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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22 **Statement of authorship:** TA and PM conceived and designed the study with input from all
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 24 statistical analyses with input from WF. MB made the figures with help from MV and PM. TP
 25 and HB provided bioacoustic expertise. PM drafted the first version of the manuscript. TA,
 26 BK and PM revised and finalized the manuscript with input from all authors.

27 **Data availability statement:** The data used in this study were collected from publicly
 28 available databases. All data and computer code used to generate the results, as well as
 29 supplementary figures and tables will be freely available at <https://osf.io/fa9ky/>.

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 $\pm$ 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 $\log(\text{male body mass}) - \log(\text{female body mass})$ or as $\log(\text{male wing length}) - \log(\text{female wing length})$. 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 ($\sim 1^\circ$ 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 $\pm$ SD = $4,030 \pm 1,626$ 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

405 vocal performance (e.g. aerial displays), and habitat properties that influence sound
 406 transmission and degradation. In general, our study calls for large-scale empirical studies on
 407 acoustic signal frequency in other animal groups as independent replication studies.

Acknowledgements

We are grateful to all contributors and the administrators of the xeno-canto database (<https://www.xeno-canto.org/>) and Macaulay Library (<https://www.macaulaylibrary.org/>). Without their contribution, this study would not have been possible. We thank Barbora Blažková for help with the collection of song recordings of endangered species and Liam Revell for help with visualising phylogenies. This study was supported by the Czech Science Foundation project 17-24782S to TA and by the Max Planck Society to BK.

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.

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

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

441 Bro-Jørgensen, J. & Beeston, J. (2015). Multimodal signalling in an antelope: Fluctuating
442 facemasks and knee-clicks reveal the social status of eland bulls. *Anim. Behav.*, 102, 231–
443 239.

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

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

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

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

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

455 Catchpole, C.K. (1987). Bird song, sexual selection and female choice. *Trends Ecol. Evol.*, 2,
456 94–97.

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

459 Christie, P.J., Mennill, D.J. & Ratcliffe, L.M. (2004). Pitch shifts and song structure indicate
460 male quality in the dawn chorus of black-capped chickadees. *Behav. Ecol. Sociobiol.*, 55,
461 341–348.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

495 Goolsby, E.W., Bruggeman, J. & Ané, C. (2017). Rphylopars: fast multivariate phylogenetic
496 comparative methods for missing data and within-species variation. *Methods Ecol. Evol.*,
497 8, 22–27.

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

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

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

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

506 Ives, A.R. (2019). R²s for correlated data: phylogenetic models, LMMs, and GLMMs. *Syst.*
507 *Biol.*, 68, 234–251.

508 Ives, A.R. & Li, D. (2018). rr2: an R package to calculate R²s for regression models. *J. Open*
509 *Source Softw.*, 3, 1028.

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

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

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

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

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

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

- 524 Menezes, J.C.T. & Santos, E.S.A. (2020). Habitat structure drives the evolution of aerial
525 displays in birds. *J. Anim. Ecol.*, 89, 482– 493.
- 526 Mikula, P., Tószögyová, A., Hořák, D., Petrusková, T., Storch, D. & Albrecht, T. (2020).
527 Female solo song and duetting are associated with different territoriality in songbirds.
528 *Behav. Ecol.*, 31, 322–329.
- 529 Morton, E.S. (1975). Ecological sources of selection on avian sounds. *Am. Nat.*, 109, 17–34.
- 530 Morton, E.S. (1977). On the occurrence and significance of motivation structural rules in
531 some bird and mammal sounds. *Am. Nat.*, 111, 855– 869.
- 532 Nakagawa, S. & Freckleton, R.P. (2008). Missing inaction: the dangers of ignoring missing
533 data. *Trends Ecol. Evol.*
- 534 Nemeth, E. & Brumm, H. (2010). Birds and anthropogenic noise: are urban songs adaptive?
535 *Am. Nat.*, 176, 465–475.
- 536 Odom, K.J., Hall, M.L., Riebel, K., Omland, K.E. & Langmore, N.E. (2014). Female song is
537 widespread and ancestral in songbirds. *Nat. Commun.*, 5, 3379.
- 538 Ophir, A.G., Schrader, S.B. & Gillooly, J.F. (2010). Energetic cost of calling: general
539 constraints and species-specific differences. *J. Evol. Biol.*, 23, 1564–1569.
- 540 Padgham, M. (2004). Reverberation and frequency attenuation in forests – implications for
541 acoustic communication in animals. *J. Acoust. Soc. Am.*, 115, 402–410.
- 542 Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401, 877–
543 884.
- 544 Paradis, E. (2011). *Analysis of Phylogenetics and Evolution with R*. Springer, Berlin.

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

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

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

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

555 Price, J.J., Earnshaw, S.M. & Webster, M.S. (2006). Montezuma oropendolas modify a
556 component of song constrained by body size during vocal contests. *Anim. Behav.*, 71,
557 799–807.

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

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

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

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

566 Riede, T., Eliason, C.M., Miller, E.H., Goller, F. & Clarke, J.A. (2016). Coos, booms, and

567 hoots: The evolution of closed-mouth vocal behavior in birds. *Evolution*, 70, 1734–1746.

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

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

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

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

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

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

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

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

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

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

588 16, 2326–2331.

589 Slabbekoorn, H. & Peet, M. (2003). Birds sing at a higher pitch in urban noise. *Nature*, 424,
590 267.

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

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

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

599 Tietze, D.T., Martens, J., Fischer, B.S., Sun, Y.-H., Klusmann-Kolb, A. & Päckert, M.
600 (2015). Evolution of leaf warbler songs (Aves: Phylloscopidae). *Ecol. Evol.*, 5, 781–798.

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

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

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

608 Valcu, M., Dale, J. & Kempenaers, B. (2012). rangeMapper: a platform for the study of
609 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 ($\sim 1^\circ$ 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

Figure 2