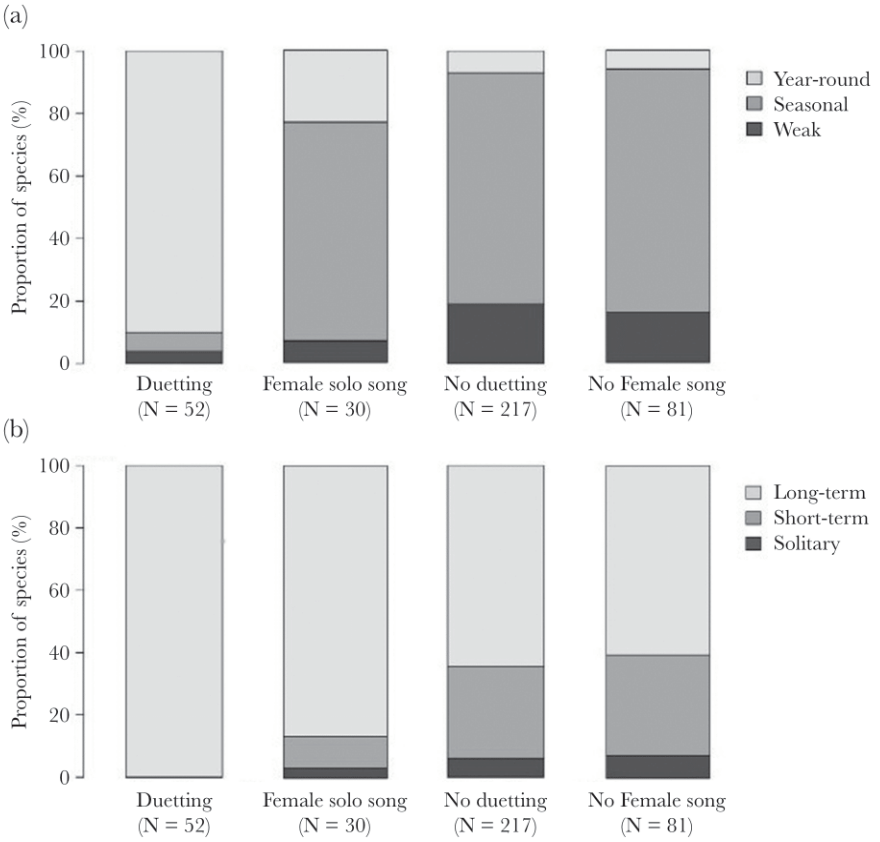

Figure 1
Associations between female song categories (duetting, female solo song, no duetting, and no female song) and the level of (a) territoriality (weak, seasonal, year-round) and (b) social bonds (solitary, short-term, long-term). Each barplot represents different female song category and the height of differently colored bars within each category refers to the proportion of species associated with different levels of territoriality and social bonds. The number *N* represents the number of species in each group.

species involved in our study exhibit slow pace of life from a global perspective (Jetz et al. 2008), and inhabit subtropical to tropical environments, where singing females are often reported (Tobias et al. 2016). Although both duetting species and species with female solo song establish mainly long-term social bonds, female solo song–duetting comparison revealed a negative association between presence of female solo song and territoriality. Subsequent analysis found that duetting species exhibited mainly the year-round territoriality while female solo song predominated in species that are territorial only seasonally although the proportion of year-round territorial species was still relatively high (>20%) in latter group. Species wherein females do not produce song exhibited on average lower level of territoriality than species with solo singing (in univariable model only) and duetting females. Interestingly, neither in multivariable models nor in univariable models cooperative breeding and environmental productivity was associated with the distribution of duetting and female solo song. Hence, level of territoriality rather than other social and environmental factors is the immediate driver affecting the distribution of female song performances in our sample of subtropical to tropical birds.

We found that female solo song emerged mainly in species exhibiting seasonal territoriality while duetting species were characterized by the presence of year-round territoriality. This indicates that the 2 vocal performances probably evolved in association with different selection pressures or different levels of the selection factors (Langmore 1998). Price (2015) suggested that sexual dimorphism in singing is often the result of losses in females rather than gains in males. Then, instead of being an evolutionary precursor of duetting, presence of female solo song may also emerge in bird lineages which expanded to areas where year-round territoriality is costly, for example, from tropical to temperate zones. However, further studies are needed to focus in greater detail on factors and large-scale patterns of female solo song in birds. Either way, despite the fact that duetting and female solo song are often clumped together under the “female song” category in multitaxonomic studies (e.g., Najar and Benedict 2015; Webb et al. 2016), our results suggest that the 2 vocal performances should be considered as independent composites of female song (also see Odom et al. 2015).

Although it is difficult to disentangle effects of territoriality and social bonds on duetting, it seems that the presence of duetting is more closely associated with presence of year-round territoriality than presence of long-term social bonds (though this was not explicitly tested; also see Tobias et al. 2016). We argue that although the presence of duetting is slightly better predicted by long-term social bonds than year-round territoriality, absence of duetting was almost always linked to the loss of year-round territoriality while majority of nonduetting species still establish long-term social bonds. For instance, duets were completely lacking in some of species-rich families inhabiting South Africa such as old-world buntings (Emberizidae), finches (Fringillidae), swallows and martins (Hirundinidae), and pipits and wagtails (Motacillidae) with no

Table 1

Results of multivariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with species-level phylogeny (the maximum credibility phylogenetic tree) as a random effect for (a) duetting (vs. no duetting, i.e., no female song and female solo song) ($N = 269$ species), (b) duetting (vs. no female song) ($N = 133$ species), (c) female solo song (vs. duetting) ($N = 82$ species), (d) female solo song (vs. no female song) ($N = 111$ species) as response variables and life-history traits (territoriality, social bonds, and cooperative breeding) and an environmental variable ($\mathrm{NDVI}_{\max}$) as explanatory variables in songbirds of the South Africa and Lesotho

Predictor	Posterior mean	95% CI	Lambda	pMCMC
(a)				
Territoriality	455.42	231.16–701.29	0.645	**<0.001**
Social bonds[a]	—	—		—
Cooperative breeding	31.09	−111.77–173.11		0.653
$\mathrm{NDVI}_{\max}$	120.66	−154.84–402.10		0.376
(b)				
Territoriality	412.89	203.70–625.62	0.815	**<0.001**
Social bonds[a]	—	—	—	—
Cooperative breeding	58.35	−102.72–211.62		0.458
$\mathrm{NDVI}_{\max}$	225.80	−45.11–506.46		0.087
(c)				
Territoriality	−273.22	−517.45–−56.75	0.997	**0.006**
Social bonds	−59.93	−211.85–109.18		0.439
Cooperative breeding	−28.33	−266.44–204.10		0.821
$\mathrm{NDVI}_{\max}$	−42.06	−427.07–398.03		0.823
(d)				
Territoriality	186.50	−63.04–435.89	0.755	0.110
Social bonds	126.81	−131.47–405.33		0.321
Cooperative breeding	84.92	−236.30–390.08		0.550
$\mathrm{NDVI}_{\max}$	310.73	−186.44–881.82		0.212

Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda), and pMCMC values are reported. Statistically significant results are highlighted by bold.

[a] Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable.

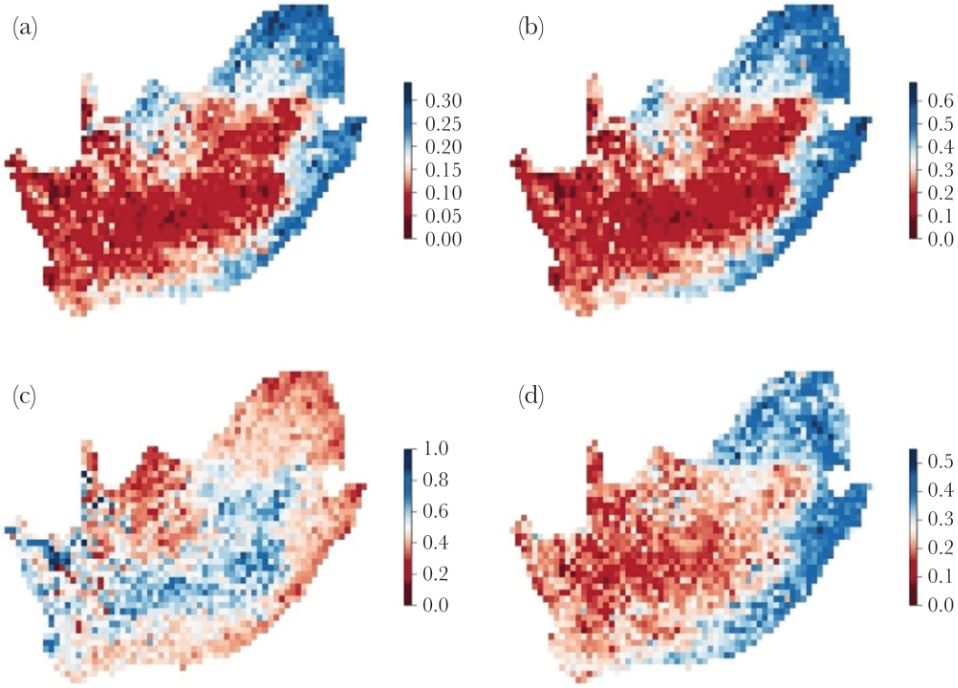

Figure 2

Geographical patterns in the proportion of species having (a) duets vs. no duets ($N = 269$ species), (b) duets vs. no female song ($N = 133$ species), (c) female solo song vs. duetting ($N = 82$ species), and (d) female solo song vs. no female song ($N = 111$ species).

year-round territorial species, whereas all batises and wattle-eyes (Platysteiridae) together with bush-shrikes (Malaconotidae) defend their territories year-round and also sing in duets. This close duetting–year-round territoriality association suggests that, similarly to findings of Tobias et al. (2016) on a global scale, variation in duetting among songbirds of South Africa could be explained by uneven taxonomic and geographical distribution of year-round territoriality rather than by other factors.

We found no direct association between distribution of any of female song composites and environmental productivity. Although South Africa represents a relatively small area in terms of a global perspective, the local productivity shows a clear and strong gradient with huge regional differences in $NDVI_{max}$; hence, the lack of this association should not be attributed to only low variation in the environmental variable evaluated. However, social behavior of birds has inevitably evolved in accordance with environmental conditions. For instance, distribution of year-round territoriality coincides to some level with the distribution of highly productive areas in South Africa (Supplementary Figure S1). Similarly, an inverse association between NDVI and clutch sizes indicates that South African songbirds inhabiting more productive areas exhibit slower pace of life (Hořák et al. 2015). In productive and relatively stable environments, species can reside and breed over the prolonged season as they are not forced to migrate, promoting sedentary lifestyles and affecting also birds' willingness to defend their territories over long periods (Ricklefs 1969; Price et al. 2009; Odom et al. 2015; Tobias et al. 2016). Under such conditions, source and mate defense may become too demanding, thus promoting singing in both partners (Tobias et al. 2016). Taken from the opposite side, these premises indicate that species inhabiting less productive and more variable environment are expected to exhibit lower levels of territoriality, favoring the presence of female solo song or no song in female birds over duetting. It is also possible that some other unmeasured environmental factor, or more probably a combination of several of them, forms specific conditions facilitating the emergence of duetting. Altogether, our results indicate that the strength of selection favoring year-round territoriality may be spatially variable also within tropical and subtropical regions, such as over the territory of South Africa.

Both female solo song and duetting are associated with higher levels of territoriality and long-term social bonds, suggesting that they may have evolved as byproduct of cooperative breeding (Seddon and Tobias 2003). However, we did not find any effect of cooperative breeding neither on female solo song nor on duetting. This may indicate that, at least in our sample of species, the evolution of singing in females and cooperative breeding is independent. Other possibility is that the absence of such association in South African species could be linked to low variability in this trait and presence of cooperative breeding only in few avian clades in the area.

In conclusion, our study finds some evidence that species of South Africa and Lesotho wherein female birds produce solo song exhibited, on average, a lower level of territoriality than duetting species but, simultaneously, a higher level of territoriality than species with nonsinging females. Hence, we suggest that female solo song and duetting may represent independent composites of female song whose evolution is driven by different levels of the territoriality. We propose that the actual value of individual territory, and consequently a bird's willingness to defend these territories, is the primary driver of the evolution of female solo song and duetting in South African songbirds. Although our study focuses on a restricted region and limited set of species, our results may have implications for furthering understanding of bird song evolution in general, and female solo song in particular. For instance, it may help us to explain why female solo song is more common than duetting in nontropical (e.g., temperate zone) songbird species.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Conflict of interest: The authors declare no competing interests.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Mikula et al. (2019).

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REFERENCES

Alerstam TA, Hedenström A, Åkesson S. 2003. Long-distance migration: evolution and determinants. Oikos. 103:247–260.

Ballance LT, Pitman RL, Reilly SB. 1997. Seabird community structure along a productivity gradient: importance of competition and energetic constraint. Ecology. 78:1502–1518.

Benedict L. 2008. Occurrence and life history correlates of vocal duetting in North American passerines. J Avian Biol. 39:57–65.

Cain KE, Langmore NE. 2015. Female and male song rates across breeding stage: testing for sexual and nonsexual functions of female song. Anim Behav. 109:65–71.

Catchpole CK. 1987. Bird song, sexual selection and female choice. Trends Ecol Evol. 2:94–97.

Chong DLS, Mougin E, Gastellu-Etchegorry JP. 1993. Relating the global vegetation index to net primary productivity and actual evapotranspiration over Africa. Int J Remote Sens. 14:1517–1546.

Dale J, Dey CJ, Delhey K, Kempenaers B, Valcu M. 2015. The effects of life history and sexual selection on male and female plumage colouration. Nature. 527:367–370.

Darwin C. 1871. The descent of man. London: J. Murray.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol. 7:214.

Garamszegi LZ, Pavlova DZ, Eens M, Møller AP. 2007. The evolution of song in female birds in Europe. Behav Ecol. 18:86–96.

Gelman A, Rubin DB. 1992. Inference from iterative simulation using multiple sequences. Statist Sci. 7:457–472.

Gilbert WM, Carroll AF. 1999. Singing in a mated female Wilson's warbler. Wilson Bull. 111:134–137.

Goward SN, Dye DG. 1987. Evaluating North American net primary productivity with satellite observations. Adv Space Res. 7:165–174.

Hackett SJ, Kimball RT, Reddy S, Bowie RC, Braun EL, Braun MJ, Chojnowski JL, Cox WA, Han KL, Harshman J, et al. 2008. A phylogenomic study of birds reveals their evolutionary history. Science. 320:1763–1768.

Hadfield JD. 2010a. MCMCglmm course notes. Available at http://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Hadfield JD. 2010b. MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. J Stat Softw. 33:1–22.

Hadfield JD, Nakagawa S. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. J Evol Biol. 23:494–508.

Hall ML. 2004. A review of hypotheses for the functions of avian duetting. Behav Ecol Sociobiol. 55:415–430.

Hall ML. 2009. A review of vocal duetting in birds. Adv Stud Behav. 40:67–121.

Harrison JA, Allan DG, Underhill LG, Herremans M, Tree AJ, Parker V, Brown CJ. 1997. The atlas of southern African birds. Vol. 2: passerines. Johannesburg, South Africa: BirdLife South Africa.

Hockey PAR, Dean WRJ, Ryan PG. 2005. Roberts – birds of southern Africa. VIIth ed. Cape Town, South Africa: The Trustees of the John Voelcker Bird Book Fund.

Hořák D, Tószögyová A, Storch D. 2015. Relative food limitation drives geographical clutch size variation in South African passerines: a large-scale test of Ashmole's seasonality hypothesis. Glob Ecol Biogeogr. 24:437–447.

del Hoyo J, Elliott A, Sargatal J, Christie DA, de Juana E. 2015. Handbook of the birds of the world alive. Barcelona, Spain: Lynx Edicions. Available at http://www.hbw.com/.

Ives AR, Helmus MR. 2011. Generalized linear mixed models for phylogenetic analyses of community structure. Ecol Monogr. 81:511–525.

Jetz W, Rubenstein DR. 2011. Environmental uncertainty and the global biogeography of cooperative breeding in birds. Curr Biol. 21:72–78.

Jetz W, Sekercioglu CH, Böhning-Gaese K. 2008. The worldwide variation in avian clutch size across species and space. PLoS Biol. 6:e303.

Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. 2012. The global diversity of birds in space and time. Nature. 491:444–448.

Justino DG, Maruyama PK, Oliveira PE. 2012. Floral resource availability and hummingbird territorial behaviour on a Neotropical savanna shrub. J Ornithol. 153:189–197.

Krieg CA, Getty T. 2016. Not just for males: females use song against male and female rivals in a temperate zone songbird. Anim Behav. 113:39–47.

Lafage D, Secondi J, Georges A, Bouzillé J-B, Pétillon J. 2014. Satellite-derived vegetation indices as surrogate of species richness and abundance of ground beetles in temperate floodplains. Insect Conserv Diver. 7:327–333.

Langmore NE. 1998. Functions of duet and solo songs of female birds. Trends Ecol Evol. 13:136–140.

Lassau SA, Hochuli DF. 2008. Testing predictions of beetle community patterns derived empirically using remote sensing. Divers Distrib. 14:138–147.

Lepage D. 2018. Avibase: bird checklists of the world – South Africa. Available at http://avibase.bsc-eoc.org/checklist.jsp?region=za&list=howardmoore. Accessed July 12, 2018.

Logue DM, Hall ML. 2014. Migration and the evolution of duetting in songbirds. Proc Biol Sci. 281:20140103.

Maher CR, Lott DF. 2000. A review of ecological determinants of territoriality within vertebrate species. Am Midl Nat. 143:1–29.

Mikula P, Tószögyová A, Hořák D, Petrusková T, Storch D, Albrecht T. 2019. Female solo song and duetting are associated with different territoriality in songbirds. Dryad Digital Repository. doi:10.5061/dryad.fn2z34tpr

Morton ES. 1996. A comparison of vocal behavior among tropical and temperate passerine birds. In: Kroodsma DE, Miller EH, editors. Ecology and evolution of acoustic communication in birds. New York: Cornell University Press. p. 258–268.

Najar N, Benedict L. 2015. Female song in New World wood warblers. Front Ecol Evol. 3:139.

Odom KJ, Hall ML, Riebel K, Omland KE, Langmore NE. 2014. Female song is widespread and ancestral in songbirds. Nat Commun. 5:3379.

Odom KJ, Omland KE, Price JJ. 2015. Differentiating the evolution of female song and male-female duets in the New World blackbirds: can tropical natural history traits explain duet evolution? Evolution. 69:839–847.

Paruelo JM, Epstein HE, Lauenroth WK, Burke IC. 1997. ANPP estimates from NDVI for the central grassland region of the United States. Ecology. 78:953–958.

Price JJ. 2009. Evolution and behavioral life-history correlates of female song in the New World blackbirds. Behav Ecol. 20:967–977.

Price JJ. 2015. Rethinking our assumptions about the evolution of bird song and other sexually dimorphic signals. Fron Ecol Evol. 3:40.

Price JJ, Lanyon SM, Omland KE. 2009. Losses of female song with changes from tropical to temperate breeding in the New World blackbirds. Proc Biol Sci. 276:1971–1980.

R Development Core Team. 2013. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ricklefs RE. 1969. The nesting cycle of songbirds in tropical and temperate regions. Living Bird. 8:165–175.

Robinson A. 1948. The biological significance of bird song in Australia. EMU. 48:291–315.

Schmidt H, Karnieli A. 2002. Analysis of the temporal and spatial vegetation patterns in a semi-arid environment observed by NOAA AVHRR imagery and spectral ground measurements. Int J Remote Sens. 23:3971–3990.

Seddon N, Tobias JA. 2003. Communal singing in the cooperatively breeding subdesert mesite *Monias benschi*: evidence of numerical assessment? J Avian Biol. 34:72–80.

de Silva HG, Marantz CA, Perez-Villafana M. 2004. Song in female *Hylorchilus* wrens. Wilson Bull. 116:186–188.

Slater PJ, Mann NI. 2004. Why do the females of many bird species sing in the tropics?. J Avian Biol. 35:289–294.

Tobias JA, Sheard C, Seddon N, Meade A, Cotton AJ, Nakagawa S. 2016. Territoriality, social bonds, and the evolution of communal signaling in birds. Front Ecol Evol. 4:74.

Webb WH, Brunton DH, Aguirre D, Thomas D, Valcu M, Dale J. 2016. Female song occurs in songbirds with more elaborate female coloration and reduced sexual dichromatism. Front Ecol Evol. 4:22.

Supplementary Information

Table S1. Results of univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with species-level phylogeny (the maximum credibility phylogenetic tree) as a random effect for (a) duetting (vs. no duetting, i.e. no female song and female solo song) (N = 269 species), (b) duetting (vs. no female song) (N = 133 species), (c) female solo song (vs. duetting) (N = 82 species), (d) female solo song (vs. no female song) (N = 111 species) as a response variables and life-history traits (territoriality, social bonds and cooperative breeding) and an environmental variable ($NDVI_{max}$) as explanatory variables in songbirds of the South Africa and Lesotho. Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda) and pMCMC values are reported. Statistically significant results are highlighted by bold.

Predictor	**Posterior mean**	**95% CI**	**lambda**	**pMCMC**
(a)				
Territoriality	402.20	158.82–629.07	0.758	**<0.0001**
Social bonds*	–	–	–	–
Cooperative breeding	55.94	-49.79–169.80	0.923	0.274
$NDVI_{max}$	134.80	-62.80–348.86	0.998	0.163
(b)				
Territoriality	376.73	157.43–589.84	0.960	**<0.0001**
Social bonds*	–	–	–	–
Cooperative breeding	72.56	-55.10–210.89	0.874	0.248
$NDVI_{max}$	205.04	-10.19–456.26	0.996	0.052
(c)				
Territoriality	-277.74	-511.67–-67.84	0.908	**0.001**
Social bonds	-112.00	-262.40–38.40	0.994	0.139
Cooperative breeding	-52.09	-287.19–166.76	0.953	0.622
$NDVI_{max}$	-40.77	-423.25–317.69	0.999	0.803
(d)				
Territoriality	223.27	27.98–437.88	0.705	**0.014**
Social bonds	195.89	-5.25–464.46	0.724	0.054
Cooperative breeding	76.82	-215.08–379.71	0.727	0.572
$NDVI_{max}$	377.74	-74.13–940.43	0.653	0.097

*Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable

Table S2. Results of multivariable and univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with species-level phylogeny (100 randomly selected phylogenetic trees, their combined output is reported) as a random effect for (a) duetting (vs. no duetting, i.e. no female song and female solo song) (N = 269 species), (b) duetting (vs. no female song) (N = 133 species), (c) female solo song (vs. duetting) (N = 82 species), (d) female solo song (vs. no female song) (N = 111 species) as a response variables and life-history traits (territoriality, social bonds and cooperative breeding) and an environmental variable ($\mathrm{NDVI}_{\mathrm{max}}$) as explanatory variables in songbirds of the South Africa and Lesotho. Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda) and pMCMC values are reported. Statistically significant results are highlighted by bold.

Predictor	**Posterior mean**		**95% CI**		**lambda**		**pMCMC**	
	Multivariable	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**
(a)								
Territoriality	458.23	291.86	226.26–703.37	81.46–490.81	0.632	0.715	**<0.0001**	**<0.0001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	28.83	40.39	-112.10–172.36	-56.71–148.51		0.899	0.673	0.396
$\mathrm{NDVI}_{\mathrm{max}}$	125.87	114.70	-143.26–410.58	-56.98–315.19		0.963	0.353	0.170
(b)								
Territoriality	410.65	374.25	207.88–619.87	156.89–590.14	0.977	0.878	**<0.0001**	**<0.0001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	56.57	65.12	-100.25–214.44	-63.53–205.52		0.945	0.464	0.312
$\mathrm{NDVI}_{\mathrm{max}}$	226.83	197.90	-37.67–509.71	-19.60–443.86		0.975	0.085	0.062
(c)								
Territoriality	-270.83	-275.74	-509.34–-51.98	-502.53–-63.85	0.987	0.796	**0.006**	**0.002**
Social bonds	-60.19	-111.43	-217.29–101.76	-260.12–37.77		0.933	0.443	0.141
Cooperative breeding	-31.20	-52.05	-270.60–198.06	-281.49–166.54		0.937	0.787	0.629
$\mathrm{NDVI}_{\mathrm{max}}$	-37.44	-47.42	-451.20–377.33	-447.29–344.14		0.946	0.827	0.799
(d)								

Territoriality	188.06	221.40	-46.24–440.05	29.51–432.89	0.786	0.754	0.104	**0.012**
Social bonds	123.18	198.24	-130.56–401.81	-20.18–450.40		0.667	0.335	0.055
Cooperative breeding	89.73	78.36	-220.76–402.16	-207.90–372.86		0.702	0.535	0.558
NDVI$_{max}$	300.60	367.48	-205.54–856.41	-95.32–905.34		0.695	0.224	0.104

*Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable

Tables S3-S5. Every species in our dataset was represented by a single sample within which variation was not considered. A potential consequence of this approach is that the random effect of the species could potentially be confounded with the residuals (Hadfield 2010). To avoid this obstacle and test the robustness of these results, we also performed the same models with different parameterization using family-level phylogeny. We used an inverse-gamma prior for random effects and an uninformative prior for residual variance in all MCMCglmms. The choice of priors was determined by the character of our data, sometimes containing a small number of samples for some variable categories. All models were run for 5,000,000 iterations with elimination of the first 20,000 iterations as a burn-in period and thinning to every 5000th iteration. For models with a maximum credibility phylogenetic tree, we used the Gelman-Rubin statistic to check for convergence of multiple MCMC chains runs in parallel. The iteration chains mixed well, exhibiting no observable autocorrelation. The Gelman-Rubin statistic threshold was established to 1.09 in all models.

References

Hadfield JD. 2010. MCMCglmm course notes. Available at http://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Table S3. Results of multivariable and univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with family-level phylogeny (the maximum credibility phylogenetic tree) as a random effect for (a) duetting (vs. no duetting, i.e. no female song and female solo song) (N = 269 species), (b) duetting (vs. no female song) (N = 133 species), (c) female solo song (vs. duetting) (N = 82 species), (d) female solo song (vs. no female song) (N = 111 species) as a response variables and life-history traits (territoriality, social bonds and cooperative breeding) and an environmental variable (NDVI_{max}) as explanatory variables in songbirds of the South Africa and Lesotho. Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda) and pMCMC values are reported. Statistically significant results are highlighted by bold.

Predictor	**Posterior mean**		**95% CI**		**lambda**		**pMCMC**	
	Multivariable	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**
(a)								
Territoriality	463.38	408.14	238.33–729.25	137.11–667.40	0.360	0.589	**<0.001**	**<0.001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	14.47	34.94	-117.85–156.52	-55.17–140.45		0.801	0.833	0.454
NDVI_{max}	110.13	100.40	-144.32–387.75	-67.86–298.04		0.832	0.396	0.217
(b)								
Territoriality	412.47	363.87	230.18–650.76	118.46–588.66	0.570	0.710	**<0.001**	**<0.001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	48.64	71.45	-101.60–205.34	-33.16–212.95		0.769	0.496	0.169
NDVI_{max}	185.68	136.17	-45.45–462.91	-50.49–367.89		0.896	0.114	0.116
(c)								
Territoriality	-314.39	-282.32	-563.23–-102.21	-510.77–-83.53	0.671	0.765	**<0.001**	**<0.001**
Social bonds	-61.48	-116.96	-236.47–101.23	-257.39–33.31		0.823	0.488	0.122
Cooperative breeding	-4.62	-12.90	-250.54–206.77	-207.40–180.00		0.826	0.980	0.898
NDVI_{max}	-18.70	-64.42	-511.52–366.85	-478.10–249.50		0.845	0.918	0.671
(d)								
Territoriality	237.13	237.17	7.60–507.24	34.21–465.40	0.762	0.742	0.060	**0.008**

Social bonds	114.25	185.70	-127.98–447.22	-37.02–430.91		0.479	0.416	0.086
Cooperative breeding	170.98	135.89	-139.99–477.11	-137.48–405.90		0.607	0.219	0.267
NDVI$_{max}$	210.48	272.18	-259.87–775.81	-147.38–776.89		0.527	0.446	0.233

*Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable

Table S4. Results of multivariable and univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with family-level phylogeny (100 randomly selected phylogenetic trees, their combined output is reported) as a random effect for (a) duetting (vs. no duetting, i.e. no female song and female solo song) (N = 269 species), (b) duetting (vs. no female song) (N = 133 species), (c) female solo song (vs. duetting) (N = 82 species), (d) female solo song (vs. no female song) (N = 111 species) as a response variables and life-history traits (territoriality, social bonds and cooperative breeding) and an environmental variable ($\mathrm{NDVI}_{\mathrm{max}}$) as explanatory variables in songbirds of the South Africa and Lesotho. Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda) and pMCMC values are reported. Statistically significant results are highlighted by bold.

Predictor	**Posterior mean**		**95% CI**		**lambda**		**pMCMC**	
	Multivariable	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**
(a)								
Territoriality	448.48	308.93	211.35–685.02	96.45–515.31	0.467	0.555	**<0.001**	**<0.001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	12.58	35.90	-119.44–146.57	-54.23–138.02		0.841	0.837	0.419
$\mathrm{NDVI}_{\mathrm{max}}$	113.38	99.16	-132.39–377.81	-65.14–296.38		0.843	0.368	0.221
(b)								
Territoriality	411.00	358.71	199.02–622.64	119.76–587.41	0.397	0.701	**<0.001**	**<0.001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	48.25	70.70	-95.71–197.02	-36.84–196.87		0.820	0.500	0.179
$\mathrm{NDVI}_{\mathrm{max}}$	189.20	133.84	-55.22–456.34	-47.86–352.20		0.837	0.118	0.129
(c)								
Territoriality	-314.50	-278.92	-556.02–-89.87	-503.43–-62.32	0.706	0.701	**0.002**	**<0.001**
Social bonds	-60.85	-116.15	-229.96–109.36	-269.15–32.94		0.850	0.476	0.131
Cooperative breeding	-5.49	-7.36	-235.85–221.95	-205.32–183.05		0.853	0.968	0.949
$\mathrm{NDVI}_{\mathrm{max}}$	-20.65	-67.94	-427.01–389.75	-414.72–253.03		0.850	0.827	0.656
(d)								

Territoriality	231.73	231.69	-16.98–496.42	28.70–452.63	0.706	0.758	0.062	**0.010**
Social bonds	113.71	181.72	-150.49–403.11	-30.73–439.26		0.601	0.404	0.074
Cooperative breeding	173.23	139.81	-125.23–477.43	-115.86–417.28		0.623	0.240	0.257
$NDVI_{max}$	194.76	275.07	-325.32–751.95	-166.64–787.60		0.596	0.453	0.206

*Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable

Table S5. The autocorrelation values among stored samples (Lag 5000) from the posterior of multivariable and univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with family-level phylogeny (the maximum credibility phylogenetic tree) as a random effect. The level of the independence of the samples in the posterior was very high, which means that the iteration chains mixed well. The values of autocorrelation refer to the following models: (a) duetting vs. no duetting (i.e. no female song and female solo song), (b) duetting vs. no female song, (c) female solo song vs. duetting, (d) female solo song vs. no female song.

Model	**Territoriality**	**Social bonds**	**Cooperative breeding**	**NDVI$_{max}$**	**Family**	**Units**
Multivariable						
(a)	-0.0051	–	-0.0062	0.0151	-0.0434	-0.0381
(b)	-0.0252	–	0.0473	-0.0029	0.0068	-0.0407
(c)	-0.0565	-0.0235	-0.0160	0.0308	-0.0046	-0.0103
(d)	0.0119	-0.0030	-0.0501	-0.0053	0.0975	0.0071
Univariable						
(a)	-0.0244	–	–	–	0.0579	-0.0367
(a)	–	–	0.0231	–	0.0208	0.0392
(a)	–	–	–	-0.0226	0.0204	-0.0355
(b)	-0.0007	–	–	–	-0.0054	0.0271
(b)	–	–	-0.0004	–	0.0439	0.0281
(b)	–	–	–	0.0455	-0.0406	0.0596
(c)	-0.0262	–	–	–	-0.0007	-0.0035
(c)	–	-0.0588	–	–	-0.0299	-0.0331
(c)	–	–	-0.0006	–	0.0094	-0.0001
(c)	–	–	–	-0.0164	0.0067	0.0054
(d)	-0.0561	–	–	–	-0.0056	-0.0323
(d)	–	0.0266	–	–	-0.0118	0.0476
(d)	–	–	0.0027	–	0.0552	-0.0421
(d)	–	–	–	0.0142	0.0027	0.0039

Tables S6-S7. In addition to models where territoriality, social bonds and cooperative were coded as continuous variables, we prepared a set of models where these variables were coded as categorical variables. We performed these models using both species- and family level phylogeny. We used an inverse-gamma prior for random effects and an uninformative prior for residual variance in all MCMCglmms. The choice of priors was determined by the character of our data, sometimes containing a small number of samples for some variable categories. All models were run for 5,000,000 iterations with elimination of the first 20,000 iterations as a burn-in period and thinning to every 5000th iteration. We used a maximum credibility phylogenetic tree and the Gelman-Rubin statistic to check for convergence of multiple MCMC chains runs in parallel. The iteration chains mixed well, exhibiting no observable autocorrelation. The Gelman-Rubin statistic threshold was established to 1.09 in all models.

Table S6. Results of multivariable and univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with species-level phylogeny (the maximum credibility phylogenetic tree) as a random effect for (a) duetting (vs. no duetting, i.e. no female song and female solo song) (N = 269 species), (b) duetting (vs. no female song) (N = 133 species), (c) female solo song (vs. duetting) (N = 82 species), (d) female solo song (vs. no female song) (N = 111 species) as a response variables and life-history traits (territoriality, social bonds and cooperative breeding) and an environmental variable (NDVI$_{max}$) as explanatory variables in songbirds of the South Africa and Lesotho. In contrast to model results reported in Table 1 and Table S1–S5 where territoriality, social bonds and cooperative breeding were treated as continuous variables, here, these variables were coded as categorical predictors. Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda) and pMCMC values are reported. Statistically significant results are highlighted by bold.

Predictor	**Posterior mean**		**95% CI**		**lambda**		**pMCMC**	
	Multivariable	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**
(a)								
Territoriality (seasonal)	-543.94	-495.72	-829.61–-266.11	-750.70–-222.68	0.478	0.520	**<0.001**	**<0.001**
Territoriality (weak)	-484.12	-442.66	-778.30–-216.51	-701.41–-168.25			**<0.001**	**<0.001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	23.27	47.42	-122.91–158.28	-42.51–152.87		0.873	0.743	0.308
NDVI$_{max}$	108.76	134.80	-151.79–423.57	-62.80–348.86		0.998	0.390	0.163
(b)								
Territoriality (seasonal)	-503.85	-459.35	-709.04–-258.84	-706.13–-220.39	0.427	0.667	**<0.001**	**<0.001**
Territoriality (weak)	-433.40	-414.69	-662.13–-199.02	-688.84–-196.43			**<0.001**	**<0.001**
Social bonds*	–	–	–	–	–	–	–	–
Cooperative breeding	44.12	60.69	-126.83–199.02	-53.34–191.26		0.877	0.567	0.290
NDVI$_{max}$	249.50	205.04	-48.04–510.83	-10.19–456.26		0.996	0.078	0.052
(c)								
Territoriality (seasonal)	473.97	418.56	187.33–754.17	174.04–683.88	0.757	0.732	**0.002**	**<0.001**
Territoriality (weak)	123.12	231.42	-257.74–559.20	-56.02–568.40			0.543	0.129
Social bonds (short)	-414.51	-115.30	-1242.35–396.92	-831.40–554.80		0.867	0.327	0.769
Social bonds (long)	-555.22	-349.80	-1288.72–172.76	-938.40–183.80			0.118	0.257

Cooperative breeding	15.05	-38.06	-200.68–243.80	-242.16–149.41		0.873	0.851	0.673
NDVI_{max}	124.89	-40.77	-358.91–590.10	-423.25–317.69		0.999	0.569	0.803
(d)								
Territoriality (seasonal)	-166.05	-208.83	-510.35–147.59	-505.01–48.61	0.686	0.728	0.290	0.118
Territoriality (weak)	-590.36	-618.66	-1270.55–83.49	-1259.45–-85.99			0.065	**0.010**
Social bonds (short)	-276.37	208.84	-1104.90–507.99	-335.52–945.22		0.658	0.443	0.500
Social bonds (long)	-85.52	381.09	-873.69–625.25	-140.52–1033.26			0.814	0.141
Cooperative breeding	106.84	147.12	-262.64–455.41	-130.23–419.45		0.689	0.518	0.259
NDVI_{max}	306.75	377.74	-262.49–915.19	-74.13–940.43		0.653	0.292	0.097

*Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable

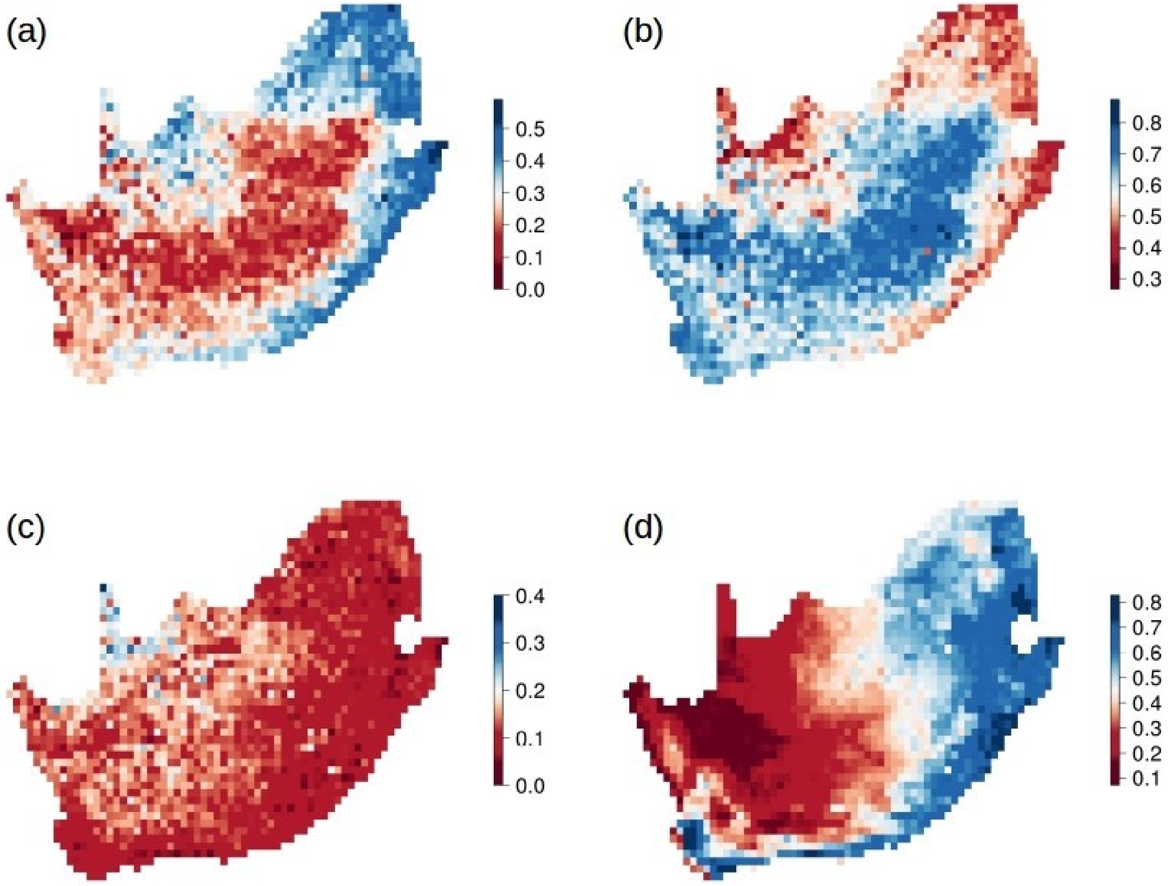
Table S7. Results of multivariable and univariable models based on a Markov chain Monte Carlo technique for generalized linear mixed-effect models with family-level phylogeny (the maximum credibility phylogenetic tree) as a random effect for (a) duetting (vs. no duetting, i.e. no female song and female solo song) (N = 269 species), (b) duetting (vs. no female song) (N = 133 species), (c) female solo song (vs. duetting) (N = 82 species), (d) female solo song (vs. no female song) (N = 111 species) as a response variables and life-history traits (territoriality, social bonds and cooperative breeding) and an environmental variable ($NDVI_{max}$) as explanatory variables in songbirds of the South Africa and Lesotho. In contrast to model results reported in Table 1 and Table S1–S5 where territoriality, social bonds and cooperative breeding were treated as continuous variables, here, these variables were coded as categorical predictors. Estimates of the posterior mean with 95% credible intervals (lower and upper CI), posterior mode of the phylogenetic signal (lambda) and pMCMC values are reported. Statistically significant results are highlighted by bold.

Predictor	**Posterior mean**		**95% CI**		**lambda**		**pMCMC**	
	Multivariable	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**
(a)								
Territoriality (seasonal)	-605.91	-544.80	-886.33–-308.21	-855.18–-263.23	0.555	0.595	**<0.001**	**<0.001**
Territoriality (weak)	-530.94	-476.81	-812.50–-262.51	-758.20–-196.01			**<0.001**	**<0.001**
Social bonds*	–	–	–	–		–	–	–
Cooperative breeding	-3.58	34.73	-159.54–150.52	-54.01–139.34		0.796	0.956	0.422
$NDVI_{max}$	89.24	100.40	-183.35–354.43	-67.86–298.04		0.832	0.532	0.217
(b)								
Territoriality (seasonal)	-520.81	-483.23	-775.70–-296.78	-766.18–-230.24	0.471	0.621	**<0.001**	**<0.001**
Territoriality (weak)	-448.59	-431.60	-698.67–-221.69	-684.87–-179.20			**<0.001**	**<0.001**
Social bonds*	–	–	–	–	–	–	–	–
Cooperative breeding	31.83	66.67	-134.34–190.09	-46.27–192.70		0.762	0.675	0.211
$NDVI_{max}$	217.53	136.17	-44.55–480.98	-50.49–367.89		0.896	0.110	0.116
(c)								
Territoriality (seasonal)	487.79	425.73	241.67–744.20	175.46–669.43	0.660	0.644	**<0.001**	**<0.001**
Territoriality (weak)	163.27	256.19	-352.42–605.40	-32.28–583.88			0.402	0.100
Social bonds (short)	-411.74	-250.10	-1458.01–403.11	-1271.20–545.80		0.775	0.394	0.631
Social bonds (long)	-537.66	-448.40	-1318.37–362.18	-1309.70–331.10			0.177	0.261
Cooperative breeding	22.28	-8.88	-187.25–209.85	-203.81–181.99		0.773	0.777	0.918

NDVI$_{max}$	159.67	-64.42	-224.39–573.48	-478.10–249.50		0.845	0.420	0.671
(d)								
Territoriality (seasonal)	-204.35	-208.83	-537.77–121.21	-505.01–48.61	0.686	0.721	0.189	0.118
Territoriality (weak)	-770.66	-618.66	-1689.02–-42.04	-1259.45–-85.99			**0.032**	**0.010**
Social bonds (short)	-192.49	208.84	-1205.64–976.49	-335.52–945.22		0.584	0.681	0.500
Social bonds (long)	-66.17	381.09	-1128.72–999.23	-140.52–1033.26			0.908	0.141
Cooperative breeding	205.42	147.12	-168.16–501.09	-130.23–419.45		0.600	0.249	0.259
NDVI$_{max}$	172.18	272.18	-523.10–689.03	-147.38–776.89		0.527	0.568	0.233

*Because all species singing in duets belonged to one social-bond (long-term) category, it was not possible to establish social bonds as an explanatory variable

Figure S1. Geographical patterns in the proportion of particular territoriality type: (a) year-round territoriality, (b) seasonal territoriality, (c) no territoriality; and environmental conditions: (d) NDVI$_{max}$ across local assemblages (0.25° × 0.25° grid cell) of South African songbirds (N = 163 species).



LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

Publications in ISI-indexed peer-reviewed journals catalogued by the Web of Science published during the doctoral study but not included in the thesis.

Tryjanowski, P., Kosicki, J., Hromada, M. & **Mikula, P.** 2019. The emergence of tolerance of human disturbance in Neotropical birds. *Journal of Tropical Ecology* 36: 1–5.

Mikula, P., Nelson, E., Tryjanowski, P. & Albrecht, T. 2019. Antipredator behaviour of old-world tropical lizard, common agama *Agama agama*, in an urban environment. *Amphibia-Reptilia* 40: 389–393.

Morelli, F., **Mikula, P.,** Benedetti, Y., Bussière, R. & Tryjanowski, P. 2018. Cemeteries support avian diversity likewise urban parks in European cities: Assessing taxonomic, evolutionary and functional diversity. *Urban Forestry & Urban Greening* 36: 90–99.

Mikula, P., Díaz, M., Albrecht, T., Jokimäki, J., Kaisanlahti-Jokimäki, M.-L., Kroitero, G., Møller, A.P., Tryjanowski, P., Yosef, R. & Hromada, M. 2018. Adjusting risk taking to the annual cycle of long-distance migratory animals. *Scientific Reports* 8: 13989.

Mikula, P., Díaz, M., Møller, A. P., Albrecht, T., Tryjanowski, P. & Hromada, M. 2018. Migratory and resident waders differ in risk taking on the wintering grounds. *Behavioural Processes* 157: 309–314.

Mikula, P., Csanády, A. & Hromada, M. 2018. A critical evaluation of the exotic bird collection of the Šariš Museum in Bardejov, Slovakia. *ZooKeys* 776: 105–118.

Morelli, F., **Mikula, P.**, Benedetti, Y., Bussière, R., Jerzak, L. & Tryjanowski, P. 2018. Escape behaviour of birds in urban parks and cemeteries across Europe: evidence of behavioural adaptation to human activity. *Science of the Total Environment* 631: 803–810.

Mikula, P., Hadrava, J., Albrecht, T. & Tryjanowski, P. 2018. Large-scale assessment of commensalistic–mutualistic associations between African birds and herbivorous mammals using internet photos. *PeerJ* 6: e4520.

Mikula, P., Šaffa, G. Nelson, E. & Tryjanowski, P. 2018. Risk perception of vervet monkeys *Chlorocebus pygerythrus* to humans in urban and rural environments. *Behavioural Processes* 147: 21–27.

Tryjanowski, P., Morelli, F., **Mikula, P.**, Krištín, A., Indykiewicz, P., Grzywaczewski, G. & Jerzak, L. 2017. Bird diversity in urban green space: A large-scale analysis of differences between parks and cemeteries in Central Europe. *Urban Forestry & Urban Greening* 27: 264–271.

Dylewski, Ł., **Mikula, P.**, Tryjanowski, P., Morelli, F. & Yosef, R. 2017. Social media and scientific research are complementary – YouTube and shrikes as a case study. *The Science of Nature* 104: 48.

Mikula, P., Morelli, F., Lučan, R., Darryl, J. & Tryjanowski, P. 2016. Bats as prey of diurnal birds: a global perspective. *Mammal Review* 46: 160–174.

Singing male of common rosefinch *Carpodacus erythrinus*. Želnava, Czech Republic, 2020. © Tomáš Albrecht