LETTER

Elevational differentiation accelerates trait evolution but not speciation rates in Amazonian birds

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Abstract

The importance of ecologically mediated divergent selection in accelerating trait evolution has been poorly studied in the most species-rich biome of the planet, the continental Neotropics. We performed macroevolutionary analyses of trait divergence and diversification rates across closely related pairs of Andean and Amazonian passerine birds, to assess whether the difference in elevational range separating species pairs – a proxy for the degree of ecological divergence – influences the speed of trait evolution and diversification rates. We found that elevational differentiation is associated with faster divergence of song frequency, a trait important for pre-mating isolation, and several morphological traits, which may contribute to extrinsic post-mating isolation. However, elevational differentiation did not increase recent speciation rates, possibly due to early bursts of diversification during the uplift of the eastern Andes followed by a slow-down in speciation rate. Our results suggest that ecological differentiation may speed up trait evolution, but not diversification of Neotropical birds.

Keywords

Amazon, Andes, diversification rates, ecological speciation, elevation, passerine birds, trait evolution.

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INTRODUCTION

The evolution of reproductive isolation is influenced by many factors, including rates of gene flow, strength of geographic barriers and strength of divergent natural selection (Harvey *et al*. 2017). These mechanisms may eventually lead to the evolution of reproductive barriers, through the accumulation of genetic differences. While genetic differentiation is expected to result in the formation of reproductive isolation for any set of populations not exchanging genes, this process can be accelerated under strong divergent selection mediated by environmental differences – a process termed 'ecological speciation' (Schluter 2000). As the strength of ecologically driven divergent selection increases, the amount of time required to evolve reproductive isolation declines (Schluter 2000; Price 2008). This potential to speed up the diversification process could be an important driver of species richness gradients, but most studies on the role of ecological divergence in accelerating evolution and diversification have been focused on species-poor faunas of island archipelagoes (e.g. Galapagos finches) and on temperate regions at high latitudes. However, our current understanding of the role of ecological divergence in accelerating evolution in species-rich habitats of the continental tropics is limited.

Several studies have demonstrated the importance of ecologically mediated divergent selection for speciation. In animals, adaptation to distinct foraging niches has resulted in divergence of morphological traits such as body size, body shape and nuptial colouration, which has led to the evolution of reproductive isolation (Nagel & Schluter 1998; Boughman 2001; Nosil & Crespi 2006). For instance, the extraordinary phenotypic diversification of ovenbirds (Furnariidae), Hawaiian honeycreepers (Drepanidini), Galapagos finches (Geospiza)

and vangas (Vangidae) may have occurred through the action of divergent selection pressures for habitat use, leading to extensive diversification of bill morphology, which facilitated the rapid specialisation of foraging strategies among lineages (e.g. Burns *et al*. 2002; Lovette *et al*. 2002; Claramunt 2010; Jønsson *et al*. 2012).

Other forces – such as sexual selection – are likely to interact with environmental conditions in driving phenotypic diversification of traits important for species recognition (Schluter 2001). For example, several aspects of the song structure and performance in passerine birds are known to be directly influenced by both habitat divergence (e.g. Seddon 2005; Weir *et al*. 2012; Derryberry *et al*. 2018) and sexual selection (e.g. Price 1998; Slabbekoorn & Smith 2002; Mason *et al*. 2017). Female preference for aspects of male song may evolve in such a way as to maximise male song transmission through the environment. Therefore, populations exposed to different environments might have experienced accelerated rates of evolution for both female preferences and male song traits, facilitating pre-mating isolation.

The tropics are often believed to harbour a greater number of ecological sources of divergent selection than high latitude biomes. If true, then tropical regions might generate greater ecological opportunity, leading to faster trait divergence and speciation than high latitudes (e.g. Ecological Opportunity Hypothesis *sensu* Schluter 2016). However, comparative analyses across closely related avian species demonstrate less divergence in climatic niche towards the equator, which is also associated with reduced evolutionary rates in body size and song divergence (Lawson & Weir 2014). Recent speciation rates are also estimated to be slower in the tropics (Wiens 2007; Weir & Schluter 2008). Together, these results question the validity of the Ecological Opportunity

Hypothesis as an explanation for high-species richness in the continental tropics.

Here, we analysed whether elevational differentiation plays an important role in accelerating trait divergence and diversification rates across closely related passerine birds from one of the most species-rich tropical regions of the planet, the Amazon basin (Hurlbert & Jetz 2007), which includes lowland regions and eastern flank of the Andes. We calculated evolutionary rates of song and morphometrics between 115 pairs of species distributed along an elevational gradient from sea level to 5000 m, and we used Brownian motion and Ornsetin–Ulhenbeck models of trait evolution to test whether evolutionary rates vary as a function of elevational difference separating members of each species pair. We chose to analyse song and morphometrics, due to the importance of these traits in the formation of reproductive isolation. Song divergence is associated with pre-mating reproductive isolation in birds (see Wilkins *et al.* 2013), and morphometric traits are believed to be fine-tuned to the ecological niche in birds (e.g. Miles & Ricklefs 1984; Miles *et al.* 1987; Smith *et al.* 1997). Divergence in morphometrics is thus likely to lead to extrinsic post-mating reproductive isolation mediated by local adaptation to environmental conditions at different elevations. If ecological differences associated with elevation influence trait divergence, we expect to find faster evolution of song and morphology in species pairs with greater differences in their elevational ranges. Similarly, we tested if increased ecological opportunity, driven by elevational differentiation, resulted in faster speciation rates along the eastern tropical Andes.

METHODS

Data collection

Our study was confined to the humid Amazonian basin (including the eastern slope of the tropical Andes whose drainages flow into the Amazon river, but not the southern Andes below 18° S latitude or western Andean slopes) and adjacent lowland regions of the Guiana shield (Fig. 1). This region is collectively referred to as the Amazon basin throughout this paper. We identified 160 passerine sister species pairs from the Amazon basin using available molecular phylogenies possessing at least 80% species for a genus or clade (see Appendix S1). We also included six phylogroups, within sisters, which represent intraspecific splits between a high and low-elevation taxon.

We used the field guide of the Birds of Peru (Schulenberg *et al.* 2010) as our primary source for gathering information of altitudinal ranges per each species, because of its description specificity. When a species in our data set did not occur in Peru or its elevational range was not described, we used other field guides or available data sets (e.g. Stotz *et al.* 1996; Ridgely & Greenfield 2001; McMullan & Donegan 2010; Del Hoyo *et al.* 2018). We then calculated the absolute difference of the mid-point of elevational range between the two species or subspecies within each phylogroup. We excluded species pairs in which one or both species' elevational range spanned from less than 500 m to more than 1600 m or whose elevational range (maximum minus minimum elevation) exceeded
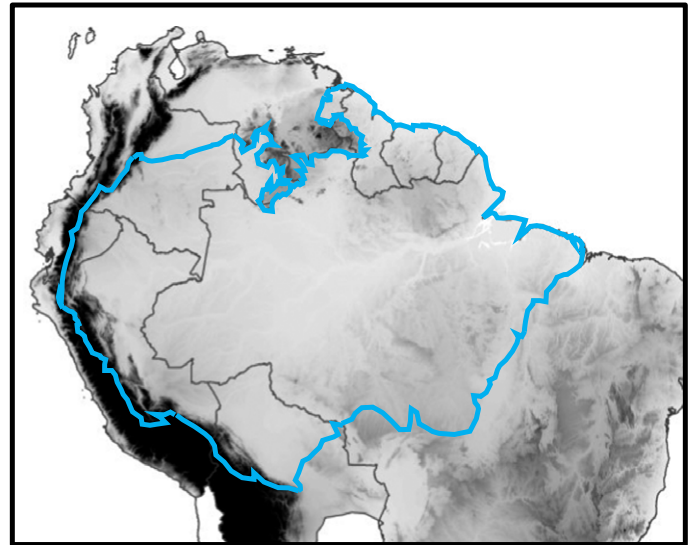
Figure 1 Map of the study region, outlined in blue, includes the Guiana shield, Amazonian biome and adjacent eastern slopes of the Andes (from Bogotá-Colombia to Cochabamba-Bolivia) that drain into the Amazon basin.

2000 m. This eliminated species with broad elevational ranges spanning lowland and highland regions, whose mid-point elevations provide a less meaningful comparison. We also used a metric of proportional elevational overlap (amount of elevational overlap (meters) divided by the average elevational range) between sister pairs, where values are capable of ranging from 0 (no overlap) to 1 (both species overlap completely).

One hundred and twenty-five of the 160 species pairs in our data set are allopatric or parapatric. Only 35 are sympatric. Importantly, many of our species pairs that overlap broadly in elevation are fully allopatric (75 allopatric sister pairs overlap in elevation by 50% or more), often with rivers (lowlands) or arid valleys (Andes) separating sisters. Elevational overlap is thus not synonymous with sympatric overlap in our data.

Time estimation

Mitochondrial sequences of cytochome b (cyt *b*) and NADH dehydrogenase subunit 2 (ND2) genes, with at least 500 bp, were obtained for one individual per species or phylogroup from GenBank for each of the 160 taxon pairs (see Appendix S2). Sequences were aligned in MEGA7 (Kumar *et al.* 2016). We fixed the topology of the tree connecting our taxon pairs (relationships between oscine families from Barker *et al.* 2015 and between suboscines families from Prum *et al.* 2015; within family relationships were determined from species-level molecular phylogenetic studies cited in Appendix S1). BEAST v2.5 (Bouckaert *et al.* 2014) was used to estimate branch lengths along this fixed topology, using a relaxed-clock model (with rate variation following a log-normal distribution, a Yule speciation prior, and a GTR-gamma model of sequence evolution with a mean rate of 0.01035 and standard deviation around that rate of 0.0035 following Weir & Schluter 2008). The analysis was run for 17 million generations, with trees

sampled every 1000 generations following a two million generation burnin. Sampled trees were used to generate the maximum sum of clade credibility tree, with median branch lengths (Fig. S1). Node ages for each sister pair were obtained from the maximum sum of clade credibility tree.

Song analysis

Overall, 1096 song files were obtained from our own field recordings and the online databases xeno-canto (http://www.xeno-canto.org) and Macaulay Library (http://macaulaylibrary.org) for 106 of the 160 sister pairs (sister pairs without song data were not sampled). Avian vocalisations were analysed using the software Raven Pro 1.4 (http://www.birds.cornell.edu/raven) for 1 to 5 individuals (mean = 4.14) per species, with individuals sampled from the same general geographic region when possible. We followed previously published methods (Weir *et al.* 2012) to obtain six measures of song frequency (low frequency, high frequency, first and third quartile frequency, centre frequency and delta frequency), as well as song length and number of notes per song (Fig. S2). Frequency measurements were taken from the fundamental song (in hertz) and song duration was measured in seconds. All measurements were log-transformed and a principal component analysis (PCA) was performed with the *prcomp* function in R 3.4.1 (R Core Team 2017), using the correlation matrix. Mean values for each species were obtained separately for PC1 and PC2 and Euclidean distances between taxa within a pair were calculated for each of these PCs. Measurement errors were calculated from the variances across individuals. We applied the variance of the other species, within a phylogroup, to species with a single song file.

Morphometric analysis

We took morphometric measurements of 616 museum specimens for 1–3 individual adult males (mean = 2.58) per species belonging to the same general geographic region for 98 of the 160 sister pairs (based on availability of data at the museums visited). We took length measurements of the bill: total bill length (from the start point of the culmen to the tip of the bill), depth (distance between the upper and lower closed mandible at the anterior end of the nostrils) and width (distance of the widest lateral mandible at the anterior end of the nostrils); wing arc; central feather of the tail; tarsus (measured from the junction to the last scale); and hallux' claw (Fig. S3). Bill and tarsus were measured with a Marathon digital caliper (8in/200 mm), and a flexible stainless-steel ruler (12in/30 cm) was used to measure the wing and tail. Because we did not include a metric measurement of body size, we corrected our morphological analysis for this later variable using a PCA approach. The first principal component (PC1) of the log-transformed morphometric measurements (in mm) represents overall body size variation and the remaining PCs are largely size-independent (Berner 2011). We analysed each remaining PC, that explained more than 2% of the variation, separately. When only one specimen of a species was available, measurement errors were calculated using the variance of the other member of the sister pair.

Patterns across the elevational gradient

We used the dated BEAST phylogeny to perform phylogenetically corrected regressions with the R package Caper v0.5 (Orme *et al.* 2013), to test whether the association between mean PC trait values and the mid-point elevational range (in meters) for individual species (i.e. not species pairs) showed significant trends. Following Freckleton *et al.* (2002), we used generalised least-square models and transformed branch lengths with maximum-likelihood estimates of Pagel's lambda (λ).

Evolutionary rates and gradient effect

We used the R package EvoRAG (Weir & Lawson 2015), to compare Brownian motion (BM) and Ornstein–Uhlenbeck (OU) null models – in which a single rate of evolution, β, is estimated for all sister pairs regardless of their difference in elevation – to models in which β varies as a linear function of the absolute difference in the mid-point elevational range separating each of the species in a pair. In addition to β estimated by the BM and OU models, the OU model estimates a constraint parameter, α, which represents the 'pull' towards an optimal trait value, that in the EvoRAG modelling framework is the intermediate trait value between a sister pair. For our linear OU models testing the effect of elevational difference, only β changes with elevation difference while α was held constant. We also fitted more complex models that tested the effect of elevational difference separately for oscines and suboscines, which represent two major groups within passerines. The former culturally inherit certain aspects of their song and the latter which rarely do. Models were compared with Akaike Information Criterion and Akaike Weights. Simulations with avian data sets of our size have previously shown that an AIC difference in models of *c*. 2 is comparable to an alpha cut-off of 0.05 in a frequentist approach (Weir & Lawson 2015), so we used this value here as indicating substantial support between competing models. These models were fitted separately to PC1 and PC2 for song data and to PC1 to PC6 for morphometric data.

The EvoRAG method assumes that species pairs are statistically independent and that differences in rates of trait evolution occur only due to the effect of the gradient in elevational differentiation being tested. Sister pairs that have similar or different levels of elevational differentiation are each drawn from a wide range of passerine bird families and do not show strong phylogenetic clumping (Fig. S1), suggesting that any differences in rate between families that may exist is not biasing our analyses with respect to elevational difference.

Ages distribution and Speciation rates

If elevational differentiation promotes faster rates of speciation, then we expect that speciation rate will increase with mid-point elevational difference and decrease with proportional range overlap. We tested these predictions using the likelihood modelling approach in Weir & Schluter (2007), which compares the actual distributions of sister species ages to those simulated under a wide range of speciation and extinction rates. Sister species' age distributions were

simulated following identical methods as in Weir & Schluter (2007) – to which we direct the reader for further details. Sister species age distributions were simulated for 17 different values of speciation rate (0.04, 0.05, 0.06, 0.15, 0.2 0.25, 0.3, 0.35 and 0.4), each of which had 12 different values of extinction rate ranging from 0 to 0.99 times the rate of speciation. We then fitted a model that allowed both speciation and extinction rates to vary linearly across the gradient. The model also estimated a lag-time to species recognition, which corrects for the fact that speciation is not a point event in time but a process, and that most recent incipient speciation events are not yet recognised by taxonomists. For each combination of speciation and extinction rate simulated, we applied 21 different mean lag-times ranging from 0 to 2 million years. The lag-time was estimated as a parameter in the model and was held constant across both gradients of elevational differentiation (e.g. difference in mid-point and range overlap).

RESULTS

Song

PC1 explained 65% of the variation and was heavily weighted by the six measurements of frequency (Table S1). We interpret PC1 as representing song frequency. PC2 explained 22% of the variation and was heavily weighted by song duration and number of notes. PC2 represents song length. For song frequency (PC1), models with an effect of elevational differentiation were strongly supported over models without elevational difference, as evidenced by > 99% of the Akaike Weight partitioned amongst the various elevational difference models (Table S3). The OU model with a linear effect of elevational difference had the best fit (Akaike weight of 0.9) (Table S3) and supported a substantial increase in evolutionary rate of song frequency with increasing difference in elevation (rates increase 8.8 times for every 2000 m of elevational differentiation; Fig. 2). In contrast, the evolution of song length (PC2) was best fitted by models in which elevational difference was not included (Table S3, Fig. 2). Restricting these analyses to sister pairs which lacked sympatric overlap gave similar patterns (Table S3). Results of model fitting using the proportion of elevational overlap are found in table S4.

Morphometrics

PC1 was positively loaded towards all morphometric measurements, explaining 73% of the variation and represents body size. Each remaining PC was positively loaded towards a particular trait: PC2 represented bill width; PC3, tail length; PC4, bill length; PC5, wing length; and PC6, bill depth. PC2 to PC6 explained 9, 7, 3, 2.8 and 2.5% of the variation, respectively (Table S2). We found no evidence of body size evolving as a consequence of the ecological differentiation between sister pairs. The best fitting model of evolution for PC1 was Brownian motion without a gradient effect. In contrast, the evolutionary rates of all remaining PCs were best fitted by models including elevational difference with total Akaike weights of all models which included elevational differentiation ranging from 0.78 to > 0.99 depending on the trait. For wing length and bill depth,

the best model included elevational difference with separate rates for oscines and suboscines. Wing length evolved faster with increasing elevational differentiation for suboscines sister pairs. The pattern was more complicated for bill depth in suboscines with similar elevational mid-points evolving faster than oscines whereas rates were faster for oscines when elevational differentiation was > 700 m (Table S3, Fig. 2). Restricting these analyses to sister pairs which lacked sympatric overlap gave similar patterns (Table S3). Results of model fitting using the proportion of elevational overlap are found in table S4.

Patterns across the elevational gradient

Elevational trends of characters are shown in Fig. 3. For our song analysis, PC1 (song frequency) had a borderline significant increase with elevation (slope = $-2.44 \times 10^{-4} \pm 9.97 \times 10^{-5}$ SE, $P = 0.0152$), while PC2 (song length) did not show any significant trend along the elevational gradient (slope = $8.30 \times 10^{-5} \pm 7.12 \times 10^{-5}$ SE, $P = 0.245$). For morphometric analyses, PC1 (body size) had a borderline significant increase with elevation (slope = $1.9426 \times 10^{-4} \pm 8.7 \times 10^{-5}$ SE, $P = 0.02679$) as predicted by Bergman's rule of increase body size in colder environments (Bergmann 1847). Three of the remaining morphometric PCs had significant elevational trends (bill width: slope = $-1.45 \times 10^{-4} \pm 3.32 \times 10^{-5}$ SE, $P = 1.98 \times 10^{-5}$; tail length: slope = $1.06 \times 10^{-4} \pm 2.89 \times 10^{-5}$ SE, $P = 0.0003$; bill length: slope = $-1.26 \times 10^{-4} \pm 2.45 \times 10^{-5}$ SE, $P = 6.88 \times 10^{-7}$) and two PCs lacked elevational trends (wing length: slope = $7.54 \times 10^{-6} \pm 2.01 \times 10^{-5}$ SE, $P = 0.707$; bill depth: slope = $2.94 \times 10^{-6} \pm 2.10 \times 10^{-5}$ SE, $P = 0.889$).

Speciation rates

Maximum-likelihood estimates of speciation rate declined significantly with mid-point elevational difference and increased significantly with elevational overlap (Fig. 4). Extinction rate estimates did not differ significantly with elevational differentiation and are not shown.

DISCUSSION

We found that evolutionary rates for key morphological and behavioural traits are positively correlated with elevational difference and overlap proportion between diverging pairs of sister species from the Amazon basin and adjacent Andean slopes. While many studies have found an important role for ecological divergence in accelerating trait evolution in specific species (e.g. Schluter 1996; Jiggins et al. 2001; Grant & Grant 2008; Matsubayashi et al. 2010; Tobias et al. 2014), this is one of the first broad-scale comparative projects to determine whether ecological divergence is more generally associated with accelerated evolution of traits important to speciation in one of the most species-rich regions, the Amazon basin (see also Funk et al. 2006; Lawson & Weir 2014). However, we did not find that accelerated trait evolution was associated with faster speciation rates.

A number of factors could result in accelerated trait evolution with increasing elevational differentiation. Traits may
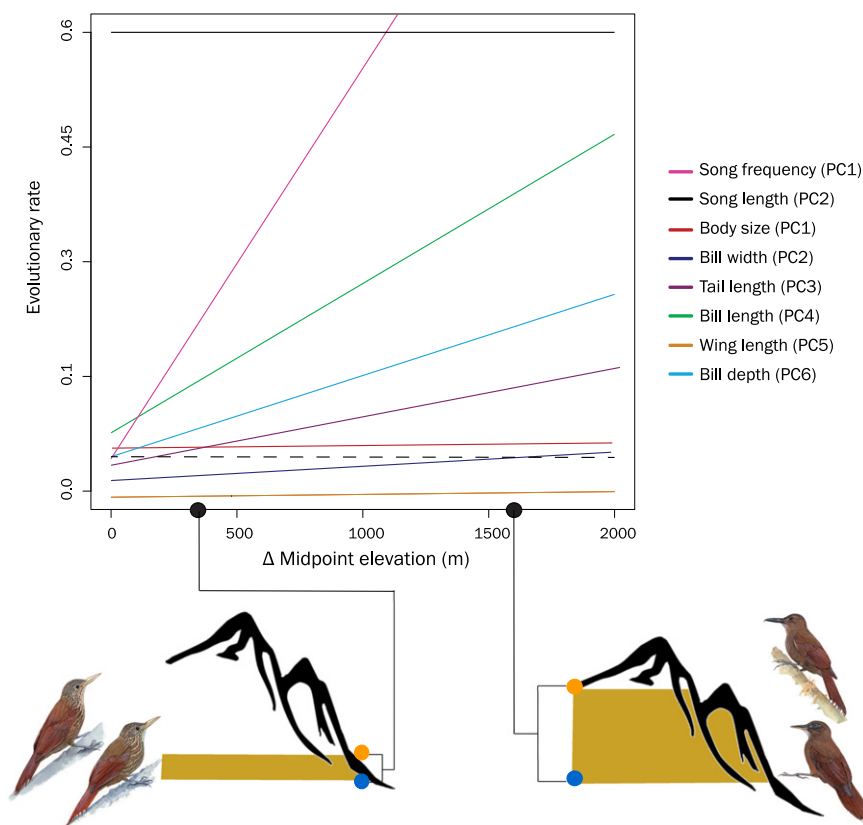
Figure 2 Change in evolutionary rates of traits as a function of mid-point elevational difference between sister species pairs of birds from the Amazon basin and adjacent Andean slopes. Maximum-likelihood estimates for the best fitting model are shown for songs (frequency (PC1) in pink and length (PC2), in black) and morphometrics (body size (PC1) in red, bill width (PC2) in blue, tail length (PC3) in purple, bill length (PC4) in green, wing length (PC5) in orange and bill depth (PC6) in turquoise). All characters, except song length and body size, best fit models in which rates of evolution increased with increasing difference in mid-point elevation. Song frequency bill width, tail length, bill length and wing length were best fitted by Ornsetin–Ulhenbeck (OU) models, with other models best fit by Brownian Motion (BM). ML estimates for morphometrics are shown with a solid line for oscines and with a dashed line for suboscines', wing length and bill depth, because those traits best supported a model with separate rates between these groups. At the bottom-right, we show an example of a morphologically differentiated sister species pair–*Dendrocincla merula* and *D. tyrannina* – with a Euclidean distance for bill length of 0.3 and difference in mid-point elevational ranges of 1550 m. In contrast, the bottom-left corner illustrates a sister species pair–*Dendroplex kienerii* and *D. picus*– with weak morphometric differentiation (Euclidean distance for bill length = 0.18) that occur at similar elevations (Δ mid-point elevational range = 400 m). Bird illustrations were taken from the book "Birds of Peru" (Schulenberg *et al.* (2010), with the permission of Princeton University Press.

show elevational trends, in which case sister pairs which differentiate in elevation should diverge more rapidly than sister pairs at the same elevation. We saw clear evidence of this in several of our traits. Bill width (Fig. 3d) and bill length (Fig. 3f) decreased, while tail length (Fig. 3e) increased significantly with increasing elevation. These trends are likely to be directly or indirectly related to ecological factors that also vary with elevation. For example, differentiation of bill size and shape is strongly associated with prey size (e.g. Kleindorfer *et al.* 2006). Though not tested for the Andes, insect prey size (almost all passerine birds feed on insects) has been shown to decline with elevation in other montane systems (e.g. Janes 1994), likely due to slower rates of insect growth and development in colder temperatures (Hodkinson 2005).

Song frequency showed a borderline significant decline with elevation (Fig. 3a). Different aspects of bird song are believed to be fine-tuned across environments in order to communicate effectively. Frequency is a structural component of song that is highly influenced by habitat but not by cultural learning (e.g. Nemeth *et al.* 2001; Boncoraglio & Saino 2007). Birds

sing at lower frequencies in tropical forests than either temperate forests or open tropical habitats, likely because tropical forests with dense vegetation possess louder ambient noise and result in greater signal attenuation (Seddon 2005; Kirschel *et al.* 2009; Weir *et al.* 2012). Most of our sister pairs occupy forest habitats. Canopy height declines and density of vegetation typically increases with elevation as one leaves lowland *terra firme* forests and enters montane cloud forests. Dense cloud-forest vegetation is expected to differentially filter out high pitches, thus selecting for lower-pitched sounds, which transmit further and with less distortion (Tobias *et al.* 2010). As a result, species pairs diverging in elevation are likely to experience different forest densities and thus different selective pressures optimising sound transmission, leading to song frequency divergence.

We also found faster evolutionary rates for wing length and bill depth with differences in elevation, but these traits showed no general increase or decrease in length with elevation (Fig. 3g, 3h). For traits that do not show elevational trends, it is possible that different clades of birds respond
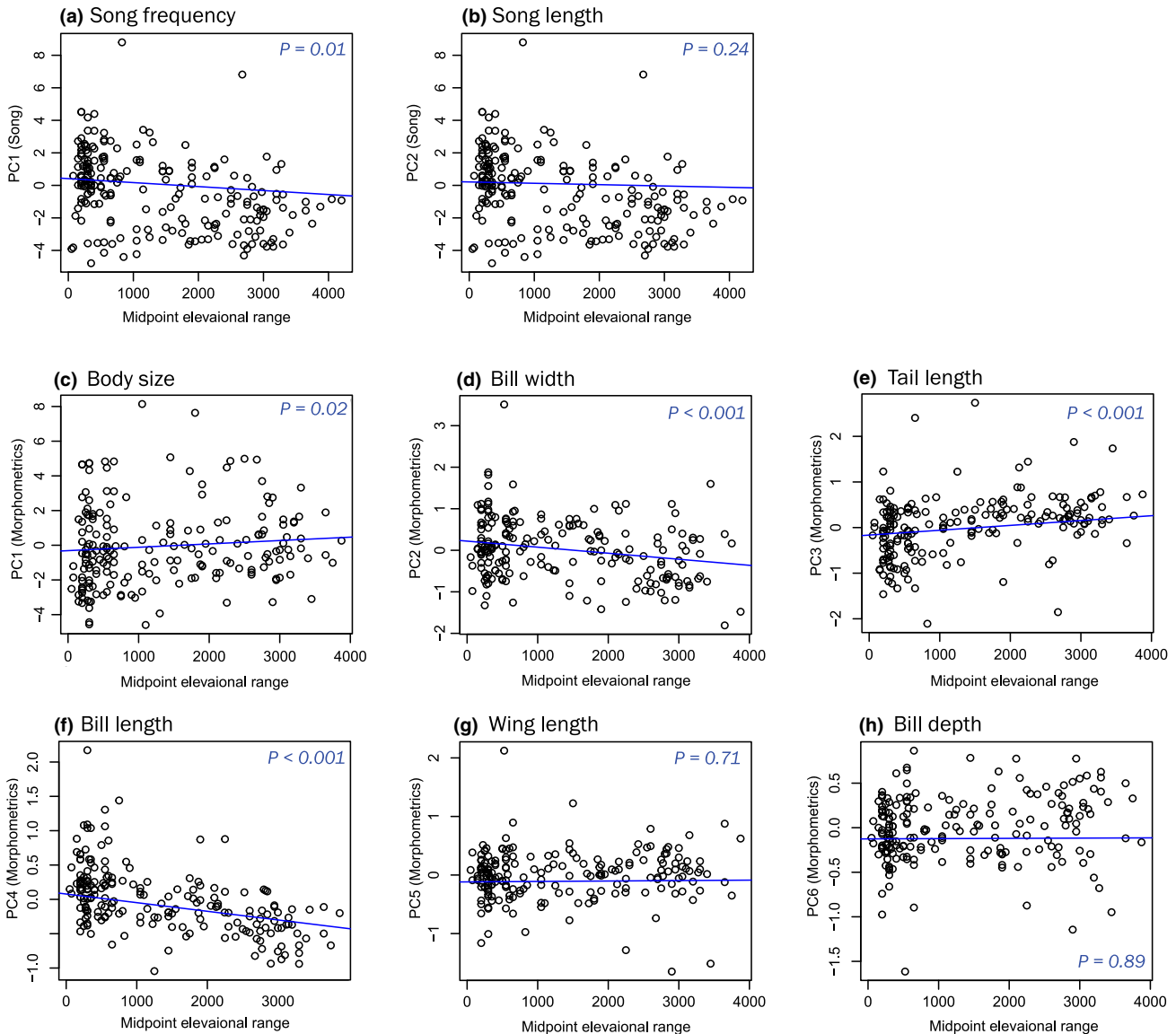
Figure 3 Mean PC values for behavioral and morphometric traits for individual species as a response of increasing elevation. Song traits are shown in (a and b) and morphometric traits in (c–h). Lines represent phylogenetically corrected regressions.

differently to elevational gradients in their trait values, with no overall elevational trend across all passerine bird groups. Although song length (Fig. 3b) and body size (Fig. 3c) showed borderline significant trends of trait values with elevational mid-point, such trends are not associated with accelerated trait evolution. Bergman's rule predicts that body size in endotherms should increase in colder environments (Bergmann 1847), possibly as a way to reduce heat loss (though this explanation is not universally accepted, e.g. Watt *et al.* 2010; Meiri 2011; Olalla-Tárraga 2011) and appears to hold true across elevational and latitudinal gradients in many parts of the world (e.g. Bulgarella *et al.* 2007; Lawson & Weir 2014). We found a borderline significant increase in body size with elevation in passerine birds (compared with Remsen 1993; Gutiérrez-Pinto *et al.* 2014; Freeman 2017, who found no effect), though the effect is minimal (Fig. 3c) and apparently not sufficient to drive a

significant increase in rates of body size evolution with increasing elevational difference. Other factors, such as sexual selection and competition, may constrain the evolution of body size across elevational gradients in the tropics (Freeman 2017).
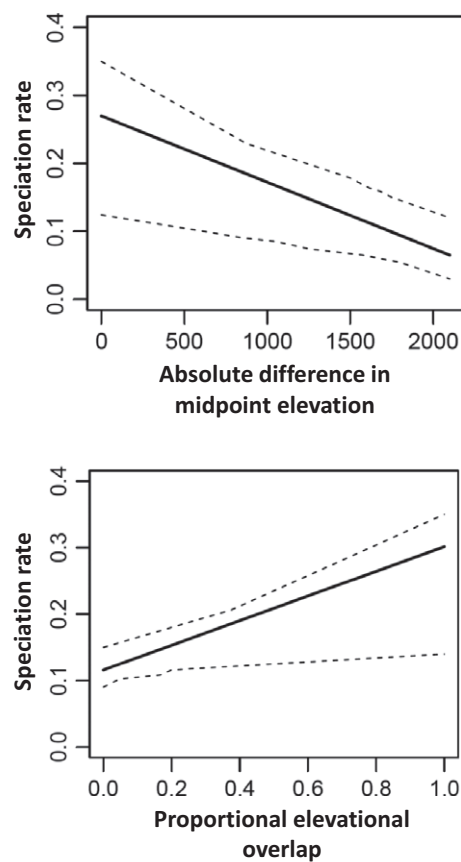
Speciation

Speciation is a two-step process that is generally initiated when populations become isolated by a geographic barrier to gene flow and is completed when populations evolve reproductive isolation. Many geographic barriers that initiate speciation and that operate within the Amazon basin are unlikely to drive substantial ecological differentiation that might accelerate trait evolution and reproductive isolation. For example, in the Amazonian lowlands, wide rivers are known to be important barriers to gene flow, with many closely related

species differentiating on opposing river banks (Mayr 1969; Haffer 2008; Leite & Rogers 2013; Weir *et al*. 2015). Arid river valleys play a similar role in the Andes, with cloud-forest populations of birds being fragmented by these barriers and differentiating on opposite sides of them. However, such geographic barriers are unlikely to be associated with strong divergent ecological selection pressures that would cause populations on opposing river banks or valley sides to experience elevated rates of trait divergence (Lawson & Weir 2014). The resulting species produced across these barriers usually represent only regional replacements rather than ecologically distinct lineages that can interact in broad sympatry (e.g. Weir 2009; Caro *et al*. 2013; Weir *et al*. 2015; Winger & Bates 2015). Recent studies show that lowland pairs differentiating across lowland Amazonian rivers often require millions of years to evolve reproductive isolation (Weir & Price 2011; Weir *et al*. 2015; Pulido-Santacruz *et al*. 2018), suggesting that the speciation process is protracted.

In contrast, the orogeny of the Andes created a series of ecologically distinct elevational zones (Cracraft 1985), which provided the potential for ecological opportunity to rapidly accelerate the speciation process for species pairs that differentiated in elevation. Elevational differentiation could accelerate both pre-mating and post-mating reproductive isolation. Pre-mating isolation would be promoted through divergent selection for different song features (or plumage colours, not tested here) that may optimise a males' attractiveness to females in different habitats at different elevational zones. Here, we found evidence for accelerated song frequency evolution with increasing elevational difference (measured either as difference in mid-point or as proportion of overlap). Frequency is a key aspect of song that is likely to be important for pre-mating isolation (e.g. Slabbekoorn & Smith 2002; Seddon 2005; Tobias *et al*. 2010). Also, direct adaptation to differences in the abiotic and biotic environments at different elevational zones could drive extrinsic post-mating isolation, whereby intermediate hybrids are unfit. Here, we found that a number of morphological features, including bill dimensions believed to be fine-tuned to the foraging niche (e.g. Derryberry *et al*. 2011), are evolving faster in pairs separated in elevation. Divergence in these traits likely contributes to extrinsic post-mating isolation. Together, our results for song and morphometrics demonstrate that the evolution of reproductive isolation, marking the completion of speciation, is likely accelerated in sister pairs undergoing elevational differentiation and suggests that ecological speciation is occurring within a subset of species among the Amazon basin. Only 10% of sister species pairs from the Amazon basin (including the eastern slope of the Andes) differ in their mid-point elevations by at least 1000 m, suggesting that, while ecological speciation mediated by elevational differentiation may play a role, it is unlikely to be a key driving factor behind most speciation events.

We next addressed whether rates of speciation are also accelerated along a gradient of elevational differentiation by applying a birth-death model to distributions of sister species pairs with different levels of elevational differentiation. Contrary to the expectation that species diverging in elevation will experience faster speciation rates, we found that sister species differing in elevation experienced a decline in speciation rate with differentiation in mid-point elevation and an increase in speciation rate with elevational overlap (Fig. 4). The lack of an accelerated speciation rate with elevational differentiation may indicate that faster evolution in song and morphometrics experienced by sister pairs which differ in elevation may not translate into faster speciation rates, and by consequence, may have limited influence on patterns of species richness (see also Rabosky & Matute 2013). However, the patterns uncovered may have resulted from greater opportunity for lowland to highland transitions when the eastern Andes were rapidly uplifted between 2 and 5 Ma (Gregory-Wodzicki 2000). Uplift of the eastern cordilleras of the Andes may have rapidly transported lowland populations to high elevations where they would have experienced distinct ecological pressures compared to their lowland counterparts and would have diverged in song and morphometrics rapidly. The slower rate of Andean uplift over the past 2 million years may have resulted in fewer opportunities for lowland to highland transitions, resulting in the apparent slower diversification rate of pairs that differ in elevation. Therefore, it seems plausible that elevational differences could lead to bursts of rapid diversification during

Figure 4 Change in speciation rates as a function of mid-point elevational difference between sister species of birds from the Amazon basin and adjacent Andean slopes. Maximum-likelihood estimates (solid bold line) and rates within 1.92 log-likelihood units of the maximum-likelihood value (approximately equivalent to a 95% confidence interval: dashed lines) are shown.

periods of rapid Andean uplift, but that such bursts have not occurred recently and are thus not reflected in the speciation rate estimates of our birth-death model fits. These fits assume constant rates of speciation through time and a burst of low to high transitions associated with periods of rapid orogeny would violate this constant rate assumption.

CONCLUSIONS

Speciation in birds from the Amazon basin may generally involve limited input from ecologically mediated divergent selection, especially for those species groups which closely track their preferred climatic environment or elevation through time. Many speciation events simply involve gradual differentiation on opposite sides of rivers or similar barriers, in which limited ecological difference occurs on either side of the barrier (e.g. Weir *et al.* 2015). However, our results suggest that for those species pairs which differentiate in elevation, the rapid evolution of traits important for reproductive isolation is driven by ecologically mediated divergent selection associated with elevational differentiation. This accelerated divergence is likely to have contributed importantly to the evolution of reproductive isolation for lineages differentiating in elevation during the uplift of the eastern cordilleras, but our results failed to demonstrate that elevational differentiation resulted in faster overall rates of speciation.

AUTHORSHIP

VELA and JTW designed the study; VELA collected the data and carried out analyses; VELA and JTW wrote the manuscript.

DATA ACCESSIBILITY SECTION

Data available from the Figshare Repository: http://doi.org/10.6084/m9.figshare.7627199.

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SUPPORTING INFORMATION

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