

On the predictability of phenotypic divergence in geographic isolation

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Abstract

Do related populations that are separated by barriers predictably evolve differences from one another over time, or is such divergence idiosyncratic and unpredictable? We test these alternatives by investigating patterns of trait evolution for 54 sister pairs of Andean forest birds that live in similar environments on either side of the arid Marañón Gap, a strong dispersal barrier for humid montane species. We measured divergence in both sexual (song and plumage) and ecological (beak size and beak shape) traits. Sexual traits evolve in a clock-like fashion, with trait divergence positively correlated with genetic distance ($r = 0.6\text{--}0.7$). In contrast, divergence in ecological traits is uncorrelated or only loosely correlated with genetic distance ($r = 0.0\text{--}0.3$). Thus, for geographically isolated Andean montane forest birds that live in similar environments, divergence is predictable in sexual traits, but not for ecological traits. This means that sexual trait divergence occurs independently of adaptive ecological divergence within the mega-diverse tropical Andean avifauna. Last, we show that variation in genetic divergence across a biogeographic barrier is associated with traits that are proxies for species' opportunities for dispersal (low elevation limit and elevational niche breadth), but not with traits that are proxies for species' dispersal abilities (hand-wing index and foraging strata).

Keywords: allopatry, mate choice, reproductive isolation, signal evolution, song evolution, species recognition

How predictable is the evolution of trait differences between geographically isolated populations? Given sufficient time, isolated populations will inevitably evolve differences, but the tempo of this divergence remains uncertain. Ernst Mayr famously depicted allopatric speciation as an orderly process of differentiation: the longer two populations are geographically isolated, the more their phenotypes diverge (Mayr, 1942). Eventually, the two populations are sufficiently different that they remain distinct upon secondary contact and thus have finished the speciation process (Mayr, 1947; Tobias et al., 2020). In this model, phenotypic divergence predictably accumulates with increasing time in a clock-like fashion.

An alternative is that phenotypic divergence is contingent, idiosyncratic, and unpredictable (e.g., Nosil, 2008). Indeed, plentiful case examples suggest the relationship between time and phenotypic divergence may be idiosyncratic even for related species living within a common geographic region (Román-Palacios & Wiens, 2018). For example, several lineages of passerine birds have colonized the Galapagos Islands. Some, like Darwin's finches, rapidly evolved different ecological and sexual traits on different islands and underwent rapid speciation (Lamichhaney et al., 2015). However, others, like mockingbirds and flycatchers, exhibit reduced levels of phenotypic differentiation between island populations, and also have much lower speciation rates (Arbogast et al., 2006; Carmi et al., 2016). For passerine birds on the Galapagos,

there appears no single “tempo” of phenotypic differentiation. Instead, different lineages may have different properties that accelerate or dampen rates of divergence. Substantial attention has been devoted to studying variation in the rate of speciation over macroevolutionary time scales using species-level phylogenies (Rabosky & Matute, 2013; Wagner et al., 2012; Weber & Agrawal, 2014). Additionally, numerous phylogeographic and hybrid zone studies have examined the early stages of population divergence of individual species complexes (Ottenburghs et al., 2015; Poelstra et al., 2014; Semenov et al., 2017; Toews et al., 2016). However, fewer studies have assessed whether there is predictability in the tempo of differentiation at early stages of divergence through comparative research across many recently divergent lineages (Peñalba et al., 2017, 2019; Winger & Bates, 2015).

Here we assess the predictability of rates of phenotypic differentiation in geographic isolation for 54 sister pairs of tropical Andean birds co-distributed across the the Marañón River valley in northern Peru, a major biogeographic barrier (Figure 1). Specifically, we quantify divergence in sexual and ecological traits for each sister pair of related populations that live in humid montane forests on either side of the Marañón. The desert scrub habitat of the Marañón River valley constitutes a strong barrier to individual movement between montane forest birds on either side (Fjeldsa & Krabbe, 1990; Parker et al., 1985; Villeumier, 1969; Winger & Bates, 2015; Winger et al.,

Received August 23, 2022; revisions received October 27, 2022; accepted November 25, 2022

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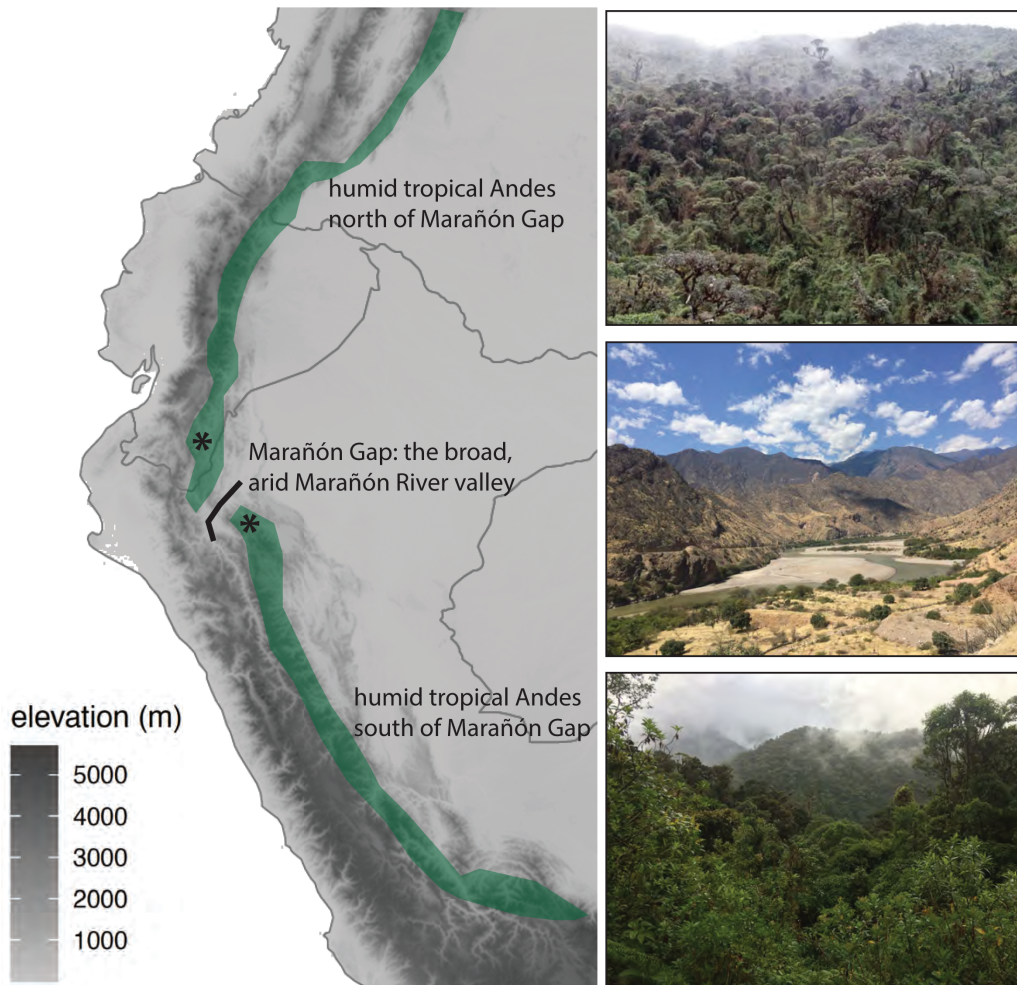


Figure 1. Map of western South America showing the location of the arid Marañón Gap (middle photo), with a schematic showing the approximate distribution of humid tropical Andean forest both north (top photo) and south (bottom photo) of the Marañón Gap. For simplicity, we highlight tropical Andean forest on the Amazon slope only. Asterisks show locations where playback experiments were conducted. Photographs by BMW (top; Tapichalaca Reserve, ~2500 m) and JH (middle: near Celendín, ~1000 m; bottom: Abra Patricia Reserve, ~2100 m).

2015). Humid tropical forest birds tend to be extremely poor dispersers (Moore et al., 2008; Salisbury et al., 2012; Sheard et al., 2020), and are unlikely to cross the ~30 km of low-elevation arid environment from humid montane forests north of the Marañón to humid forests south of the Marañón, or vice versa. These species are restricted to montane forest, making it equally unlikely that individuals could cross via a circuitous route through the lowland humid forest of the Amazon basin. This “Marañón Gap” is the biggest break in the otherwise mostly continuous ribbon of humid montane forests that occurs along the Amazonian slope of the tropical Andes from Venezuela to Bolivia (Weigend, 2002; Young & Reynel, 1997). Moreover, the Marañón Gap has likely existed for the entirety of the Andean orogeny, and indeed may have been substantially broader in the past (Bicudo et al., 2019). The Marañón Gap has thus long served as a barrier to individual dispersal of montane forest species, likely resulting in limited gene flow between populations on either side.

Genetic evidence indicates that many montane bird populations north and south of the Marañón Gap have been isolated for several million years (Winger & Bates, 2015). There are many examples of populations on either side exhibiting such high levels of phenotypic and genetic divergence that they are

ranked as distinct species. Yet dozens of other populations of montane birds show little to no genetic and phenotypic divergence across the Marañón Gap (Schulenberg et al., 2010). The large number of montane taxa distributed in similar habitats on both sides of the Marañón Gap, with variable levels of genetic and phenotypic divergence, makes this an excellent system for determining how trait evolution proceeds in geographic isolation (Winger, 2017; Winger & Bates, 2015). Our study addresses the tempo of phenotypic evolution in ecologically similar environments, in contrast to the more common approach of studying phenotypic evolution across strong environmental gradients where divergent natural selection is likely to be strong. In such cases, divergence in ecological and sexual traits may not be independent, as ecological divergence may drive sexual trait divergence (Linck et al., 2020; Moritz et al., 2000; Schluter, 2000), making the two processes difficult to disentangle. In contrast, the ecological similarity of our study system means that divergent natural selection is likely to be weak, allowing us to examine the predictability of ecological and sexual trait divergence in a system where these traits have the opportunity to evolve independently.

Our primary goal was to test the predictability of trait divergence over time. Does divergence predictably accrue in

a clock-like fashion, or are rates of divergence unpredictable and contingent on lineage-specific properties? We measured phenotypic divergence by using field playback experiments to measure vocal behavioral discrimination between sister pairs on either side of the Marañón Gap, in concert with plumage and morphological measurements from museum specimens. To test for a phylogenetic component to rates of phenotypic evolution, we asked whether rates of trait evolution differed between two sister clades of passerines, oscines, and suboscines. These analyses test whether genetic divergence predicts phenotypic divergence, and are focused on understanding observed variation in phenotypic divergence among lineages occupying similar environments.

Our secondary goal was to explain observed variation in genetic divergence. To accomplish this, we tested whether traits related to dispersal ability (relative wing length and foraging strata) and dispersal opportunity (breadth of elevational range and low elevation limit) predicted genetic divergence (Burney & Brumfield, 2009; Claramunt et al., 2012; Gadek et al., 2018; Sheard et al., 2020). Taken together, our study represents a multidimensional analysis interrogating how populations diverge from one another in geographic isolation.

Methods

Sister pair selection

We analyzed a dataset of 54 sister pairs; each sister pair comprises a montane bird population living north of the Marañón and a related montane bird population living south of the Marañón (Supplementary Table S1). We focused on sister pairs that (1) had available tissue samples or genetic data from locations close to the Marañón Gap on both the northern and southern sides, (2) were reasonably common, and (3) use song to defend territories. The latter two criteria were important to allow us to conduct large samples of field playback experiments. The intersection of these three criteria led to the choice of taxa; the 54 sister pairs we analyze here comprise ~20% of the passerine sister pairs that live in montane forest on both sides of the Marañón. We obtained measurements of genetic divergence for 51 sister pairs, song divergence using field playback experiments for 36 sister pairs, plumage divergence for all 54 sister pairs, and beak divergence for 50 sister pairs. We had complete trait data for 30 sister pairs. We intentionally sampled sister pairs across the full breadth of differentiation: 38 sister pairs are currently ranked as conspecific and 16 as distinct species (Remsen et al., 2022). Sister pairs were approximately evenly split between suboscines, which have innate songs ($n = 25$) and oscines, which exhibit vocal learning ($n = 29$).

Genetic divergence

We calculated genetic divergence for 51 of our 54 sister pairs, using 1041 bp of the mitochondrial gene ND2 from samples collected both north and south of the Marañón in Peru and Ecuador. Genetic samples were unavailable for species in three sister pairs. For 15 of 51 species pairs, we generated 41 new ND2 sequences from tissue samples provided by museum collections following standard Sanger sequencing procedures described in Winger and Bates (2015) and augmented these with appropriate sequences from previously published studies (Supplementary Table S2). Genetic data for the remaining 36 sister pairs came from previously published studies (see

Supplementary Table S2 for full sample information). Genetic sample sizes per sister pair ranged from 2 to 58 (median = 14), with 86% of sister pairs represented by multiple sequences per side of the Marañón. We estimated genetic divergence using GTR-gamma models in RAXML 7.2.8 (Stamatakis et al., 2008) following Winger and Bates (2015). Sister pairs in our dataset range in genetic divergence from 0% to 16% divergent in mtDNA (i.e., isolated for roughly 0–6 million years under the assumption of approximately 2.5%–2.9% per million years for divergence in ND2 (Lerner et al., 2011; Smith & Klicka, 2010). While recognizing the limitations of a single-locus molecular clock, potential lineage-specific variation in rates of mtDNA evolution, and gene tree discordance (García-Moreno, 2004; Lanfear et al., 2010; Nabholz et al., 2016), in the comparative context of this study we interpret mitochondrial divergence as a reasonable proxy for the relative amount of time since the two populations in a sister pair stopped exchanging genes. This assumption is supported by previous genome-wide sequencing of birds in this system that documented mtDNA divergence serves as a proxy for time since the cessation of last gene flow (Winger, 2017).

Song divergence (behavioral discrimination)

We conducted 519 field playback experiments to measure song divergence for 36 sister pairs (mean = 13.8 territories tested per sister pair, range = 5–27). On the north side of the Marañón, GAM conducted playback experiments in extreme southeastern Ecuador, primarily at Reserva Tapichalaca (Provincia de Zamora-Chinchipec; coordinates = $-4.50, -79.13$), ~120 km north of the Marañón River (282 playback experiments on 28 sister pairs completed in February–March 2017). On the south side of the Marañón, BGF and JH conducted playback experiments in the Abra Patricia region in northern Peru (Región de Amazonas; coordinates = $-5.70, -77.81$), ~80 km south of the Marañón River (215 playback experiments on 28 sister pairs completed in July 2019). For 20 sister pairs, we conducted experiments both north and south of the Marañón. For the remaining 16 sister pairs, we conducted experiments on one side of the Marañón.

Playback experiments measured behavior in response to two treatments—song from the local population (sympatric treatment) and song from the population on the other side of the Marañón River (allopatric treatment). Our general methods have been described previously (Freeman et al., 2017, 2022a). Here we describe the key details. We downloaded high quality natural vocalizations from the online archives of xeno-canto.org and the Macaulay Library of Natural Sounds ($n = 5.70 \pm 1.90$ recordings/treatment, mean \pm standard deviation). We began an experiment when we detected an individual or pair of one of our sister pairs. We placed a wireless speaker (UE Roll or JBL Charge 2+) at least 15 m from the bird (typically 15–25 m from the bird), then broadcast song at natural volumes (~80 dB) using a smartphone while standing ~10 m from the speaker. For each treatment, we broadcast song for 2 min, then observed behavioral responses for 5 min. The key behavioral response we analyzed was closest approach to the speaker, either perching or in flight, measured in horizontal distance to the speaker. If the bird(s) approached to within 5 m of the speaker before the conclusion of the 2 min of song playback, we stopped playback, as we considered this to represent an obvious strong approach response. Birds typically left the area (to more than 15 m from the speaker) and/or ceased vocalizing at an elevated rate at the end of the 5 min

of behavioral observation. If not, we waited until they left the area and/or returned to pre-playback vocal activity. We then proceeded to the second treatment. In this experimental design, the sympatric treatment serves as a positive control. We alternated the order of treatments (sympatric vs. allopatric) between experiments within a sister pair. We did not find any evidence that the order of treatments explained variation in response to playback in our data: the estimate for the effect of treatment order was close to zero (estimate \pm standard error = 0.0077 ± 0.023 , $p = .74$) in a linear model to predict whether territorial birds approached the speaker.

We quantified song divergence for each sister pair by calculating the proportion of tested territories where birds ignored allopatric song. We defined ignoring allopatric song as failing to approach within 15 m of the speaker in response to allopatric song. We focused on territorial birds that ignored allopatric song because these represent clear cases of strong behavioral discrimination against allopatric song. Responses to allopatric song were symmetric across the Marañón. For example, when northern birds ignored southern song, southern birds also ignored northern song (for 15 sister pairs with five or more experiments both north and south of the Marañón, the correlation between song divergence north vs. south of the Marañón was Pearson's $r = 0.94$; $p < .001$, [Supplementary Figure S1](#)). We therefore included all experiments, whether performed north or south of the Marañón, when calculating song divergence for each sister pair.

Plumage divergence

We measured plumage divergence for all 54 sister pairs using a plumage scoring system ([Tobias et al., 2010](#)). Plumages were scored by BMW, AEM, and OJ on the basis of examining a series of museum specimens. This approach uses categorical assignments of plumage similarity based on human vision; previous work in a subset of our study taxa found that plumage divergence estimated using the plumage scoring system was tightly correlated with plumage divergence estimated using spectrometric measurements and a model of the avian visual system (Pearson's $r = 0.84$; [Winger & Bates, 2015](#)). Human vision assessments of plumage may be especially reliable for suboscines, which appear to lack UV vision ([Seddon et al., 2010](#)). Thus, plumage scoring by humans appears to be a reasonable method to measure plumage divergence in this study system, though we recognize that unlike our methods for song divergence, this method does not assess a bird's response to plumage differences.

Beak divergence

We calculated two components of beak divergence—beak size divergence and beak shape divergence—for 51 of 54 sister pairs using morphometric measurements collected by BMW, AEM, and OJ from 1,008 museum specimens. Our aim was to measure 10 individuals (five adult males and five adult females) per population (north vs. south of the Marañón); our final morphological dataset contained 9.9 individuals per population (4.3 adult females and 5.5 adult males). We measured three functional traits (all in mm) for each specimen: (1) bill length from nares to bill tip; (2) bill width (at the nares); (3) bill depth (at the nares). We additionally measured tarsus length, wing chord, and tail length, but we focus on beak measurements for this analysis. For each sister pair, we ran a principal component analysis (PCA) on logged trait values, excluding individuals that had missing values for at least one

trait. This analysis places individuals from both north and south of the Marañón in a morphospace that was essentially two-dimensional (on average, 84% of variation per sister pair is explained by PC1, with an additional 11.4% of variation explained by PC2). We calculated divergence in beak size (PC1) and beak shape (PC2) for each sister pair as the difference in absolute value of the species' means along these PC axes. However, these raw differences in species means are biased estimates of the true differences in species means. To account for this bias incurred by sampling error, we estimated bias-corrected divergence in beak size and beak shape for each sister pair using pooled sample variance calculated for all species (see [Freeman et al., 2022b](#) for a demonstration of this bias and how using pooled sample variance accounts for this bias).

Dispersal ability and dispersal opportunity

We tested whether four ecological traits related to dispersal explain variation among sister pairs in their genetic divergences. The first two are related to the dispersal ability of individuals: the hand-wing index ([Kipp, 1959](#)) and foraging strata. We expect that species with longer, pointier wings (higher hand-wing index values) to have greater dispersal ability than species with short stubby wings (low hand-wing index values ([Claramunt et al., 2012](#); [Sheard et al., 2020](#)), and that species foraging in the canopy have greater dispersal ability than understory dwellers ([Burney & Brumfield, 2009](#)). We extracted hand-wing index values from a recently published global dataset ([Sheard et al., 2020](#)), and quantified species' preferred foraging strata using the EltonTraits database ([Wilman et al., 2014](#)): we assigned terrestrial foragers a score of 1 and canopy foragers a score of 4.

The last two traits are related to dispersal opportunity, that is, the opportunity for populations to expand their ranges via dispersal: species' low elevation limits, and species elevational niche breadth. Here we expect that species living at relatively low elevations and with broader elevational distributions will have a greater opportunity to disperse across the Marañón Gap compared to species living at relatively high elevations or in narrow elevational distributions. The Marañón Gap is both narrower and more humid in the low elevation foothills of the east slope of the Andes, potentially providing greater opportunity for low elevation species to cross the gap. Additionally, species with broader elevational distributions may have greater flexibility in climate and habitat, which could allow them to cross the gap. We used distributional data from eBird, a global citizen science project ([Sullivan et al., 2009](#)) to define species' low elevational limits. Using the June 2022 version of the basic eBird dataset ([eBird Basic Dataset, 2022](#)), we extracted records for each species from north and south of the Marañón Gap. We filtered records and only included stationary checklists, area checklists, and traveling checklists with a distance of 3 km or less. We quantified each population's low elevation limit as the value of the 10% quantile, its high elevation limit as the value of the 90% quantile, and elevational niche breadths as the elevational range between species' low and high elevation limits. We defined the elevational niche breadth of the sister pair as the mean elevational niche breadth of the two populations, and the low elevation limit of the sister pair as the minimum low elevation limit of the two populations.

Statistical analysis

We performed all statistical analyses in R (R Development Core Team, 2022). We first compared the strength of the correlation between genetic divergence and the divergence of each trait across the Marañón Gap. To do so, we calculated the Spearman's correlation between genetic distance and trait divergence for each trait for each of 30 sister pairs for which we had complete trait divergence information. For each trait, we also calculated the Spearman's correlation between genetic distance and trait divergence using all sister pairs for which we had data for that trait ($n = 36\text{--}54$).

Next, we modeled the relationship between genetic (predictor variable) and trait divergence (outcome variable) using non-linear and linear models. We evaluated whether oscines and suboscines differed in evolutionary rates by using F tests to compare a full model with a "clade" term (suboscine/oscine) to a reduced model that lacked this clade term (the "anova" function in R). Behavioral song discrimination is bounded by 0 and 1, and we followed previous studies (Freeman et al., 2022a; Weir & Price, 2019) in fitting non-linear regressions (Michaelis-Menten models). Specifically, we fit a reduced model (song discrimination \sim genetic divergence/ $[\beta + \text{genetic divergence}]$) and a full model (song discrimination \sim genetic divergence/ $[\beta + x * \text{clade} + \text{genetic divergence}]$). β measures the genetic divergence value at which song discrimination equals 0.5, and x is an indicator variable that equals 1 for suboscines and 0 for oscines. We fit linear regressions to plumage and morphometric divergence (trait divergence \sim genetic divergence for the reduced model and trait divergence \sim genetic divergence:clade for the full model). We did not force linear regressions through the origin. Our *a priori* predictions were that suboscines would have faster divergence in vocal behavioral discrimination, following previous work (Freeman et al., 2017, 2022a) while oscines, which tend to have more complex coloration, would have faster divergence in plumage (Marcondes & Brumfield, 2019; Price-Waldman et al., 2020). We had no *a priori* predictions for differences between clades in rates of beak size or beak shape evolution.

Last, we tested whether traits linked to dispersal ability (hand-wing index and foraging strata) and dispersal opportunity (low elevation limit and elevational niche breadth) predicted genetic divergence by calculating Spearman's correlation for each trait. Our *a priori* predictions were that (1) hand-wing index is negatively correlated with genetic divergence; (2) foraging strata is negatively correlated with genetic divergence; (3) low elevation limit is positively correlated with genetic divergence; and (4) elevational niche breadth is negatively correlated with genetic divergence.

Results

Sexual signal evolution is more predictable than morphological evolution

We found evidence that divergence in sexual signals (song and plumage) was more tightly correlated with genetic distance than was divergence in beak size and shape. To directly compare correlations between different traits, we calculated correlations for the subset of sister pairs with complete trait data ($n = 30$). Sexual trait divergence was correlated with genetic distance (Figure 2a and b: song: $r = 0.67$ [95% CI = 0.46–0.87], $p = 5.9 \times 10^{-5}$; plumage: $r = 0.64$ [95% CI = 0.39–0.89], $p = .00012$). In contrast, beak divergence was not correlated with genetic distance (Figure 2c and d: beak size

divergence: $r = 0.12$ [95% CI = -0.24 to 0.48], $p = .53$; beak shape divergence: $r = 0.045$ [95% CI = -0.41 to 0.49], $p = .36$). Results were similar when considering all sister pairs for which we had data for a particular trait, with sample sizes of sister pairs ranging from 33 for song divergence to 51 for plumage divergence (Supplementary Figure S2). Again, sexual trait divergence was strongly correlated with genetic distance (song: $n = 33$; $r = 0.70$ [95% CI = 0.52–0.88], $p = 5.1 \times 10^{-6}$; plumage: $n = 51$; $r = 0.64$ [95% CI = 0.44–0.84], $p = 4.3 \times 10^{-7}$). In this larger sample, beak size divergence was correlated with genetic distance ($n = 47$, $r = 0.33$ [95% CI = 0.071–0.59], $p = .023$), while beak shape was not correlated with genetic distance ($n = 47$, $r = 0.22$ [95% CI = -0.11 to 0.55], $p = .13$).

Oscines and suboscines have similar rates of trait divergence

We found little evidence that rates of trait divergence differ between oscines and suboscines (Supplementary Figure S3). As predicted, song divergence evolved faster in suboscines and plumage divergence evolved faster in oscines; however, these relationships were weak and not statistically significant (for song: $F = 3.20$, $p = .084$; for plumage: $F = 1.61$, $p = .21$). We had no *a priori* prediction for differences in rates of trait divergence for morphometric traits, and found similar rates of divergence between oscines and suboscines for both beak size ($F = 0.30$, $p = .59$) and beak shape ($F = 1.83$, $p = .18$).

Dispersal opportunity traits are more tightly correlated with genetic divergence than are dispersal ability traits

We found evidence that traits that are proxies for dispersal opportunity were correlated with genetic divergence (Figure 3c and d: low elevation limit: $r = 0.37$ [95% CI = 0.092–0.64], $p = .0082$; elevational niche breadth: $r = -0.33$ [95% CI = -0.59 to -0.080], $p = .016$). In contrast, we found no evidence that traits that are proxies for dispersal ability were correlated with genetic divergence (Figure 3a and b: for hand-wing index: $r = -0.15$ [95% CI = -0.46 to 0.17], $p = .31$; for foraging strata: $r = 0.039$ [95% CI = -0.28 to 0.36], $p = .79$).

Discussion

Geographically isolated populations have the opportunity to diverge from one another in their traits. A key question is to what degree is there a "tempo" of trait divergence, where the amount of divergence is predictable given the amount of time in isolation. Here, we report evidence that sexual traits are quite predictable in their evolution, while ecological traits are less so, analyzing a comparative dataset of 54 sister pairs of passerine birds that live in similar environments on either side of a common biogeographic barrier. The result that the tempo of sexual trait divergence is likely decoupled from environmentally adaptive trait divergence is consistent with a previous assessment of a smaller number of taxa in this system ($n = 16$; Winger & Bates, 2015). However, in this prior study the tempo of trait divergence in vocal characters was inconclusive, likely owing to difficulties of quantifying bioacoustic trait divergence in different taxa. The extensive playback experiments performed here allow a robust assessment of behavioral discrimination in song, and provide strong evidence that the sexual traits of song and plumage evolve in a more clock-like fashion than do morphometric traits, at least over the time scales analyzed here.

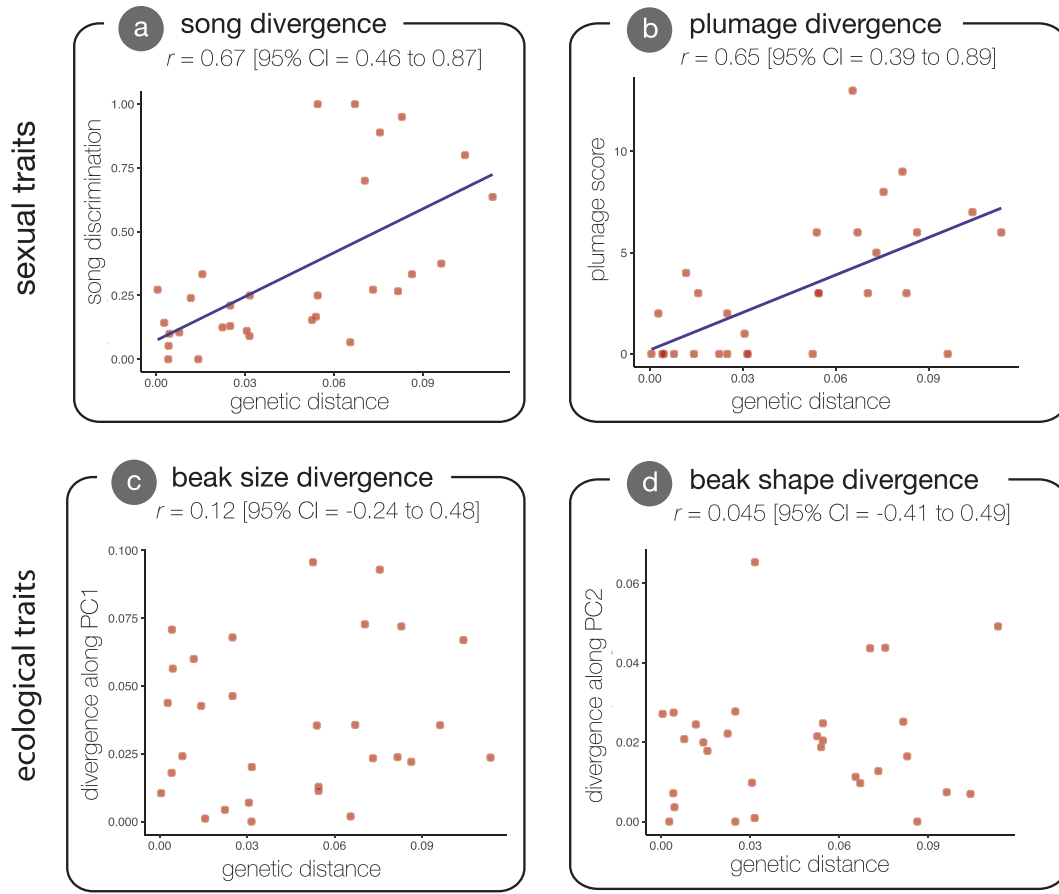


Figure 2. Sexual trait divergence is correlated with genetic distance (a and b), but divergence in ecological traits is not (c and d). We report univariate Spearman's correlations with 95% confidence intervals for the subset of sister pairs with complete trait information ($n = 30$); because the dataset is the same for each trait, the correlations between different traits are directly comparable. Trendlines illustrate statistically significant correlations. In these analyses, we put genetic divergence on the x-axis because we are asking whether genetic divergence predicts trait divergence.

The expanded species set also enabled us to test whether patterns of trait divergence differ between the two major clades of passerines—suboscines and oscines. We find that lineage-specific factors do not strongly modify rates of trait divergence in this system. We conclude that trait evolution, at least for sexual traits, is more predictable than contingent in our study system.

What drives trait divergence in this system?

We specifically chose to study the tempo of trait divergence in geographic isolation in a system where isolated populations live in a similar abiotic and biotic environment. Populations north and south of the Marañón Gap live in humid montane forest where most species, at least for birds, are shared between north and south. This similarity should mean that sister pairs in our dataset experience minimal divergent natural selection based on factors such as climate, habitat, or food resources. Morphometric divergence was subtle and idiosyncratic, consistent with a lack of strong divergence in ecological selection pressure. Yet we find that sister pairs nonetheless diverge significantly over time in the sexual traits of song and plumage. Reflecting the predictable divergence in song and plumage with time, we find a strong positive correlation between song divergence and plumage divergence (Spearman's $r = 0.55$ [95% CI = 0.26–0.84], $p = .00049$). This result is counter to the “transfer hypothesis” which posits that selection favors divergence

in just one signaling modality (Mason et al., 2014; Shutler & Weatherhead, 1990).

There are three possible explanations for what could drive this observed divergence in sexual traits. First, despite overall environmental similarity, sister pairs may still experience some divergent natural selection. Second, populations isolated in identical environments can still experience different trajectories of natural or sexual selection based on the stochastic order of mutations or other stochastic aspects of selection (i.e., mutation-order speciation, where populations diverge due to differences in the order by which advantageous mutations occur). Such mutation-order speciation is expected to particularly apply to signaling traits (Langerhans & Riesch, 2013; Mendelson et al., 2014; Schluter, 2009), and could explain our finding that sexual trait divergence outpaces morphometric trait divergence. Third, genetic drift could influence trait divergence. Though it is difficult to rule out any role of drift or divergent selection, mutation-order divergence provides a natural framework for explaining how plumage and song traits come to be favored in geographically isolated populations that experience similar environments, and mutation-order speciation is consistent with the clock-like divergence patterns in these traits shown here (Winger & Bates, 2015).

Implications for speciation

The typical model of speciation in birds is that geographically isolated populations that diverge in traits linked to

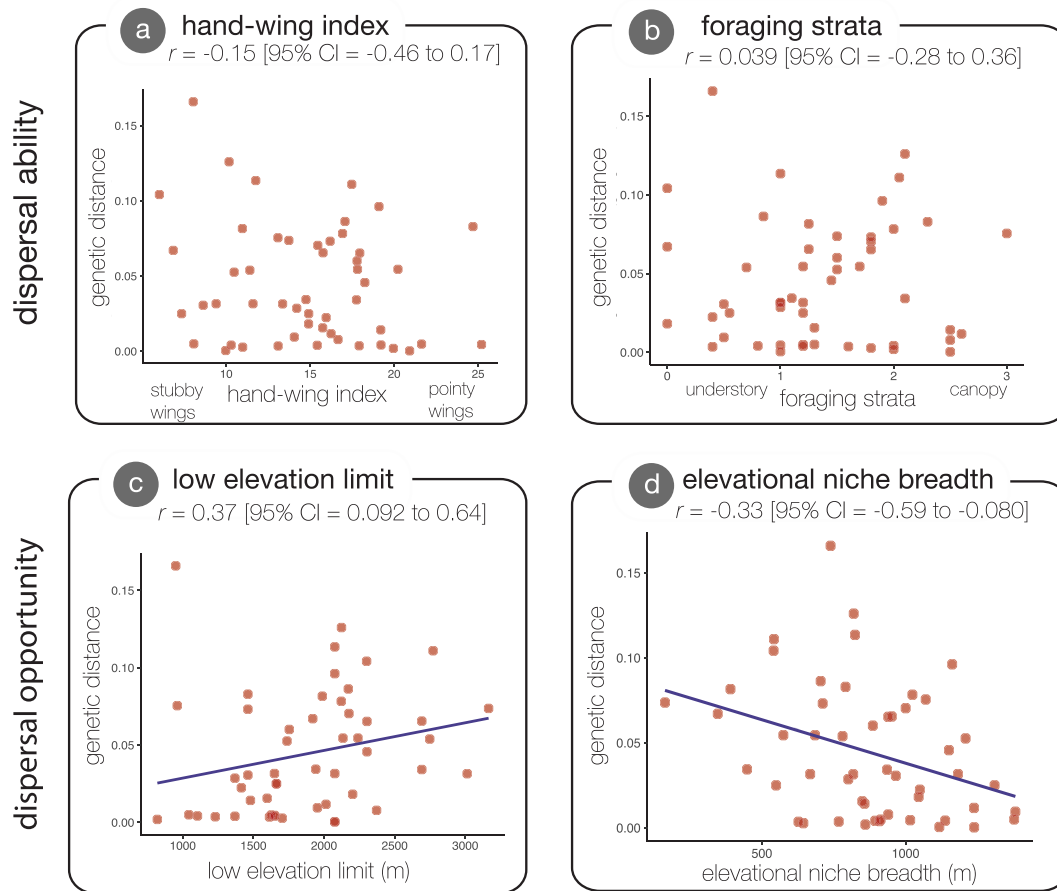


Figure 3. Genetic distance is not correlated with metrics of dispersal ability (a and b), but is weakly but significantly correlated with metrics of dispersal opportunity (sample sizes: $n = 51$ for each comparison). We report univariate Spearman's correlations with 95% confidence intervals. Trendlines illustrate statistically significant correlations. In these analyses, we put genetic divergence on the y-axis because we are asking whether dispersal-related traits predict genetic divergence.

reproductive barriers remain distinct upon secondary contact (Mayr, 1942). Song and plumage are the two major traits that can promote premating isolation, and here we show that both song and plumage have predictable relationships with genetic divergence. Previous research examining introgression throughout the nuclear genome on a subset of sister pairs in this study system found that recent gene flow was detectable between pairs with low mtDNA divergence but absent between pairs with high mtDNA divergence (Winger, 2017), implying that the mtDNA distances used here are a reasonably proxy for the relative amount of time since the cessation of gene flow. Whereas much attention has been placed in recent years on instances in which phenotypic divergence proceeds despite genetic introgression (e.g., Poelstra et al., 2014, Semenov et al., 2017, Toews et al., 2016), our results suggest that divergence in sexual traits may be tied to the evolution of reproductive barriers and cessation of gene flow in this system.

The divergence in sexual traits that we document could be a cause or a consequence of speciation. It would be a cause of speciation if divergence in sexual traits in allopatry led to increased premating barriers and reduced gene flow upon secondary contact during periods when the Marañón Gap was a weaker barrier to dispersal. Alternatively, it would be a consequence of speciation if populations that evolved post-mating barriers in isolation had reduced hybrid fitness upon

secondary contact, with reinforcement then driving the evolution of divergence in traits or preferences (Demko et al., 2019; Hudson & Price, 2014). Our study does not directly test these alternatives. However, we draw on multiple lines of evidence to speculate that divergence in sexual traits takes place in allopatry rather than through reinforcement. In Andean cloud forest birds, plumage and song variation is often highly discrete rather than continuous across their linear geographic ranges (Remsen, 1984) with relatively few examples of stable hybrid zones (but see Céspedes-Arias et al., 2021; Luzuriaga-Aveiga et al., 2021) that are thought to be associated with reinforcement-driven evolution of postmating reproductive isolation. Additionally, Winger (2017) found that sister pairs with evidence for recent genomic introgression across the Marañón Gap tended to lack phenotypic differentiation, whereas introgression was not evident in taxa with notable plumage or vocal differences.

Dispersal opportunity vs. dispersal ability

We found substantial variation in genetic divergence of high elevation forest birds across the Marañón Gap. In other systems, genetic differentiation across biogeographic barriers is related to dispersal ability, with reduced genetic differentiation in species that are identified as better dispersers (e.g., canopy species, species with more pointed wings) (Burney & Brumfield, 2009; Cuervo, 2013; Sheard et al., 2020;

Yamaguchi, 2022). However, these same traits have minimal explanatory power in our analysis. In contrast, we find that genetic differentiation is reduced in species with broad elevational ranges and with lower low elevation limits. Similar results have been found for bird species across other Andean barriers (Cuervo, 2013). We interpret these patterns as indicating that species with broader elevational ranges and lower low elevation limits have had greater opportunity to disperse across the barrier over long time periods. In addition, a broader elevational range suggests more generalized habitat requirements, which likely facilitate more dispersal through the dry Marañón Gap. Therefore, we interpret our results as evidence that dispersal opportunity shapes patterns of genetic differentiation more so than intrinsic dispersal ability.

Conclusion

Evolutionary biologists have long pondered whether the evolution of differences between allopatric populations is predictable. Here, we provide empirical data to show that trait evolution can be predictable or not in tropical montane birds, depending on the trait. We find predictable divergence for sexual traits (song and plumage), while morphometric divergence is more idiosyncratic. This is consistent with the observation that isolated populations in our study system live in similar environments, and so may experience minimal divergent selection on ecomorphological traits. In contrast, sexually selected traits diverge predictably with time: nearly all species with deep genetic divergences across a common biogeographic barrier have substantial divergence in song, plumage, or both. We were unable to explain variation in genetic divergence using ecological correlates of dispersal ability, but genetic divergence was somewhat correlated with ecological correlates of dispersal opportunity. We conclude that sexual trait divergence, which predictably accrues with time in geographic isolation, is an important component of diversification in the tropical Andes, Earth's hottest biodiversity hotspot.

Supplementary material

Supplementary material is available online at *Evolution* (<https://academic.oup.com/evolut/qpac040>)

Data availability

Raw data, summarized data, and code are available on Dryad (<https://doi.org/10.5061/dryad.xwdbvr1hc>). New sequences generated for this study are archived in GenBank (OP791918–OP791958); see [Supplementary Table S2](#) for full information on genetic sequences analyzed in this study.

Funding statement

B.G.F. gratefully acknowledges support from postdoctoral fellowships from the National Science Foundation (Award No. 1523695), Banting Canada (379958), and the Biodiversity Research Centre. O.J. and A.E.M. were supported by the National Science Foundation (GRFP DGE-1247192).

Conflict of interest: We have no conflicts of interest to declare.

Acknowledgments

For providing loans of tissue samples newly sequenced for this study, we thank the Louisiana State University Museum of Natural Science Collection of Genetic Resources, the University of New Mexico Museum of Southwestern Biology (Division of Birds), and The Field Museum, Bird Division. For assistance with lab work we thank Melanie Florkowski, Rachael Herman, Caleb Kaczmarek, and Raquel Marchan Rivadeneira. We thank Steven W. Cardiff for facilitating access to specimens at the Louisiana State University Museum of Natural Science. We thank the many people who have archived their song recordings on xeno-canto and the Macaulay Library of Natural Sounds as well as the curators who maintain these invaluable collections. We thank Gloria Jilahuanco Huamansulca, ECOAN, and the Jocotoco Foundation for logistical assistance in the field. Ralf Yorque provided cogent comments that improved this manuscript.

References

- Arbogast, B., Drovetski, S., Curry, R., Boag, P., Seutin, G., Grant, P., Grant, B., & Anderson, D. (2006). The origin and diversification of Galapagos mockingbirds. *Evolution*, 60, 370–382.
- Bicudo, T. C., Sacek, V., de Almeida, R. P., Bates, J. M., & Ribas, C. C. (2019). Andean tectonics and mantle dynamics as a pervasive influence on amazonian ecosystem. *Scientific Reports*, 9, 1–11.
- Burney, C. W., & Brumfield, R. T. (2009). Ecology predicts levels of genetic differentiation in Neotropical birds. *American Naturalist*, 174, 358–368.
- Carmi, O., Witt, C. C., Jaramillo, A., & Dumbacher, J. P. (2016). Phylogeography of the vermilion flycatcher species complex: multiple speciation events, shifts in migratory behavior, and an apparent extinction of a Galápagos-endemic bird species. *Molecular Phylogenetics and Evolution*, 102, 152–173.
- Céspedes-Arias, L. N., Cuervo, A. M., Bonaccorso, E., Castro-Farias, M., Mendoza-Santacruz, A., Pérez-Emán, J. L., Witt, C. C., & Cadena, C. D. (2021). Extensive hybridization between two Andean warbler species with shallow divergence in mtDNA. *Ornithology*, 138, 1–28.
- Claramunt, S., Derryberry, E. P., Remsen, J. V., & Brumfield, R. T. (2012). High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1567–1574.
- Cuervo, A. M. (2013). *Evolutionary assembly of the Neotropical montane avifauna*. Louisiana State University Doctoral Dissertations.
- Demko, A. D., Sosa-López, J. R., & Mennill, D. J. (2019). Subspecies discrimination on the basis of acoustic signals: a playback experiment in a Neotropical songbird. *Animal Behaviour*, 157, 77–85.
- eBird Basic Dataset. 2022. *Version: EBD_relJun-2022*. Cornell Lab of Ornithology.
- Fjeldsa, J., & N. Krabbe. 1990. *Birds of the High Andes*. Zoological Museum, University of Copenhagen.
- Freeman, B. G., Montgomery, G. A., & Schluter, D. (2017). Evolution and plasticity: divergence of song discrimination is faster in birds with innate song than in song learners in Neotropical passerine birds. *Evolution*, 71, 2230–2242.
- Freeman, B. G., Rolland, J., Montgomery, G. A., & Schluter, D. (2022a). Faster evolution of a pre-mating reproductive barrier is not associated with faster speciation rates in New World passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 289, 20211514.
- Freeman, B. G., Weeks, T., Schluter, D., & Tobias, J. A. (2022b). The latitudinal gradient in rates of evolution for bird beaks, a species interaction trait. *Ecology Letters*, 25, 635–646.
- Gadek, C. R., Newsome, S. D., Beckman, E. J., Chavez, A. N., Galen, S. C., Bautista, E., & Witt, C. C. (2018). Why are tropical mountain

- passes “low” for some species? Genetic and stable-isotope tests for differentiation, migration and expansion in elevational generalist songbirds. *Journal of Animal Ecology*, 87, 741–753.
- García-Moreno, J. (2004). Is there a universal mtDNA clock for birds? *Journal of Avian Biology*, 35, 465–468.
- Hudson, E. J., & Price, T. D. (2014). Pervasive reinforcement and the role of sexual selection in biological speciation. *Heredity*, 105 S, 821–833.
- Kipp, F. (1959). Der Handflügel-Index als flugbiologisches Maß. *Die Vogelharte*, 20, 77–86.
- Lamichaney, S., Berglund, J., Almén M. S., Maqbool, K., Grabherr, M., Martínez-Barrio, A., Promerová M., Rubín, C. J., Wang, C., Zamani, N., Grant, B. R., Grant, P. R., Webster, M. T., & Andersson, L. (2015). Evolution of Darwin’s finches and their beaks revealed by genome sequencing. *Nature*, 518, 371–375.
- Lanfear, R., Ho, S. Y. W., Love, D., & Bromham, L. (2010). Mutation rate is linked to diversification in birds. *Proceedings of the National Academy of Sciences*, 107, 20423–20428.
- Langerhans, R., & Riesch, R. (2013). Speciation by selection: a framework for understanding ecology’s role in speciation. *Current Zoology*, 59, 31–52.
- Lerner, H. R. L., Meyer, M., James, H. F., Hofreiter, M., & Fleischer, R. C. (2011). Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Current Biology*, 21, 1838–1844.
- Linck, E., Freeman, B. G., & Dumbacher, J. P. (2020). Speciation and gene flow across an elevational gradient in New Guinea kingfishers. *Journal of Evolutionary Biology*, 33, 1643–1652.
- Luzuriaga-Aveiga, V., Ugarte, M., & Weir, J. T. (2021). Distinguishing genomic homogenization from parapatric speciation in an elevationally replacing pair of *Ramphocelus* tanagers. *Molecular Ecology*, 30, 5517–5529.
- Marcondes, R. S., & Brumfield, R. T. (2019). Fifty shades of brown: macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. *Evolution*, 73, 704–719.
- Mason, N. A., Shultz, A. J., & Burns, K. J. (2014). Elaborate visual and acoustic signals evolve independently in a large, phenotypically diverse radiation of songbirds. *Proceedings of the Royal Society B: Biological Sciences*, 281, 2014–0967.
- Mayr, E. (1942). *Systematics and the origin of species*. Columbia University Press.
- Mayr, E. (1947). Ecological factors in speciation. *Evolution*, 1, 263–288.
- Mendelson, T. C., Martin, M. D., & Flaxman, S. M. (2014). Mutation-order divergence by sexual selection: diversification of sexual signals in similar environments as a first step in speciation. *Ecology Letters*, 17, 1053–1066.
- Moore, R. P., Robinson, W. D., Lovette, I. J., & Robinson, T. R. (2008). Experimental evidence for extreme dispersal limitation in tropical forest birds. *Ecology Letters*, 11, 960–968.
- Moritz, C., Patton, J. L., Schneider, C. J., & Smith, T. B. (2000). Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*, 31, 533–563.
- Nabholz, B., Lanfear, R., & Fuchs, J. (2016). Body mass-corrected molecular rate for bird mitochondrial DNA. *Molecular Ecology*, 25, 4438–4449.
- Nosil, P. (2008). Ernst Mayr and the integration of geographic and ecological factors in speciation. *Biological Journal of the Linnean Society*, 95, 26–46.
- Ottenburghs, J., Ydenberg, R. C., Van Hooft, P., Van Wieren, S. E., & Prins, H. H. T. (2015). The Avian Hybrids Project: gathering the scientific literature on avian hybridization. *Ibis*, 157, 892–894.
- Parker, T., T. Schulenberg, G. Graves, & M. Braun. 1985. The avifauna of the Huancabamba region. In *Ornithological Monographs No. 36: Neotropical Ornithology* (pp. 169–197). American Ornithologists’ Union.
- Peñalba, J. V., Joseph, L., & Moritz, C. (2019). Current geography masks dynamic history of gene flow during speciation in northern Australian birds. *Molecular Ecology*, 28, 630–643.
- Peñalba, J. V., Mason, I. J., Schodde, R., Moritz, C., & Joseph, L. (2017). Characterizing divergence through three adjacent Australian avian transition zones. *Journal of Biogeography*, 44, 2247–2258.
- Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Müller I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G., & Wolf, J. B. W. (2014). The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science*, 344, 1410–1414.
- Price-Waldman, R. M., Shultz, A. J., & Burns, K. J. (2020). Speciation rates are correlated with changes in plumage color complexity in the largest family of songbirds. *Evolution*, 74, 1155–1169.
- Rabosky, D. L., & Matute, D. R. (2013). Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds. *Proceedings of the National Academy of Sciences*, 110, 15354–15359.
- R Development Core Team. (2022). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing.
- Remsen, J. V. Jr. (1984). High incidence of “leapfrog” pattern of geographic variation in andean birds: implications for the speciation process. *Science*, 224, 171–173.
- Remsen, J. V. Jr., J. Areta, E. Bonaccorso, S. Claramunt, A. Jaramillo, D. Lane, J. F. Pacheco, J. Pérez-Emán, M. B. Robbins, F. G. Stiles, & K. J. Zimmer. (2022). *A classification of the bird species of South America*. American Ornithologists’ Union. <http://www.museum.lsu.edu/~Remsen/SACCBaseline.html>
- Román-Palacios, C., & Wiens, J. J. (2018). The tortoise and the finch: testing for island effects on diversification using two iconic Galápagos radiations. *Journal of Biogeography*, 45, 1701–1712.
- Salisbury, C. L., Seddon, N., Cooney, C. R., & Tobias, J. A. (2012). The latitudinal gradient in dispersal constraints: ecological specialisation drives diversification in tropical birds. *Ecology Letters*, 15, 847–855.
- Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford University Press.
- Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, 323, 737–741.
- Schulenberg, T. S., D. F. Stotz, D. F. Lane, J. P. O’Neill, & T. A. Parker. 2010. *Birds of Peru*. Princeton University Press.
- Seddon, N., Tobias, J. A., Eaton, M., & Ödeen A. (2010). Human vision can provide a valid proxy for avian perception of sexual dichromatism. *Auk*, 127, 283–292.
- Semenov, G. A., Scordato, E. S. C., Khaydarov, D. R., Smith, C. C. C. R., Kane, N. C., & Safran, R. J. (2017). Effects of assortative mate choice on the genomic and morphological structure of a hybrid zone between two bird subspecies. *Molecular Ecology*, 26, 6430–6444.
- Sheard, C., Neate-Clegg, M. H.C., Alioravainen, N., Jones, S. E. I., Vincent, C., MacGregor, H. E. A., Bregman, T. P., Claramunt, S., & Tobias, J. A. (2020). Ecological drivers of global gradients in avian dispersal inferred from wing morphology. *Nature Communications*, 11, 1.
- Shutler, D., & Weatherhead, P. J. (1990). Targets of sexual selection: song and plumage of wood warblers. *Evolution*, 44, 1967–1977.
- Smith, B. T., & Klicka, J. (2010). The profound influence of the Late Pliocene Panamanian uplift on the exchange, diversification, and distribution of New World birds. *Ecography*, 33, 333–342.
- Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, 57, 758–771.
- Sullivan, B. L., Wood, C. L., Iliff, M. J., Bonney, R. E., Fink, D., & Kelling, S. (2009). eBird: a citizen-based bird observation network in the biological sciences. *Biological Conservation*, 142, 2282–2292.
- Tobias, J. A., Ottenburghs, J., & Pigot, A. L. (2020). Avian diversity: speciation, macroevolution and ecological function. *Annual Review of Ecology, Evolution, and Systematics*, 51, 533–560.
- Tobias, J. A., Seddon, N., Spottiswoode, C. N., Pilgrim, J. D., Fishpool, L. D. C., & Collar, N. J. (2010). Quantitative criteria for species delimitation. *Ibis*, 152, 724–746.

- Toews, D. P. L., Taylor, S. A., Vallender, R., Brelsford, A., Butcher, B. G., Messer, P. W., & Lovette, I. J. (2016). Plumage genes and little else distinguish the genomes of hybridizing warblers. *Current Biology*, 26, 2313–2318.
- Villeumier, F. (1969). Pleistocene speciation in birds living in the high Andes. *Nature*, 223, 1179–1180.
- Wagner, C. E., Harmon, L. J., & Seehausen, O. (2012). Ecological opportunity and sexual selection together predict adaptive radiation. *Nature*, 487, 366–369.
- Weber, M. G., & Agrawal, A. A. (2014). Defense mutualisms enhance plant diversification. *Proceedings of the National Academy of Sciences*, 111, 16442–16447.
- Weigend, M. (2002). Observations on the biogeography of the Amotape-Huancabamba zone in northern Peru. *Botanical Review*, 68, 38–54.
- Weir, J. T., & Price, T. D. (2019). Song playbacks demonstrate slower evolution of song discrimination in birds from Amazonia than from temperate North America. *PLoS Biology*, 17, e3000478.
- Wilman, H., Belmaker, J., Simpson, J., Rosa, C. de la, Rivadeneira, M. M., & Jetz, W. (2014). EltonTraits 1.0: species-level foraging attributes of the world's birds and mammals. *Ecology*, 95, 2027.
- Winger, B. M. (2017). Consequences of divergence and introgression for speciation in Andean cloud forest birds. *Evolution*, 71, 1815–1831.
- Winger, B. M., & Bates, J. M. (2015). The tempo of trait divergence in geographic isolation: avian speciation across the Marañón Valley of Peru. *Evolution*, 69, 772–787.
- Winger, B. M., Hosner, P. A., Bravo, G. A., Cuervo, A. M., Ariztizábal N., Cueto, L. E., & Bates, J. M. (2015). Inferring speciation history in the Andes with reduced-representation sequence data: an example in the bay-backed antpittas (Aves; Grallariidae; *Grallaria hypoleuca* s. l.). *Molecular Ecology*, 24, 6256–6277.
- Yamaguchi, R. (2022). Intermediate dispersal hypothesis of species diversity: new insights. *Ecological Research*, 30, 1–315.
- Young, K., & C. Reynel. 1997. Huancabamba region, Peru and Ecuador. In S. Davis, V. Heywood, O. Herrera MacBryde, and A. Hamilton (Eds.). *Centres of plant diversity: a guide and strategy for their conservation* (pp. 465–469). WWF and IUCN.