

Evolution and plasticity: Divergence of song discrimination is faster in birds with innate song than in song learners in Neotropical passerine birds

Benjamin G. Freeman,^{1,2,3} Graham A. Montgomery, and Dolph Schluter^{1,2}

¹Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

²Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

³E-mail: freeman@zoology.ubc.ca

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Plasticity is often thought to accelerate trait evolution and speciation. For example, plasticity in birdsong may partially explain why clades of song learners are more diverse than related clades with innate song. This “song learning” hypothesis predicts that (1) differences in song traits evolve faster in song learners, and (2) behavioral discrimination against allopatric song (a proxy for premating reproductive isolation) evolves faster in song learners. We tested these predictions by analyzing acoustic traits and conducting playback experiments in allopatric Central American sister pairs of song learning oscines ($N = 42$) and nonlearning suboscines ($N = 27$). We found that nonlearners evolved mean acoustic differences slightly faster than did learners, and that the mean evolutionary rate of song discrimination was 4.3 times faster in nonlearners than in learners. These unexpected results may be a consequence of significantly greater variability in song traits in song learners (by 54–79%) that requires song-learning oscines to evolve greater absolute differences in song before achieving the same level of behavioral song discrimination as nonlearning suboscines. This points to “a downside of learning” for the evolution of species discrimination, and represents an important example of plasticity reducing the rate of evolution and diversification by increasing variability.

KEY WORDS: Mate choice, phenotypic plasticity, reproductive isolation, signal evolution, song evolution, species recognition.

Learning can accelerate trait evolution and speciation. This is because learned traits evolve due to both genetic and cultural evolution, and cultural evolution may substantially increase overall rates of change (Verzijden et al. 2012; Yeh and Servedio 2015). Learning can impact mate choice and hence speciation, both when mate preferences are learned (e.g., sexual imprinting), and when mating displays are learned (e.g., learned song). If learned mate choice traits diverge faster than innate mate choice traits, learning might accelerate speciation by speeding up evolutionary rates of reproductive isolation (Laland 1994; Irwin and Price 1999; Lachlan and Servedio 2004). The idea that learning promotes speciation is a special case of the more general hypothesis that phenotypic plasticity in traits may speed the rate of evolution. Theory predicts that plasticity can often (but not always) facili-

tate trait evolution and speciation (Price et al. 2003; Paenke et al. 2007; Pfennig et al. 2010; Nonaka et al. 2015), but empirical studies evaluating how plasticity alters the tempo of evolution are scarce (Pfennig and McGee 2010).

Birdsong is a classic example of a trait used in mate choice where both the preference and the trait itself are, in part, learned (Edwards et al. 2005; Verzijden et al. 2012). In three avian orders (the hummingbirds, parrots, and oscine passerines), male individuals learn from adult tutors the songs they use in mate choice displays (Nottebohm 1972), and species richness is much higher in these three clades of song learners compared to related clades with innate song (Nottebohm 1972; Fitzpatrick 1988). Due to this correlation between song learning and species richness, birdsong has been held up as a case example of how learning can

speed diversification (hereafter, the “song learning” hypothesis; Nottebohm 1972; Fitzpatrick 1988; Edwards et al. 2005). Three clades of song learning birds are not enough to test statistically the association between learning and diversification, motivating new tests. Here, we test predictions of the song learning hypothesis applied to the passerines, which consist of two sister clades: the oscines, or songbirds, which learn their songs and are extraordinarily diverse (~ 4650 species, representing ~ 45% of all extant bird species), and their sister clade, the suboscine passerines (~ 1250 species) (Jetz et al. 2012), in which song learning is mostly or entirely absent (Tobias et al. 2012; Touchton et al. 2014).

The song learning hypothesis assumes that song learning promotes the rapid evolution of novel song variants that serve as premating barriers between populations. In birds, speciation is initiated in allopatry (Barraclough and Vogler 2000; Price 2008), and theoretical population genetic models of song evolution demonstrate that song learning accelerates song divergence and hence allopatric speciation over a wide range of plausible conditions (Lachlan and Servedio 2004). Once divergent songs have evolved in allopatry, song learning could generate culturally based reproductive isolation upon secondary contact in oscine passerines. For example, in 1981, an immigrant medium ground finch (*Geospiza fortis*) that was especially large and sang an unusual song arrived to the island of Daphne Major in the Galapagos (Grant and Grant 2009). The descendants of this immigrant individual bred with each other and not with resident medium ground finches. In this case, reproductive isolation was based in large part on the distinctive songs of males, which male chicks learned and female chicks sexually imprinted upon. Similarly, two allopatric subspecies of zebra finch mate assortatively in captivity due in large part to female preference for their father’s song phenotype, a preference that is generated via sexual imprinting (Clayton 1990).

The song learning hypothesis makes two clear predictions that can be tested with comparative data. First, allopatric populations of song learners (oscines) should evolve acoustic differences in song faster than allopatric populations of nonlearners (suboscines). Second, these acoustic differences should constitute a premating barrier to reproduction. That is, birds are predicted to perceive differences between local versus allopatric song, and behaviorally discriminate against allopatric song (e.g., due to imprinting generating a preference for local song). If so, the song learning hypothesis predicts that song learners (oscines) should evolve behavioral discrimination against allopatric song faster than nonlearners (suboscines). We tested these two predictions in a large sample of allopatric sister pairs of Neotropical oscines and suboscines of varying divergence times (measured using genetic distance in mtDNA) by (1) measuring acoustic traits to analyze evolutionary rates of acoustic divergence, and (2) conducting field

playback experiments to measure evolutionary rates of behavioral song discrimination. This study is a rare empirical investigation of how phenotypic plasticity is associated with trait divergence and the evolution of premating barriers to reproduction.

Methods

PLAYBACK EXPERIMENTS TO MEASURE SONG DISCRIMINATION

We conducted playback experiments in Costa Rica and Panama in March–April 2015 and February–April 2016 to measure song discrimination in 69 allopatric sister pairs of Neotropical passerines (42 oscines and 27 suboscines). This total includes both populations classified as distinct species ($n = 31$; 20 oscines and 11 suboscines) and as subspecies ($n = 38$; 22 oscines and 16 suboscines; Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.3hp02>). In total, we conducted playback experiments on 74 populations comprising 69 sister pairs (five of which were reciprocal, see below), with a sample size of 12.2 ± 2.8 territories per population (mean \pm standard deviation; range = 5–21). Our playback experiments simulate secondary contact between related allopatric populations. Because we define song discrimination as ignoring allopatric song (see below), we use song discrimination by territorial birds as a proxy for mate discrimination (Isler et al. 1998; Uy et al. 2009). That is, we assume that populations that ignore allopatric song are likely to have evolved substantial premating reproductive isolation based on song (Payne 1986; Baker and Baker 1990; Alström and Ranft 2003; Patten et al. 2004).

Playback experiments followed a standard methodology (McGregor et al. 1992; Pegan et al. 2015). Briefly, each experiment measured the behavioral response of a territorial bird to two treatments: (i) a song from the local population (sympatric treatment) and (ii) a song from the foreign, allopatric population (allopatric treatment). We alternated treatment order between territories to examine possible sequence effects of playback treatments. We used large banks of natural vocalizations archived at xeno-canto.org and the Macaulay Library at the Cornell Lab of Ornithology to maximize independence of replicates ($n = 6.6 \pm 1.7$ recordings/treatment, mean \pm SD), and used a single recording in each treatment. We used good quality recordings in playback experiments (most recordings were graded “A” on xeno-canto, or rated three or more stars on Macaulay Library), and did not normalize amplitudes. Each treatment consisted of placing a wireless speaker (UE Roll or JBL Charge 2+) within a territory, broadcasting song at natural amplitudes (approximately 80 dB at 1 m from the speaker) for two minutes, and observing behavioral responses both during the two minutes of playback and a subsequent five-minute observation period. We recorded multiple

behavioral responses to playback (closest approach to speaker, latency to approach speaker, latency to vocalize, numbers of songs, and number of responding individuals) for each treatment period. However, for this study, we focus only on closest approach to the speaker (m), a reliable indicator of behavioral response to playback (Peters et al. 1980; Martin and Martin 2001; Searcy et al. 2006; Freeman 2015; Freeman et al. 2016). We measured closest approach to speaker by eye, or by pacing the distance between the birds' closest approach and the location of the speaker. At the beginning of each treatment, territory owners were within hearing distance out of sight (> 15 m distant from the speaker) or, more uncommonly, visible > 15 m distant from the speaker. If a bird was still responding to playback at the conclusion of the first treatment (e.g., if the territorial bird(s) remained within 15 m of the speaker, or continued to vocalize at an elevated rate), we waited until two minutes after it stopped responding (moved > 15 m away but stayed within hearing distance, and ceased vocalizing at an elevated rate) before initiating the second treatment.

In our experimental design, the sympatric treatment serves as a positive control—we expect territorial birds to respond aggressively to sympatric song playback. Thus, in our analysis we only included experiments where the putative territory owner approached to within 15 m of the speaker in response to the sympatric treatment (typically to within 5 m of the speaker), reasoning that such birds were actively defending territories. Because cases where a territory owner responded to the allopatric treatment but not the sympatric treatment were rare ($< 2\%$ of experiments), this method should not bias our estimates of song discrimination. Our aim was to use behavioral response to playback experiments as a measure of species discrimination relevant to premating reproductive isolation based on song. Thus, we define song discrimination for this study as instances where the territory owner(s) ignored allopatric song, defined as a failure to approach within 15 m of the speaker in response to the allopatric treatment. We calculated song discrimination for a sister pair as the proportion of territories that discriminated against allopatric song.

Our method of measuring song discrimination is prone to false positives; in individual experiments, birds that approach to within 15 m of the speaker out of curiosity or happenstance are considered as “responders.” Although the vast majority of individuals approaching to within 15 m of the speaker in response to a treatment were clearly aggressively defending their territories, for some species (both oscines and suboscines) it was difficult to distinguish between a weak aggressive territorial defense and a bird that was curiously investigating a novel sound (B.G.F. and G.A.M. pers. obs). For this comparative study, we therefore use a common “all or none” currency to measure song discrimination rather than species-specific metrics of discrimination. Though this approach will inevitably include some false positives, we

have no a priori expectation that suboscines and oscines will differ in their rates of false positives. Finally, we note that a consequence of measuring song discrimination in this manner is that we will underestimate the true rate of song discrimination. That is, a method that generated fewer false positives would lead to higher estimates of song discrimination; our inferred evolutionary rates of behavioral discrimination are thus minimum estimates.

Birdsong functions in both mate choice and territorial defense. Biologists often equate territorial defense with males, but female song is widespread in tropical birds and females of many tropical species participate in territorial defense (Odom et al. 2014). Indeed, we observed multiple individuals aggressively respond (i.e., approach the speaker) to playback experiments in approximately 50% of all treatments where at least one bird approached to within 15 m of the speaker (453 out of 907 sympatric treatments, and 244 out of 471 allopatric treatments). Species varied in their propensity for multiple individuals to respond to playback; for many taxa, multiple birds approached the speaker in nearly all cases (e.g., most antbirds, wrens, and warblers), while responses by multiple birds were less common in other species (e.g., thrushes and woodcreepers). When we observed multiple individuals respond to playback ($n = 697$ instances), we observed two birds 80% of the time ($n = 556$), and groups of three or more birds less frequently (20% of the time; $n = 141$). Most of the species we studied are sexually monomorphic, and we were thus unable to identify the sex of the responding birds. In the few species where males and females obviously differ in plumage (e.g., antbirds), multiple responding individuals always included both males and females (presumably mated pairs or family groups). We think it is likely that multiple responding individuals in monomorphic species also constituted mated pairs (or family groups). If so, our dataset consist of species where both males and females participate in territorial defense.

Our playback experiments measure discrimination against allopatric song in the context of territorial defense. We argue that our data describing territorial responses to allopatric song are a reasonable (and likely conservative) proxy for inferring premating reproductive isolation between populations. This is because selection on females choosing mates is likely stronger than on birds (both males and females) engaging in territorial defense, as the cost of responding to a novel signal by mounting an aggressive territorial defense is presumably small relative to the cost of making a mistake in mate choice. As a result, the response function to song is typically broader for birds engaging in territorial defense compared to females choosing mates (Searcy and Brenowitz 1988; Patten et al. 2004; Seddon and Tobias 2010; Danner et al. 2011; Curé et al. 2012). That is, if a territorial bird (typically considered to be a male, although in this study it also could be a female) ignores a song in a territorial context, it is likely that a female would also discriminate against that song in

a mate choice context. Consequently, we assume that sister pairs where a majority of territorial individuals fail to approach the speaker (for this study, song discrimination scores > 0.5) have likely evolved a degree of premating reproductive isolation based on song.

GENETIC DISTANCES

We obtained homologous mitochondrial DNA sequences (primarily ND2 and cytochrome *b*) from GenBank for most sister pairs ($n = 63$ sister pairs; 37 oscines and 26 suboscines, see Table S1 for information on the mtDNA markers used in each case). We then calculated uncorrected sequence divergence (p-distances) between sister pairs in MEGA5 (Tamura et al. 2011). Mitochondrial DNA sequences are often considered to represent neutral loci that evolve in a relatively clock-like fashion, with approximately 2% divergence per million years (Weir and Schluter 2008), such that mitochondrial genetic distances serve as a proxy for the time elapsed since sister species last shared a common ancestor. If mitochondrial haplotypes are not selectively neutral (Dowling et al. 2008; Ribeiro et al. 2011), using mitochondrial genetic distances to evaluate evolutionary rates in suboscines and oscines will be biased if rates of mitochondrial evolution differ between oscines and suboscines. However, there is no a priori expectation that rates of mitochondrial divergence differ between suboscines and oscines. We tested this assumption by comparing mitochondrial genetic distances to genetic distances from multilocus trees downloaded from birdtree.org (Jetz et al. 2012) with the Hackett backbone including only taxa with genetic data. This method produced 21 sister pairs (15 oscines and six suboscines) with matched estimates for mtDNA divergence (from GenBank) and multilocus divergence (from Jetz et al. 2012). The two measures of genetic distance were tightly correlated ($r^2 = 0.78$, Fig. S1), and the best-fit linear model did not include clade identity ($df = 1$, $F = 1.19$, $P = 0.28$). Thus, our use of mitochondrial genetic distances appears to be appropriate for this dataset.

STATISTICAL ANALYSIS OF SONG DISCRIMINATION

All statistical analyses were carried out in R (R Development Core Team 2014). We analyzed evolutionary rates of song discrimination in a hypothesis-testing framework. Our null hypothesis was simply that evolutionary rates of song discrimination do not differ between oscines and suboscines; our alternate hypothesis was that evolutionary rates of song discrimination do indeed differ between these two groups of passerines. Our response variable, song discrimination, is a proportion bounded by 0 and 1. Further, we expect song discrimination to be 0 when genetic distance equals 0 (i.e., no discrimination to local, sympatric song). We used Michaelis–Menten curves, fit using the “nls” function, to model evolutionary rates of song discrimination given

genetic distance. Our null model fit the formula $y = ax/(b + x)$ to the full dataset, where a is the asymptote and b is a measure of the rate of increase. Our alternative model fit the formula $y = ax/(b + c\delta + x)$, where δ is an indicator variable of clade identity (in our case, $\delta = 1$ for suboscines and $\delta = 0$ for oscines) and c is the difference in rate between the two groups, such that $b + c$ estimates the rate of increase for suboscines and b alone estimates the rate of increase for oscines. By fitting the same asymptote “ a ” to the two groups we allow rates of increase (but not the asymptote) to differ between clades. We assume that, at very large genetic distances, both clades will eventually converge upon the same high level of song discrimination and have similar asymptotes.

Last, visual inspection of histograms of song discrimination scores suggested that suboscine song discrimination scores were bimodally distributed while oscine song discrimination scores were not (see Results). We formally evaluated whether suboscine and oscine song discrimination distributions differed in their number of modes using the “mclust” package (Fraley et al. 2012), which fits Gaussian mixture models and uses Bayesian Information Criteria (BIC) to measure relative support for a one-mode versus bimodal distribution of discrimination values.

VOCAL TRAITS ANALYSIS

We analyzed 1018 songs from 126 populations representing 63 of 69 sister pairs (8.1 ± 1.7 songs per population; mean \pm SD) in the software Raven Pro 1.5 (BioacousticsResearchProgram 2014). We did not include sister pairs in this analysis that had small sample sizes of audio recordings (< 5 songs/population), or where we could not accurately define individual notes in Raven Pro because variation in note morphology was continuous rather than discrete. We measured acoustic traits for the same recordings used in playback experiments, supplementing these with additional recordings downloaded from xeno-canto (<http://xeno-canto.org>) and the Macaulay Library at the Cornell Lab of Ornithology (<http://macaulaylibrary.org>) to boost sample sizes. For a representative song from each recording, we measured seven song variables (total note count, mean note rate, mean note length, peak frequency, low frequency, mean note frequency range, and total song frequency range) following Mason et al (2014). We measured minimum and maximum frequencies of notes by eye on spectrograms, and used waveforms to aid in delimiting temporal measures (i.e., note length). Calculating minimum and maximum frequencies by eye (as opposed to using threshold approaches) has been shown to generate bias in studies examining the role of anthropogenic noise on song frequencies (Zollinger et al. 2012; Ríos-Chelén et al. 2017). Our method to measure frequency is unlikely to bias the results of this study, as we have no a priori expectation that frequency measurements taken by eye will systematically differ between oscines and suboscines, and

because we did not analyze recordings that had substantial anthropogenic noise (which makes it difficult to accurately measure frequency by eye). Within Raven, we used a Hann spectrogram window with 512 samples, a time grid with an overlap of 50 percent and a hop size of 256 samples, and a frequency grid with discrete Fourier transform set at 512 and grid spacing of 86.1 Hz.

To analyze patterns of variation in acoustic traits in oscines and suboscines, we log-transformed total note count and ran a principal components analysis (PCA) using the correlation matrix based on the centered and scaled dataset in which all variables were set to mean of zero and a standard deviation of one. This method places all recordings of all populations in the same acoustic space. In this analysis, PC1, PC2, and PC3 explained 44.2%, 28.4%, and 9.9% of variation, respectively. We calculated absolute acoustic divergence for each sister pair as the distance between population means along PC1, and fit Brownian motion models to examine how absolute acoustic divergence in sister pairs evolves as a function of genetic distance (Martins 1994). To minimize skew of residuals, we transformed our data as $\log(\text{absolute acoustic divergence} + 1)$. We then evaluated whether suboscines and oscines differed in their evolutionary rates of absolute acoustic divergence by comparing AIC values of a model that fit a single rate to the entire dataset (single slope model) versus a model that fit different rates for oscines and suboscines (two-slopes model); we calculated AIC values in the EvoRAG package (Weir and Lawson 2015).

Analyzing absolute trait divergences between oscines and suboscines is problematic if these two clades systematically differ in their degree of within-population trait variation. To investigate this possibility, we quantified within-population variation in acoustic space as the standard deviation among individuals for each population along PC1, PC2, and PC3, and used *t*-tests to determine if these two metrics of variation differed between oscines and suboscines. We then calculated the pooled standard deviation for each sister pair, and quantified standardized acoustic divergence within each sister pair as the distance between population means along PC1 expressed in units of pooled standard deviations. We again fit Brownian motion models to examine how standardized acoustic divergence in sister pairs evolves as a function of genetic distance following Martins (1994), and transforming our data as $\log(\text{absolute acoustic divergence} + 1)$ to minimize skew of residuals. We then used EvoRAG (Weir and Lawson 2015) to compare AIC values of a model that fit a single rate to the entire dataset (single slope model) versus a model that fit different rates for oscines and suboscines (two-slopes model). Last, we investigated the correlation between standardized acoustic divergence and behavioral song discrimination from playback experiments, and used fitted Michaelis–Menten curves and the “anova” function to test whether suboscines and oscines differed in their rela-

tionship between standardized acoustic divergence and behavioral song discrimination.

Results

Our results contradict the predictions of the song learning hypothesis. We found that suboscines evolved mean differences in song at a slightly faster rate than did oscines (Fig. 1; a single slope model has less support than a two-slopes model; $\Delta\text{AIC} = 2.91$). Moreover, evolutionary rates of song discrimination are much faster in suboscines (nonlearners) than in oscines (song learners; Fig. 2)—a model with different rates of divergence in suboscines and oscines was a significantly better fit to our data than a model fitting the same rate to the two clades ($df = 1$, $F = 10.13$, $P = 0.0023$). Our models estimate that allopatric sister pairs of suboscines evolve song discrimination roughly four times faster than do oscines. For example, suboscines evolved a song discrimination value of 0.5 after ~ 1 million years (genetic distance = 1.9%), whereas oscines evolved the same value only after ~ 4 million years (genetic distance = 8%).

Our analysis of acoustic traits suggest that the observed fourfold faster evolutionary rate of song discrimination in suboscines is linked to the lower amount of within-population song variation in suboscines relative to oscines. Four results point to this conclusion. First, absolute song divergence increases with increasing genetic distance similarly for suboscines than oscines (but slightly faster for suboscines; Fig. 1). Second, oscines were significantly more variable in song within populations than were suboscines, with oscine populations 79%, 59%, and 54% more variable than suboscine populations along PC1, PC2, and PC3, respectively (Fig. 3; for PC1: $t = 6.67$, $df = 122.7$, $P \ll 0.001$; for PC2: $t = 5.15$, $df = 120.1$, $P \ll 0.001$; for PC3: $t = 3.28$, $df = 119.5$, $P = 0.0014$). Third, these previous two results imply that at a given genetic distance, suboscine populations are far more divergent in song than are oscines when divergence is scaled by within-population variation. This is indeed the case. Suboscines evolve standardized acoustic divergence, when difference is expressed in units of pooled standard deviations, much faster than do oscines (Fig. 4; a two-slopes model has considerably more support than a single slope model; $\Delta\text{AIC} = 7.28$).

Finally, song discrimination in oscines and suboscines is more closely tied to standardized divergence in song rather than to the absolute difference. That is, oscines and suboscines have a similar relationship between song discrimination and standardized acoustic divergence (Fig. 5, $df = 1$, $F = 0.081$, $P = 0.77$), but song discrimination is marginally higher in suboscines than oscines when using absolute acoustic divergence (Fig. S2, $df = 1$, $F = 3.2$, $P = 0.079$). This finding indicates that oscines and suboscines appear to follow the same “rules” for perceiving and

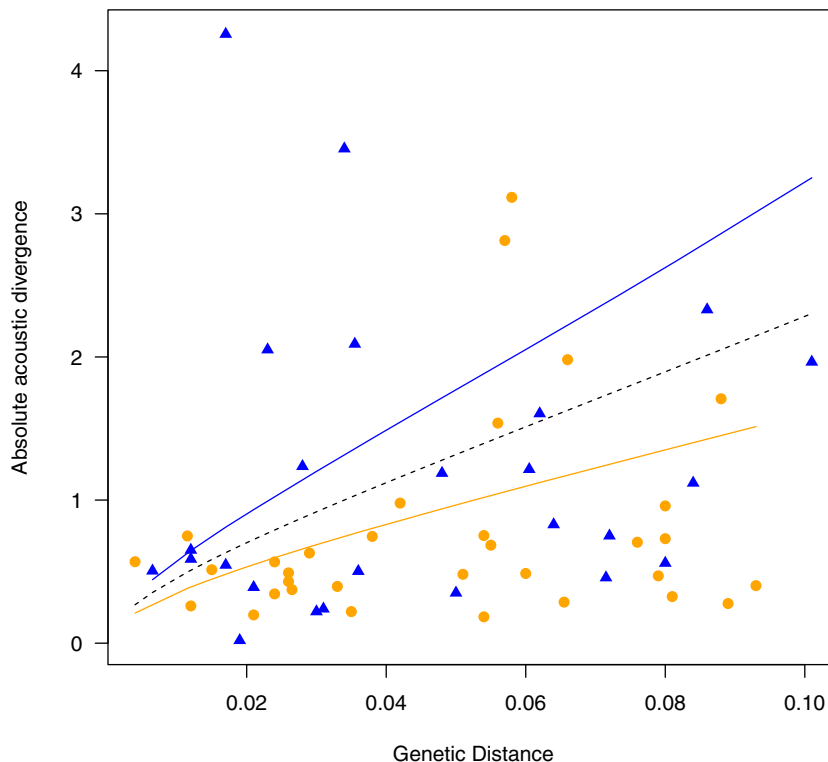


Figure 1. Evolutionary rates of absolute acoustic divergence in suboscine (blue triangles) and oscine (orange circles) sister pairs. Absolute acoustic divergence is measured as the distance between population means in acoustic space (along PC1) within a sister pair, and genetic distances are measured from homologous mitochondrial DNA sequences. The trend lines illustrate predictions from the best-fit Brownian Motion model for both the entire dataset (dashed line) and for oscines and suboscines (colored lines). Absolute acoustic divergence significantly increases with genetic distance at a slightly faster rate for suboscines than for oscines.

discriminating against allopatric song when within-population variation is incorporated. Song discrimination in both oscines and suboscines is high when mean acoustic differences between populations become separated by about four standard deviations and overlap in song traits between populations along PC1 in multivariate acoustic space is minimal. In sum, the greater within-population song variation means that oscines must evolve greater absolute differences in song before achieving the same level of discrimination as suboscines. This points to “a downside of learning” for the evolution of species discrimination that is not incorporated into the song learning hypothesis.

Lower within-population variation in suboscine song, combined with roughly similar rates of absolute song divergence in suboscines and oscines, implies that the evolution of song discrimination in suboscines should occur faster than in oscines. That is, oscines should require longer evolutionary time than suboscines to make the transition from low discrimination to high discrimination. This logic may explain the observed difference in the distribution of discrimination between sister pairs of suboscines and oscines (Fig. 6). Suboscine sister pairs show either low or high song discrimination, indicating greater consistency between territorial individuals within a population in their behavioral response

to allopatric song. In contrast, oscines commonly had intermediate discrimination scores, indicating substantial within-population variation in behavioral response to allopatric song. Gaussian mixture models provide statistical support for this observation; for suboscine sister pairs, the best-fit model had two components ($\Delta\text{BIC} = 9.32$), providing strong support for the inference that the suboscine distribution is sampled from two distributions with distinct means (Fig. S3A), while for oscines, models with one and two components had similar fits (the one component model had a slightly better fit; $\Delta\text{BIC} = 0.92$, see Fig. S3B), indicating that oscine song discrimination scores fit a unimodal distribution relatively well (Fig. 6B).

Discussion

Birdsong is an important part of avian mate choice—populations that sing different songs typically do not interbreed (Baker and Baker 1990; Grant and Grant 1996). Thus, any factor that accelerates song evolution may speed up speciation rates in birds. The capacity of individuals to learn their songs (present in three clades of birds; the hummingbirds, parrots, and oscine songbirds) has been hypothesized to be one such factor, explaining in part why song

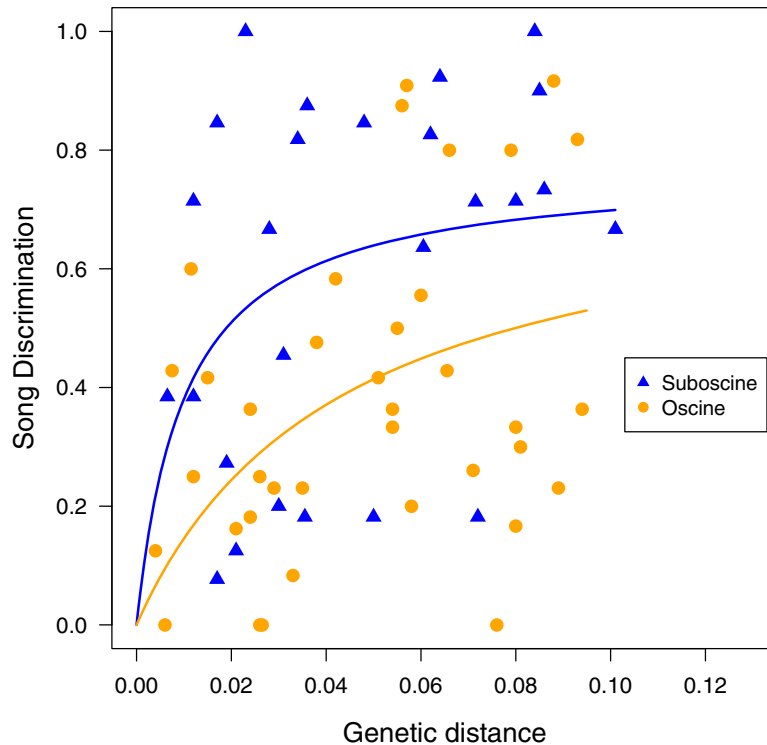


Figure 2. Evolutionary rates of song discrimination for sister pairs of suboscine (blue triangles) and oscine (orange circles) passerines, with model predictions plotted from the best-fit Michaelis–Menten curve. Each point represents an allopatric sister pair. Song discrimination is the proportion of territories in a population that failed to approach the speaker in response to playback of its allopatric sister population; song discrimination scores above 0.5 indicate sister pairs in which the majority of territories ignored allopatric song. Genetic distances are measured from homologous mitochondrial DNA sequences. Contrary to the prediction of the song learning hypothesis, suboscines (innate song) have significantly faster evolutionary rates of song discrimination in geographic isolation than do oscines (learned song).

learning clades harbor high species richness. This “song learning” hypothesis predicts that, when comparing sister pairs of related, geographically isolated populations, song learning oscines evolve differences in song that are relevant to species recognition faster than do nonlearning suboscines. Contrary to these predictions, we found that, in a large set of allopatric sister pairs of Neotropical passerines, (1) the evolutionary tempo of mean acoustic divergence was somewhat faster in suboscines than oscines (Fig. 1), and (2) nonlearning suboscines evolve behavioral song discrimination significantly faster than do song-learning oscines (Fig. 2). These results call into question the hypothesis that oscines’ capacity to learn songs explains why they are far more diverse than suboscines.

Our principal finding is that song learning is associated with slower evolution of song discrimination in Neotropical passerines. The slower evolutionary tempo of behavioral song discrimination in song learning oscines appears to result in large part from their greater variability in song within populations. This inference is supported by the following results. First, as mentioned above, suboscines and oscines had similar evolutionary rates of abso-

lute song divergence (Fig. 1). Second, nonlearning suboscines showed significantly less within-population variation in acoustic space than do song learning oscines (Fig. 3). Third, this difference in within-population variation results in suboscines having significantly faster evolutionary rates of standardized acoustic divergence (Fig. 4). Finally, behavioral song discrimination is more closely tied to standardized song differences than to absolute differences in song (Fig. 5, Fig. S2). In sum, our results are consistent with a model wherein plasticity in mating display traits increases trait overlap between related populations, thereby slowing the rate at which pre-mating barriers to reproduction evolve between isolated populations.

COMPARISONS WITH SIMILAR STUDIES

This study is a rare empirical investigation of how learning, a type of phenotypic plasticity, is associated with divergence in a trait important for mate choice (birdsong). We can compare our results to two previous studies that have investigated patterns of song evolution in oscines and suboscines. Our results are similar to the findings of a previous analysis of passerine sister pairs that

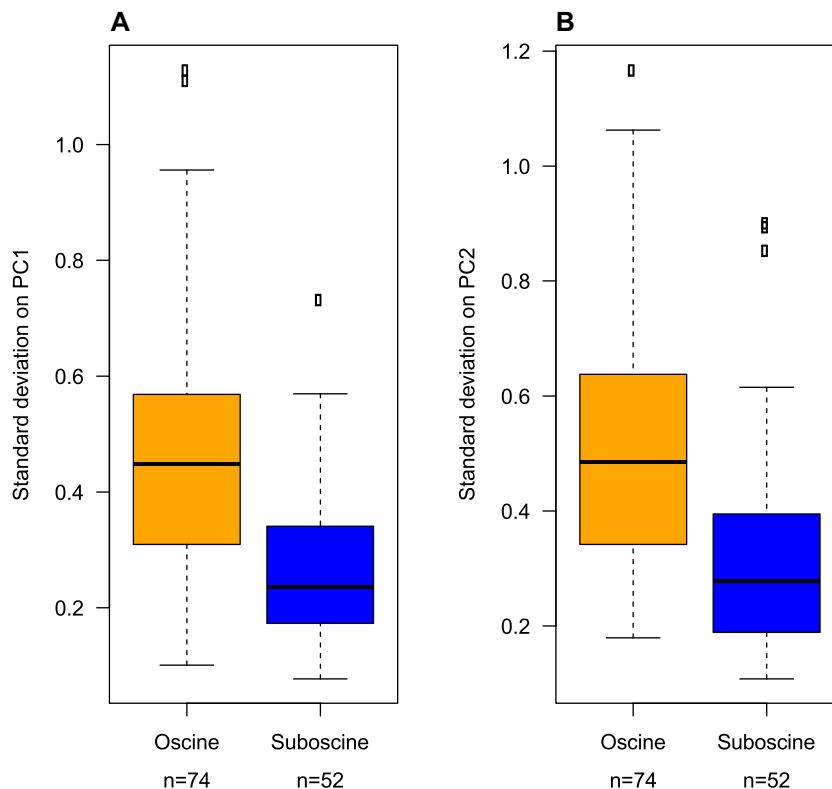


Figure 3. Suboscines show greater within-population variation in acoustic space than oscines. When all populations (mean = 8.0 recordings/population) are analyzed in the same multivariate space, oscine populations are 78% more variable than are suboscine populations along PC1 (A) and 61% more variable along PC2 (B).

found evolutionary rates of divergence in syllable diversity and song length were similar in tropical oscines and suboscines (Weir and Wheatcroft 2011). In contrast, a recent comparative analysis found rates of song evolution to be higher in a diverse Neotropical clade of song learners (oscines; thraupids) compared to a similarly diverse codistributed clade of nonlearners (suboscines; furnariids; Mason et al. 2017). These conflicting results may result from differences in phylogenetic scope: patterns of song evolution may differ at the tips of phylogenetic trees (sister pair studies that consider only the recent past) compared with deeper phylogenetic scales (whole clade analyses). Last, further research is needed to test if patterns of song divergence vary across the latitudinal gradient; temperate zone sister pairs of oscines show faster divergence in syllable diversity (but not song length) compared to temperate zone suboscines, though this finding was based on a small sample size of temperate zone sister pairs of suboscines (Weir and Wheatcroft 2011).

Comparative studies of birdsong measure acoustic divergence in a variety of ways. Studies can analyze divergence in single acoustic traits or employ multivariate analyses that consider many dozens of acoustic traits. We used a multivariate approach analyzing seven acoustic traits, as multiple song traits are typically important in species recognition, and almost any song trait

can be used in species recognition (Peters et al. 1980). We find that both oscines and suboscines show strong behavioral discrimination when sister pairs differ in acoustic space by around four or more standard deviations along PC1 (Fig. 5). This suggests that oscines and suboscines use similar rules based on within population variation in song traits to determine when allopatric song is sufficiently different from local song that it is ignored. However, relatively few sister pairs in our data have distances larger than around four standard deviations, and there are many sister pairs that show high song discrimination despite low acoustic divergence (Fig. 5). More detailed acoustic analyses that consider additional acoustic traits would be useful to better investigate the putative threshold of \sim four standard deviations that describes how behavioral discrimination is related to divergence in multi-dimensional acoustic space.

THE EVOLUTIONARY TEMPO OF PREMATING REPRODUCTIVE ISOLATION

Our playback experiments created “experimental secondary contact.” That is, our experiments offer insight into how naive individuals might respond to allopatric song at the initiation of secondary contact. We used behavioral song discrimination as a proxy for pre mating reproductive isolation based on song. As such, we

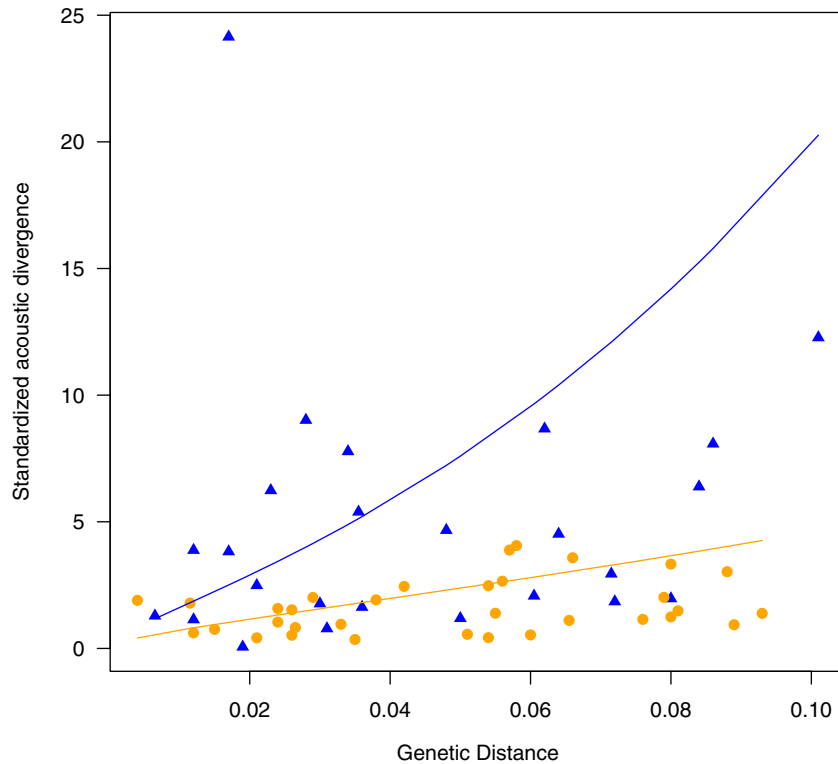


Figure 4. Evolutionary rates of standardized acoustic divergence are significantly faster in suboscine sister pairs (blue triangles) than in oscine sister pairs (orange circles). Standardized acoustic divergence is measured as the distance between population means along PC1 within a sister pair expressed in pooled standard deviations, and genetic distances are measured from homologous mitochondrial DNA sequences. The trend lines illustrate predictions from the best-fit Brownian Motion model.

interpret low levels of song discrimination to indicate a lack of pre-mating reproductive isolation based on voice, and high levels of song discrimination as stronger pre-mating reproductive isolation. It is unknown what level of song discrimination in allopatry would be sufficient to generate pre-mating reproductive isolation based on song were secondary contact to occur (Hudson and Price 2014). Here, we assume that sister pairs where the majority of territorial birds we tested ignored allopatric song (song discrimination values greater than 0.5) have evolved considerable pre-mating reproductive isolation based on song. If so, our data provide an approximation of the tempo by which pre-mating reproductive isolation based on song evolves in Central American passerines. In our dataset, it takes on the order of one million years in allopatry (genetic distance = 1.9%) for sister pairs of Central American suboscines to evolve pre-mating reproductive isolation based on song (song discrimination = 0.5), and 4 million years for sister pairs of oscines (genetic distance = 8%). In contrast, sister pairs of Amazonian suboscines that are estimated to be relatively old (~ 3–4 million years) have not yet evolved song differences that constitute pre-mating barriers to isolation, and hybridize at the headwaters of major river drainages (Weir et al. 2015). The discrepancy between our data and these old Amazonian hybrid zones may indicate that Amazonian suboscines evolve song discrimination much

more slowly than Central American suboscines, or that song discrimination values between allopatric populations need to be greater than 0.5 to suggest the evolution of a pre-mating barrier to reproduction.

Our estimates of the tempo of pre-mating reproductive isolation in tropical passerines can be compared to published data estimating how long it takes for secondary contact following allopatric speciation to occur in this avifauna. Weir and Price (2011) analyzed divergence times between sympatric and allopatric sister species (and phylogroups) to estimate that it takes roughly three million years after lineages split to attain secondary sympatry in both Neotropical oscines and suboscine. Because we estimate that suboscines evolve considerable pre-mating reproductive isolation based on song in allopatry after one million years, we suggest that suboscines, but not oscines, are likely to have evolved substantial pre-mating reproductive isolation based on song in allopatry prior to secondary contact. If so, the buildup of suboscine diversity within regions may be limited more by factors that influence range expansion and population persistence rather than by the evolution of pre-mating reproductive isolation (Rabosky and Matute 2013; Price et al. 2014).

Our estimates of the tempo of pre-mating reproductive isolation in tropical passerines are also relevant to the debate on

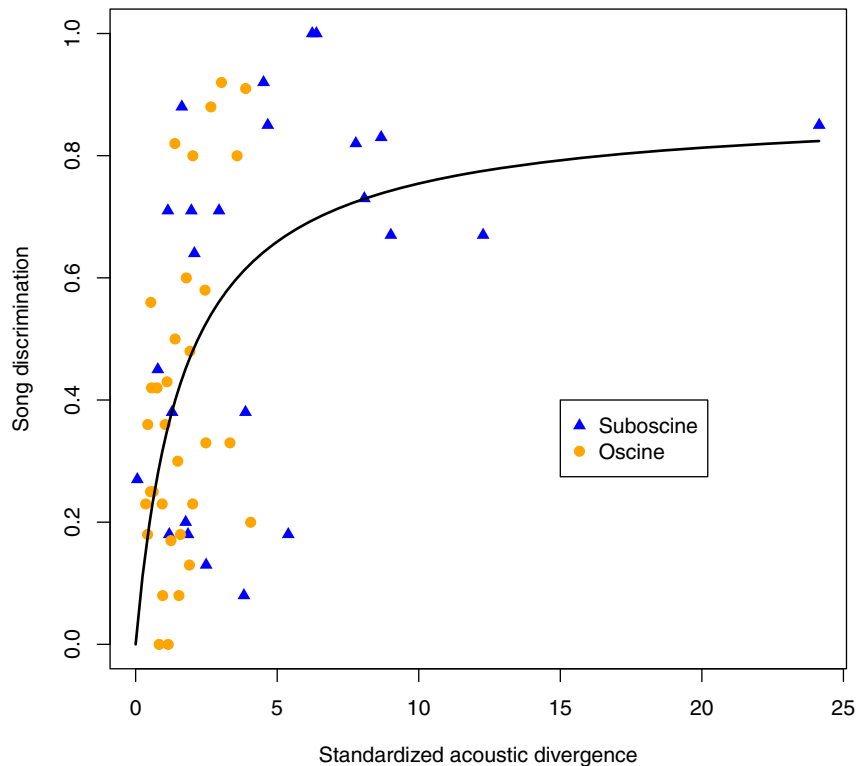


Figure 5. Oscines (orange circles) and suboscines (blue triangles) have a similar relationship between song discrimination and standardized acoustic divergence. Sister pairs with standardized acoustic divergence greater than around four have high song discrimination, but song discrimination is more variable at low values of standardized acoustic divergence. The trendline illustrates predictions from the best-fit Michaelis-Menten model.

how and when interactions with close relatives drive trait divergence (Schluter 2000; Pfennig and Pfennig 2012; Tobias et al. 2014; Freeman 2015). Sympatric species of both suboscines and oscines typically differ substantially in song (Isler et al. 1998; Seddon et al. 2008), a pattern that could reflect divergence in allopatry, reproductive character displacement or reinforcement upon secondary contact, or a combination of these two processes (Wilkins et al. 2013). As noted above, allopatric sister pairs of suboscines are likely often to have evolved substantial premating reproductive isolation based on song (and high values of standardized acoustic divergence) in allopatry prior to secondary contact, at least when considering sister pairs that occur in Central America. This suggests that sympatric species of suboscines may differ in song in large part due to divergence in allopatry rather than solely as a consequence of character displacement (though interactions in sympatry can also influence suboscine song evolution, including promoting song convergence; Seddon and Tobias 2010; Tobias et al. 2014).

In contrast, oscine sister pairs in our dataset often failed to evolve premating reproductive isolation based on song in allopatry, even over periods of time longer (> four million years) than that necessary for Neotropical oscines to achieve secondary con-

tact following speciation (Weir and Price 2011). That sympatric oscines nevertheless are typically divergent in song (Grant and Grant 1996; Price 2008) suggests that reproductive character displacement or reinforcement upon secondary contact may play a relatively greater role in the evolution of oscine song, supporting the idea that learning facilitates reinforcement by increasing assortative mating (Irwin and Price 1999; Servedio et al. 2009). Relevant to this speculation, there are many examples of oscine sister pairs where around half of tested territories ignore allopatric song while the other half respond (Fig. 6, Fig. S3). Oscine sister pairs with intermediate song discrimination have already diverged somewhat in song and species recognition; this variation in discrimination could facilitate reinforcement upon secondary contact (in contrast to suboscines, which show either low or high song discrimination in allopatry, Fig. 6, Fig. S3).

Conclusion

The oscine passerines are the most remarkable radiation within birds, and many classic examples of rapid diversification fall within this clade, including Darwin's Finches (Grant and Grant 2009), North American wood-warblers (Lovette et al. 2010),

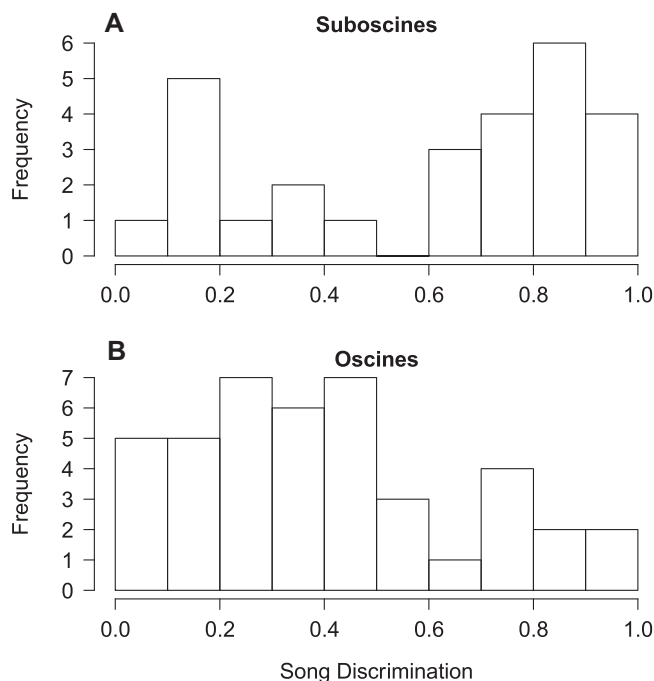


Figure 6. Histograms of song discrimination scores for sister pairs of suboscines (A) and oscines (B). Suboscines ($n = 27$) have a bimodal distribution with sister pairs having either low or high song discrimination, with few intermediate cases; oscines ($n = 42$) show a unimodal distribution (see also Fig. S3).

Hawaiian honeycreepers (Lerner et al. 2011), and “capuchino” seedeaters (Campagna et al. 2012). Oscines learn their songs, and this ability has been hypothesized to partially explain why oscines comprise nearly half of all extant birds and why oscines are four times more diverse than their sister clade, suboscines, which have innate song. The “song learning” hypothesis predicts that the learned behavioral trait of birdsong accelerates evolutionary rates of song divergence and species discrimination in oscines, leading to faster speciation. However, we found that the tempo of song discrimination is significantly faster in allopatric sister pairs of Central American suboscines (nonlearners) compared to oscines. Our results demonstrate that the observed faster diversification of oscines than suboscines is unlikely to be due to song learning promoting faster rates of song evolution and species discrimination in allopatry. Instead, the reasons why oscines are so much more diverse than suboscines are likely due to differences in life-history traits (e.g., Kennedy et al. 2014), or ecological (rather than reproductive) consequences of plasticity, such as if oscines’ greater capacity to learn facilitates adaptation that promotes range expansion or population persistence (e.g., Fitzpatrick 1988).

The discrepancy between the expectation that song learning promotes divergence and our finding that song learning reduces divergence appears to be mostly due to the largely overlooked impact of learning on trait variation. We found that learning is

associated with increased acoustic variation within populations, and, for a given amount of absolute divergence in song traits, with reduced discrimination against a signal from a related, allopatric population. These results point to “a downside to learning” for speciation: learning increases trait variation that slows evolutionary rates of discrimination against signals important for pre-mating reproductive isolation.

AUTHOR CONTRIBUTIONS

BGF designed this study; GAM and BGF conducted fieldwork; BGF and DS carried out analyses; BGF wrote the first draft and DS and GAM provided extensive comments and edits.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Genetic distances in mitochondrial DNA in sister pairs are highly correlated with branch length distances from multi-locus phylogenies (multi-locus data downloaded from birdtree.org).

Figure S2. Suboscine sister pairs (blue triangles) show a trend for a faster rate of song discrimination given absolute acoustic divergence than do oscines (orange circles).

Figure S3. Density estimation for suboscines (A) and oscines (B) from the “mclust” package (Fraley et al. 2012), which fits Gaussian mixture models to measure relative support for the number of distributions with equal variances (“components”) that are sampled from to generate the observed univariate distribution.