Full Title: Consequences of divergence and introgression for speciation in Andean cloud forest birds

Running Head: Introgression and speciation in Andean birds

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Abstract

Divergence with gene flow is well documented and reveals the influence of ecological adaptation on speciation. Yet it remains intuitive that gene exchange inhibits speciation in many scenarios, particularly among ecologically similar populations. The influence of gene flow on the divergence of populations facing similar selection pressures has received less empirical attention than scenarios where differentiation is coupled with local environmental adaptation. I used a paired study design to test the influence of genomic divergence and introgression on plumage differentiation between ecologically similar allopatric replacements of Andean cloud forest birds. Through analyses of short-read genome-wide sequences from over 160 individuals in 16 co-distributed lineages, I found that plumage divergence is associated with deep genetic divergence, implicating a prominent role of geographic isolation in speciation. By contrast, lineages that lack plumage divergence across the same geographic barrier are more recently isolated or exhibit a signature of secondary genetic introgression, indicating a negative relationship between gene flow and divergence in phenotypic traits important to speciation. My results suggest that the evolutionary outcomes of cycles of isolation and divergence in this important theatre of biotic diversification are sensitive to time spent in the absence of gene flow.

Introduction

A persistent question in speciation research is the extent to which populations can differentiate while they are exchanging genes (Mayr 1963; Coyne and Orr 2004; Nosil 2008). For differentiation to occur in the face of gene flow, local selection pressures must be strong enough to overcome the homogenizing force of gene flow. Numerous instances of divergence with gene flow have now been revealed, often among populations that exist across different environments and which exhibit phenotypic differences associated with ecological adaptation (Svensson 2012; Castillo et al. 2015). These studies highlight the potential for local ecological adaptation, as opposed to geographic isolation, to be a primary driver of speciation (Nosil et al. 2009; Schluter 2009; Pinho and Hey 2010). Yet, for a non-trivial portion of biodiversity, gene exchange likely inhibits speciation (Templeton 1981; Burney and Brumfield 2009; Kisel and Barraclough 2010; Claramunt et al. 2012; Cutter and Gray 2016). This may be particularly true for populations that exist across environments with similar selection pressures, because a favorable mutation in one population is likely to be favorable in neighboring populations (Schluter 2009; Nosil and Flaxman 2011; Langerhans and Riesch 2013). Previous authors have therefore suggested that divergence (and ultimately speciation) of ecologically similar populations should require substantial periods of time in the absence of gene flow (Price 2008, 2010; Nosil and Flaxman 2011).

Phenotypic differentiation among populations with conserved ecology is often evident in sexual or social signals such as color pattern (West-Eberhard 1983; Svensson 2012; Martin and Mendelson 2014; Mendelson et al. 2014). In such cases, phenotypes are hypothesized to diverge via different evolutionary responses to uniform ecological selection pressures, a process termed mutation-order speciation (Mani and Clarke 1990; Schluter 2009; Langerhans and Riesch 2013; Mendelson et al. 2014). Under this hypothesis, natural or sexual selection (as opposed to drift) may still drive phenotypic evolution within populations, but the phenotypic differences between populations are not indicative of divergent adaptation. Rather, differentiation is a consequence of stochasticity in the favorable mutations that arise in geographical isolation. There is a strong expectation that gene flow

will prevent divergence under mutation-order conditions, a prediction that has generally been supported by population genetic simulations (Nosil and Flaxman 2011; Anderson and Harmon 2014; Mendelson et al. 2014). However, in contrast to widespread empirical demonstration of divergence in the face of gene flow among ecologically disparate populations (Nosil 2008; Schluter 2009; Marques et al. 2016), the relationship between gene flow and trait divergence among ecologically similar populations has received scarce empirical attention. Consequently, though allopatric divergence of ecologically conserved populations is hypothesized to be an important aspect of the generation of biodiversity (Wiens 2004; Hua and Wiens 2013; Langerhans and Riesch 2013), the population genetic parameters regulating such divergence remains poorly understood.

Here, I test empirically the relationship between gene flow and phenotypic differentiation among codistributed, ecologically-similar sister pairs of cloud forest Andean birds found on either side of a biogeographic barrier, the Marañón Valley of Peru (Fig. 1). Many cloud forest Andean birds have latitudinally expansive but elevationally narrow geographic ranges that track specific bands of habitat along Andean slopes (Graves 1988; Weir et al. 2008; Gutiérrez-Pinto fet al. 2012; Valderrama et al. 2014). These taxa often exhibit sharply delimited geographic variation in phenotype, especially in plumage or song, typically corresponding to dispersal barriers such as arid valleys that interrupt the latitudinal continuity of humid montane forest (Chapman 1923; Remsen 1984; Graves 1991; Bonaccorso 2009; Lutz et al. 2013; Benham et al. 2014; Winger et al. 2015). The largest and most prominent barrier is the valley of the Marañón River in northern Peru in concert with the nearby low elevation pass known at the North Peruvian Low (Parker et al. 1985; Weigend 2002; Winger and Bates 2015). The environments on either side of the Marañón valley are largely similar to one another (humid montane forest), whereas the interior of the broad, low elevation valley is arid and cloaked in dry scrub and woodland (Fjeldsa et al. 1999; Weigend 2002; Killeen et al. 2007; Särkinen et al. 2012; Oswald et al. 2016). Thus, the Marañón region presents a topographical and ecological barrier to dispersal for humid forest taxa that otherwise have broad latitudinal distributions along the east slope

of the Andes (Parker et al. 1985; Weir 2009; Winger and Bates 2015). Despite this distributional gap, many cloud forest bird species are found on both sides of the Marañón with no apparent phenotypic differences, reflecting the ecological similarity on either side of the barrier (Winger and Bates 2015). However, other cloud forest bird species exhibit marked plumage differences on either side of the Marañón (Graves 1982; Parker et al. 1985; Fjeldså and Krabbe 1990), suggesting that isolation across the barrier has led to divergence. This system, wherein many codistributed taxa differ in their degree of phenotypic divergence across a common dispersal barrier, provides a powerful opportunity to test the population genetic parameters that govern the differentiation of plumage – a trait well known to be important for avian speciation (Price 2008)— among ecologically similar populations.

Winger and Bates (2015) studied 16 lineages of cloud-forest passerine birds distributed across the Marañón. They found that divergence at a mitochondrial gene (ND2) predicted plumage differentiation between adjacent sister populations across the Marañón (Fig. S2A). Substantial plumage divergence between populations was indicative of relatively deep genetic divergence, whereas a lack of plumage divergence tended to indicate greater genetic similarity. Based on this pattern of mtDNA divergence, Winger and Bates (2015) proposed that plumage divergence in this system is regulated principally by the duration of time populations are isolated in the absence of gene flow, as opposed to variation in rates of plumage evolution. However, with only a single mtDNA locus, Winger and Bates (2015) were limited in their ability to test the influence of gene flow on divergence. For example, sister populations with plumage differences may show divergence in mtDNA markers despite having experienced gene flow in other regions of the genome, as has recently been suggested in Amazonian birds isolated across riverine barriers (Weir et al. 2015). Introgression in the nuclear genome would suggest that substantial divergence in traits important to speciation has proceeded or persisted in the face of gene flow, even when ecological conditions are similar. Conversely, if introgression was limited to mitochondrial loci, then the observed low mtDNA

divergence among populations that are identical in plumage could belie a deeper history of isolation and imply stasis in phenotypic evolution.

In this paper, I gather genome-wide sequence data from the 16 taxa in the study design conceived by Winger and Bates (2015). The taxa are from eight genera (representing four families) of passerine birds (Table 1). In each genus, I sampled a pair of sister allospecies that replace one another geographically at the Marañón (Fig. 1); each allospecies pair forms a "superspecies." The superspecies are distinct in plumage color or pattern but are ecologically similar in gross habitat association and elevation on either side of the barrier. Most superspecies have diverged little, if at all, in morphometric traits that are commonly considered proxies for ecological divergence (e.g., Seddon et al. 2013) including bill, wing, leg and tail dimensions (Winger and Bates 2015). In each genus I also sampled a third species whose range extends across the Marañón but that shows little or no plumage differentiation on either side of the barrier (Fig. 1). I refer to these taxa as "monotypic species." The study design thus includes 8 superspecies and 8 monotypic species which, to avoid taxonomic confusion, I refer to as the 16 "lineages". In other words, a "lineage" in this context is either a superspecies or a monotypic species, but always contains individuals found on both sides of the Marañón (Fig. 1).

Through this paired study design, I test whether divergence in plumage among ecologically similar populations within each of 8 superspecies required an absence of gene flow, or whether divergence in phenotype among ecologically similar populations has occurred (or persisted) despite genetic introgression. The goal of this study is not to test whether initial population divergence occurred in the face of gene flow. Rather, I assume that geographic isolation has played some role in divergence, and test how subsequent introgression has influenced differentiation in plumage. Specifically, I test whether allospecies pairs with pronounced plumage divergence have "hidden" histories of introgression (Huang 2016; Zarza et al. 2016) or whether they have been entirely isolated for prolonged periods of time as suggested by Winger and Bates (2015).

I also explore patterns of genetic divergence and introgression in the lineages that lack plumage differentiation (monotypic species) across the Marañón to test the historical processes that have maintained similarity in plumage across the barrier. Lineages that are uniform in plumage on both sides of the Marañón may lack plumage differentiation because of the recency of range expansion across the barrier, ongoing gene flow across the barrier, or as a result of more discrete episodes of introgression that could serve to maintain phenotypic similarity among ecologically similar but geographically structured populations. Recent ancestry versus rampant gene flow are notoriously difficult to distinguish (Cruickshank and Hahn 2014). Therefore, in lineages that are genetically very similar across the Marañón I do not attempt to distinguish gene flow from recent isolation as both scenarios have the same implication: there has not been sufficient time in the absence of gene flow for phenotypes to diverge. However, if there has been sufficient isolation such that populations on either side of a barrier are genetically structured, then gene flow occurring subsequent to isolation can be identified more reliably (Rosenzweig et al. 2016). Winger and Bates (2015) showed that three monotypic species have relatively deep mtDNA differentiation (~2-5.5% average pairwise divergence) across the Marañón despite their plumage similarity, with two monotypic species exhibiting genetic divergence higher than that of some superspecies (Fig. S2A). Here, I test whether the monotypic species that have genetic structure across the Marañón also exhibit a signature of post-divergence introgression. If introgression occurred in these more deeply diverged monotypic species but not in the superspecies, this would provide evidence that gene flow has disrupted plumage divergence. If no introgression occurred in the monotypic species, this would suggest that their populations have not been isolated across the Marañón for long enough for plumage differences to evolve. If introgression has occurred both among superspecies and among monotypic species, this would suggest that divergence in phenotypic characters important to speciation cannot be predicted by commonalities of population history such as gross levels of genomic divergence and introgression, even within a geographically and ecologically controlled study design.

Methods

Study System and Design.— Each of 8 genera in the study design (Table 1) contain two sampled "lineages": a superspecies (differentiated in plumage across the Marañón) and a monotypic species (not differentiated in plumage across the Marañón). Two monotypic species (*Chlorornis riefferii* and *Pyrrhomyias cinnamomea*) are classified as different genera than their corresponding superspecies (*Cnemathraupis* and *Nephelomyias*, respectively), but phylogenetic analyses indicate that each are closely related sister species to the genus of the superspecies (Ohlson et al. 2009; Tello et al. 2009; Sedano and Burns 2010).

Winger and Bates (2015) sampled 3-20 mtDNA sequences from both sides of the Marañón valley in each monotypic species and superspecies to verify the sister relationships of populations separated by the Marañón within each lineage and to compare genetic differentiation across the valley. Here, I sample genome-wide loci from a subset of the 522 individuals included in Winger and Bates (2015). For each of the 16 lineages, I sampled 2-5 individuals from populations adjacent to the Marañón valley on either side, and, where possible, 1-3 individuals from populations further away the barrier to the north and south for a total of 181 individuals.

DNA extraction and sequencing.— Genomic DNA samples were extracted from muscle tissue or blood as in Winger and Bates (2015). Genomic libraries were prepared with a Genotyping-by-Sequencing (GBS) protocol (Elshire et al. 2011), which uses a restriction enzyme to sample short stretches of DNA from throughout the genome. Library preparation and sequencing were contracted to the Cornell University Institute for Genomic Diversity. The PstI restriction enzyme was used and 100 basepair reads were sequenced on two lanes (95 samples each) of a HiSeq 2000 (Illumina, San Diego, CA, USA).

Bioinformatics.— Raw reads were filtered, assembled, and genotyped in *pyRAD* v3 (Eaton and Ree 2013; Eaton 2014). I retained clusters of 50 basepairs or longer, I required clusters to have a minimum depth of 6 reads, and I used a clustering threshold of 88% sequence similarity. In exploratory analyses, I found that results were qualitatively similar when using a clustering threshold of 85% similarity and a minimum depth of coverage of 10 reads, which resulted in a smaller dataset. Additional parameter settings and filters are described in the Supplementary Information. As described below, I constructed intra-generic or intra-lineage alignments in *pyRAD* for downstream analyses.

Phylogeny building.— I generated maximum-likelihood phylogenies from concatenated alignments of GBS sequences of each of the 16 lineages to examine intraspecific relationships and genetic structure. I used three individuals from the other lineage in the genus as outgroups. For example, three individuals of the monotypic species *Iridosornis analis* were used as outgroups for the phylogeny of the superspecies *I. rufivertex* + *I. reinhardti*, and three individuals from the *Iridosornis* superspecies were used as the outgroup for the *I. analis* phylogeny (Fig. 1). For the *Grallaria* superspecies, I used a sample of a different species not included in the study design (*Grallaria quitensis*) as an outgroup, because Winger et al. (2015) showed that this species, and not the monotypic species *G. squamigera* in the study design, is the closest relative of the *Grallaria* superspecies and GBS sequence data were available. I built phylogenies using *RAxML* v8.2 (Stamatakis 2014) using the GTR-γ nucleotide substitution model and the rapid bootstrap search algorithm, and performed 100 bootstrap replicates to assess nodal support.

Genetic Structure.— I used *Structure* v2.3.4 (Pritchard et al. 2000; Falush et al. 2003) to identify populations within each lineage for subsequent analyses of differentiation and introgression and to assess admixture between populations separated by the Marañón. *Structure* uses Bayesian inference to assign a given number of genetic clusters (K) to individuals. For each of the 16 lineages, I used *pyRAD* to generate alignments of putatively unlinked SNPs by sampling a biallelic SNP from

loci that were present in every individual in a lineage. I ran each *Structure* analysis for K = 1-6 clusters and replicated runs five times each, using the admixture model with correlated allele frequencies. I ran each replicate for 500,000 generations and discarded 50,000 generations as "burnin." I used *Structure Harvester* (Earl and vonHoldt 2012) to evaluate the optimal clustering scheme in each lineage. I examined the probabilities that individuals found on the north and south sides of the Marañón were from separate genetic clusters under the best supported clustering schemes. As lineages differed in the number of shared loci available for analysis (Table 1), I repeated analyses on a random subset of 500 SNPs for every lineage (corresponding to the number of SNPs in the lineage with the least amount of data) to test whether differences in the amount of data among lineages impacted results.

I calculated pairwise F_{ST} within each of 16 lineages as a measure of differentiation across the Maranon. I calculated Weir and Cockerham's (Weir and Cockerham 1984) F_{ST} using the R package *hierfstat* (Goudet and Jombart 2015), after using *vcfR* (Knaus and Grünwald 2017) to convert pyRAD output files. In some cases, population structure unrelated to the Marañón was present within a lineage due to divergences across other arid valleys in Peru such as the Huallaga or Apurímac rivers or the high Andes that separate the humid east and west slopes of northern Ecuador (Fig. 1; Winger and Bates 2015; Winger et al. 2015). Therefore, I used *Structure* to identify which individuals to include in a pairwise north-south comparison across the Marañón. For example, if *Structure* identified an individual from far away from the Marañón as better assigned to a third population representing a divergence event across a different barrier, I excluded that individual so as not to conflate divergence across other barriers with divergence across the Marañón. I restricted loci to those shared across both the superspecies and the monotypic species in each genus (Table 1). I repeated calculations with different alignments that were generated using the following conditions: 1) when using a random subset of 500 loci, 2) within each lineage rather than across all individuals in the genus and 3) when calculated with alignments that only included individuals from populations near to the Marañon on

either side, defined as northwest of the Huallaga river and south of the Zamora river (Fig. 1), rather than more broadly defined populations guided by the *Structure* analysis. Pairwise F_{ST} results were similar using any of these alignments, so I present here F_{ST} based on the *Structure*-defined populations using the genus-level alignments as representative of trans-Marañón differentiation.

Winger and Bates (2015) used pairwise GTR-corrected distances and a relaxed molecular clock to estimate trans-Marañón divergence in each lineage (Fig. S2A). Here, I calculated pairwise F_{ST} on the mtDNA sequences in Winger & Bates (2015) in *Arlequin* v3.5 (Excoffier and Lischer 2010). I also calculated Nei's standard genetic distance D_S (Nei 1972) across the Marañón with GBS loci in *hierfstat* and with mtDNA sequences in *Arlequin*.

Winger and Bates (2015) calculated trans-Marañón Mahalanobis distance in plumage color in each lineage with data from spectrophotometric measurements of museum specimens. Measurements were taken from 10 plumage patches from 4-6 specimens from either side of the Marañón in each of the 16 lineages (total specimens = 184). Mahalanobis distance, unlike Euclidean distance, accounts for correlation among variables. Winger and Bates (2015) calculated trans-Marañón Mahalanobis distance for each of 10 patches using three standard colorimetric variables (hue, chroma and brightness) as multivariate data, which they found to be similar to Mahalanobis distance in avian tetrahedral color space. Following Winger and Bates (2015), I use here the mean Mahalanobis distance (across 10 patches) of three colorimetric variables as an evolutionary distance in plumage across the Marañón for each lineage.

Introgression Analyses — To evaluate trans-Marañón introgression I calculated four-taxon *D*-statistics ("ABBA/BABA" tests; Durand et al. 2011; Eaton and Ree 2013). *D*-statistics use the pattern of alleles present across four divergent lineages to infer introgression. Given three populations P1, P2, P3 and an outgroup O with a pectinate phylogeny (((P1,P2), P3), O), *D*-statistics test for introgression

between taxa P2 and P3 by comparing the number of sites with allele pattern ABBA or BABA, where A represents the ancestral allele and B represents a derived allele.

$$D(P_1, P_2, P_3, O) = \frac{\sum C_{ABBA}(i) - C_{BABA}(i)}{\sum C_{ABBA}(i) + C_{BABA}(i)} \quad (1)$$

Roughly equal numbers of ABBA and BABA sites should be present as a result of incomplete lineages sorting (ILS). However, if introgression has occurred between P2 and P3, then an excess of ABBA sites is expected and the *D*-statistic will be positive. The linear geographic ranges and population structure of Andean birds conveniently enable the design of ABBA/BABA tests to infer introgression across geographic barriers, as long as lineage histories of divergence are represented by a pectinate three-taxon phylogeny ((P1,P2), P3), where P2 and P3 represent populations on either side of the barrier in question (Winger et al. 2015). To test for introgression between sister populations across the Marañón, P2 and P3 represent populations close to the Marañón on either side, and P1 represents a population further away from the Marañón on the north or south side (Fig. 3). Therefore, a P1 population must be sampled that is more closely related to P2 on the same side of the Marañón than P2 is to P3 on the opposite side (Fig. 3). If populations on either side of the Marañón are too closely related to one another with respect to other sampled populations (i.e., sister populations or a single panmictic population), then the test would not distinguish shared ancestry (ILS) from similarity due to introgression. I examined the *RAxML* phylogenies (Fig. S1) to determine whether a ((P1,P2), P3) topology as described above could be sampled from each lineage. The outgroup O was sampled from three individuals of the other lineage in each genus (except for the *Grallaria* superspecies) as described for the *RAxML* analysis. Some lineages had multiple individuals available for each P1, P2 and P3 population. Therefore, I pooled individuals in each population and used *pyRAD* to calculate the *D*-statistic using allele frequencies at each site, rather than counts of allele patterns, which allows for the inclusion of heterozygous sites (Durand et al. 2011; Eaton and Ree 2013). For each test I performed 100 bootstraps and converted Z scores for each test to p-values with Holm-Bonferroni

correction (Eaton and Ree 2013; Eaton et al. 2015). In 6 lineages, sampling allowed for two different 4-taxon testing schemes to be constructed from the same lineage by rearranging whether P1 and P2 represented populations on the north or south sides of the Marañón (Fig. 3). In these lineages, I calculated *D*-statistics and assessed significance for both testing schemes.

To determine the amount of trans-Marañón introgression that occurred in each lineage, I calculated the *f* statistic of Green et al. (2010), which measures the proportion of the genome that has been shared by introgression. The *f* statistic is calculated by dividing the numerator of the *D*-statistic (equation 1) for the test in question with the numerator of a *D* test where P2 is replaced by a second sample of P3, that is, (((P1,$P3_a$), $P3_b$), O):

$$f = \frac{S(P_1,P_2,P_3,O)}{S(P_1,P_{3a},P_{3b},O)} \quad (2)$$

where S is the numerator of equation 1. This *f* statistic compares the difference between ABBA and BABA sites detected between P2 and P3 with the difference in ABBA and BABA between two P3 samples, thereby estimating the fraction of the genome shared by introgression. Martin et al. (2015) found that *f* and related statistics were more robust indicators of introgression than the *D* statistic. The *f* reported here is the same as $\hat{f}_G$ of Martin et al. (2015). I also calculated $\hat{f}_{hom}$ of Martin et al. (2015), which replaces P2 with a replicated P3 population, rather than using two separate samples of P3, i.e., (((P1,P3), P3), O); this test therefore assumes that the upper bound of introgression is complete homogenization of the genome.

To further test trans-Marañón introgression I used *TreeMix* v.1.12 (Pickrell and Pritchard 2012) to generate a maximum-likelihood phylogeny of individuals and infer admixture for each lineage. *TreeMix* uses a likelihood procedure to add admixture events (migration "edges") to a bifurcating topology. Topologies with migration edges indicate ancestry derived from multiple parental populations. The weight of a migration edge is related to the proportion of alleles in a

population derived from migration. I built trees separately for each monotypic species and superspecies, using the same outgroups described above for the *D*-statistics. I defined populations geographically as close-, mid- or far-distance from the Marañón based on the Huallaga and Apurímac river barriers on the south side of the Marañón and the east or west slope on the north side (Fig. 1), following Winger and Bates (2015). I used a custom script provided by Eaton et al. (2015) to select a single biallelic SNP from each locus for which at least one individual in each population and outgroup was genotyped. I inferred topologies with 0-5 migration events and evaluated whether migration occurred by comparing the likelihood of trees with and without migration. I then evaluated the statistical significance of an edge by testing whether its weight was significantly different than zero using *TreeMix's* jackknifing procedure over blocks of 10 SNPs.

Results

GBS Informatics.— After exploratory analyses, I excluded 18 low coverage or potentially contaminated samples that failed to cluster adequately, leaving 163 individuals in the study (Table S1). These individuals had a total of 428M raw reads (mean 2.6M per sample ± SD 1.4M), which were filtered and clustered to 55K ± 22K putative loci per individual with depth of coverage of 6 or greater (mean depth of coverage = 15X; Table S1). The number of shared loci in each complete coverage alignment varied across lineages and genera (Table 1). In six of the eight genera, the superspecies alignments had fewer shared loci than the monotypic species alignments, probably because the high divergence between their sister allospecies pairs resulted in fewer reads clustering together as putative loci (Huang and Knowles 2014; Harvey et al. 2016). Nevertheless, within these datasets, superspecies still had greater genetic divergences than monotypic species (see below), making GBS loci a conservative test for divergence in this context.

Maximum-Likelihood Phylogenies.— All eight superspecies contained reciprocally monophyletic lineages on opposite sides of the Marañón with bootstrap values of 100 (Fig. S1). Two monotypic species (*Chlorornis riefferii* and *Hemispingus frontalis*) contained reciprocally monophyletic lineages separated by the Marañón (Fig. S1). In five monotypic species (*Grallaria squamigera, Thripadectes holostictus, Ochthoeca frontalis*, *Iridosornis analis*, *Leptopogon superciliaris*), clades were not entirely monophyletic with respect to the Marañón, but nevertheless geographic structure across this barrier was evident in their phylogenies. The remaining monotypic species (*Pyrrhomyias cinnamomea*) showed little evidence of lineage sorting across the Marañón (Table 2, Fig. S1).

Genetic Structure.— At the optimal K, individuals from the north and south sides of the Marañón assigned to separate clusters with a probability of 1.0 in all eight superspecies (Fig. S1). Four of the monotypic species showed no evidence of genetic clustering with respect to the Marañón. The other four monotypic species had optimal clustering schemes that segregated individuals on either side of the Marañón (Table 2; Fig. S1). However, relative to the superspecies, the difference in log likelihoods of K=1 and K > 1 were slight, suggesting that structure in these monotypic species is weak. The monotypic species *Thripadectes holostictus*, *Grallaria squamigera* and *Chlorornis riefferii* had distinct clusters representing northern and southern populations, but some individuals from close to the barrier showed mixed ancestry (Fig. S1). Results were similar when using only 500 SNPs for each lineage, indicating that comparisons among lineages were not biased by the number of loci in the alignment.

Superspecies had higher trans-Marañón F_{ST} and genetic distance than monotypic species, both within each genus and across genera (Fig. 2, Table 2). The relationship between genetic divergence and plumage divergence was stronger for the GBS loci than the mitochondrial locus both in a categorical framework (superspecies vs. monotypic species) and quantitative (genetic distance or F_{ST} vs. pairwise Mahalanobis distance in plumage, Figs. 2, S2). The three monotypic species with trans-

Marañón clustering in the *Structure* analyses also had F_{ST} values approaching that of the superspecies (Fig. 2). Nei's genetic distance also showed generally similar patterns as F_{ST}, though with some overlap between superspecies and monotypic species (Fig. S2). These patterns of divergence were maintained when the datasets were reduced to a random subset of 500 loci for each genus (not shown).

ABBA/BABA tests.— All eight superspecies and four of eight monotypic species had topological patterns (P1,P2),P3) that enabled four-taxon *D*-statistic tests (Table S2). The phylogenetic relationships among populations of the monotypic species tended to be more poorly supported than that of the superspecies, but in four monotypic species there was sufficient structure across the Marañón to determine (P1,P2),P3) topologies (Fig. S1). The remaining four monotypic species had no genetic structure with respect to the Marañón, making the *D* statistic inappropriate in this context (see Methods). Consistent with Martin et al. (2015), I found that *D* and *f* (i.e., $\hat{f}_G$ of Martin et al. 2015) are moderately correlated (Pearson's correlation coefficient = 0.64, p = 0.005), and that D tends to overestimate introgression relative to *f* (Fig. S3). Results from the two methods for calculating *f* ($\hat{f}_G$ and $\hat{f}_{hom}$) were highly correlated (Pearson's correlation coefficient = 0.92, p <0 .001) and therefore $\hat{f}_G$ of Martin et al. (2015) is reported here as *f*, the proportion of genomic introgression (Fig. 4).

In the *Ochthoeca* superspecies, one of two schemes resulted in a significant *D*-statistic ($p < 0.001$; Table 1) and a high *f* statistic (Fig. 4), indicating introgression. However, these results should be considered with caution due to a unique sampling consideration (see Discussion). The *Grallaria* superspecies also had a significant *D*-statistic ($p < 0.001$; Table 1) and the *Hemispingus* superspecies was marginally significant ($p = 0.010$; Table 1). The *D*-statistics in the remaining six superspecies were not significantly different than zero at $p < 0.01$. Regardless of *p*-values, all tests for superspecies had very low levels of *f* (except the one scheme for *Ochthoeca*), indicating that if introgression occurred it involved only a small fraction of the genome (Fig. 4). By contrast, the four monotypic species tested had significant *D*-statistics and higher values of *f* (Fig. 4). The remaining four

monotypic species were not tested because they were too genetically similar across the Marañón (see Methods).

TreeMix.— In six of the eight superspecies, the results of the *TreeMix* analyses did not provide evidence for introgression across the Marañón. In the *Grallaria* superspecies, a migration event between populations separated by the Marañón provided a modest improvement in log likelihood score of the tree (3.8). However, the weight (proportion of alleles derived from migration) was low (0.097) and not significantly different than zero ($p > 0.05$). In the *Ochthoeca* superspecies, a migration event across the Marañón improved the likelihood of the tree modestly, but only on a topology that also included an unlikely migration event with the outgroup species, making interpretation of this result difficult. *TreeMix* analyses of six of the eight monotypic species also did not reveal any trans-Marañón migration events. The remaining two monotypic species showed improvement in likelihood score with trans-Marañón migration: *Chlorornis riefferii* and *Grallaria squamigera* had improvements in likelihood of 27.1 and 10.5, respectively, with trans-Marañón migration edges (Fig. 5). The weights of the migration edges in both of these species' topologies were significantly different than zero ($p < 0.001$).

TreeMix is better at detecting gene flow that occurs over a discrete, short period of time and performs more poorly when populations have prolonged periods of gene flow or are at equilibrium (Pickrell and Pritchard 2012). The results for the monotypic species are consistent with this assessment: the *TreeMix* topologies of monotypic species that had no or very weak population structure with respect to the Marañón as revealed by the *Structure* and *RAxML* analyses were not improved by migration edges, whereas the topologies of monotypic species with greater structure across the Marañón were improved by a migration event (Table 2).

Discussion

Numerous studies have found evidence for phenotypic divergence in the face of gene flow (Pinho and Hey 2010; Feder et al. 2013; Seehausen et al. 2014). In many of these scenarios, natural selection is hypothesized to drive divergent adaptation of populations to disparate environments, which raises the possibility that geographic isolation is not necessary for speciation if ecological selection pressures are strong enough. Modern analyses have revealed "hidden" genetic introgression even among populations assumed to have diverged in an allopatric model, such as populations of subtropical birds found in different mountain ranges (Zarza et al. 2016). However, the influence of introgression on the divergence of traits important to speciation, such as plumage signals in birds, has rarely been studied in a comparative framework. Analyses are particularly lacking in systems wherein phenotypic differences among populations are not obviously coupled with environmental variation and thus speciation may be less likely to proceed via adaptive divergence (West-Eberhard 1983; Svensson 2012; Langerhans and Riesch 2013; Mendelson et al. 2014).

Through analysis of thousands of short-read loci, I found that levels of genomic differentiation of avian sister populations of cloud-forest birds separated by the Marañón Valley of Peru predicts their plumage divergence, both within and across genera (Fig. 2), indicating a relationship between gross levels of genome divergence and differentiation in a phenotypic trait important to speciation. The relationship between genetic divergence and plumage divergence using GBS loci is consistent with, but more pronounced than, results from a previous study of mtDNA divergence in these lineages (Fig. S2, Winger and Bates 2015).

My results further suggest a negative relationship between levels of genetic introgression and plumage divergence. The allospecies pairs in each genus that had marked plumage differences (the superspecies) show little to no evidence of gene flow occurring subsequent to geographic isolation. There are two potential exceptions. The *Grallaria* superspecies showed potential evidence of introgression in the ABBA/BABA and *TreeMix* tests, involving a relatively small fraction of the genome (Fig. 4, Table S2). The *Ochthoeca* superspecies showed higher introgression (Fig. 4), but this

requires additional sampling to determine if introgression occurred directly between these two taxa. A closely related but unsampled species, *Ochthoeca jelskii*, is found parapatric to *O. diadema* and *O. pulchella* in drier habitats. Phylogenetic analyses suggest that *O. jelskii* may be most closely related to *O. diadema*, with *O. pulchella* sister to *O. jelskii* + *O. diadema* (García-Moreno et al. 1998). Eaton and Ree (2013) and Eaton et al. (2015) showed that in this phylogenetic scenario, if *O. pulchella* and *O. jelskii* have exchanged genes, then the *D*-statistic could falsely recover introgression between *O. pulchella* and *O. diadema* because of the shared history between *O. diadema* and *O. jelskii.* A more sophisticated five-taxon *D*-statistic test would be useful to test the source of introgression (Eaton and Ree 2013; Pease and Hahn 2015), but sampling of *O. jelskii* was not available.

Conversely, the monotypic species — those lineages that lack plumage divergence with respect to the Marañón — exhibit genetic relationships involving a more complex combination of divergence and gene exchange. In four of the monotypic species (*Leptopogon superciliaris*, *Pyrrhomyias cinnamomea*, *Ochthoeca frontalis*, and *Iridosornis analis*), analyses indicate a general lack of genetic differentiation with respect to the Marañón (Fig. 2, Fig. S1). This pattern of genetic similarity could be a consequence of two non-mutually exclusive processes: recent colonization of populations across the Marañón or substantial gene exchange across the barrier. *Leptopogon superciliaris* and *Iridosornis analis* are typically found at lower elevations than the other lineages in the study, where the Marañón is a weaker barrier to dispersal, making ongoing gene exchange perhaps a greater possibility in these lineages than in the others. However, distinguishing initial population splitting from ongoing gene flow with confidence at such shallow levels of divergence is difficult, and will require demographic modelling of denser and wider sampling from throughout these species' ranges (e.g., Toews et al. 2016). Regardless, the lack of genetic structure in these four monotypic species suggests that substantial ongoing gene flow or very recent isolation underlies the lack of plumage differentiation in half of the 8 monotypic species.

The other four monotypic species (*Grallaria squamigera*, *Chlorornis riefferii*, *Hemispingus frontalis* and *Thripadectes holostictus*) exhibit a genetic signature consistent with a period of divergence followed by secondary introgression across the Marañón. Like the superspecies, these four lineages have pronounced geographic structure with respect to the Marañón as indicated by maximum-likelihood phylogenies and *Structure* analysis (Table 2, Fig. S1), and relatively high F_{ST} values and genetic distance in mtDNA and GBS loci (approaching or exceeding the divergences in the superspecies; Figs. 2, S2). Unlike the superspecies, however, these monotypic species also show a strong signal of secondary introgression between populations separated by the Marañón as revealed by the ABBA/BABA tests (Table 2, Fig. 4). In two monotypic species, the *TreeMix* tests also supported the ABBA/BABA results (Fig. 5), though in general the *TreeMix* results were more difficult to interpret (see Results).

Periods of divergence and introgression among tropical montane organisms are not surprising given the potential for barriers such as the Marañón to become more or less porous through time (Ramírez-Barahona and Eguiarte 2013). However, the relationship between secondary introgression and phenotypic divergence remains poorly understood in this and other systems (Huang 2016). In the speciation literature, the influence of gene flow on phenotype has primarily been documented in the context of discovering loci that are resistance to gene flow and involved in the maintenance of reproductive isolation (Kronforst et al. 2013; Martin et al. 2013; Cruickshank and Hahn 2014; Larson et al. 2014; Toews et al. 2016; Vijay et al. 2016). By contrast, in this system, secondary introgression observed between deeply structured populations may have served to homogenize incipient differences in plumage, thereby preventing speciation. However, this study did not identify the genes underlying plumage differences or test for introgression specifically in those regions, so the direct impact of introgression of plumage cannot be assessed. Rather, the *f* statistic is proportional to the overall degree of introgression between taxa (Martin et al. 2015). Thus, an alternative possibility is that plumage traits in lineages with introgression did not diverge sufficiently to preclude mating during secondary

contact, thereby enabling gene exchange and homogenizing incipient phenotypic differentiation. In other words, gene flow may have occurred because plumage had not differentiated sufficiently to act as a premating isolating mechanism, rather than plumage becoming homogenized by gene flow. Regardless of the historical process, my results suggest that a pattern of secondary introgression is associated with lower plumage divergence, whereas marked plumage divergence is associated with isolation. Notably, the two superspecies with potential introgression (*Grallaria* and *Ochthoeca*) have the lowest plumage divergence across the Marañón of any superspecies in the study (Fig. 2), suggesting a possible relationship between introgression and degree of plumage divergence, as opposed to simply introgression and presence versus absence of plumage divergence.

Given the linearity of species geographic ranges in the Andes, the ABBA/BABA tests provided a convenient framework for detecting introgression across the Marañón independent of ILS. However, two potential caveats bear mentioning. First, the test assumes divergence between P1 and P2 such that "AB" or "BA" alleles in (P1,P2) will be common. However, the geographic barriers separating P1 and P2 (Fig. 3) may be weak (Winger et al. 2015) and P1 and P2 in some lineages were poorly structured (Fig. S1). Genetic mixing of P1 and P2 could dilute a signal of introgression between P2 and P3 because there would be fewer loci with alternate "A" and "B" alleles in P1 and P2. Second, the accurate detection of introgression between P2 and P3 depends on the topology (((P1,P2),P3)O) accurately representing population histories of divergence. The ML phylogenies of each of the 12 lineages for which I performed ABBA/BABA tests were consistent with this pectinate topology (Fig. S1). However, if any of these species' inferred phylogenies do not accurately represent divergence history and populations assigned as P2 and P3 are each other's closest relatives with respect to P1 (Fig. 3), then ILS could be falsely interpreted as secondary admixture. This caveat highlights the difficulty of confidently distinguishing introgression from ILS when populations are shallowly or recently diverged, even with specialized tests.

Overall, my results support the assessment of Winger and Bates (2015) that plumage divergence in this system is associated with substantial genetic isolation, but shed light on the processes responsible for this result by more explicitly demonstrating how genomic introgression is related to stasis in plumage divergence. The observed genetic isolation could be purely a consequence of prolonged geographic isolation. Alternatively, if geographic isolation occurred for long enough for both plumage signals and mate preferences for plumage signals to diverge, then premating isolation based on plumage in concert with competitive exclusion could also be involved in preventing gene exchange and maintaining allopatry between sister allospecies during hypothetical periods of secondary contact (West-Eberhard 1983; Gröning and Hochkirch 2008; Safran et al. 2013). Experimental field studies of mate preference and its relationship to plumage characters (e.g., Uy et al. 2009; Greig et al. 2015; Safran et al. 2016b) and other characters important to avian speciation, especially vocalizations, are required to test the likelihood of these alternatives. Additionally, if secondary contact occurred but premating isolation was incomplete, hybrid incompatibilities arising due to "byproduct" genomic divergence could have precluded introgression, masking a history of secondary contact (Sobel et al. 2009; Feder et al. 2013; Singhal and Moritz 2013). However, I consider prolonged allopatry to be the most likely scenario for maintaining isolation given that none of the allospecies pairs are known to have contact zones (with the possible exception of *O. pulchella* and *O. diadema* in northern Peru; Museum of Southwestern Biology unpublished specimen data).

The existence of purely allopatric speciation is uncontroversial. However, much recent speciation research has emphasized the potential for local ecological adaptation to drive speciation in the face of gene flow (Nosil et al. 2009; Schluter 2009; Harrison and Larson 2014). Although subtle adaptive morphological differentiation along ecological gradients has been documented in Andean cloud forest birds (Milá et al. 2009; Benham and Witt 2016), much of the species diversity in the Andes and other tropical montane regions is thought to have evolved through cycles of population isolation, divergence, and dispersal across physical barriers that bisect ecologically similar regions

(Diamond 1973; García-Moreno and Fjeldså 1999; Freeman 2015; Winger et al. 2015). Given that the humid east slope of the Andes hosts one of the most diverse avifaunas — and biotas — on earth (Myers et al. 2000; Rahbek and Graves 2001), inferring the population genetic parameters that underlie the evolutionary outcomes of cycles of population expansion, divergence and secondary contact is important for understanding broader temporal patterns in the build-up of biodiversity. My results suggest that the outcomes of these cycles may be quite sensitive to disruptions from gene flow and that incipient differences may frequently collapse rather than persist upon secondary contact. This result stands in contrast to recent speciation genomic research on avian hybrid zones that has demonstrated the maintenance of plumage differences despite extensive introgression (Ellegren et al. 2013; Baldassarre et al. 2014; Poelstra et al. 2014; Toews et al. 2016) and raises the question, is the persistence of discrete (i.e., non-clinal) differences in plumage in the face of gene flow the exception or the rule in avian speciation? In other words, our perception of reinforcement as an important aspect of speciation (e.g., Hudson and Price 2014) may be inflated by an emphasis on studies of stable hybrid zones, whereas the prevalence of population fusion during secondary contact may be more poorly documented (Templeton 1981; Rabosky 2013; Cutter and Gray 2016). Further documentation of levels of genomic introgression of diverse taxa along the speciation continuum will help illuminate how and when gene flow disrupts versus promotes speciation (Roux et al. 2016).

This study also highlights that we know fairly little about the factors that promote speciation in cases where population divergence is not maintained by obviously divergent selection pressures (Schluter 2009; Nosil and Flaxman 2011; Mendelson et al. 2014). For example, social selection may be involved in the plumage divergence of populations separated by the Marañón, but the mechanisms of social selection that drive and maintain phenotypic divergence in ecologically similar scenarios have received less attention than cases with an obvious link between phenotype, fitness in the environment, and mate choice (West-Eberhard 1983; Prum 2010; Martin and Mendelson 2012; Mendelson et al. 2014). How long must populations facing similar selection pressures be isolated to

evolve differences in social signals important to speciation (Winger and Bates 2015; Cutter and Gray 2016; Zamudio et al. 2016)? Are differences in social signals that evolved under uniform selection pressures sufficient to curtail gene exchange during secondary contact, or are hybrid incompatibilities also necessary to achieve reproductive isolation (Nosil and Flaxman 2011; Mendelson et al. 2014; Ono et al. 2017)? Additionally, although the environments on either side of the Marañón are largely similar in macroecological features such as habitat and climate, there likely are subtle differences in environment and community structure that could potentially influence the selective environment for mate choice. This complication demands the question, do organisms found in such putatively similar environments evolve differences in social signals purely as a function of mate preference, or is local adaptation to subtle ecological variation not considered here also involved in trait divergence (Safran et al. 2016a)? I suggest that further study of the drivers of genomic and phenotypic differentiation during the first stage of speciation – geographic isolation – across a range of ecological contexts will be important to complement research programs on later stages of speciation, such as hybridization, for understanding how sexual and natural selection interact with molecular evolution to drive speciation.

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Tables

Table 1. GBS alignment statistics. Shared Loci (Genus) = Number of loci shared across all members of a genus, used for F_{ST} and genetic distances calculations. Shared Loci (Lineage) = number of loci shared across only individuals within a lineage (used for maximum-likelihood phylogenies and *Structure* analyses). Lineage (SNPs) = number of putative unlinked SNPs used in *Structure* analyses. A single biallelic SNP was sampled from each locus.

Family	**Genus**	**Lineage**	***N* Individuals (Lineage)**	**Shared Loci (Genus)**	**Shared Loci (Lineage)**	**Lineage SNPs**
Thraupidae	***Cnemathraupis***	superspecies (***C. aureodorsalis*** + ***C. eximia***)	7	420	1712	935

		monotypic (***Chlorornis riefferii***)	12	420	7686	5244
Grallariidae	***Grallaria***	superspecies (***G. przewalskii*** + ***G. hypoleuca***)	14	756	2095	1776
		monotypic (***G. squamigera***)	13	756	3739	2174
Thraupidae	***Hemispingus***	superspecies (***H. xanthophthalmus*** + ***H. verticalis)***	9	772	2652	1662
		monotypic (***H. frontalis***)	11	772	12294	6829
Thraupidae	***Iridosornis***	superspecies (***I. reinhardti*** + ***I. rufivertex***)	9	429	1205	802
		monotypic (***I. analis***)	9	429	5606	3724
Tyrannidae	***Leptopogon***	superspecies (***L. taczanowskii*** + ***L. rufipectus***)	8	264	1036	664
		monotypic (***L. superciliaris***)	10	264	905	630
Tyrannidae	***Nephelomyias***	superspecies (***N. ochraceiventris*** + ***N. lintoni***)	8	207	1920	964
		monotypic (***Pyrrhomyias cinnamomea***)	11	207	1282	1044
Tyrannidae	***Ochthoeca***	superspecies (***O. pulchella*** + ***O. diadema***)	11	229	940	743
		monotypic (***O. frontalis***)	11	229	1231	830
Furnariidae	***Thripadectes***	superspecies	8	2900	5652	3450

	(***T. scrutator*** **+** ***T. flammulatus***)				
	monotypic (***T. holostictus***)	11	2900	11182	5588

Table 2. Summary of results. Reciprocal monophyly refers to whether lineages were reciprocally monophyletic with respect to the Marañón in the RAxML phylogeny (a test of geographic structure of GBS loci; Fig. S1). F_{ST} refers to the F_{ST} across GBS loci of populations separated by the Marañón. The *Structure* column indicates whether the *Structure* analyses suggested that populations on either side of the Marañón were from separate genetic clusters, and whether these clusters had 100% assignment of individuals or mixed ancestry (see *Structure* plots in Fig. S1). "*TreeMix* Migration" indicates whether the *TreeMix* tests suggested trans-Marañón introgression. "*f*" indicates the proportion of genomic introgression inferred from the ABBA/BABA tests (a single value in lineages with one (P1,P2),P3,O)) scheme or a mean value for lineages with two schemes; Fig. 3, Table S2). In the right hand column, an interpretation of population history is offered based on the collective evidence from these tests.

Lineage	**Reciprocal monophyly?**	***F***	***Structure?***	***TreeMix* Migration?**	***f***	**Interpretation**
SUPERSPECIES						
Cnemathraupis aureodorsalis + eximia	Yes	0.85	Yes (100%)	No	0.000	Isolation
Grallaria przewalskii + hypoleuca	Yes	0.58	Yes (100%)	No	0.068	Isolation
Hemispingus xanthophthalmus +	Yes	0.45	Yes (100%)	No	0.032%	Isolation
Iridosornis reinhardti + rufivertex	Yes	0.38	Yes (100%)	No	0.003	Isolation
Leptopogon taczanowksti + rufipectus	Yes	0.54	Yes (100%)	No	0.013	Isolation
Nephelomyias ochraceiventris + lintoni	Yes	0.69	Yes (100%)	No	0.017	Isolation
Ochthoeca pulchella + diadema	Yes	0.25	Yes (100%)	?	0.260	Isolation + potential
Thripadectes scrutator + flammulatus	Yes	0.49	Yes (100%)	No	0.013	Isolation
MONOTYPIC SPECIES						
Cnemathraupis (Chlorornis) riefferii	Yes	0.21	Yes (mixed)	Yes	0.111	Isolation + introgression
Grallaria squamigera	No	0.19	Yes (mixed)	Yes	0.658	Isolation + introgression
Hemispingus frontalis	Yes	0.07	Yes (100%)	No	0.640	Isolation + introgression
Iridosornis analis	No	0.00	No	No	NA	Gene flow or recent isolation
Leptopogon superciliaris	No	0.01	No	No	NA	Gene flow or recent isolation
Nephelomyias (Pyrrhomyias)	No	0.00	No	No	NA	Gene flow or recent isolation
Ochthoeca frontalis	No	0.07	No	No	NA	Gene flow or recent isolation
Thripadectes holostictus	No	0.21	Yes (mixed)	No	0.568	Isolation + introgression

Figure 1. Modified with permission from Winger and Bates (2015). A) an example of a sister allospecies pair (superspecies) with plumage divergence across the Marañón Valley (*Iridosornis rufivertex* and *I. reinhardti*). B) *I. analis*, a congener of the allospecies pair in A, shows no plumage divergence on either side of the Marañón and is referred to as a monotypic species. Pairs of superspecies and monotypic species from seven other genera (for 16 total "lineages") were included in this study (Table 1). C) Sampling localities of the 163 individuals included in the analyses from all 16 lineages. Squares are proportional to the number of samples from that site. The arid or semi-arid valleys of the upper Zamora, Huallaga, and Apurímac rivers act as additional dispersal barriers to Andean cloud forest birds (Weir 2009; Winger et al. 2015).

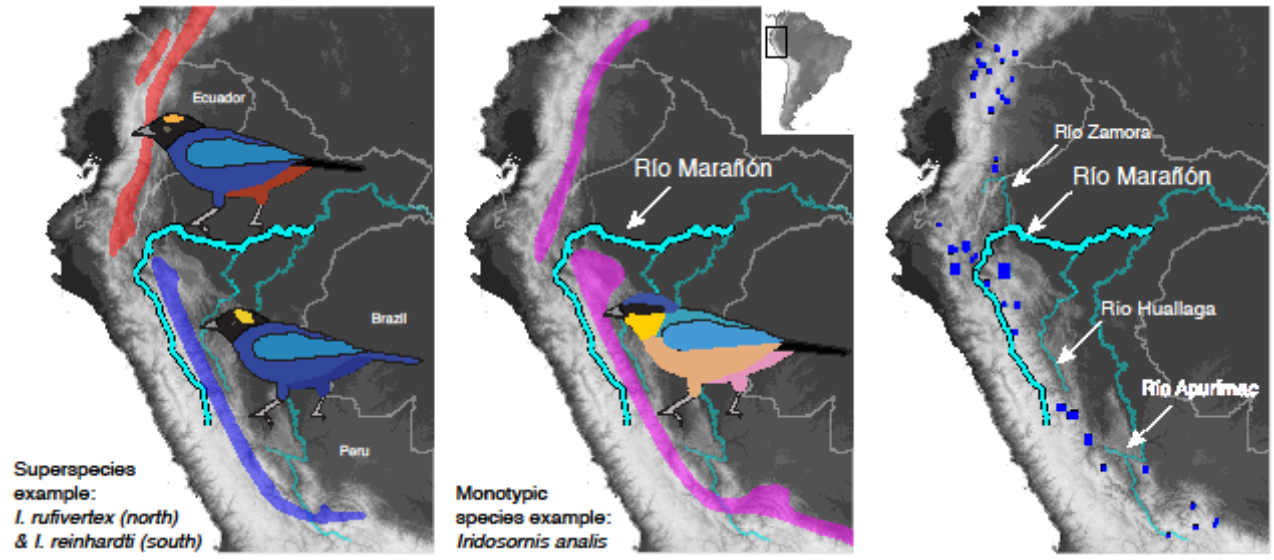

Figure 2. Trans-Marañón F_{ST} of GBS loci in each of 16 lineages (8 superspecies in gray and 8 monotypic species in white). Top, F_{ST} versus a multivariate estimate of trans-Marañón plumage distance in each lineage. Plumage distances are from Winger and Bates (2015) and are log Mahalanobis distances of hue, chroma and mean brightness averaged across 10 plumage patches measured from museum specimens with a spectrophotometer. F_{ST} is strongly related to plumage divergence, both on a continuous scale (plumage distance, top, $r = 0.87$, $p < 0.001$), and according to

the categorical classification of superspecies or monotypic species (boxplot, bottom). The boxplot summarizes the median and quantiles of F_{ST} in monotypic species and superspecies.

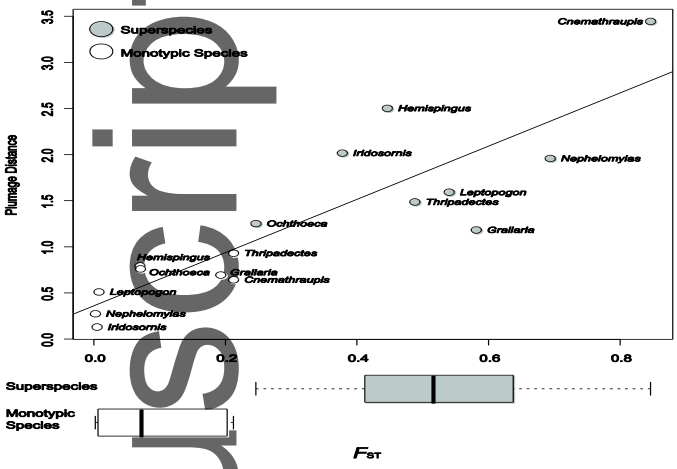

Figure 3. Schematic of how *D*-statistics were used to test for introgression across the Marañón. The test uses the ratio of alleles ABBA or BABA in a four-taxon pectinate phylogeny (((P1, P2), P3), O), where O is an outgroup, to reveal introgression between populations P2 and P3. Two different testing schemes were used, depending on the phylogenetic relationships of the populations and the available sampling, to test for trans-Marañón introgression in each lineage. Scheme 1 requires a P1 population sampled from northern Ecuador that is sister to a P2 population north of the Marañón, whereas Scheme 2 requires a divergent P1 population in central or southern Peru that is sister to a P2 close to the Marañón on the south side. In both cases, populations P1 and P2 must be each other's closest relatives with respect to P3.

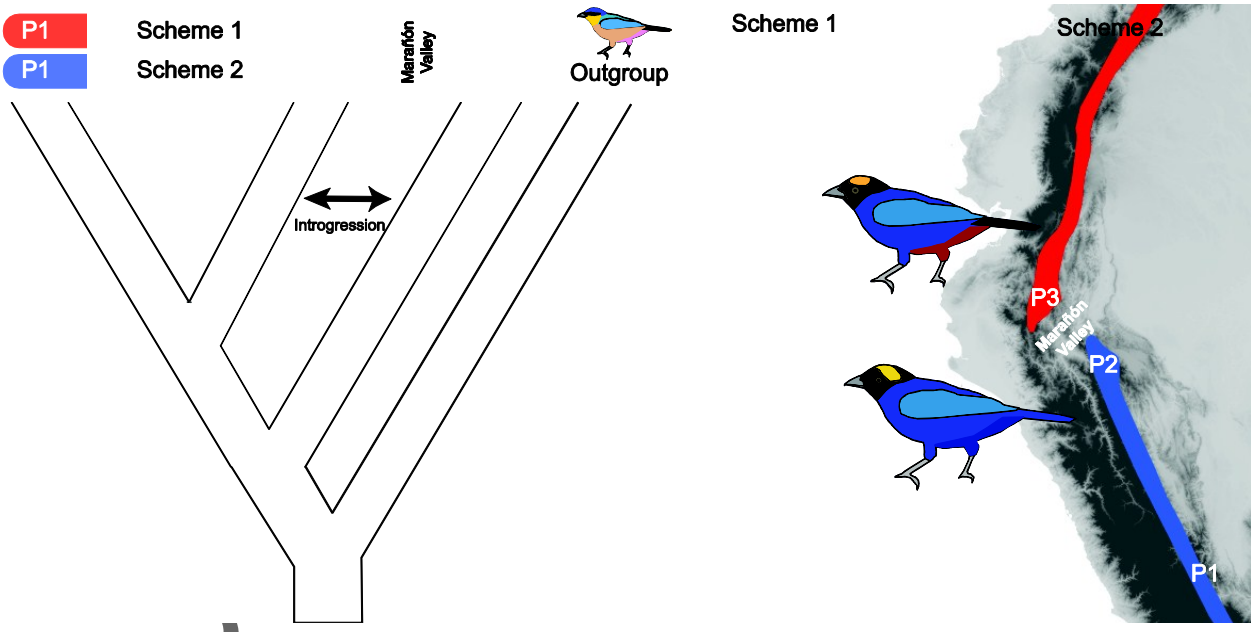

Figure 4. The proportion of the genome that experienced introgression across the Marañón (*f*; equation 2) from the ABBA/BABA tests. The black and gray circles indicate *f* values from ABBA/BABA tests that were designed with two different schemes for the assignment of populations P1, P2 and P3 (illustrated in Figure 3). Squares indicate the mean *f* across two testing schemes. Not all lineages had appropriate topological relationships or sampling to perform both testing schemes, hence there is only one *f* value for six lineages. Four of the monotypic species (*Leptopogon, Nephelomyias*, *Ochthoeca*, and *Iridosornis*) were not included in this test because the populations in each of these lineages are too genetically similar to one another across the Marañón for this test to be appropriate (see text); in these lineages, recent ancestry cannot presently be distinguished from substantial introgression.

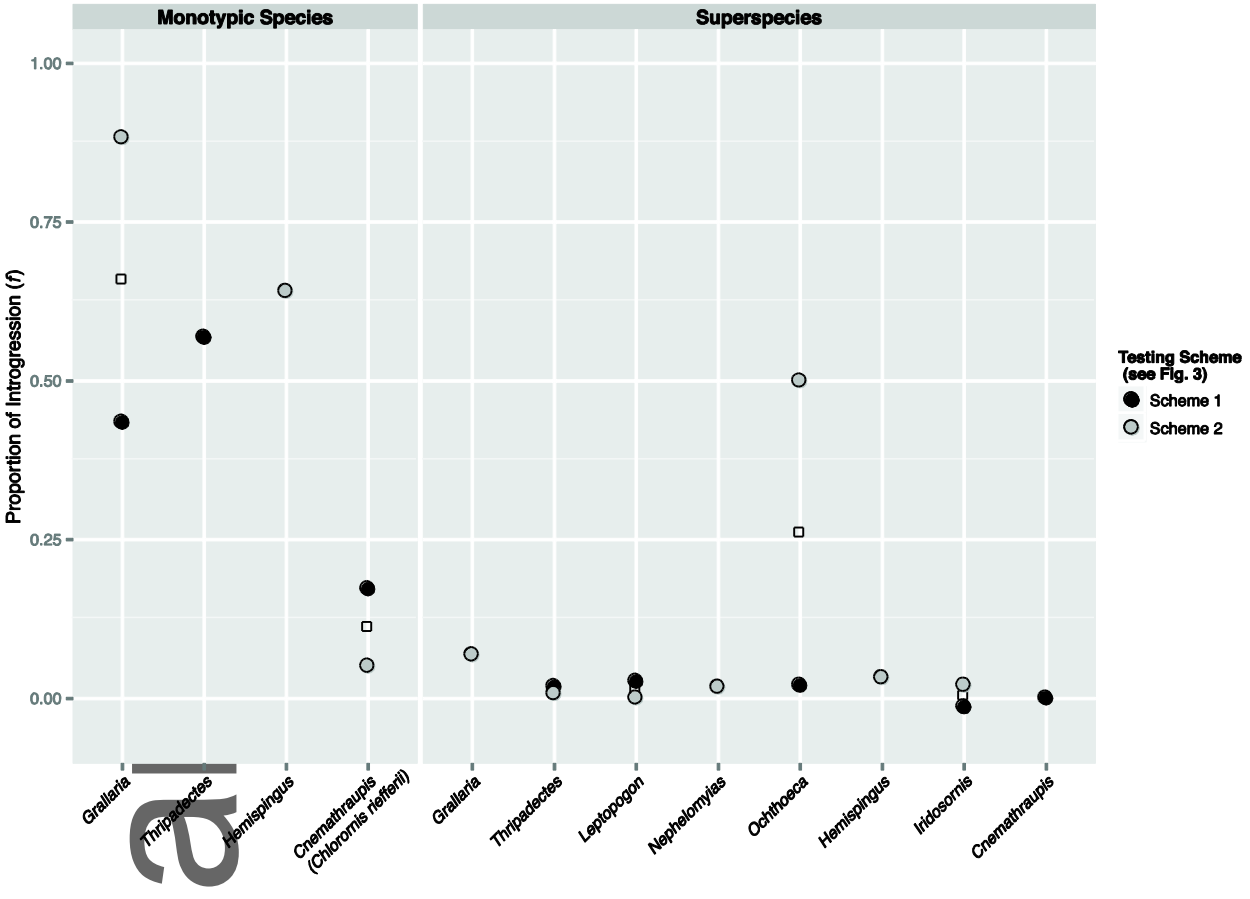

Figure 5. *TreeMix* topologies with migration. The monotypic species *Chlorornis riefferii* (which is sister to the *Cnemathraupis* superspecies and paired with that genus for the purpose of this study; Sedano and Burns 2010) and *Grallaria squamigera* experienced trans-Marañón introgression according to the *TreeMix* tests. The arrows indicate ancestry derived by migration from a population elsewhere in the topology, with the colors referring to the proportion of migration inferred (labelled as percent admixture). In the case of *Chlorornis riefferii*, the population nearest the Marañón on the north side is inferred to have 19% of its ancestry derived from a migration event from the south side of the barrier. The population of *Grallaria squamigera* nearest the Marañón on the south side is inferred to have 42% of its ancestry derived from populations on the north side. Population labels "Close", "Mid", and "Far" refer to sampling distances from the Marañón (Fig. 1): Close, near to the Marañón (south of the Zamora River on the north side and north of the Huallaga river on the south side); Mid, a medium distance from the Marañón (the east slope of the Andes north of the Rio Zamora

on the north side and between the Huallaga and Apurímac rivers on the south side); and Far, further from the Marañón (the west slope of the Andes of northern Ecuador on the north side, and south of the Apurímac River on the south side).