Article title: Selective sweeps and the role of divergent and parallel selection in Amazonian and Andean bird diversification

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Data accessibility statement: Code and an example dataset of called SNPs for this work are provided on GitHub (https://github.com/Weir-Lab/sisterspecies_sweeps/). Raw sequencing reads are archived on the National Center for Biotechnology Information Sequence Read Archive (PRJNA665047, PRJNA600749, PRJNA433557, PRJNA421731, and PRJNA725153).

Abstract

Both divergent and parallel selection can contribute to genomic and population differentiation, and thus speciation. However, the relative importance of each type of selection in driving speciation is poorly understood, especially in the context of environmental change. We compared selective sweeps and time of divergence in avian sister species pairs from the Amazonian lowlands that each underwent the same set of shared climatic cycles in parallel since speciating (*n*=9 pairs) with species pairs from different elevations and environments (*n*=8 pairs). Using phylogenetic multilevel Bayesian modeling, we inferred divergent selection in elevationally-differentiated pairs, while the lowland-restricted species pairs exhibited patterns consistent with parallel selection acting during glacial cycles as the key driver of selective sweeps. Parallel selection may play the key role in driving speciation in the species-rich lowlands where wide rivers are often the only barriers separating newly diverging species in similar environments.

Introduction

Natural selection leading to adaptation is a fundamental force shaping genomic differentiation and biological diversity (Darwin & Wallace 1858; Maan & Seehausen 2011; Ogden & Thorpe 2002; Price 2008; Schluter 2000). This genomic change may ultimately lead to the formation of new species due to the build-up of genetic incompatibilities that arise as closely related species differentially adapt (Campagna & Toews 2022; Nosil *et al.* 2009). Divergent natural selection, driven by environmental differences, can fast-track the formation of reproductive barriers between populations – a process often referred to as ecological speciation (Nosil 2012; Rundle & Nosil 2005; Schluter 2009). Populations can also differentiate under parallel selective forces when exposed to non-divergent environmental changes (Cerca 2023). In this case, each population adapts to the same environmental changes, but in different ways, resulting in genomic differentiation that may lead to speciation, especially if adaptations are disrupted in hybrid individuals. This process is often referred to as mutation order speciation (Mani & Clarke 1990; Nosil & Flaxman 2011). While both divergent and parallel selection are believed to operate in nature and contribute to the speciation process, little is understood about their relative importance in driving speciation and the build-up of species richness (but see Anderson & Weir 2022; Thompson *et al.* 2019). While divergent selection has been more studied and has often been proposed to be a dominant driver of trait evolution, recent comparative analyses across thousands of terrestrial vertebrate species suggested that only 10% of species had diverged in key traits (e.g., body size and bill shape) due to divergent (Anderson & Weir 2022). Here, we use a genomic approach to study the prevalence of positive selection and tease apart the contributions of parallel and divergent selection to genetic differentiation.

The Amazon basin provides an ideal laboratory to investigate both the contributions of divergent and parallel selection on diversification. Spanning more than six million square

kilometers with an elevation span from near sea level to over six thousand meters along the eastern slope of the Andes, the Amazon basin harbors an extensive array of habitat types. Broad expanses of rainforest cover the lowland plains. Ascending from the lowlands, a series of elevational zones carpet the eastern flank of the tropical Andes. Lowland rainforests extend to the foothill forest zone, which occurs from about 600 to 1,300 meters, followed by the epiphyte encrusted subtropical montane forest zone at 1,300 to 2,400 meters, above which the temperate montane forest zone occurs to treeline (about 3,300 m), with paramo and puna grasslands above treeline (Ridgely & Greenfield 2001) (Fig. 1). Each of these elevational zones has produced endemic species that specialize in habitats and ecological conditions within those zones and together, these zones encompass a region of exceptionally high species richness.

Many environmental factors vary with elevation, including both climatic variables (e.g., precipitation, temperature, solar radiation, wind speed, oxygen pressure, atmospheric CO_2, etc.) and biotic aspects (e.g., niche breadth, forest height, foraging strata, species diversity, competition strength, etc.) (Smith *et al.* 2014). These factors could, therefore, generate divergent selective forces for populations of a species – or newly diverged species – living at different elevations. In contrast to populations or species pairs differing in elevational zone along the Andean slopes, lowland-restricted rainforest species may be subject to strong parallel selection pressures across their ranges. For example, wide rivers often form dispersal barriers that bisect the ranges of widespread populations of lowland birds and other terrestrial species, leading to genetically differentiated populations on opposing riverbanks (Boubli *et al.* 2015; Dalapicolla *et al.* 2024; Naka & Brumfield 2018; Roncal *et al.* 2015; Weir *et al.* 2015). In many cases, these populations have become distinct species, each endemic to different Amazonian interfluves. However, environmental conditions do not generally differ appreciably on opposite sides of rivers. This lack of strong environmental differentiation suggests that parallel selection could be

a key factor driving *in situ* speciation within lowland forest species. Parallel selection may also operate within elevational zones of the Andes. When those elevational zones become bisected by arid river valleys or other barriers, populations occurring at the same elevational zones on either side of these barriers may diverge genetically due to parallel selection pressures.

For species already adapted to particular environmental conditions, parallel selection may act slowly to produce further genomic differentiation. However, sudden environmental changes could strengthen parallel selection pressures, resulting in an increased genomic divergence between allopatric populations or closely related species. Environmental change over the past several million years was most heavily influenced by the mid-to-late Pleistocene glacial cycles (Cracraft *et al.* 2020; Ramírez-Barahona & Eguiarte 2013; Rull 2011). Unlike high latitude regions, only the highest elevations of the Amazon basin – the higher reaches of the Andes – were directly glaciated. Nevertheless, glacial cycles influenced temperature and possibly rainfall across the Amazon basin. Elevational zones of the Andes shifted downslope during glacial periods. While most Andean species may have simply tracked their preferred environmental conditions by moving downslope and upslope during and after glacial periods, lowland species, often surrounded by river barriers on multiple sides, had nowhere else to migrate. Lowland populations may have been forced to adapt *in situ* to changing conditions. In this way, parallel selection driven by repeated glacial cycles over the past 700 kyr may have substantially impacted lowland species, leading to genomic differentiation and ultimately contributing to speciation. We hypothesized that glacial cycles in the mid- to late-Pleistocene have driven substantial parallel selection in birds of the Amazonian lowlands. Meanwhile, divergent selection may be prominent between lowland birds and those of the Andean highlands because the glacial cycles would have had diverging effects when species were from different elevations.

Here, we harness the power of genome-wide data to assess whether closely related pairs of avian species show signatures of either parallel or divergent natural selection. Positive selection – either parallel or divergent – may produce selective sweeps in the genome where the target of selection, and linked nearby regions, become fixed or highly differentiated between populations (high fixation index, F_{ST}), while genetic diversity within populations (nucleotide polymorphism, π) is reduced (Boitard *et al.* 2012; Hejase *et al.* 2020; Stephan 2016). Genomic signatures of strong selective sweeps often stand out as 'islands of differentiation' in F_{ST} and 'valleys of genetic diversity' in π, relative to the genomic background signature of these metrics (Wolf & Ellegren 2017). We calculated the number of such regions in the genomes for a series of closely related species or genetically differentiated populations within a species ("species pair" hereafter) (Fig. 2). In order to test the effect of elevational differentiation on the accumulation of selective sweeps through time, we divided our species pairs into two categories: lowland-restricted pairs, in which both species in a pair occur in the lowlands of the Amazon (i.e., generally below 1000 m; n=9 pairs); and elevationally-differentiated pairs, where the two species in each pair are differentiated in the elevational zones they occupy (n=8 pairs).

Both parallel and divergent selection produce selective sweeps. These may occur at the same genes or different genes for the two species in a species pair under both types of selection. It is thus not possible to differentiate which sweeps resulted from divergent versus parallel selection simply by looking at the genomic signatures. Instead, we used a different strategy to tease apart the role of parallel and divergent selection in Amazonian diversification. The major glacial cycles that are expected to have resulted in repeated bursts of parallel selection for populations or species occurring at the same elevations operated over the past 0.7 million years. We quantified the number of selective sweep signatures for a series of species pairs of different ages since they split from a common ancestor, but all of which are older than 0.7 Ma. All of

these pairs, thus, experienced all seven major glacial cycles that occurred during the mid-to-late Pleistocene. We predict that if glacial cycles driving bursts of parallel selection are primarily responsible for adaptive genomic differentiation in lowland-restricted pairs, we would see a nearly flat relationship between the number of selective sweeps and age of species pair – since all lowland-restricted pairs experienced the same number of bursts of parallel environmental change. Species pairs that are several million years old would have the same number of sweeps as those that are just one million years old. This prediction for lowland species pairs assumes that the effect of divergent selection in general and of parallel selection during earlier periods before the mid-to-late Pleistocene glacial cycles was negligible. If it were not negligible, then the number of selective sweeps would increase with species age rather than remain flat, with a slope near 0 in value. In contrast to lowland-restricted species pairs, we expect that divergent selection will have its largest impact on species pairs adapting to distinct elevational zones. Older pairs will have had more time to adapt under divergent selection and, therefore, we predicted that the number of selective sweeps in elevationally differentiated species pairs would increase with increasing age of species pairs, thus slopes would have positive values above 0, and be steeper than in species exhibiting parallel selection. Older species pairs in this latter category will have more selective sweeps than similar age species pairs restricted to the lowlands.

Methods

Study species

We examined genomic divergence of 17 closely related allopatric species pairs: 9 lowland-restricted pairs, in which both species occur at low elevations in the Amazon basin; and 8 pairs at different elevations, in which one species belongs lower in the Amazonian lowlands than the other in the Andean highlands. In the latter category, both species of the *Chlorospingus* pair are Andean, but one occurring at higher elevations, *C. parvirostris*, than its sister species, *C. flavigularis* (Supplementary Fig S1). To explore the differences in elevation, we collated elevational information for each species from either a book specializing in Amazonian birds (Schulenberg *et al.* 2007) when available, or the Birds of the World online resource if not (Del Hoyo *et al*. 2020b, a; Marantz *et al.* 2020; Snow 2020b, a; Snow & De Juana 2020; Snow & Sharpe 2020; Zimmer *et al*. 2020). At least four individuals per species were analyzed (mean = 9.5; range = 4 to 24). Divergence time of species pairs spans from 0.7 Ma to about 6 Ma, representing different stages of the speciation continuum.

Blood or tissue samples of most bird species from the tropical Andes were collected in the Manu Biosphere Reserve in the Department of Cusco or at a variety of locations in the Department of San Martín, Peru. Samples of lowland-restricted species were mostly obtained from Matto Grosso, Brazil. Complementary tissue samples were obtained from Museu Paraense Emilio Goeldi (MPEG), the Louisiana State University Museum of Natural Science (LSUMNS), and the Field Museum of Natural History (FMNH).

Library preparation and sequence data

A total of 323 individuals were analyzed among 34 species (17 species pairs). DNA was extracted from blood or tissue samples using E.N.Z.A tissue or blood extraction kits (Omega Bio-Tek). Genotyping-By-Sequencing (GBS) libraries (Elshire *et al.* 2011) were generated for all samples. The Cornell Institute of Genomic Diversity generated libraries for several lowland species in the dataset and these were sequenced on the Illumina HiSeq 2000 platform using 100 bp single-end sequencing. An almost identical GBS method (following the protocol modifications in Luzuriaga□Aveiga *et al.* 2021) was used to generate libraries for the remaining species. The latter were sequenced on the Illumina HiSeq 4000 platform by the Centre for

Applied Genomics at the Hospital for Sick Children (SickKids), using 100 bp paired-end sequencing. Sequences of some individuals have already been published by the Weir Lab. Details of individuals used, accession numbers, and sequencing method can be found in the Supporting Online Materials.

Sequences were demultiplexed and filtered using the function *process_radtags* of the STACKS 2.3 pipeline (Catchen *et al.* 2011), with default parameters. Reads were trimmed for adapters and low-quality sequences using Trimmomatric-0.38 (Bolger *et al.* 2014), with the following parameters: TRAILING:3 SLIDINGWINDOW:4:10 MINLEN:30. Sequence reads of individuals of each species were aligned to the reference genome of its closest relative available (Supporting Online Materials) using Bowtie2: --phred33 --very-sensitive --mp 5,1 --score-min L, -0.6, -0.6. The *mpileup* and *call* functions of BCFtools 1.12 (Li 2011) was used to call genotypes using default settings. Individual genotype calls with < 5x depth and with a genotype likelihood of < 20 were set to missing.

For each species pair we generated three different filtered datasets, each used for different purposes. Dataset 1 was used to calculate d_{XY} and π, for which variable and non-variable sites are needed. We excluded sites with more than 90% missing genotype calls and SNPs with high heterozygosity (>0.75). Dataset 2 was used to calculate F_{ST}, for which only biallelic sites are required. SNPs with a minimum of 4 genotype calls per population were retained. We excluded sites with minor allele frequency <0.05, with mean sequencing depth greater than 95th percentile, and with observed heterozygosity exceeding 0.75. Dataset 3 was used to generate Site Frequency Spectra (SFS). We used identical filtering parameters as in dataset 1, except we removed genotype calls with < 10x depth, and to reduce linkage disequilibrium, we thinned dataset 3 to retain only one SNP per 10 kb.

Detection of lineage-specific selective sweeps

We first ran a relatedness analysis of individuals within species, using *ngsRelate* (Korneliussen & Moltke 2015). For this analysis, we used unfiltered VCF files of all individuals per species and kept default parameters for the estimation of Jacquard coefficients and relatedness summary statistics. Individuals with co-ancestry coefficient (theta) > 0.1, equivalent to half sibling relationship or higher (Ackerman *et al.* 2017) one of the two related individuals was removed.

Next, we examined signatures of selective sweeps in each of the 17 species pairs, using summary statistics. We used Pixy 1.2.7 (Korunes & Samuk 2021) to calculate $d_{XY,}$ using Dataset 1, and F_{ST} and π, using Dataset 2, across 250 kb non-overlapping windows. Only windows with a minimum of 10 SNPs were retained. The female *W*-chromosome was excluded because many individuals were male and we did not have enough female individuals in many comparisons to include the *W*. F_{ST}, d_{XY} and π for each window were transformed into *z*-scores by subtracting the mean across windows and then dividing it by the standard deviation across windows. Mean and standard deviations were calculated separately for each macrochromosome (chromosomes > 2.5-6 μm long (Griffin *et al.* 2007); chromosomes 1-10, 1A, 4A and Z) and jointly for the microchromosomes (chromosomes < 2.5 μm long; chromosomes 11- 29). One tailed *P*-values were calculated from *z*-scores using the *pnorm* function in R (R Core Team 2021), with lower.tail = FALSE to detect significant peaks of F_{ST}, and lower.tail = TRUE to detect significant drops of π and d_{XY}. Peaks or drops were considered significant if their *p*-value was ≤ 0.05.

Windows were considered to exhibit signatures consistent with lineage-specific selective sweeps if they possessed significantly elevated peaks in F_{ST} and significantly reduced drops in π in one (lineage-specific sweeps) or both (sweeps targeting the same window in both species) species of a pair, and with no significant drop in d_{XY}. In contrast, a simultaneous drop in d_{XY} (Fig. 2D) together with elevated in F_{ST} and π in both species of a pair would be consistent with either a selective sweep that occurred in the common ancestor or of a selective sweep that crossed species boundaries through introgression (Irwin *et al.* 2016). Selective sweep signatures may span more than one 250 Kb window. To avoid counting these as multiple sweeps, we combined windows of selection less than 1 Mb apart as a single sweep. We standardized the number of detected selective sweeps across species pairs by dividing the number of windows of selection observed by the total number of windows assessed per species pair.

Estimation of divergence times

We used FASTSIMCOAL2 (Excoffier & Foll 2011) to estimate the effective population size (N_e) of each member of a species pair, as well as divergence time from a common ancestor. We used Arlequin v3.5 (Excoffier & Lischer 2010) to generate a multidimensional site frequency spectrum (SFS) of species by re-filtering SNPs from down-samples of at least 4 (diploid) individuals per species. We used a minimum site depth cutoff of 10 per individual and manually

entered the number of non-variable sites into the SFS. We used family-specific generation times found in the literature to calibrate both per generation mutation rates, calculated from whole-genome datasets in Feng *et al.* (2020) and time of divergence in Myr (Supplementary Table S1). These mutation rates were used to calibrate our demographic models. To build an understanding of the data, we explored a linear model of $\log_{10}N_e$ and mean π in a species, with the expectation that the two values should be correlated.

Patterns of adaptive genomic evolution through time

Using linear regression, we compared how the number of detected selective sweeps accumulated through time for species pairs restricted to the lowlands versus different elevations. If elevational differentiation promotes divergent selection, we would expect to find a higher number of selective sweeps and *y*-intercept (or slope) values in species pairs that occur at different elevations versus lowland-restricted pairs. Potential phylogenetic non-independence of sister pairs was corrected using the following approach. We pruned a time-calibrated phylogeny of all birds of the world (Pulido-Santacruz & Weir 2016) to retain only one representative species for each pair in the dataset, using the *keep.tip* function in the R package APE (Paradis & Schliep 2019). A phylogenetic multilevel model with the number of detected sweeps dependent on the interaction of divergence time of each pair and category (lowland versus different elevations), and the phylogenetic variance-covariance matrix as a varying intercept was fit in the R package brms (Bürkner 2017, 2018, 2021). Specifically, we used a normal distribution to fit the model, with normally distributed priors of mean 0 and standard deviation 0.01 for the model intercept and coefficients, and Student t-distributed priors with 3 degrees of freedom for overall standard deviation and sigma. The model was run over 4 independent Hamiltonian Monte Carlo chains, using 5,000 burn-in and 15,000 sampling iterations each (60,000 iterations total). Adapt delta was raised to 0.999, and maximum tree depth was raised to 12. Trace lots, potential scale reduction factors $\hat{R}$ of 1, and posterior predictive checks over 100 draws were used to assess model fit. To assess differences in slopes between species pairs in the lowlands versus those from different elevations, we used the *hypothesis* function in brms. Specifically, we tested a directional hypothesis that the slope of the birds from different elevations was greater than the slope of birds from the same elevations.

We used linear modeling to assess the possibility that other patterns may have driven the observed selective sweeps, as opposed to ecologically-driven parallel or divergent selection. Using linear models in R, we tested models of the number of detected sweeps dependent on an interaction of either mean π or $\log_{10}N_e$ and elevational category at a species-level, highland or lowland. Another linear model was used to assess whether the accumulation of selective sweeps in highland-lowland pairs was driven by suboscine or oscine species. This was done in a manner consistent with the overall model of selective sweeps and divergence time in brms, except only lowland-highland species pairs were used, and a categorical variable of suboscine or oscine was tested in its interaction with divergence time, rather than elevational category, as previously done. Here, a directional hypothesis test was used to assess whether the suboscine and oscine species each accumulated sweeps over time at a slope greater than 0, as we predicted for the species pairs representing different elevations. We also visualized the proportion of overlap in elevational ranges between lowlands-restricted species pairs and those from different elevations, and plotted those values against the number of observed selective sweeps.

Results

The species categorized as from highland elevations had generally greater elevational ranges than lowland species (Supplementary Fig S2). Two species pairs in genera *Chlorospingus* and *Ramphocelus* had substantially overlapping elevational ranges, but the populations studied existed in allopatry (Schulenberg *et al.* 2007). The standardized number of detected selective sweeps varied between species pairs and elevational categories in a model with overall R^2 of 0.65 (95% credible interval (CI): [0.34, 0.88]) (Fig. 3). Lowland-restricted pairs had a flatter relationship between detected selective sweeps and age of the species pair (slope = $8.20\text{x}10^{-5}$, 95% CI: [$-1.62\text{x}10^{-3}$, $1.52\text{x}10^{-3}$]) than the pairs from differing elevations (slope = $3.14\text{x}10^{-3}$, 95% CI: [$7.69\text{x}10^{-4}$, $5.31\text{x}10^{-3}$]). An evidence ratio of 135 from a directional hypothesis test corresponded to a 99.3% posterior probability that the pairs from differing elevations had a higher slope than the lowland-restricted pairs. Observed π or N_e were correlated (r=0.72, adjusted R^2=0.50, p=$2.01\text{x}10^{-6}$; Supplementary Fig S3), and neither drove observations of selective sweeps, (p=0.17 and 0.40, respectively) (Supplementary Figs S4; S5). A paired t-test of pairs at

different elevations shows that highland species have consistently lower π but not $\log_{10}N_e$ relative to their lowland close relative (paired t-test: $p = 0.025$ (π), $p = 0.28$ (N_e); Fig. 4).

A limitation of our study design is the taxonomic mismatch between species categories. All species pairs from the Amazonian lowlands are suboscines, whereas species pairs at different elevations are phylogenetically more diverse, including two non-passerine pairs (motmots and woodpeckers), three oscines (tanagers and icterids) and three suboscines (woodcreepers, manakins, and flycatchers). However, we found that non-suboscine species in elevationally differentiated pairs accumulated selective sweeps at a similar pace through time as suboscine species, and both exhibited signatures of divergent selection as slopes were greater than 0 (oscine evidence ratio 44.6, 97.8% posterior probability; suboscine evidence ratio 381.2, 99.7% posterior probability) (Fig. S5). Moreover, all linear models were corrected for phylogenetic structure. As such, we do not believe that the differences in the patterns found here between lowland-restricted species pairs and pairs with elevation differentiation were driven by phylogenetic composition. In addition, no relationship was evident between the proportion of elevational range overlap and the number of selective sweeps in either category of lowlands-restricted species pairs or those from different elevations (Supplementary Fig S7).

Discussion

Among birds in the Amazonian basin, we observed a nearly flat relationship between sister species age and the number of observed selective sweeps in the lowland species pairs. Meanwhile, the dataset with one lowland and one highland species in each pair had a positive relationship between age and number of observed sweeps. This difference in slope was robust to phylogenetic structure, and independent of genome-wide π or N_e. From these observations, we inferred that elevationally differentiated species pairs exhibited a striking signature of divergent selection, while lowland-restricted species pairs were characterized by parallel selection. These differences in selective pressures are important in the context of a cyclic climatic history in the Amazonian lowlands and adjacent Andean slopes during the Pleistocene, with especially strong cycles starting 700 ka (Angel *et al.* 2017; Bräuning 2009; Liu *et al.* 2008; Nevado *et al.* 2018; Ramírez-Barahona & Eguiarte 2013; Rutherford & D'Hondt 2000; Simpson 1975). Therefore, these results demonstrate extensive parallel selection in lowland sister species that experienced

similar environmental cycles, and environmentally-driven differences in the roles of parallel and divergent selection in shaping species divergence and richness.

The strong positive increase in the number of selective sweep signatures through time in elevationally-differentiated pairs is consistent with ongoing divergent selection along elevational zones. Older pairs would have had more time to adapt to different elevational zones, resulting in a greater number of selective sweeps compared to younger pairs. This result aligns with previous studies, which demonstrated that avian species pairs with elevational differentiation evolved different phenotypic (Luzuriaga-Aveiga & Weir 2019) and physiological adaptations (Lague *et al*. 2017, 2020) in the Andes and have much faster rates of trait evolution compared to species pairs restricted to the lowlands. Divergent selection may therefore accelerate evolution, which is a finding consistent with a large body of research that links rapid adaptation to divergent selective pressures (Garcia Castillo *et al*. 2024; Schluter 2001; Wadgymar *et al.* 2022; White *et al*. 2022).

For species pairs restricted to lowland regions, the flat relationship between the number of detected selective sweeps and time of species divergence was likely generated by parallel selection. If the major climatic cycles of the past 700 kyr were primarily responsible for parallel selection compared to earlier periods, then all species pairs from the same lowland elevations older than 700 kyr (i.e., all species pairs in our dataset) should have experienced roughly the same amount of parallel selection, regardless of their differing time of divergence from a common ancestor. All pairs should thus exhibit approximately the same number of selective sweeps regardless of their differing ages. Our findings are consistent with this expectation. Moreover, this flat relationship with time also suggests that in birds of the Amazonian lowlands, cyclic parallel selection driven by mid- to late-Pleistocene glacial cycles was a more prominent evolutionary force than either divergent selection or parallel selection occurring before the mid-Pleistocene, when the climate was more stable.

A limitation of this work is that genomic architecture may have influenced the results. We analyzed F_{ST} and π in windows of at least 250 kb, thus, smaller sweeps would be undetected. The larger sweeps we were able to detect may be associated with architecture such as chromosomal inversions, which are understood to support local adaptation and selective sweeps in a range of species (Cutter & Payseur 2013; Wellenreuther & Bernatchez 2018). Centromeres

and telomeres possess low recombination rates that facilitate sweeps, which lower π and elevate F_{ST} over broad genomic regions, thereby generating strong sweep signatures that are easily detected (see Cutter & Payseur 2013). However, there are only a limited number of these genomic features, and if they were a major contributor to our detections of selective sweeps, the number of such selective sweep signatures we detected would be constant with increasing time, as sweeps in older species pairs would be overwritten by subsequent sweeps. In such a scenario, the elevationally-differentiated pairs would also exhibit a flat relationship between species pair age and the number of selective sweeps, like lowland-restricted pairs. Instead, we uncovered a strong positive slope for elevationally-differentiated pairs (Fig. 4), indicating that avian genomes are capable of accumulating detectable selective sweep signatures through time. Therefore, the approach we adopted for detecting divergent selection might be influenced by, but was not invalidated by conserved genomic architecture.

While the difference we uncovered between lowland-restricted and elevationally-differentiated sister pairs followed our expectations, caution is still needed when interpreting our results. Adaptive alleles do not always generate F_{ST} peaks when traits under selection are highly polygenic, because polygenic traits may evolve with incomplete sweeps at many loci. In such cases, the influence of selection on individual loci is likely too small to be detected (Stephan 2016). Similarly, soft sweeps acting on standing variation may generate too weak of a signature for our methods to detect, because with standing variation the target of selection will have recombined with a variety of surrounding genetic variants, which then fail to materialize as a sweep signature when the target is fixed (Fig. 2). It thus remains possible that divergent selection may have operated between lowland-restricted species pairs in primarily polygenic traits or soft sweeps. This scenario is also plausible because greater nucleotide diversity observed in the lowland species (Supplementary Fig S2) means there is a larger pool of standing genetic variants present that soft selection could act upon. Given these considerations, we can only conclude that divergent selection driven by elevational differentiation has increased the number of signatures of hard selective sweeps compared to lowland-restricted pairs.

Despite the limitations of our approach, we found a clear signal that elevationally-differentiated species pairs exhibit stronger divergent selection, in terms of increased numbers of hard selective sweeps, compared to species pairs found at the same lowland elevations. These results highlight the importance of divergent ecological selection in adaptation to different

habitats, which may cause elevated F_{ST} and differences in π between sister taxa. But does this result in a faster speciation rate or a greater build-up of high species richness? Elevational differentiation in Neotropical avian species appears to be the exception rather than the rule. More commonly, birds show signatures of niche conservatism – a process in which species track their preferred climatic environments and habitats through long periods of evolutionary time (Pyron *et al.* 2015; Wiens *et al.* 2010). Niche conservatism leads to the production of geographic replacements that occur on opposite sides of physical barriers, such as wide rivers in the Amazonian lowlands or arid river valleys in the Andes. These geographic replacement species retain similar niche requirements and continue to evolve under parallel selective pressures. A recent meta-analysis of birds, mammals, reptiles, and other groups found preliminary evidence that parallel selection was a generally stronger force in shaping trait evolution than divergent natural selection (Anderson & Weir 2022). One possible reason for this is that divergent natural selection tends to accelerate reproductive isolation based on ecological differences that are ephemeral (Anderson *et al.* 2023a; Cutter & Payseur 2013), such as, hypothetically, rainfall patterns that change over short timescales in the regions studied here (Lavado Casimiro *et al.* 2013). When those ecological differences evaporate, reproductive isolation disappears, and species collapse back into a single gene pool (Anderson *et al.* 2023b). In contrast, reproductive isolation driven by parallel selection may be more likely to generate intrinsic incompatibilities that are less likely to be ephemeral and result in species that are less prone to collapse, thus contributing more to long-term buildup of high species richness (Anderson *et al.* 2023). While we found a clear signature of divergent natural selection in rare cases where species have diverged tremendously in their niche by adapting to different elevations, we failed to find evidence of divergent selection in pairs that were restricted to the lowlands, a region of exceptionally high species richness. Our results highlight the potential importance of niche conservatism and parallel selection in diversification in one of the most species-rich environments of the planet. Our results suggest that divergent selection may accelerate evolution, but does not strongly contribute to the build-up of species richness in super-rich regions like the Amazonian lowlands.

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Tables & Figures

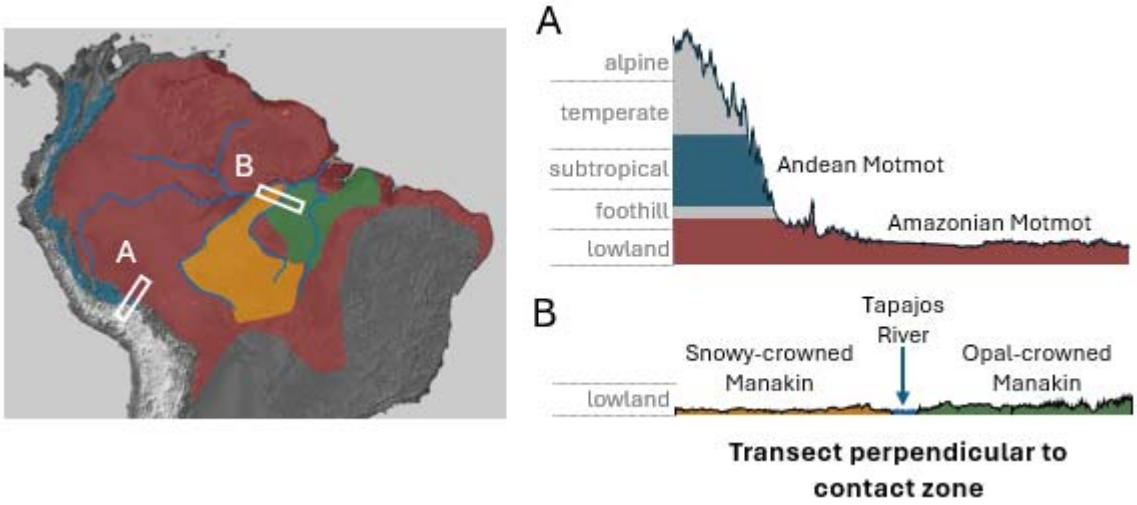

Figure 1. Geographic ranges of two example species pairs overlaid on a topographical map of South America. In A) the foothill forest zone occurs from approximately 600 to 1,300 m (foothill), the epiphyte encrusted subtropical montane forest zone from approximately 1,300 to 2,400 m (subtropical), the temperate montane forest zone to the treeline at approximately 3,300 m (temperate), and the alpine zone exists above 3,300 m. One species pair has the Amazonian Motmot (*Momotus momota*), in red, distributed in lowland forest from 0 to 700 m and its sister species, the Andean Motmot (*M. aequatorialis*), in blue, distributed in Andean cloud forest from 1000 to 2300 m. The other species pair has both species distributed in low elevation rainforest in different regions of the Amazon basin, with the Opal-crowed Manakin *(Lepidothrix iris*) distributed east of the Tapajos River and the Snowy-crowed Manakin (*L. nattereri*) distributed west of the river. Insets A and B show 250 km-long transects running perpendicular to the contact zones between each species pair. Coloured bands show the elevational profiles of each species. Shaded relief topographical base map obtained from NASA (https://www.nasa.gov). Major rivers of the Amazon basin are shown in blue.

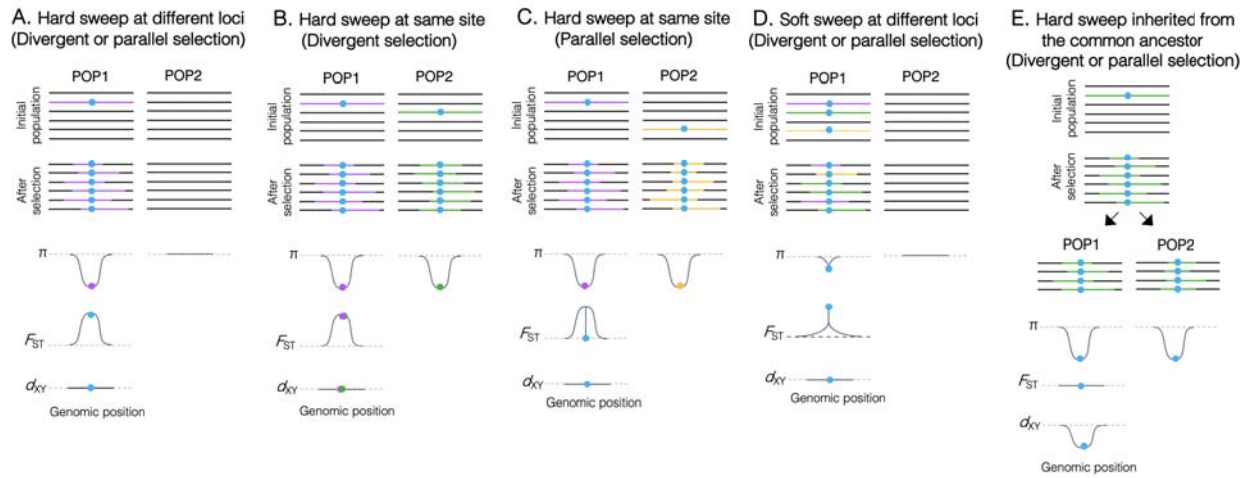

Figure 2. Hard and soft selective sweeps, with corresponding expectations of π, F_{ST} and d_{XY} under divergent and parallel selection. In hard sweeps (A–C, E), a positively selected de novo mutation (turquoise circle) arises in a single genomic background. Selection causes fixation of this mutation together with closely linked surrounding blocks of the genomic background that have not had time to experience recombination (coloured segments). The soft sweep (D) is similar, but the allele that is the target of selection is segregating in the population prior to becoming positively selected, resulting in all genomic backgrounds it is associated with increasing in frequency. The resulting patterns of π, F_{ST} and d_{XY} after selection can be used to detect potential selective sweeps. Hard sweeps at different loci (A) result in drops in π only in the species that is the target of selection for a given locus. The drop in π elevates F_{ST}. Hard sweeps at the same site (B–C) result in a drop in π in both species and an increase in F_{ST}. They produce almost identical patterns of F_{ST} and π under divergent (B) and parallel (C) selection but differ only in the value of F_{ST} at the site that is the target of selection, with the site having an F_{ST} of 1 under divergent selection (where different alleles at the site of selection are fixed), and an F_{ST} of 0 under parallel selection (where the same allele at the site of selection is fixed). Sweeps resulting from divergent or parallel selection cannot easily be discriminated. Soft sweeps at different loci (D) produce similar patterns in π and F_{ST} as hard sweeps, but the drops in π and increases in F_{ST} are less extreme, making them harder to detect. A sweep in the common ancestor of two populations (E) should result in both daughter populations inheriting low π around the selected site, but, unlike sweeps at the same site in both populations, an ancestral sweep results in a drop in F_{ST} and in d_{XY} between the daughter populations. Turquoise circles show the allele that is the target of selection. Coloured lines represent different haplotype blocks.

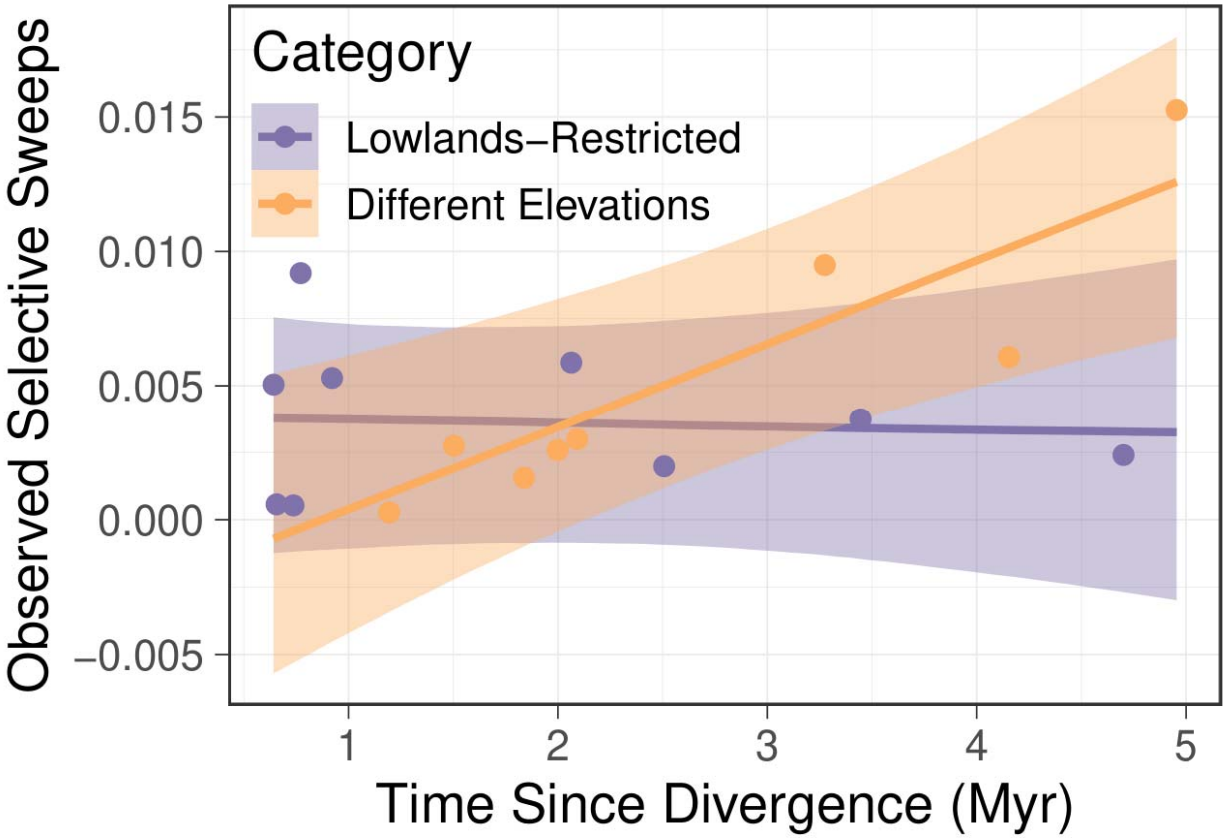

Figure 3. Number of observed selective sweeps accumulated between species pairs versus their divergence times, calculated from the nuclear genome using demographic modeling. Species pairs restricted to the Amazonian lowlands are shown with purple, and pairs occupying different elevational zones from the Amazonian lowlands towards the high Andes are shown in orange. Lines show model fits with phylogenetic relatedness accounted for. Shading shows the 95% credible intervals.

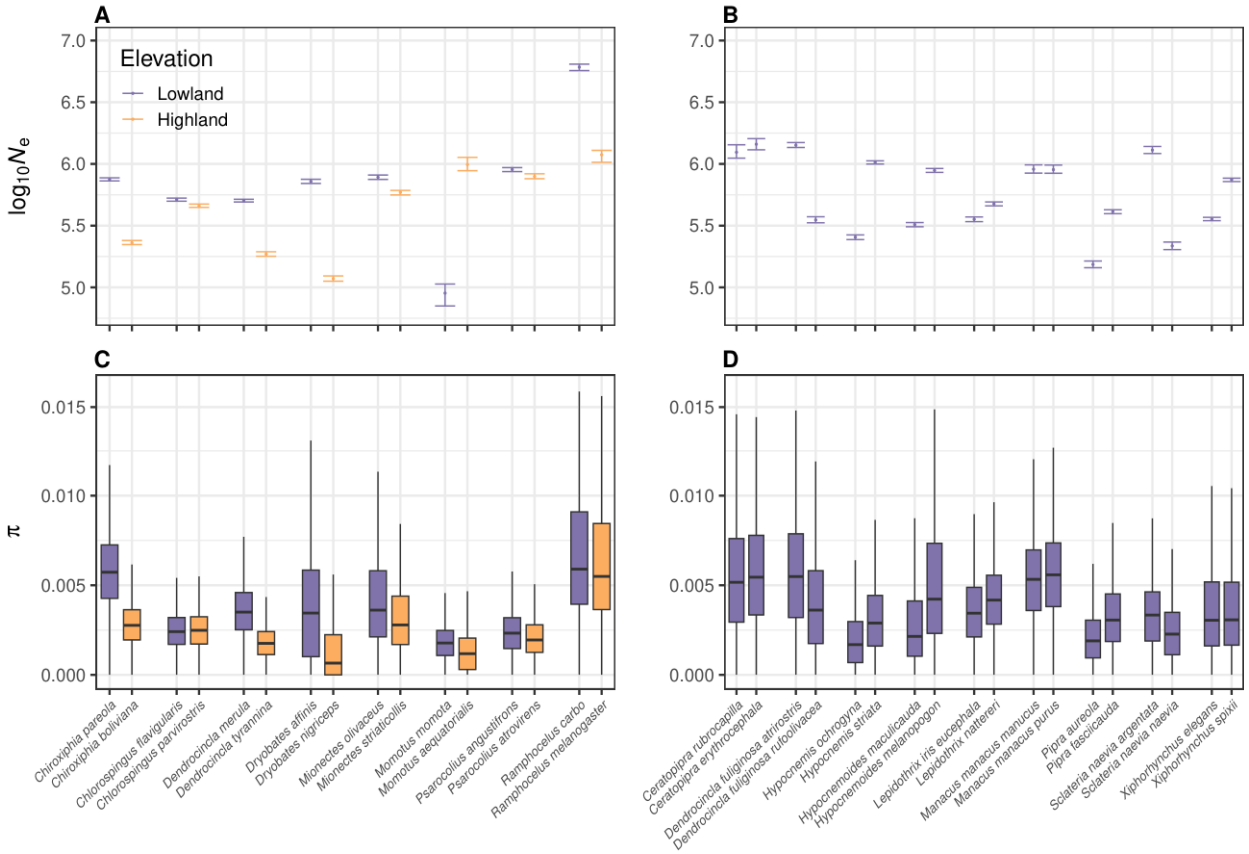

Figure 4. Estimated effective population size ($\log_{10}N_e$, top panels A and B) and box and whisker plots of average genome-wide nucleotide diversity (mean π, bottom panels C and D) across 250 Kb genomic windows. Species delineated by category, between pairs from different elevations (A and C) and lowland-restricted pairs (B and D). Lowland species are in purple, while highland species are in orange.

Supporting Information

Supplementary Table S1: Generation times and mutation rates used in coalescent modelling for major families represented in the dataset.

Family	Genus	Generation time	Mutation rate
Icteridae	*Psarocolius*	Mean = 1.83	2.096×10^{-9}
Thraupidae	*Ramphocelus*	Mean = 2.86	3.98×10^{-9}
Passerellidae	*Chlorospingus*	Mean = 2.33	2.99×10^{-9}
Thamnophilidae	*Hypocnemoides* *Hypocnemis* *Sclateria*	Mean = 2.44	4.14×10^{-9}
Dendrocolaptidae	*Xiphorhynchus* *Dendrocincla*	Mean = 2.83	4.14×10^{-9}
Tyrannidae	*Mionectes*	Mean = 1.98	3.73×10^{-9}
Pipridae	*Ceratopipra* *Pipra* *Lepidothrix* *Manacus* *Chiroxiphia*	Mean = 3.34	6.06×10^{-9}
Picidae	*Dryobates*	Mean = 2.86	6.89×10^{-9}
Momotidae	*Momotus*	Mean = 2.85	5.84×10^{-9}

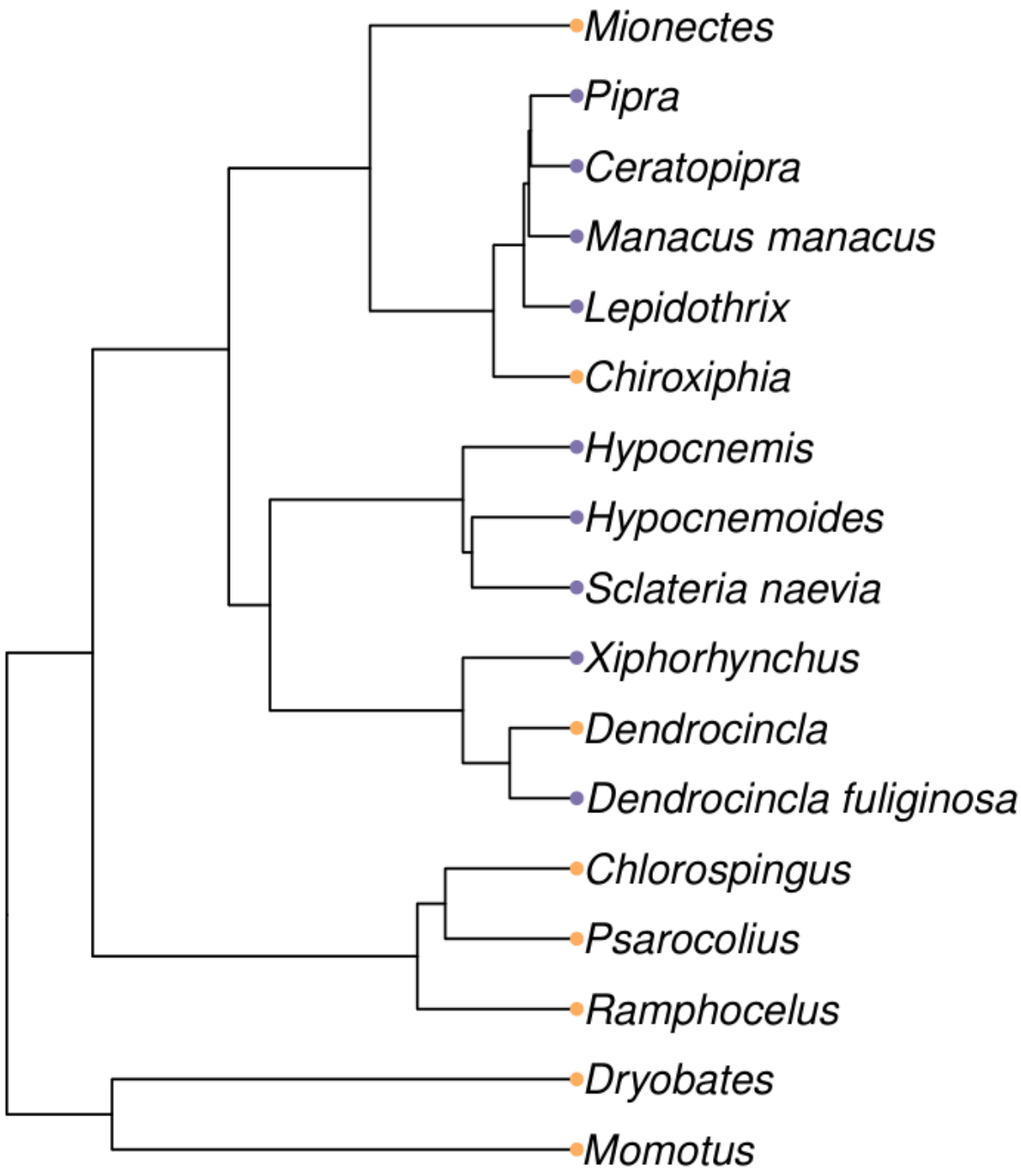

Figure S1. Phylogeny of the taxa included in the dataset. Tips of the tree represent sister species or subspecies pairs, colour-coded by their elevational category: purple for lowland-restricted pairs and orange for pairs at different elevations. *Dendrocincla fuliginosa* from the lowlands represents two subspecies, *D. f. atrirostris* and *D. f. rufoolivacea*. *Dendrocincla* at different elevations represents *D. merula* and *D. tyrannina.*

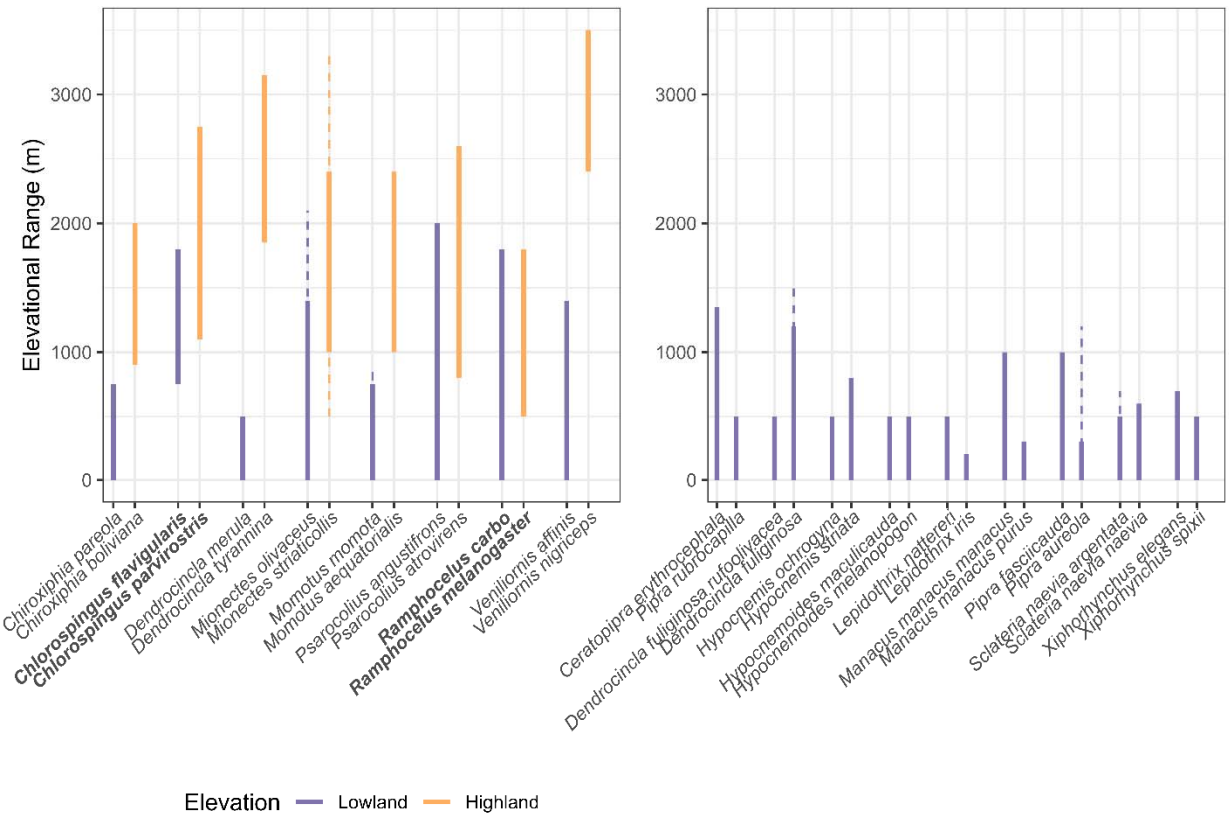

Figure S2. Elevational ranges for species in the present study. For species where both a core range and maximum and minimum observations were recorded, dashed lines were used to show the extreme ends of a range. However, this information was not available consistently. While genera *Chlorospingus* and *Ramphocelus* (bold) had largely overlapping species elevational ranges, they were allopatric and likely experienced different historical environments.

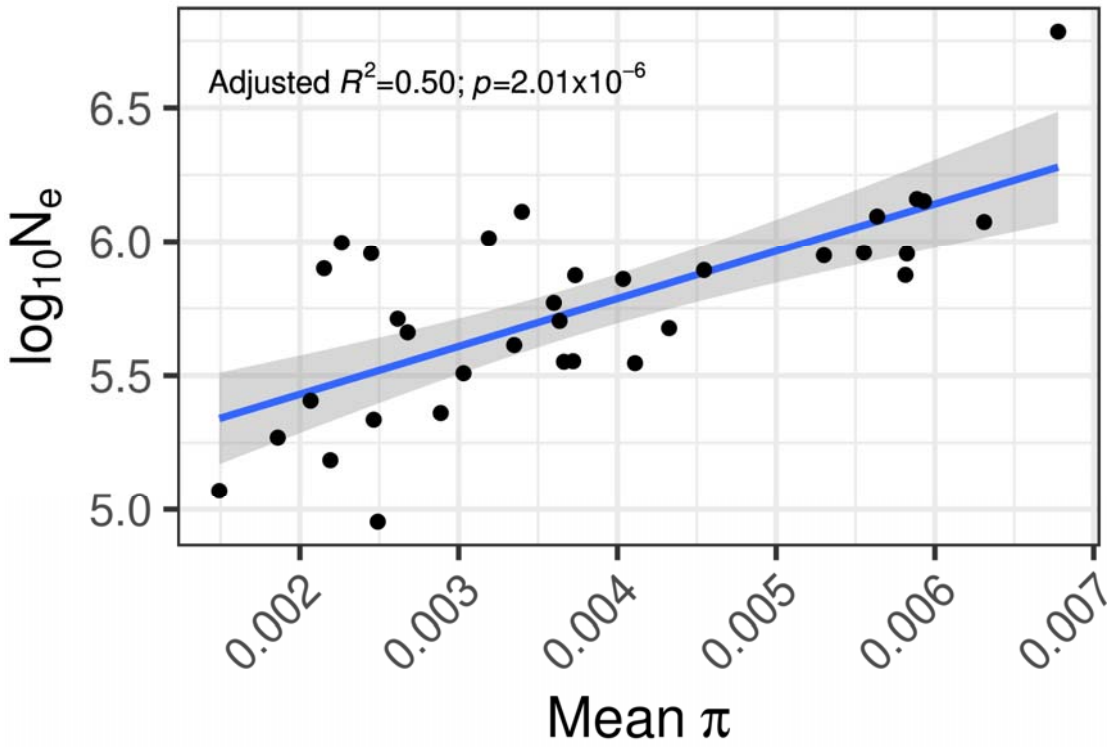

Figure S3. Linear regression between effective population size (N_e) and mean genome-wide nucleotide diversity (π).

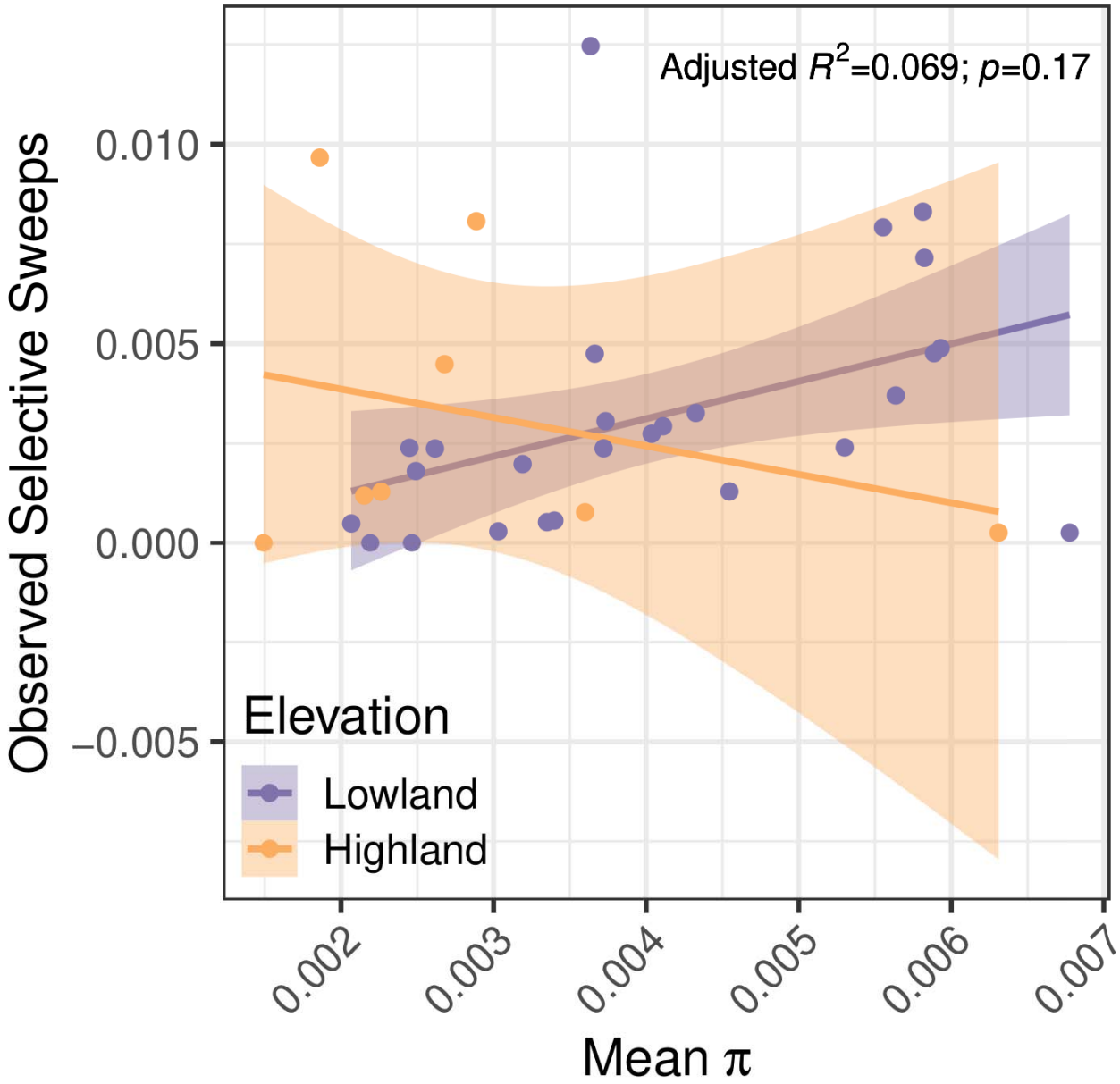

Figure S4. Regression analyses between number of lineage-specific windows of selection and average genome-wide nucleotide diversity, π, for individual species (not species pairs). Here, mean π interacted with elevational category of lowland or highland. Species from the Andean highlands are shown in orange, and lowland Amazonian species are shown in purple. This model was not significant (p=0.17).

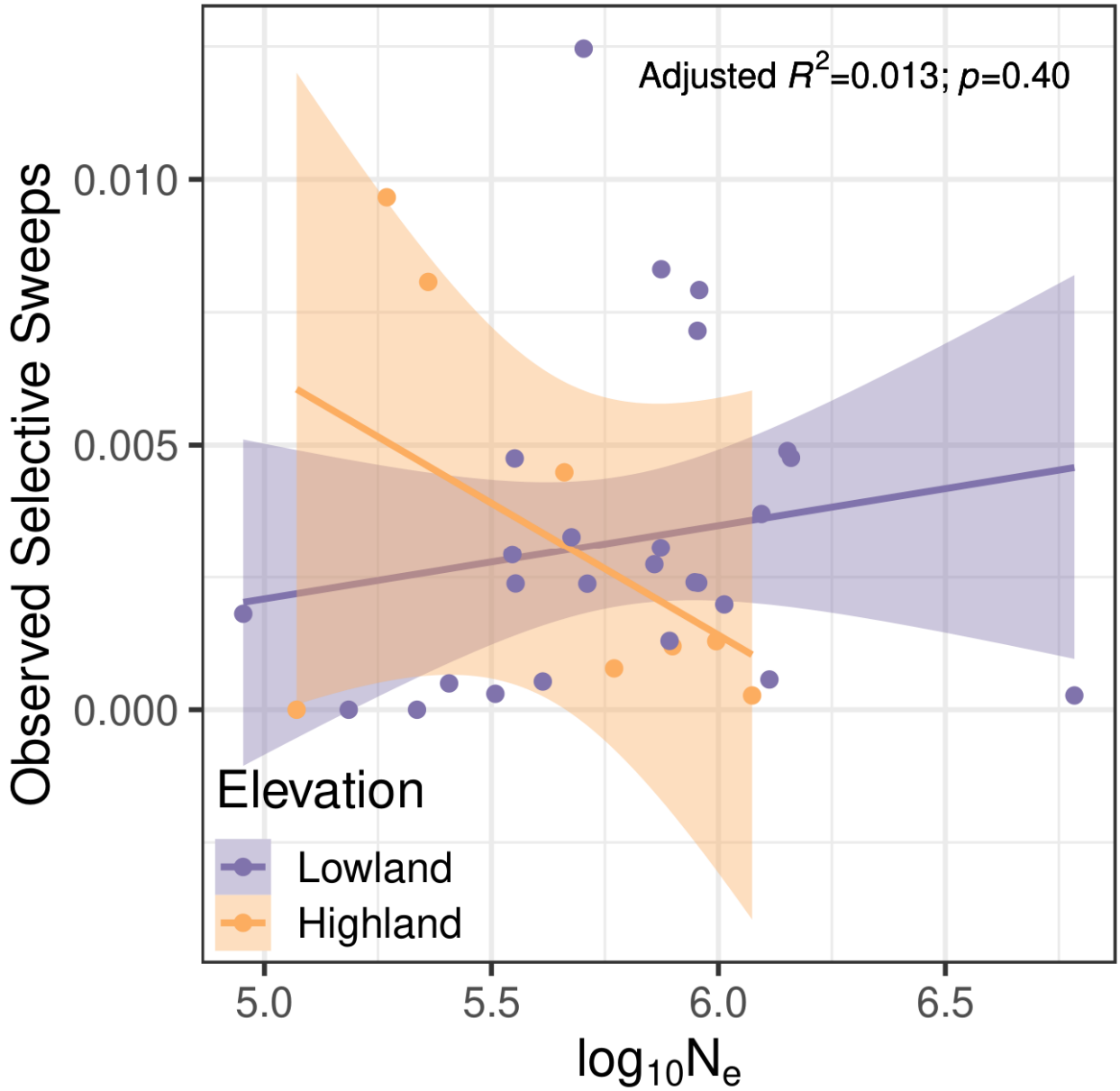

Figure S5. Regression analyses between the number of lineage-specific windows of selection and $\log_{10}$ effective population size ($\log_{10}N_e$) for individual species. Here, $\log_{10}N_e$ interacted with elevational category of lowland or highland. Species from the Andean highlands are shown in orange, and lowland Amazonian species are shown in purple. This model was not significant (p=0.40).

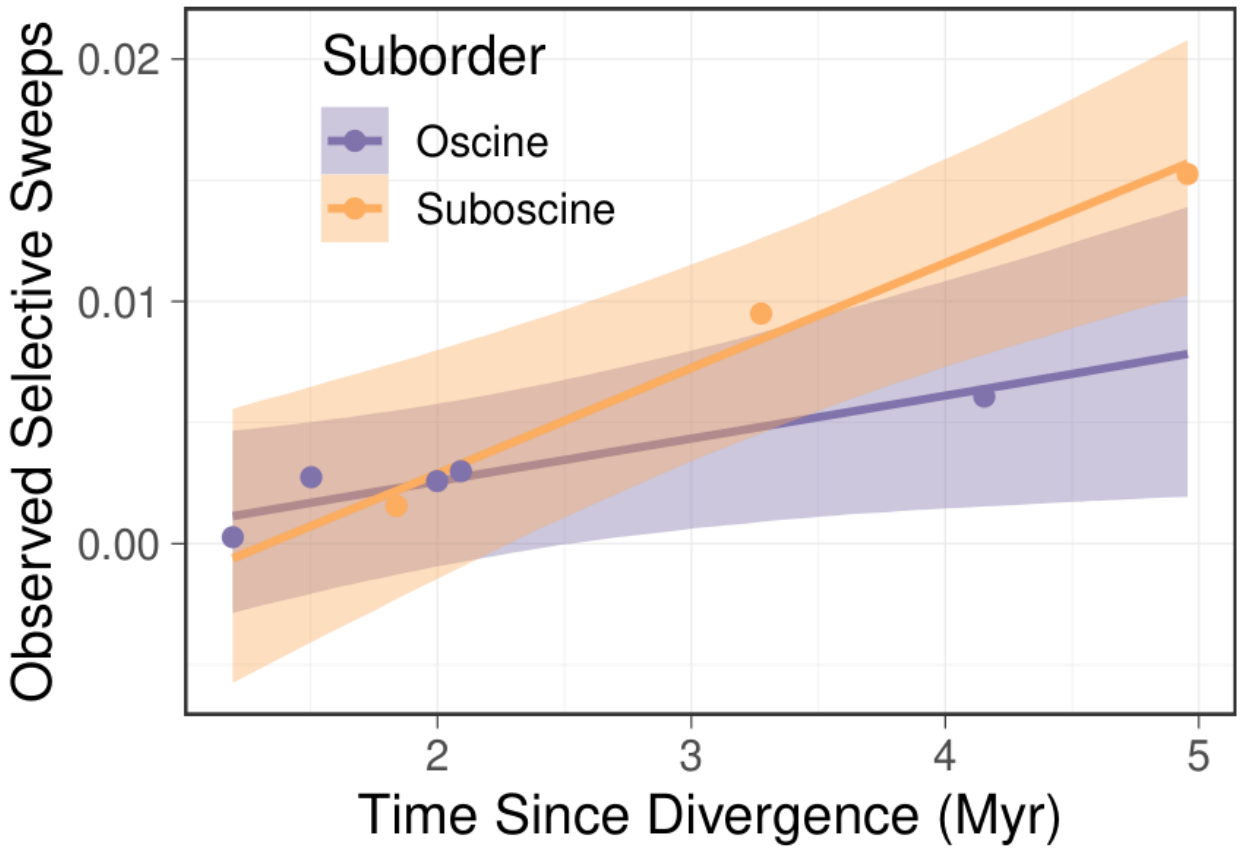

Figure S6. Number of observed selective sweeps accumulated between species pairs versus their divergence times, calculated from the nuclear genome using demographic modeling for only elevationally differentiated species pairs. Oscine species pairs are shown in purple, and suboscine pairs are shown in orange. Lines show model fits with phylogenetic relatedness accounted for. Shading shows the 95% credible intervals.

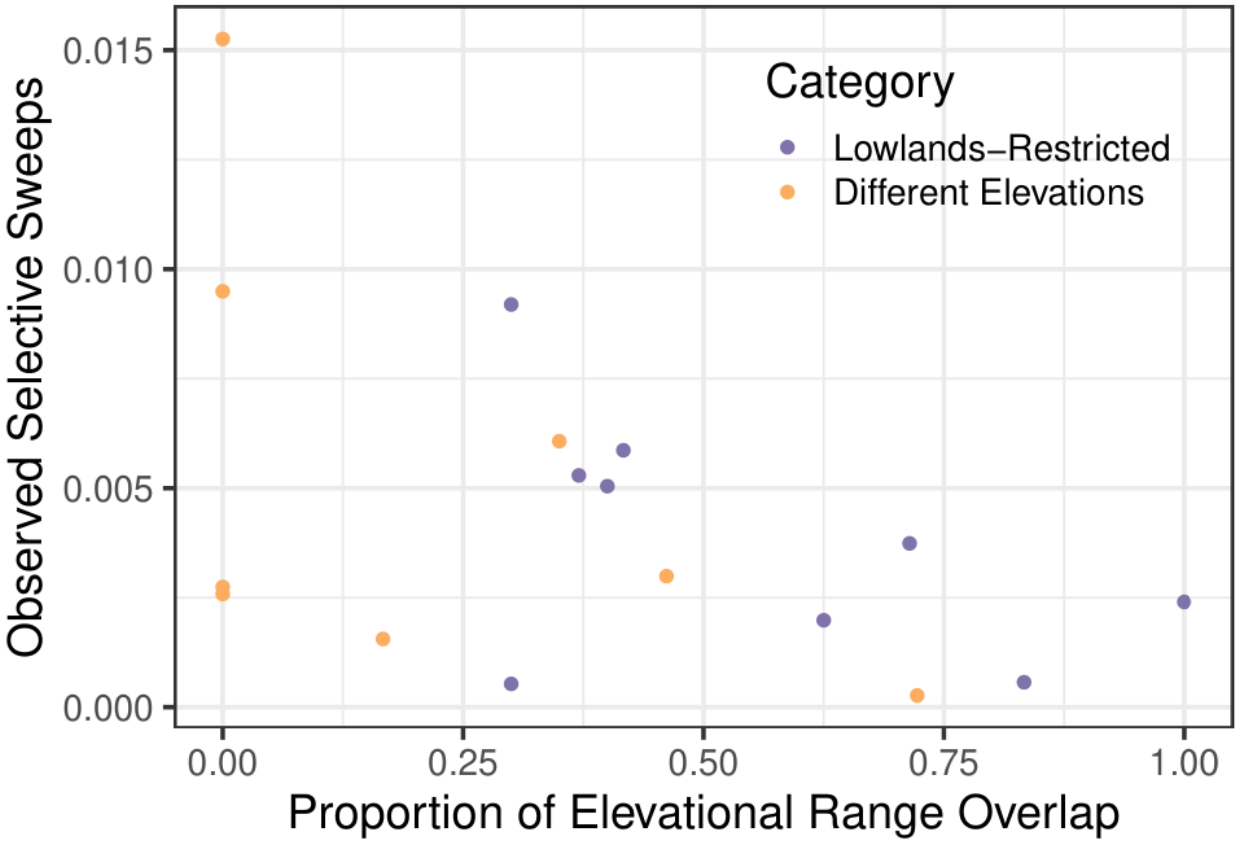

Figure S7. Proportion of elevational range overlap for each sister species pair, within lowlands-restricted or different elevation categories, against observed selective sweeps.