

# THE ROLE OF ECOLOGICAL CONSTRAINT IN DRIVING THE EVOLUTION OF AVIAN SONG FREQUENCY ACROSS A LATITUDINAL GRADIENT

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Just as features of the physical and biotic environment constrain evolution of ecological and morphological traits, they may also affect evolution of communication systems. Here we analyze constraints on rates of vocal evolution, using a large dataset of New World avian sister taxa. We show that species breeding in tropical forests sing at generally lower frequencies and across narrower bandwidths than species breeding in open habitats, or at high latitudes. We attribute these restrictions on birdsong frequency to the presence of high-frequency insect noise and greater degradation of high-frequency sounds in tropical forests. We fit Ornstein–Uhlenbeck models to show that recent evolution of song frequency has been more greatly constrained in tropical forests than elsewhere, that is, songs have shown less tendency to diverge over time in tropical forests, consistent with inferred acoustic restrictions. In addition, we find that song frequency has evolved more rapidly overall at high latitudes in both forest and open habitats. Besides a larger available sound window, other factors contributing to more rapid divergence at high latitudes may include an overall increased intensity of sexual selection, occupation of more divergent habitats, and the presence of fewer competing species.

**KEY WORDS:** Acoustic adaptation, evolutionary rates, latitudinal gradient, New World birds, Ornstein–Uhlenbeck model, song frequency.

Ecological opportunity is thought to be a key factor affecting the evolution of ecological traits (Schluter 2000; Yoder et al. 2010). When the number of species competing for a resource is low, ecological opportunity is high and may result in extensive trait divergence between species in response to competition. This divergence may be along various dimensions, such as size of prey consumed and the type of habitat occupied. As the number of competing species partitioning a dimension increases, ecological opportunity declines, and trait divergence becomes more difficult (Schluter 2000; Gavrillets and Losos 2009; Yoder et al. 2010).

Such effects are generally considered in the context of ecological competition (Yoder et al. 2010) but similar principles should apply to competition for channels of communication. One such channel is acoustic. Several studies have found that within sympatric communities of birds, species partition acoustic space (Nelson and Marler 1990; Seddon 2005; Luther 2009; Grant and Grant 2010). Some features of birdsong are unlikely to be constrained by this partitioning. For example, different song syllable types can be combined in an almost endless variety of ways (Weir and Wheatcroft 2011). By contrast, song frequency (i.e., pitch) should

be more constrained given there is only a finite range of frequencies over which songs can be sung. Here we ask how competition for limited acoustic space along the frequency dimension has affected evolutionary rates and bounds.

While large numbers of competing species may reduce ecological opportunity in the frequency component of acoustic space, other factors limit the frequency range available to birds. Song frequency shows both morphological and environmental correlates, implying strong selection pressures on this trait for efficient communication (Podos et al. 2004; Boncoraglio and Saino 2007; Price 2008). Morphological constraints include bill shape and body size. For example, rapid modulation of frequency across a wide bandwidth is difficult (Podos 1997), but may be easier for species with small beaks (Podos 2001). A particularly strong morphological correlate of frequency is body size, whereby large species sing at lower frequencies (Wallschläger 1980; Price 2008). The usual explanation is that the product of amplitude and frequency that maximizes transmission efficiency is at lower frequencies for larger species than smaller species (Ryan and Brenowitz 1985).

Song frequency also correlates with environmental features such as habitat structure and background noise (Morton 1975; Bradbury and Vehrencamp 1998; Boncoraglio and Saino 2007). For example, low-frequency sounds have longer wavelengths allowing them to diffract around vegetation more than high-frequency sounds (Bradbury and Vehrencamp 1998). The differential diffraction of low- and high-frequency components of birdsong results in a distorted signal and birds breeding in dense forest where song attenuation is particularly strong, tend to sing at lower frequencies presumably to optimize transmission distance and song quality (Morton 1975; Padgham 2004; Tobias et al. 2010). High-frequency insect noise in tropical forest habitats may also favor transmission of low-frequency birdsongs (Morton 1975; Ryan and Brenowitz 1985; Kirschel et al. 2009a; reviewed by Bradbury and Vehrencamp 1998). Conversely, birdsong in urban environments or near torrential rivers with loud low-frequency background noise have higher frequency songs than in other environments (Martens and Geduldig 1990, Slabbekoorn and den Boer-Visser 2006; Nemeth and Brumm 2010; Cardoso and Atwell 2011), although the extent to which this represents a plastic versus evolutionary response is not well understood.

Each of these morphological and environmental correlates of song frequency may provide important constraints on the frequency space within which birds can evolve. The stronger these constraints, the smaller the available frequency space available, and the more difficult it should be for newly formed species to diverge in song frequency. To test these predictions, we fit an Ornstein–Ulhenbeck model of evolution (Hansen 1997; Butler and King 2004; Weir and Wheatcroft 2011) to song frequency for a large dataset of 116 closely related avian sister taxa belonging to communities that differ in both biotic and abiotic constraints.

The Ornstein–Ulhenbeck model allows estimation of both the rate of evolution and the degree of evolutionary constraint for these communities. We test whether evolutionary constraints are greater (and rates of evolutionary divergence slower) in forest (which strongly attenuates high frequencies) versus grassland (which has low attenuation), in communities with high background insect din versus quiet communities, and in regions with high versus low avian species diversity. If song frequency space is a limited resource, then we expect that evolutionary constraint should be highest and rates of evolution lowest in those environments that have the highest species richness (the tropics) and in which song transmission is most greatly affected (dense forests with strong signal attenuation and loud insect din).

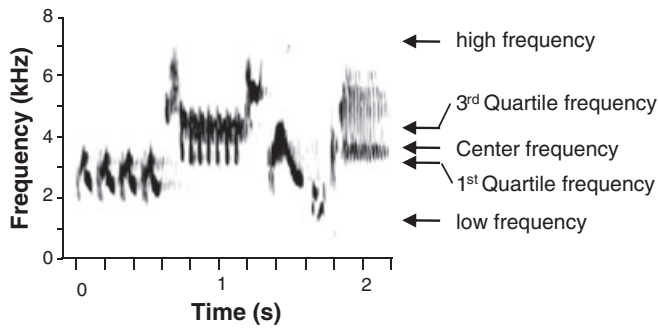
## Methods

### DATA

#### *Sister pairs*

We identified 116 phylogenetically independent sister pairs (sister species and phylogroup splits within species) of passerine birds from molecular phylogenetic trees for the New World for which we could obtain recordings of their songs. For choice of pairs and further details about numbers of songs sampled per species see Weir and Wheatcroft (2011). The latitudinal position of a pair was defined as the average of the absolute midpoint latitudinal measured for entire breeding range of the two taxa in the pair. While there are some difficulties in the use of midpoint latitudes to condense the notion of a species entire geographic range into a single measurement (Weir and Schluter 2007; Weir and Wheatcroft 2011), we avoided the most problematic cases by excluding species with latitudinal ranges greater than 45°, and species pairs whose absolute midpoint latitudes differed by more than 25°. Latitudes were measured from GIS shapefiles of each species geographic range (Ridgely et al. 2003).

We used sister pairs both to control for any phylogenetic contribution to the overall latitudinal pattern of frequency usage and also to obtain estimates of the rate of frequency divergence between closely related species. As described in Weir and Schluter (2007), genetic distances were obtained using the GTR- $\gamma$  model from mitochondrial cytochrome b sequences (except for six species from southern South America, where due to the lack of cytochrome b sequences we used other protein-coding mitochondrial genes with similar rates of evolution). Following Weir and Wheatcroft (2011), we only included sister taxa with genetic distances greater than 0.75%. The data are included as supplemental information. We also obtained node ages for sister pairs from a phylogeny generated in BEAST 1.5.4 (Drummond and Rambaut 2006) using cytochrome b sequences, a relaxed clock (with rate variation following a log-normal distribution and Yule speciation prior) with the GTR- $\Gamma$  model of sequence evolution,



**Figure 1.** Six frequency measures were used in this analysis as illustrated on a sonogram of Lincoln's sparrow (*Melospiza lincolnii*). The sixth measure, bandwidth, is the difference between high and low frequency as is not illustrated on the graph.

and a mean molecular rate of 0.0105 estimated for birds (Weir and Schluter 2008). To do this, we partially fixed the topology (suborders, families, genera, and sister pairs were constrained to be monophyletic). We ran the analysis for 20 million generations, sampled trees every 1000 generations following a burn-in of 5 million generations, and then constructed a maximum sum of clade credibility tree (included in supporting online dataset along with GenBank accession numbers). We ran all sister pair analyses twice, first using genetic distances and second, using branch length separating sister pairs from a relaxed clock tree. They each gave very similar results and parameter values and throughout the text we present results only for the analysis based on genetic distances.

### Song frequency

Recordings were obtained by the authors in the field, from the Macaulay Library of Natural Sounds (<http://macaulaylibrary.org>), the Xeno-canto database (<http://www.xeno-canto.org>), and from commercially available audio publications. From these songs JTW measured six aspects of song frequency (Fig. 1) using RAVEN 1.4 (website: [www.birds.cornell.edu/raven](http://www.birds.cornell.edu/raven)): highest and lowest frequency (kHz), center frequency (the frequency at which the energy in the song is divided equally above and below), first quartile frequency (the frequency below which 25% of song energy occurs), third quartile frequency (the frequency below which 75% of song energy occurs), and bandwidth (highest minus lowest frequency). All measurements were obtained for the fundamental frequency (i.e., first harmonic).

### Body mass

We obtained available measures of mass from Dunning (2008) for 171 of the 232 taxa. Mass was log transformed and all analyses using mass were restricted to these 171 taxa. For most species, we used average mass of males and females. For some species, measurements from one of the sexes were not available, and measurements for the available sex were used.

### Allopatry/Sympatry

We classified sister pairs as sympatric only if their ranges overlapped by more than about 100 km (i.e., parapatric sister pairs with a contact or narrow hybrid zone were considered allopatric; see Weir and Price 2011 for further details).

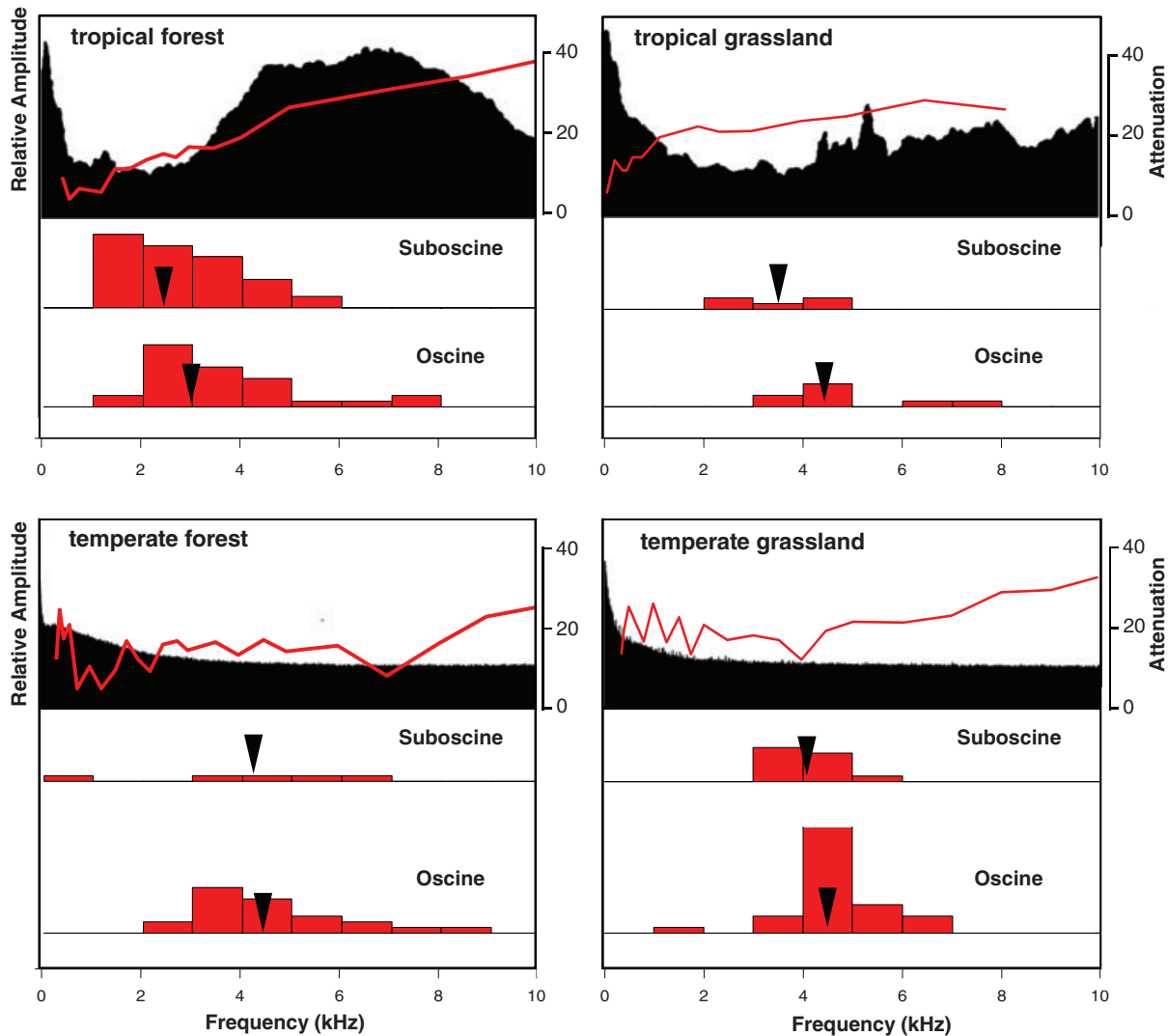
### Habitat

We classified species as belonging to forest (closed habitat) and nonforest (open habitats, including grassland, marshland, scrub, nonforested lake edge) habitats based on habitat descriptions for each species in Stotz et al. (1996) and Nature Serve (<http://www.natureserve.org>). These habitat divisions are necessarily broad: forest included a variety of tropical (<23°) and temperate (>23°) forest types with varying degrees of openness as well as forest edge whereas nonforest included scrub, grassland, savannah, marsh, and other open terrestrial habitats.

### Background noise and attenuation

We measured the power spectra (the pattern of how sound amplitude varies with frequency) of ambient, nonavian, noise in temperate forest and grassland and compared these to published spectra for a secondary Neotropical lowland wet forest (Pipeline Road, Panama), which used similar methods (Ryan and Brenowitz 1985). Ambient noise was recorded during the breeding season in May and June 2010 and 2011 in four temperate forests (primary growth redwood forest in California, secondary Douglas-fir and ponderosa pine in British Columbia, secondary mixed hemlock, pine and broadleaf forest in Ontario, and a boreal spruce forest in Quebec) and one temperate grassland (grassland and sagebrush flats with juniper in Oregon), with minimal wind during the mid- to late-morning using Sennheiser omnidirectional microphones (Sennheiser K6-ME62 and MKH-20-P48) mounted 1 m off the ground and pointed vertically upwards. For each site we obtained 2–5 min of recording, intervals with avian bird vocalizations were deleted, and power spectra were obtained in RAVEN 1.4b from the remaining intervals without avian sounds (total 30 s for each recording) but including other types of biotic or abiotic sounds. Power spectra are presented in Figure 2. As spectra were similar across the four temperate forests measured (with a flat relationship between power and frequency above 1 kHz), we present results for only the most northern latitude forest (Quebec). Recording volumes may have varied across different studies, so only the shape of power spectra can be compared across sites.

We obtained published measurements of how sounds of various frequencies degrade (excess attenuation) through lowland tropical grassland (Morton 1975), and lowland tropical wet forest (Marten et al. 1977) in central Panama, and temperate open field and temperate (hemlock) forest in Millbrook, New York (Marten



**Figure 2.** Histograms of center frequency (mean shown by black triangles) for species of New World oscine and subsong birds in tropical (midpoint latitudes between 0° and 23°) and temperate (>23°) latitudes. Power spectra show nonavian background noise (relative power of background noise versus frequency, scaled across power spectra so that the frequency with the lowest power in each are equal) for tropical wet forest and grassland (Panama, data from Ryan and Brenowitz 1985) and temperate forest (coniferous) and grassland with scattered sagebrush and juniper (this study, see text). Gray lines (red lines in online version) plot excess attenuation (dB/100 m) in tropical and temperate (coniferous) forests (attenuation data and tropical power spectra taken from Morton 1975; Marten and Marler 1977; Marten et al. 1977).

and Marler 1977). Each study played recordings of white noise in several habitat types and measured frequency attenuation at various heights from the ground and used very similar methods. We used the excess attenuation data at 0.31 m (1 ft) off the ground for the tropical grassland and 1 m off the ground for other habitat types. Morton (1975) and Marten et al. (1977) both measured attenuation in identical tropical forest habitats. Although the excess attenuation curves for tropical forest were slightly different in each study, the pattern was very similar: increasing attenuation with increasing frequency (Marten et al. 1977). Here, we include only the curve from Marten et al. (1977).

**ANALYSIS**

*Patterns across the gradient*

We conducted a multiple regression analysis of each song frequency measurement on midpoint latitude (for each species/phylogroup), mass and their interaction. These regressions were conducted both on the raw data or while simultaneously controlling for phylogeny. For the latter analyses we constructed an ultrametric phylogeny based on sequence data (see above). We then used generalized least-squares regression (e.g., Martins and Hansen 1997), with the covariance between a pair of species computed as their shared branch length in the phylogeny,

**Table 1.** Variable loadings and proportion of variance explained for the first two principal components (PC1, PC2) of song frequency measurements extracted from the correlation matrix. Q1 and Q3 refer to the first and third quartile (see Fig. 1).

Measure	PC1	PC2
Q1 frequency	0.44	0.22
Q3 frequency	0.45	0.01
Center frequency	0.45	0.13
Bandwidth	0.34	-0.65
High frequency	0.42	-0.35
Low frequency	0.34	0.62
Proportion of variation	79%	17%

as expected under the Brownian motion (BM) model of evolution (Martins and Hansen 1997). To conduct these analyses we used nlme (Pinheiro et al. 2009) and APE (Paradis et al. 2004) in R (R Development Core Team 2010).

**Evolutionary rates**

To evaluate song divergence between species pairs over time, we compared the amount of song frequency divergence between sister pairs to the genetic distance separating them. To measure frequency divergence we used a principal component analysis on the covariance matrix of the six log-transformed frequency measurements of all taxa. The first two principal components together explained 96% of the variance (Table 1). Our unit of analysis is the Euclidean distance between sister taxa, obtained from the first two principal component scores (i.e., a single multivariate Euclidean distance in bivariate space).

We modeled evolutionary change in song as an Ornstein–Uhlenbeck process (hereafter OU), a modification of the BM model for evolutionary divergence (Hansen 1997; Butler and King 2004; Weir and Wheatcroft 2011). OU models extend BM models by including a parameter  $\alpha$  that measures the extent to which divergence is constrained (i.e., shows a greater tendency to return to a central starting value than expected under BM) in addition to the rate parameter of the BM model ( $\beta$ ). This parameter,  $\alpha$ , represents the “pull” toward an optimum trait value, which in our case is the intermediate value between the sister pair. This affords every sister pair a unique optimum, independent from the optima of other sister pairs in a dataset, but a single value of  $\alpha$  is estimated across sister pairs.

We write  $D_i$  as the Euclidean distance between the songs of two taxa in sister taxon  $i$ , separated by time  $T_i$ , where genetic distance is a proxy for time. Then the likelihood computed across all sister pairs of taxa is (Weir and Wheatcroft 2011):

$$L(\beta, \alpha | D, T) = \prod_i \frac{2}{\sqrt{2V_i\pi}} \exp\left(-\frac{D_i^2}{2V_i}\right) \quad (1)$$

where  $V_i = \frac{\beta}{2\alpha}(1 - \exp^{-T_i\alpha})$ .

A large value for  $\alpha$  implies a strong force tending to retard divergence, that is a large constraint. As  $\alpha$  approaches 0, the model collapses to that of BM. Because we wished to ask if evolution rate and constraint in song varies along the latitudinal gradient, both  $\beta$  and  $\alpha$  were assumed to be linear functions of the midpoint latitude of each sister pair ( $Lat$ ):

$$V_i = \frac{b_\beta Lat_i + c_\beta}{2(b_\alpha Lat_i + c_\alpha)} (1 - \exp^{-T_i(b_\alpha Lat_i + c_\alpha)}), \quad (2)$$

where  $b_\alpha$  and  $b_\beta$  are the slope parameters and  $c_\alpha$  and  $c_\beta$  are intercept parameters. This becomes a four-parameter model ( $b_\alpha, c_\alpha, b_\beta, c_\beta$ ) for OU and two-parameter model for BM. The model in equation 2 could be used to estimate rates across any gradient by substituting  $Lat$  with any continuous variable.

The Euclidean distance between species is generally inflated by sampling and measurement error within species and this inflation becomes relatively more important when few individuals are measured and the true distance between species is small. Following Weir and Wheatcroft (2011), we correct for inflation using an approach based on the ANOVA, where for each species pair  $D^2$  in equation 1 is estimated as:

$$2(MS_{between} - MS_{within})/N_o. \quad (3)$$

Here  $MS_{between}$  is the summed group mean square estimates for PC1 and PC2,  $MS_{within}$  is the summed error mean square estimates for PC1 and PC2, and  $N_o$  is the weighted number of individuals measured within each species in a pair (Sokal and Rohlf 1995, p. 214). Equation 3 corrects a typographical error in Weir and Wheatcroft (2011). We refer to this estimate as the corrected Euclidean distance. The correction for measurement error results in some corrected Euclidean distances being negative. These were set to zero.

A model in which a single set of parameters was estimated for the entire dataset was compared to models in which separate parameter estimates were obtained for various subsets of the data including forest and nonforest, oscines and suboscines, and sympatric and allopatric. For each data subset we estimate parameters under BM (i.e., the OU model with  $\alpha$  set to a small value near 0) and OU with and without latitude. For six temperate sister pairs, one taxon was classified as in nonforest habitat and the other taxon as in forest habitat, and these six pairs were eliminated from the evolutionary rates analysis. Models were compared using the Akaike Information Criterion corrected for sample size (AICc) and Akaike weights (Burnham and Anderson 2002). The model with the lowest AICc value best explains the data. Maximum-likelihood analyses were performed in R using the nlm function in the base package with the following constraints on parameter space: the y intercepts ( $c_\beta$  and  $c_\alpha$ ) > 0, and the x intercepts  $\geq 66^\circ$  or  $\leq 0^\circ$  (i.e., to accommodate the latitudinal range of our dataset). The Hessian matrix calculated at the optimum was

inverted into the variance covariance matrix, and was then used to obtain 95% confidence intervals of model parameters. In one of our data subsets (nonforest), the Hessian was not invertible because the maximum-likelihood estimate of  $c_{\beta}$  occurred at 0, its lower bound. To estimate the Hessian for this dataset we relaxed the constraint on  $c_{\beta}$ .

These models were coded in R (R Development Core Team 2010) and will be included in the next release of the package GEIGER (Harmon et al. 2008).

## Results

### HABITAT CHARACTERISTICS

Tropical forests have both louder ambient noise and greater signal attenuation at higher frequencies than either temperate forest or temperate and tropical grassland habitats (Fig. 2). At frequencies between 4.5 and 9 kHz, ambient noise in tropical forest shows a threefold decibel increase compared with 1–4 kHz. In contrast, temperate forest and temperate and tropical grassland have relatively flat ambient noise power spectra from 2 to 10 kHz: in temperate habitats, ambient noise decreases slightly as frequency increases, whereas tropical grassland shows a slight increase (Fig. 2; the shape of spectra should be compared between environments rather than the absolute power, due to different recording sensitivities across studies).

In addition to ambient noise effects, sound in tropical forests shows greater attenuation of higher frequency (6.5–10 kHz) and similar or less attenuation at low frequencies (0–4.5 kHz) than in either temperate forests or temperate or tropical grassland habitats (Fig. 2). Thus, tropical forests have a well-defined frequency “window” between 1 and 4–5 kHz at which both ambient noise and attenuation is low, whereas the frequency range between 1 and 10 kHz in grasslands and temperate forests is less affected by either background noise or distorted attenuation.

### PATTERNS OF SONG FREQUENCY

Forest species (both oscine and suboscine) at higher latitudes sing on average at higher frequencies and over a greater bandwidth than those at lower latitudes, but nonforest species do not show a significant effect of latitude on any frequency measure (Figs. 2, 3). This result holds true if regressions are corrected for phylogeny (Fig. 3, Table 2).

For the dataset including species for which we have body mass estimates, we found a strong correlation of mass with frequency (e.g., log mass and center frequency,  $r = -0.6$ ,  $N = 171$ ). Inclusion of body mass in the model did not affect associations with latitude. First, in the nonforest habitat, no associations of latitude with any song parameter were significant whether mass is in the model ( $N = 50$ ). Second, within the forest habitat, lat-

**Table 2.** Phylogenetically corrected regressions within forest habitat.

	<i>Slope</i> ± <i>SE</i>	<i>P</i> *
Center frequency		
Latitude	81±25	<0.0001
Mass	−589±269	0.03
Latitude:Mass	−21±8	<0.0086
High frequency		
Latitude	199±47	<0.0001
Mass	426±513	0.41
Latitude:Mass	−53±15	<0.0007
Low frequency		
Latitude	26±22	0.24
Mass	−315±238	0.19
Latitude:Mass	−9±7	0.21
Bandwidth		
Latitude	174±48	<0.0005
Mass	784±521	0.14
Latitude:Mass	−44±15	<0.0048

\*Based on 117 degrees of freedom. Significant values ( $P < 0.05$ ) are in bold

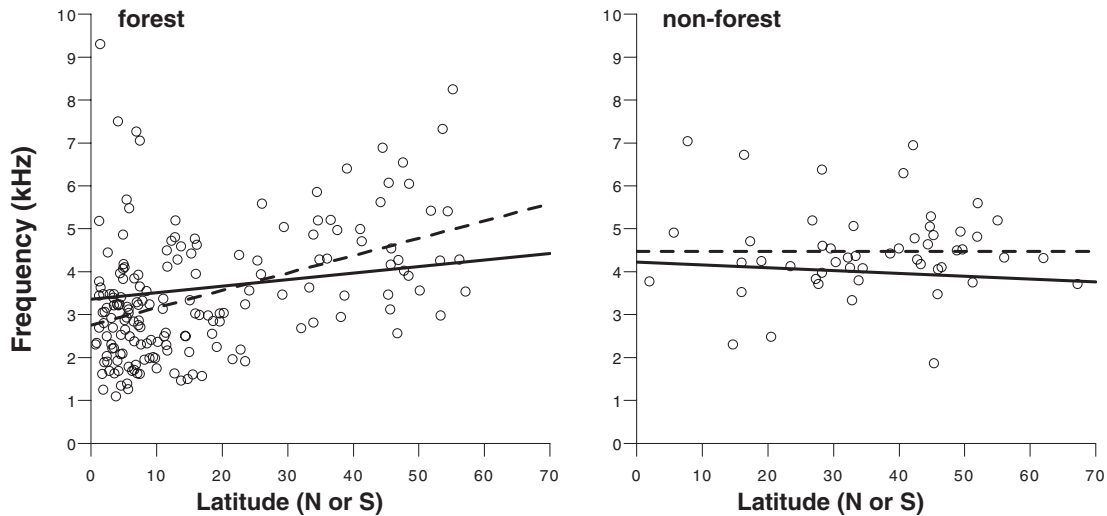
itude remains significantly correlated with bandwidth, high frequency, and center frequency when mass is in the model ( $N = 121$ , Table 2). Within forest habitat, for high and for center frequency, body mass and latitude show a significant interaction (Table 2): the negative correlation between body mass and frequency is stronger at higher latitudes.

Patterns of birdsong frequency match those expected from habitat characteristics (i.e., nonavian background noise and attenuation; Fig. 2). The energy of tropical forest birdsong ( $N = 129$ ) is concentrated between 2700 (first quartile) and 3360 Hz (third quartile), well within the tropical forest frequency window of 1–4 kHz, whereas the songs of temperate forest ( $N = 50$ ) or grassland birds at either latitude ( $N = 55$ ) have more than three-fourths of their energy above 3900 Hz (Q1). Overall, the songs of grassland and temperate forest birds had frequency measurements (Q1, Q3, center, bandwidth, high, and low frequency) roughly 1.5 times higher than the songs of tropical forest birds.

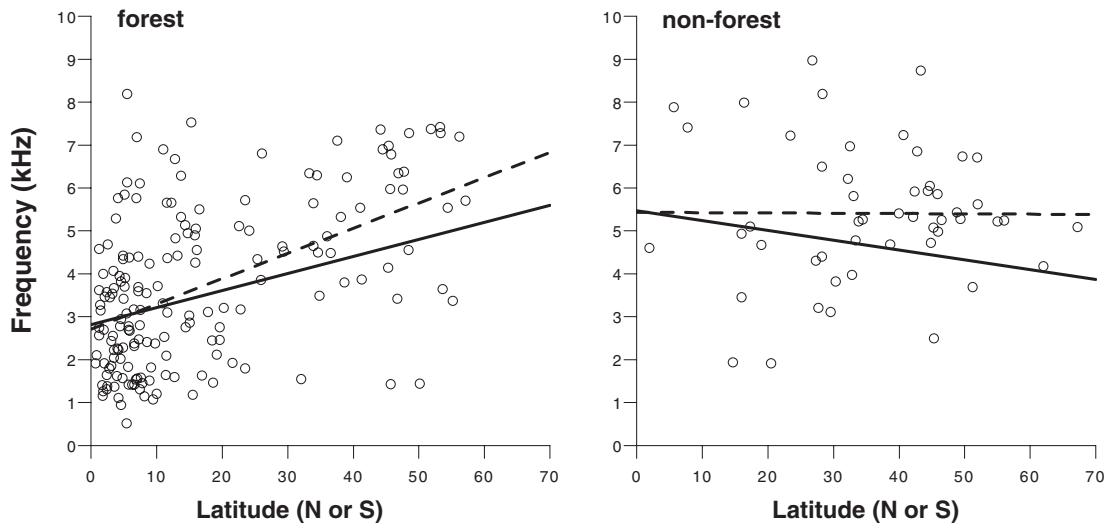
### EVOLUTIONARY RATES

We ask how the strong correlations of latitude with frequency in forest habitats are associated with evolutionary rates and constraints. In Figure 4, we plot corrected Euclidean song frequency distances (using eq. 3) against genetic distances, for each species pair. Tropical forest appears to show a bounded pattern, with species pairs that are long separated showing similar degrees of song divergence to those much more recently separated (Fig. 4, left, open circles). By contrast, species pairs in temperate habitats seem to have continued to diverge across the considered

## A) Centre Frequency



## B) Bandwidth

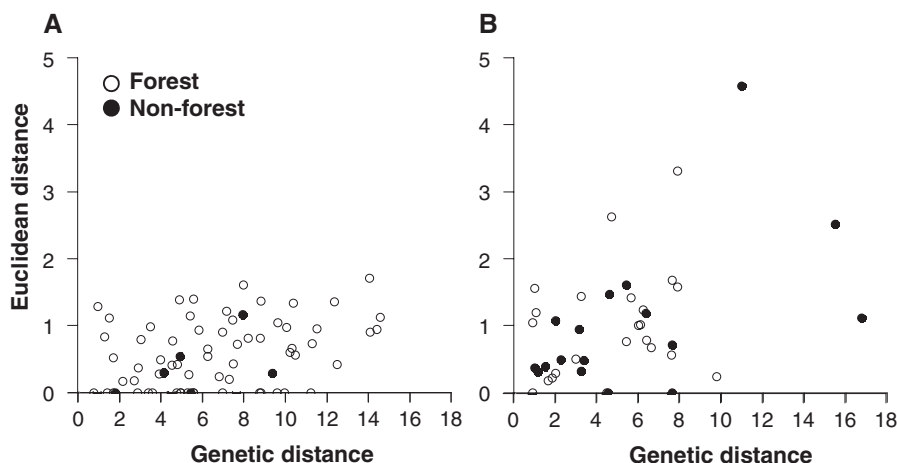


**Figure 3.** Regressions of center frequency and bandwidth on absolute latitude for forest and nonforest species. Regressions were calculated using least squares either without taking phylogeny into account (uncorrected, dashed lines) or correcting for phylogeny (generalized least squares, corrected, solid lines) using a covariance structure between species calculated using Brownian motion, with the phylogeny described in the supplemental information (Martins and Hansen 1997). (A) uncorrected forest: intercept = 2.76, slope = 0.0404,  $t = 6.39$ ,  $N = 173$ ,  $P < 0.0001$ ; corrected forest: intercept = 3.35, slope = 0.0153,  $t = 2.47$ ,  $N = 173$ ,  $P = 0.0146$ ; uncorrected nonforest: intercept = 4.47, slope = 0.000,  $t = 0.003$ ,  $N = 51$ ,  $P = 0.997$ ; corrected nonforest: intercept = 4.23, slope =  $-0.00068$ ,  $t = 0.69$ ,  $N = 51$ ,  $P = 0.492$ ; (B) uncorrected forest: intercept = 2.71, slope = 0.0588,  $t = 7.72$ ,  $N = 173$ ,  $P < 0.0001$ ; corrected forest: intercept = 2.81, slope = 0.0400,  $t = 3.767$ ,  $N = 173$ ,  $P = 0.0002$ ; uncorrected nonforest: intercept = 5.43, slope = 0.000,  $t = 0.042$ ,  $N = 51$ ,  $P = 0.97$ ; corrected nonforest: intercept = 5.47, slope = 0.023,  $t = 1.44$ ,  $N = 51$ ,  $P = 0.16$ .

time span (Fig. 4, right). In addition, although the sample size is small there is no obvious bounding in open tropical habitats (Fig. 4, left, closed circles).

To more formally test these patterns, we constructed models that allowed for evolutionary rates and constraints to be constant or to change linearly with latitude, and to vary for different model

subsets (forest or nonforest, oscine or suboscine, sympatric or allopatric). Models without latitude had no support, contributing  $<2\%$  of the Akaike weight in total. Among models including latitude, the model with the lowest AICc value estimated separate evolutionary rates and constraints for forest and nonforest species, but with a fit of OU to the forest subset and a BM to the



**Figure 4.** Corrected Euclidean distances plotted against genetic distances for sister pairs in (A) tropical (0°–23°) and (B) temperate (23°–66°) latitudes.

nonforest subset (Table 3). This best-fit model greatly outperformed all other models tested as illustrated by the large delta AICc values for other models. The eight models that separate forest from nonforest habitats (total weight 61%) have 5× more support than the eight models that separate oscines/suboscines and 6× more support than the eight models that separate sympatric from allopatric groups.

Under the best-supported model, evolutionary rate ( $\beta$ ) increased significantly with latitude in both forest and nonforest species (forest: slope = 0.022 ± 0.0058 95% confidence interval, nonforest: slope = 0.01225 ± 0.0011; Fig. 5). For forest envi-

ronments, a significant improvement in fit of an OU model over a BM model (Table 3) implies that song frequency evolution has been bounded over the time period we analyzed (i.e., ~9 million years assuming a 2% molecular clock; see Weir and Schluter 2008). The strength of bounding declines threefold with increasing latitude (slope = -0.0077 ± 0.0029 95% confidence interval; Fig. 5). Thus, within forests, frequency evolution is both more rapid and less constrained at higher latitudes, confirming the patterns apparent in Figure 4.

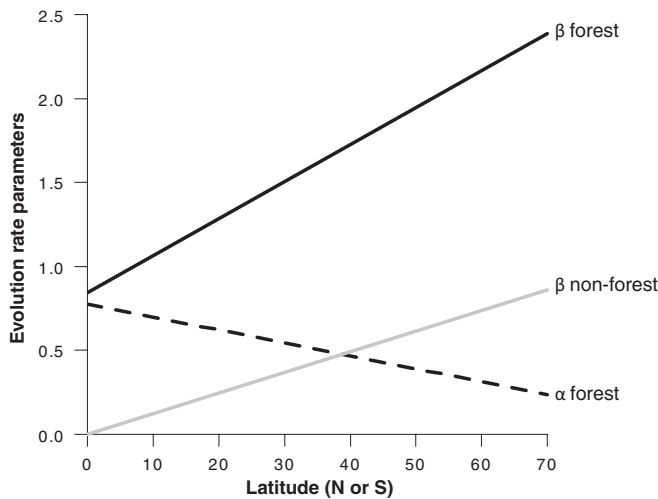
In contrast to species in forests, a BM model without bounding best-fit nonforest species, but as in forest

**Table 3.** Comparison of support for different models of frequency evolution fit to the data.  $\Delta$ AICc scores (AICc for each model – smallest AICc score) and Akaike weights (AICc weight) are used as metrics of model support. The best-fit model has the largest AICc weight and has a  $\Delta$ AICc value of 0 (bold).

	Latitude not in model			Latitude in model		
	<i>N</i>	$\Delta$ AICc	AICc weight	<i>N</i>	$\Delta$ AICc	AICc weight
All (BM)	1	17.36	0.000	2	5.91	0.027
All (OU)	2	13.91	0.000	4	2.71	0.133
Oscine (BM)/Suboscine (BM)	2	16.98	0.000	4	5.62	0.031
Oscine (BM)/Suboscine (OU)	3	20.01	0.000	6	3.53	0.088
Oscine (OU)/Suboscine (BM)	3	24.70	0.000	6	9.97	0.004
Oscine (OU)/Suboscine (OU)	4	14.58	0.000	8	8.07	0.009
Allopatric (BM)/Sympatric (BM)	2	17.70	0.000	4	8.95	0.006
Allopatric (BM)/Sympatric (OU)	3	13.00	0.000	6	25.61	0.000
Allopatric (OU)/Sympatric (BM)	3	16.37	0.000	6	3.63	0.083
Allopatric (OU)/Sympatric (OU)	4	11.12	0.002	8	8.29	0.009
Forest (BM)/Nonforest (BM)	2	19.39	0.000	4	7.53	0.012
Forest (BM)/Nonforest (OU)	3	28.11	0.000	6	5.37	0.035
Forest (OU)/Nonforest (BM)	3	11.91	0.001	6	<b>0.00</b>	<b>0.515</b>
Forest (OU)/Nonforest (OU)	4	7.48	0.012	8	5.61	0.031

*N* is the number of model parameters. BM = Brownian motion model, OU = Ornstein–Uhlenbeck model.





**Figure 5.** Evolutionary rates of song frequency across latitude under the best supported model. This best-fit model used OU for the forest subset ( $N = 87$  pairs of species) and BM for the nonforest subset ( $N = 23$  pairs). Maximum-likelihood values of  $\beta$  and  $\alpha$  are shown across the latitudinal gradient.

species, song evolution has proceeded faster at higher latitudes (Fig. 5).

## Discussion

Tropical forests in our dataset have greater ambient high-frequency background noise and more rapid attenuation of high-frequency sounds than either temperate forests or grassland habitats. This creates a frequency “window” between 1 and 4 kHz within which background noise and attenuation are relatively low. Our results show that tropical forest birds sing primarily at frequencies within this window, whereas birds breeding in tropical grasslands or at high latitudes make use of higher frequencies when they sing (Figs. 2, 3, see also Morton 1975; Ryan and Brenowitz 1985). The correspondence between ambient noise and sound attenuation with song frequency is strong, and has been observed in other systems, such as urban versus rural comparisons (Slabbekoorn and den Boer-Visser 2006; Cardoso and Atwell 2011). However, a more detailed analysis of background noise and attenuation across a greater diversity of forest and nonforest habitat types than measured in this study would be valuable.

Habitat-based differences in song frequency usage are reflected in the patterns of evolutionary divergence between sister species. Uniquely within tropical forests, song evolves in a more constrained manner than in open habitats or at higher latitudes. In contrast to BM, where expected trait divergence between two species increases indefinitely through time, expected trait divergence when evolution is bounded ( $\alpha > 0$ ) increases rapidly initially, then slows as it approaches an asymptote set by  $\alpha$ . This

bounding is evident in the observed levels of frequency divergence of tropical sister species, which do not appear to increase with increasing sequence divergence (Fig. 4). In contrast, songs continue to diverge with increasing sequence divergence at high latitudes. Our results suggest that a narrower sound window in tropical forest constrains the extent to which tropical forest species can diverge (i.e., they evolve within a smaller total frequency space).

While insect din and attenuation may constrain tropical forest birdsong to lower frequencies, within this low-frequency window, the entire animal community could further affect divergence. All other things being equal, under a theory of character displacement, species should become evenly dispersed across available resource space, thereby minimizing competition between them (Slatkin 1980). Thus, the fewer the species that compete for the same resource space, the greater the average distance in trait space we should observe between them. The multitude of birds and other animals in tropical forests should provide a generally constraining influence on divergence between any one pair of species (i.e., there is less free space to evolve into), with much less constraint in less speciose environments (grasslands and temperate forests). The constraint could operate at two levels. First, species may compete directly for acoustic space (Nelson and Marler 1990). Evidence that this happens in the tropics comes from observations that even co-occurring species with similar songs use signal space disjunctly (Luther 2009). Second, species could compete ecologically (in traits other than song frequency), undergo ecological character displacement, and then the ecological differences (e.g., in size or habitat) lead to acoustic divergence (Kirschel et al. 2009b). In theory, this latter possibility could be tested by comparing sister species that separate into forest and nonforest habitats. In practice, shifts between forest and nonforest habitats are rare in our dataset (only six [5%] of 116 pairs, which were excluded from diversification rate estimates). However, these six pairs do not seem to be associated with exceptionally large Euclidean distances in song frequency.

In contrast to the results for birdsong frequency, a recent analysis using the OU model on a largely overlapping dataset, failed to show any evidence of constrained evolution in song syntax (i.e., song syllable diversity and song length; Weir and Wheatcroft 2011). Unlike song frequency, a strong constraint on song syntax is not expected, because species can combine song elements in a large variety of ways. Indeed, avian songs range from very short songs with few syllable types to very long songs with many syllable types. The contrast between frequency and syntax is useful, because it illustrates how competition for acoustic space can result in unbounded evolution through time in traits such as song syntax that are not strongly resource limited, and strongly bounded evolution in traits like frequency that we a priori expect to be more limited.

In addition to greater constraints on song frequency evolution in tropical forests, characteristics of song frequency show significantly faster evolution over the past several million years at higher latitudes than at lower latitudes, and this is apparent within both forest and nonforest habitat. Similar findings have been reported for song syllable diversity (Weir and Wheatcroft 2011) and plumage color (Martin et al. 2010) in birds. The generality of the finding that sexually selected traits like song and color show greater recent evolution at higher latitudes demands a common explanation. The greater boundedness of song frequency evolution in tropical forests could result in slower rates of song frequency evolution there. However, it is not clear that similar constraints should apply to syllable complexity (for which constrained evolution is not supported; Weir and Wheatcroft 2011), or for color (for which constrained evolution has not been tested). Weir and Wheatcroft (2011) consider two other possibilities. The first is that sexual selection pressures or natural selection costs may differ between the temperate and the tropics, leading to greater exaggeration of traits in the temperate in general. If exaggeration happens in different directions or in different traits for closely related taxa, then divergence is expected (Irwin 2000; Irwin et al. 2001). Irwin et al. (2008) note that singing across a larger bandwidth is one way to increase song complexity; others include increasing syllable diversity. Thus, if both members of a sister pair are selected to increase complexity, and only one does so by increasing bandwidth, the pair will diverge in aspects of song frequency. Consistent with this explanation, frequency bandwidth increases on average from tropical to temperate latitudes (Figs. 2, 3), as do number of syllable types per song (Weir and Wheatcroft 2011).

A second explanation for more rapid divergence in sexually selected traits in the temperate is again related to acoustic adaptation. Our estimates suggest a strong effect of habitat (forest vs. nonforest) on the rate of frequency evolution (Table 3), with forests having faster evolutionary rates than nonforest environments at all latitudes (Fig. 5). Transitions between microhabitat and forest types have been shown to drive song divergence in tropical forests (e.g., perch height, Seddon 2005; bamboo-terra firme differentiation, Tobias et al. 2010). These transitions have yet to be evaluated in temperate habitats, but plausibly they have stronger effects on the evolution of song traits in temperate regions. For example, coniferous and deciduous forest in temperate latitudes show different levels of excess attenuation at different frequencies (Marten et al. 1977), implying shifts between these kinds of forest types could drive more rapid frequency divergence in temperate compared to tropical forests. In contrast, we expect transitions between microhabitats within nonforest habitats to result in less frequency divergence due to the greater structural simplicity of these environments, though direct tests are needed.

Our coarse-grained division into forest and nonforest habitat types suggest that the range of song frequencies used by New World passerine birds is correlated with the background noise and sound transmission properties of the habitat in which they breed. Furthermore, the magnitude and rate of song frequency evolution closely tracks the available character space; evolution has both been slow and more bounded in the species-rich tropical forests, where insect din and excess attenuation should select against high frequencies, than in temperate forests. While a number of other factors may affect latitudinal differences in rate and bounding, our results demonstrate that patterns of divergence of song frequency are consistent with constraints of acoustic adaptation and, thus, that habitat characteristics structure the evolution of a key discrimination trait. Rates of evolution in other species discrimination traits like avian plumage (Martin et al. 2010) and syllable structure (Weir and Wheatcroft 2011) are also estimated to be faster at high latitudes, and together with song frequency could contribute to faster estimated rates of speciation at high latitudes (Weir and Schluter 2007).

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