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Avian diversification along elevational zones in the Tropical Andes: density-dependent cladogenesis of tanagers, ecological speciation and climate-driven population genetic differentiation

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Avian diversification along elevational zones in the Tropical Andes: density-dependent cladogenesis of tanagers, ecological speciation and climate-driven population genetic differentiation

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor in Philosophy in Biology

by

Raul E. Sedano Cruz

2013

ABSTRACT OF THE DISSERTATION

Avian diversification along elevational zones in the Tropical Andes: density-dependent cladogenesis of tanagers, ecological speciation and climate-driven population genetic differentiation in isolation

by

Raul E. Sedano Cruz

Doctor of Philosophy in Biology

University of California, Los Angeles, 2013

Professor Thomas B. Smith, Chair

Examining patterns of biodiversity in the Tropical Andes provides insight into ecological and evolutionary processes leading to accumulation of lineages in montane regions. In the Tropical Andes, the dramatic changes in topographic and environmental conditions over short distances constitute an ideal system for studying morphological adaptation and geographic discontinuities. These patterns of biodiversity along elevational gradients in the Tropical Andes remains intriguing because there is no comprehensive theory that explains, species richness, high levels of endemism, and the extraordinary variation in traits that are ecologically relevant, in a

region that occupies less than 1% of global surface. In particular, I examine phylogenies, eco morphology and population genetics of birds in the Tropical Andes.

In chapter one, I use phylogenetic-based methods to assess slowdown in evolution of body size, changes among lineages in elevational-range overlap and rate-shifts in cladogenesis. Interspecific phylogenetic comparative analysis of the Core Tanager clade supports an overall density-dependent cladogenesis consistent with tenets of adaptive radiation. In chapter two, I use explicit spatial hypotheses of the distribution of the Andean Purplish-Mantled Tanager for present-day and to the past ice-age 18,000-22,000 years ago to test for concordant patterns between population genetic structure using microsatellites loci and mtDNA sequence variation, and potential effects of climate change. Topographical and environmental determinants explain a large proportion of spatial genetic variation in this tanager, but demographic history does not appear to be responding to the expansion of suitable area since last ice-age. In the third chapter, I use coalescent methods for a multilocus data set to examine demographic history between of The Olive-striped and Streak-necked Flycatcher (the montane *Mionectes*) and between populations for each of these species. The amount of differentiation between species that barely overlap ranges along elevation is similar to the differentiation observed between populations within the same species across the Andean ranges. Because divergence times broadly overlapped between and within the same species, discordant patterns of differentiation in plumage and vocal traits between populations within the same species as compared with distinctive plumage and vocal phenotypes between species cannot be simply explained by rapid differentiation due to genetic drift alone.

The dissertation of Raul E. Sedano Cruz is approved.

Victoria Sork

Michael Alfaro

Thomas Guillespie

Thomas B. Smith, Committee Chair

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2013

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CHAPTER 1

Does Body Size and Elevational-Range Variation Explain Evolutionary Diversification of Tanagers (Aves: Thraupini) in the Tropical Andes?

ABSTRACT

Patterns of morphological diversification in species-rich clades provide insight into the evolutionary processes generating diversity. I investigated spatial distribution between different elevational zones in one of these morphological characters, body size, in 93 tanagers of the Tropical Andes. The major goal of this study is to test the adaptive diversification hypothesis for the core tanagers clade, which states that rates of cladogenesis decelerate and the rate of evolution of ecological relevant traits slowdown, as the availability of newly formed suitable areas decline. Such decline in ecological opportunity is expected by the final geological uplift of the Andes to their current heights and the formation of higher elevational grounds. Pulses of elevational range overlap through time shows the contraction and expansion of ancestral ranges over the entire history of the core tanager group (7-10 million years before present). Along this process size disparity has become progressively smaller than expected under a Brownian model of size change, and overall evolution of size has slowed down later in evolutionary history. By testing different models of lineage diversification, I find evidence of a dependency of the rates of cladogenesis upon body size and elevation. The pattern of size evolution appears associated with an overall decline in cladogenesis of tanager species, suggesting that size diversity and lineage accumulation may be ecologically and spatially limited. However, elevational zonation does not predict the frequency distribution of body size in a phylogenetic framework, body sizes among core tanagers lineages evolve with unpredictable trajectories with respect to highland and lowland ranges. The lack of correlation between elevational zonation and body size among core tanagers, and between changes in elevational-range overlap and size disparity among species suggest a more complex adaptive processes than a classical view of thermoregulatory adaptation of larger organism's predicted in colder environments as in the Andean highland.

INTRODUCTION

The enormous avian diversity of the Tropical Andes is unlikely the result of uniform processes of speciation and extinction across time. While there is evidence of numerous diversification events across the Andes (Cadena *et al.* 2007; Campgna *et al.* 2011; Caro *et al.* 2013; Chaves & Smith 2011; Gutierrez-Pinto *et al.* 2012; Sedano & Burns 2010), how new morphological and lineage diversity accumulates across varying environmental compromises along elevation and latitude remains an intriguing and long-standing area of investigation (Brumfield & Edwards 2007; Cadena *et al.* 2011; Chapman 1917, 1926; Cheviron & Brumfield 2009; Claramunt 2010; Derryberry *et al.* 2011; Humboldt & Bonpland 1807; Kattan *et al.* 2004; McCormack *et al.* 2009; Remsen 1984; Sedano & Burns 2010; Vuilleumier 1978; Weir & Price 2011; Weir & Schluter 2007). Examining patterns of phenotypic variation and the tempo of lineage diversification can potentially provide insights into spatial and ecological determinants of the speciation process (Cuvier 1822; Derryberry *et al.* 2011; Kennedy *et al.* 2012; Osborn 1902; Schluter 2000; Simpson 1944; Slater *et al.* 2010; Weir & Mursleen 2013).

Researchers take the idea that newly open ecological opportunities colonized by divergent species become eventually a filled niche space, and suggest that such process may eventually slowdown the rate at which species are formed (Harmon *et al.* 2003; Phillimore & Price 2008; Stanley 1973). Thus, evidence of slowdowns in rates of diversification and evolution of ecological relevant traits is often interpreted as density-dependent cladogenesis (Barker *et al.* 2013), a dependency of speciation and extinction upon an adaptive process in accumulation of phenotypic diversity (Schluter 2000; Simpson 1944). Interspecific competition for limited ecomorphological space and pleiotropic constraints on phenotypes might also be part of broader interpretations for a slowdown in the rate at which species are formed (Foote 1997). However, it

stands to reason that spatial constraints alone can impact the process of diversification. The fine subdivision of the geographic domain itself, due to the successive partitioning of ancestral ranges during speciation events (Nelson & Platnick 1981), may eventually prevent population isolation. Over time, this mechanism often considered strictly geographic, can generate patterns of decelerating diversification through time (Pigot 2010).

Few studies have tested for slowdowns in lineage diversification and evolution of ecological relevant traits (Derryberry *et al.* 2011; Kennedy *et al.* 2012; Slater *et al.* 2010; Weir & Mursleen 2013). Two of these studies found patterns conflicting with predictions density-dependent cladogenesis, while the more recent studies found slowdowns in both diversification and trait evolution consistent with adaptive radiation. These studies only provide an indirect association between morphological traits and lineage radiation and none incorporated explicit spatial considerations on neither diversification nor trait evolution. The extend to how spatial considerations increase or decrease diversification is not well understood (Kissel & Barraclough 2010); however, spatial patterns of biological traits suggest complex relationships between morphological variation with the processes of speciation and extinction (cladogenesis), which ultimate determine the rate at which species are formed.

For instance, body size is critical to many aspects of an organism's life-history traits, such as home range (Garland *et al.* 1992; Gillespie 2002), mating success, fecundity, lifespan (Roy 2008) and metabolism (McNab 1983). Typically, when mapping size onto a phylogeny it is assumed that small and large-bodied species have equivalent probabilities of speciation and extinction (Claramunt 2010; Derryberry *et al.* 2011; Harmon *et al.* 2003; Nee *et al.* 1992; Purvis *et al.* 2000). However, extinction is not random with respect to body size (Purvis *et al.* 2000),

ancestral states of size may determine performance and fitness (Brown *et al.* 1993), which then in turn would constraint subsequent cladogenesis (Butler & King 2004; FitzJohn 2010).

Species' spatial distribution may also influence the effect of size on cladogenesis. Extinction risk depends upon the size of a species' geographic range (Purvis *et al.* 2000; Turner 1996), which would mean that species with small and large ranges equally may not have the same rates of speciation (Kissel & Barraclough 2010). Despite the general rule of smaller organisms requiring smaller ranges, spatial patterns of morphological variation suggest that reciprocal interactions between ecological relevant traits and geography may have important implications for the speciation process (Remsen 1984). For example, elevation has been thought to be an important predictor of avian diversity in the complex topography of the Tropical Andes (Blackburn & Ruggiero 2001; Brumfield & Edwards 2007; Cadena *et al.* 2011; Fjeldsa & Rahbek 2005; McNab 2009; Sekercioglu *et al.* 2008; Vuilleumier 1978). In this region, changes in habitats and climatic regimes over short distances across altitudinal zones also suggest that physiological and morphological diversity might correlate with abiotic factors that co-vary with elevation. In passerines, elevation can broadly predict body mass-specific metabolic rates (McNab 2009a); thus, body mass might be an important predictor of diversification and is expected an indicator of limits imposed on i.e. basic energetic requirements by ecology across elevational zones (Endler 1977).

Furthermore, the area of altitudinal zones is a significant predictor of the number of bird species (Kattan and Franco 2004), whether speciation rates are higher at low or high-elevational zones, possibly because area varies due to landmass effect, requires further test to examine cladogenesis sensitive to spatial scales (Kissel and Barraclough 2010). The potential interactions between size, elevation and cladogenesis suggest that studies utilizing phylogenetic-based

methods that examine the tempo of lineage accumulation should consider both trait-state and the spatial distribution of taxa when making predictions on species turnover.

The high species turnover across the highland and lowland regions of tropical Andes (Fjeldsa & Irestedt 2009; Kattan & Franco 2004; Kattan *et al.* 2004), and the striking morphological variation of Andean passerines (Blackburn & Ruggiero 2001) constitutes an ideal system to examine the potential dependency of avian diversification upon morphological diversity and species elevational distribution. I investigate in this study the diversification of tanagers as a function of body size and spatial distribution along elevation. Tanagers are one of the richest avian radiations in the Tropical Andes (Burns 1997; Klicka *et al.* 2007). They are widely distributed in lowland and highland areas from Mexico to Argentina but the largest density of species occur in the Tropical Andes. I focus on a subgroup, the core tanagers that comprise 20 genera and roughly 100 species (Burns 2002; Burns & Naoki 2004; Sedano & Burns 2010). The core tanagers clade diversified in and out of the Tropical Andes, which pattern of cladogenesis is consistent with an early burst in diversification followed by a rate-shift, a slowdown in the branching process (Sedano and Burns 2010). The high species turnover across elevation and a remarkable variation in body size (ranging 8.5 to 96 g) in the core tanager group constitutes an excellent system to explore how body size and elevation may have influenced cladogenesis, and whether or not there is a correlation between body size and elevational ranges in the core tanager clade.

The major goal of this study is to test the adaptive diversification hypothesis (sensu Schluter 2000) for the core tanager clade, which predicts slowdowns in cladogenesis and evolution of ecological relevant traits. This expectation implies the initial availability of newly open suitable areas at higher elevations, presumably associated with the formation of higher

elevational grounds by the geological uplift of the Andes to their current heights (Gregory-Wodzicki 2000). I examine the tempo of both lineage and body size diversification, using body mass as a surrogate of ecomorphological diversity. I used a time-scale genealogy based on mitochondrial DNA as the core tanagers phylogeny (Sedano & Burns 2010). Using this tree, I determine patterns of size disparity among lineages (Harmon *et al.* 2003) in order to examine potential constraints in size evolution over time, and test whether evolution of size is consistent with a decline in size diversification. I further examine evolution of size and elevational-range overlap between closely related forms to explore if the core tanagers are particularly likely to show a tendency to diversify in either sympatric or allopatric conditions along elevation. Next, I tested for a potential dependency of cladogenesis upon body size or elevation as surrogates of ecological and spatial determinants that can control the process of cladogenesis. By using quantitative state speciation and extinction comparative phylogenetic analysis (FitzJohn 2012) and geographic state speciation and extinction analysis (Goldberg *et al.* 2011), I examined cladogenesis as function of body size and elevation respectively. I then test whether elevational zonation predicts body-size frequency distribution in core tanagers, by examining hypotheses of reciprocal interactions between size and elevational ranges using the Ornstein-Uhlenbeck approach for comparative phylogentic analysis (Butler & King 2004).

METHODS

Phylogeny

A dated molecular tree containing 93 species of core tanagers out of the roughly 100 species that likely comprise the clade was generated using published sequences (Sedano & Burns 2010) for two mitochondiral (mt) genes cytochrome *b* and NADH dehydrogenase subunit 2. The

mtDNA phylogeny was time-calibrated in a Bayesian framework implemented in BEAST 1.4.8 (Drummond & Rambaut 2007), constraints were placed on certain well-supported (> 0.9 posterior probability) nodes to match a recently published higher-level topology (Sedano and Burns 2010). Branch lengths were estimated using birth-death, lognormally distributed priors in a relaxed molecular clock model (Drummond & Rambaut 2007). This ultrametric tree, a comprehensive collection of species body-masses and distributional ranges of species across elevation were used to conduct the following array of analyses: to test if body size disparity has become significantly smaller, I measure diversification in body mass using a disparity-through-time plot. I predict that changes in size diversification coincide with a decline in formation of ecological opportunity by the period of Andes final uplift. I also conducted a node-height test to determine whether size evolution has decelerated over time consistent with adaptive radiation. The dated tree was used for examining constraints in species elevational zonation by plotting changes among lineages in elevational-range overlap through time. Diversification with high levels of range overlap would support a pattern of ecological space filling up as in adaptive radiation. Further, the mtDNA phylogeny was used to conduct a rate heterogeneity test to evaluate rate-shifts that are expected consistent with a decline in cladogenesis. I also examine rate heterogeneity in cladogenesis as a function of size and elevational ranges. These traits are expected to be important ecomorphological and spatial controls of adaptive radiation in core tanagers. Finally, the core tanager phylogeny was used to examine the possibility of direct reciprocal interactions between body size and species elevational zonation as function of time using a comparative phylogentic analysis based on Ornstein-Uhlenbeck model.

Body Size

I used core tanager body mass as a surrogate of size and ecomorphological variation. Body masses were compiled for each species from a variety of sources (Dunning 2007), including data from specimens at the Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, and over 700 entries from extensive fieldwork in the northern Andes. I included data for all species but *Tangara fastuosa* (no information on size was available) of the 93 terminal tips in the phylogeny. Entries in our data set ranged from one to 240 specimens, the mode was five specimens per species. Three or more specimens were used in 88% of the species sampled, while singletons represented only seven species. Data were natural log transformed to moderate a right-skew distribution toward smaller avian body masses (Maurer 1998), while still keeping disparity proportional among observations in original morphospace.

To reduce the bias and focus on broad patterns of size change among lineages, I assessed intraspecific geographical variation in size by generating a distribution of size for each species. I generated normal distributions of log-transformed body-masses for each species represented in the data set by two or more specimens. Each synthetic distribution of body mass was made using the maximum and minimum body mass data points and by an estimate of the process of standard deviation (Vandeman 1999). Cross validation suggested that our synthetic dataset adequately addresses interspecific geographic variation. Since generating distributions of size for each species did not affect the analytical outcome, I report results of comparative analyses using the observed dataset.

Species Elevational Range

An estimate of the maximum and minimum elevation for each species was obtained from a variety of published sources (Hilty & Brown 1986; Isler & Isler 1999; Restall *et al.* 2006; Ridgely & Greefield 2001; Ridgley & Tudor 1989) and extensive records from conducted fieldwork. For analyses that required grouping species by elevational zones, each species elevation-range was assigned into categories as lowland (0-1500 m), highland (500-3050 m) or occurring at both (0-3050+ m) (Sedano & Burns 2010). Such categorical assignment follows other studies of the role of elevation in diversification of birds (Brumfield & Edwards 2007). This broad distinction of highland and lowland ranges are ecologically informative categorical groups because they tend to capture differences across altitudinal zones in three aspects: 1) thermal zonation of tropical mountains and changes in oxygen barometric pressure, 2) historical changes in vegetation structure (Hooghiemstra & van der Hammen 2004), and 3) species turnover along elevation (Chapman 1917; Kattan & Franco 2004).

Size Disparity through Time

To test whether size evolution changed among and within lineages, I measured size diversification using a disparity-through-time plot (Harmon *et al.* 2003). I examined the dispersion of size disparity in multivariate space by measuring the mean Euclidean distance among species log body masses. I plotted the relative body mass disparity against clade age, including all divergent events across the deep and numerous branches of the phylogeny. The body mass disparity through time shows the partition of size *among* and *within* subclades. Body mass disparity through time was compared to 10,000 simulations of a uniform-rate process of cladogenesis as implemented in GEIGER (Harmon *et al.* 2007) using R (R-development-Core-

Team 2008). I then calculated the area between the observed and simulated clade disparity curves, computed as the morphological disparity index (MDI) (Slater *et al.* 2010). A negative MDI indicates that body mass disparities are less than predicted under a Brownian motion process, and that size evolution is on average partitioned *among* subclades rather than *within* subclades, in which case a positive MDI is expected (Harmon *et al.* 2003). Ecologically diverging clades are expected to show less subclade disparity than clades evolving in the absence of ecological constraints.

Slowdown in Body Size Evolution

To examine whether body size evolution accelerated or slowed through time, I implemented the node-height test (Frackleton & Harvey 2006). Evolution of ecologically relevant traits is expected to slowdown as ecological space is saturated by accumulation of species. The node-height test correlates independent contrasts (IC) (Felsenstein 2008) for body size on the core tanagers phylogeny and the approximate subclade age (height) of each node at which ICs were computed. The underlying assumption on IC calculation is a uniform-rate Brownian motion process on branch lengths information. Then, a positive linear correlation between IC and node heights indicates a deceleration of body size evolution over time; in contrast, a negative correlation would indicate acceleration of size evolution following a random walk process (Frackleton & Harvey 2006).

Rate Heterogeneity

To determine whether or not a uniform rate of cladogenesis best explained the branching process of the core tanager clade, I conducted a rate heterogeneity test that allows examining if rate-shift are consistent with an overall decline in diversification as predicted by adaptive

radiation. Rate heterogeneity was examined by modelling stepwise evolutionary diversification using phylogenetic and taxonomic information of putative populations for each species in FOSSILMEDUSA (Alfaro et al. 2009). The analysis as implemented in FOSSILMEDUSA using R (R-development-Core-Team 2008) also allows for further examination of speciation and extinction rates to infer decline or increase trends from a uniform process of cladogenesis. The methods used to fit a series of increasing rate-shifts to estimate the maximum-likelihood for a set of birth-death parameters have previously been described (Santini *et al.* 2009). Models of rate-shift were restricted to six parameters to keep a 1:16 ratio between parameters and tree datapoints, model selection was implemented by estimation of the maximum likelihood in terms of information gains larger than four units (Burnham & Anderson 2004) using the Akaike Information Criterion (AIC) (Akaike 1973).

Cladogenesis and Body Mass

The pattern of diversification was examined as a function of core tanagers body masses using the Quantitative State Speciation and Extinction (QUASSE) framework (FitzJohn 2012). I expect that the probabilities of speciation and extinction in core tanagers are at least in part dependent on adaptive ecomorphology. The QUASSE framework can be used to determine whether or not body size is an important predictor of the rate for core tanager cladogenesis. The QUASSE model, instead of assuming that there is no dependency of the branching process on body sizes with a uniform-rate of cladogenesis (a traditional trait-mapping approach), allows for rate shifts given a set of functions dependent on body size (FitzJohn 2010). Several functions that describe a dependency of diversification on size were fit to the core tanager phylogeny, including the simplest uniform-rate model. A modal, sigmoidal, or stepwise function were fit to model the

probability of speciation while keeping extinction constant or, alternatively, by modeling a variable probability of extinction while keeping speciation constant (FitzJohn 2012). I examined only models with non-directional body-size evolution to prevent runoff of parameters (> 6) with respect to the tree dimension. Analyses were performed in R (R-development-Core-Team 2008) using the following packages: DIVERSITREE (FitzJohn 2012), APE (Paradis *et al.* 2004), PHYLOBASE (Bolker *et al.* 2008) and GEIGER (Harmon *et al.* 2007).

Cladogenesis and Elevational Range

I examine the pattern of diversification as a function of elevation range using the Geographic State of Speciation and Extinction analysis (GEOSSE) (Goldberg *et al.* 2011). I expect that the probabilities of speciation and extinction in core tanagers are also dependent on spatial constraints itself. The GEOSSE method required the core tanager phylogeny and species assigned to categorical elevational-ranges. Instead of assuming that species elevation-range evolved on a static branching process with a uniform rate (a traditional trait-mapping approach), the GEOSSE model allows rate-shifts in cladogenesis between elevational-ranges given a set of biogeographical assumptions (Goldberg *et al.* 2011; Goldberg *et al.* 2005; Ree *et al.* 2005). The GEOSSE framework was used to generate extant diversity of core tanager species given a phylogeny as a function of highland and lowland categorical ranges as well as lineage exchange between ranges. From GEOSSE model selection, I determined whether or not elevation is an important predictor of the rates of cladogenesis. Parameters from GEOSSE were estimated by determining the distribution of the second order AIC over the posterior distribution of 10,000 trees using MCMC. The results are reported as the proportion of tree samples from the posterior density distribution (pdd) for which a statement of a prediction is highly supported (> 0.95).

Analyses were performed in R (R-development-Core-Team 2008) as implemented in the package DIVERSITREE (FitzJohn 2010; Goldberg *et al.* 2011).

Changes in Range-Overlap Over Time

This analysis allows one to examine whether there is a correlation between elevational-range overlap among lineages and subclade-ages. I explore if related forms show a tendency to occur in either allopatry or sympatry, which may constitute an indirect measure for modes of diversification driven by either competition for ecological space or further partitioning of ecological opportunity respectively. The nested pairwise mean elevational overlap between species was estimated as the product of two matrices: the Euclidean distance between elevational ranges for every species pair, and a matrix of syntopic species pairs by zoogeographic regions (Parker *et al.* 1996), subregions (Hilty & Brown 1986; Isler & Isler 1999; Restall *et al.* 2006; Ridgely & Greefield 2001; Ridgley & Tudor 1989) and local bird lists (RNOA 2005). These data were sorted against the approximate node height for each subclade (Fitzpatrick & Turelli 2006) to represent topologically weighted averages for comparisons across internal nodes in the phylogeny (Warren *et al.* 2008). Overall, each node provides an estimate of the average overlap between lineages after a certain time since speciation. Since overlap tends to be rare and deeper nodes are more likely to exhibit overlap by chance (Fitzpatrick & Turelli 2006), I assigned each data point of elevational range and node-age in two categories: "marginal spatial overlap" as < 10% elevational range intersection *within* clades, "nontrivial" overlap as 10-50% intersection and "high" overlap as > 50% intersection (Lynch 1989). Analyses were performed in R (R-development-Core-Team 2008) using packages APE (Paradis *et al.* 2004), PHYLOBASE (Bolker *et al.* 2008); PHYLOCLIM (Heibl 2011) and GEIGER (Harmon *et al.* 2007).

Correlation Between Body Size and Elevation

Whether elevational-ranges may have constrained the body-size frequency distribution was tested using model-fitting approach, by assigning a categorical elevational-range to each branch of the phylogeny using the R-package OUCH (King & Butler 2009). Each categorical elevational range implies a distinct environmental regime for body-mass change and presumably a different evolutionary trajectory for each branch. Thus, the expected transformed body masses for each species are a weighted average, where the weights depended on how long a lineage has evolved under a certain elevational regime. The main assumption is that today's elevational categorical regimes provide some information about size diversification. OUCH allows AICc model comparison, ranging from relatively simple to relatively more complex that represent one or multiple optimum regimes of size evolution. Thus, I tested for a dependency of the rate of size evolution on elevational ranges with respect to a uniform-rate Brownian motion process as a null model. The second simplest model assumes a single unknown global regime for size change across the phylogeny with a directional term of size change over time. The last three models assume full knowledge of the elevational origin of core tanagers by fixing deeper braches to lowland, highland, or lowland/highland ancestral regimes. The model allows these regimes imposed onto branches to progressively evolve to current elevational-states. Additionally, I examined the effect of uncertainty of the phylogeny itself on testing whether elevational-ranges may have constrained evolution of body size. This was conducted performing a censored rate heterogeneity test as implemented in BROWNIE (O'Meara *et al.* 2006) by examining changes in body masses along elevational ranges across 500 post-burnin trees of the core tanager phylogeny.

RESULTS

Changes in Body Size Evolution

Later in evolutionary history disparities in body sizes *among* lineages (size disparity) have become progressively smaller over time (Fig 1-1a). In general, there are small differences of log body-masses (~ 10%) for a majority of subclades in the core tanager phylogeny (58th percentile), while fewer subclades (85th percentile) show disparities over 50% in their body-masses. Size disparity through time (dark line in Fig 1-1a) is within the lower end of the much greater variation in size disparity under simulated conditions given a phylogeny and body-masses for each taxa (grey zone in Fig 1-1a). One segment of size disparity in particular has become progressively smaller over time than expected under a Brownian motion model of size evolution. Later in evolutionary history it is unlikely that size disparity is the result of morphological evolution under a Brownian motion process; towards the present, body sizes evolving *among* lineages were more dependent upon their ancestral states (lower area under the grey zones in Fig 1a). Figure 1-1b shows the best linear fits on a scatter plot between ICs and node height, this line indicates an increase in the value of ICs with node height. Over time body size evolution is changing systematically through the numerous branches of the tree, suggesting that core tanagers are broadening morphological space.

Cladogenesis Dependency on Body Size

FOSSILMEDUSA rate heterogeneity analysis provided evidence for changes in cladogenesis via shifts in the rate of branching process for the core tanagers (Table 1-1). Furthermore, the QUASSE analysis provides evidence of a dependency in the branching process of core tanagers upon their body masses. Those core tanager species closer to a modal body

mass experience a higher rate of speciation (ΔAIC = 4.2 against the uniform-r model). The modal function is the best-fit model over a sigmoidal (ΔAIC > 10 against the modal model), or as compared to a stepwise function (ΔAIC > 4.8 against either the modal or sigmoidal model) (Table 2-1). Irrespective of the actual function that may describe a dependency of cladogenesis on body size, I found evidence against a uniform-rate process of speciation and extinction with respect to size (LRT against constant speciation-rate model chisqr = 16, p = 0.001).

Decline in Cladogenesis

FOSSILMEDUSA rate heterogeneity analysis favors a process with two rate shifts as the best fit across the phylogeny (1 Rate Break+1 in Table 1-1). By comparison of both speciation and extinction rates estimates, our analysis provides strong evidence of a decline in diversification. The initial burst in cladogenesis characterized by low extinction rate ($r_{basal\ node}$ = 0.26; $e_{basal\ node}$ =4.3483 e-08) was followed by two decelerations in cladogenesis (Fig. 2-1). The deceleration in the branching process at two nodes was the result of high speciation rates, but even higher extinction rate estimates. The first node where a rate-shift took place ($r_{node\ A}$ = 0.23; $e_{node\ A}$ = 8.2603 e-01) includes 12 *Tangara* species widely distributed across elevation (Fig. 2). The second rate-shift took place in a node that comprises eight *Tangara* species ($r_{node\ B}$ = 0.40; $e_{node\ B}$ = 3.7217 e-01), all at lower elevation range but *T. desmaresti* restricted to higher elevations (Fig. 2-1).

Cladogenesis Dependency on Highland and Lowland Ranges

The GEOSSE analysis provided evidence of a higher rate of speciation in the lowlands (SL) than in the highlands (SH) where SL-SH = 0.20962 (pdd = 0.99). Cladogenesis dependent on elevation is also indicated by slightly higher extinction rates *within* the lowlands (XL) as

compared to the highland region (XH) where XL-XH = 0.0401 (pdd = 0.79). Furthermore, model selection shows that the addition of an extra parameter of speciation "between-elevational-ranges" does not substantially improve model fit (Table 2-1). GEOSSE provides evidence of unequal rates of speciation and extinction *within* elevational ranges and suggests that lower elevations are major centers of diversification.

Partitioning of Elevational Range Overlap through Time

Changes in elevation-range overlap among lineages have occurred over the entire history of the core tanagers diversification (Fig. 1-1c). Clearly, elevational range overlaps do not correlate with node-heights ($p < 0.76$). This absence of evidence for a linear relationship between range overlap and clade-age suggests that related forms are not particularly likely to show a tendency to diversify in either sympatric or allopatric conditions. Instead, partitioning elevational-range overlap through time *among* subclades reveals pulses of expansion and contraction of ancestral ranges. This is consistent with the GEOSSE analysis, making it difficult to ascertain whether anagenesis (by gains in elevational range expansion) or cladogenesis is primarily responsible for core tanagers diversification (posterior probability < 0.5). Nevertheless, the average range overlap among subclades provides valuable insight into the overall pattern of diversification: 56% of subclades show non-trivial elevational range-overlap (10-50%), while the rest show marginal range-overlap (<10%).

Elevation and Body-size

OUCH provides evidence that specifying an optimum elevational-range as a surrogate of an evolutionary regime of size change was not a better fit for the branching process of the core tanagers phylogeny. Instead, the best model is a uniform-rate Brownian motion (BM) process of

size change. The BM was selected as a better fit among six models (ΔAICc = 10) that explicitly distinguish mean body size between highland and lowland ranges (Table 3-1). A single-rate Brownian model of trait change implies that size evolves independently of elevational ranges (Fig. 3-1). The parameter estimates are reasonable for both the body-mass (~28.5 g) at the root of the phylogeny [3.35 ln(g); sd = 0.12] and the drift parameter (0.41). The rate heterogeneity test in BROWNIE provides further support BM is substantially better than allowing subclades to have their own rate, and further implies little effect of tree uncertainty (for 65% of the trees; ΔAICc 13 and parametric bootstrap p-value 0.09). Thus results from OUCH and BROWNIE are congruent and neither supports the prevailing notion of larger-bodied species at higher elevations. Overall BM predicts extensive size convergence across highland and lowland ranges. The Node Height scatter plot also provides valuable insight in the relationship between elevation and size. Figure 1-1b shows that levels of elevational-range overlap do not particularly aggregate independent contrasts for body size. This implies that marginal elevational-range overlap among related forms does not predict rate of size evolution.

DISCUSSION

Slowdown in Body Size Evolution

The BM model of trait evolution is a fairly good approximation for describing evolution of body masses (as a surrogate of size) among core tanagers. Using this single-rate model, I found valuable patterns that deviate from a BM of trait evolution. First, the Node Height scatter plot shows that over time core tanagers seem to have broadening body size variation, but slowing down size diversification. Second, later in evolutionary history body sizes evolving *among* subclades were more dependent upon their ancestral states. The slowdown of size evolution and

progressively smaller size disparities were intensified long after the earlier burst in core tanagers diversification, providing evidence that lineages exploring new adaptive zones undergo rapid radiation (Burbrink and Pyron 2010). These two patterns coincide with the period of the final geological uplift of the Andes, which is presumably a period when initial ecological opportunity ends. Such pattern of trait evolution supports the notion of phenotypic diversity filling ecological opportunities (Frackleton and Harvey 2006), as one of the premises of adaptive radiation.

Dependency of Lineage Diversification Upon Size

In contrast to the prevailing notion that body mass alone (as a surrogate of ecomorphological diversity) is not correlated with effective cladogenesis within bird families (Nee et al. 1992), the QUASSE analysis indicates that changes in body masses were in part responsible for rate-shifts in cladogenesis of core tanagers (Thraupidae). This direct association between cladogenesis and size supports the patterns of body size disparity and slowdown evolution of size consistent with adaptive radiation. Rate-shifts estimates utilizing FOSSILMEDUSA are driven by high species turnover and consistent with an overall decline in cladogenesis. Such decline roughly coincides with a period of rapid mountain uplift (Sedano & Burns 2010) and continued long after the Andes reached their maximum elevations around 2.7 mya (Audemard & Audemard 2002; Graham 2009; Gregory-Wodzicki 2000; Hoorn *et al.* 1995). It stands to reason that novel ecological openings along elevation were limited by the final mountain uplift, slowing body size evolution as expected as niches fill over time (Foote 1997; Gavrilets & Losos 2009; Simpson 1944) under adaptive radiation (Schluter 2000). Although it is difficult to ascertain whether a dependency of cladogenesis upon body size is primarily responsible for a decline in core tanager diversification, our results suggest that body mass is an

important predictor of diversification and might be an indicator of limits imposed by ecology across elevation (Endler 1977).

Dependency of Cladogenesis Upon Elevation-Range

The GEOSSE analysis indicates that rate-shifts in cladogenesis for core tanagers can be associated with a categorical zonation of elevation between highlands and lowland regions. This dependency of cladogenesis on elevation strongly suggests that after speciation events, sister-species do not shifted their elevation-ranges independently, implying a heritable component of species elevational-range. Collectively this also suggests that elevation-ranges of extant species are probably most informative of past speciation events (Fitzpatrick & Turelli 2006; Losos & Glor 2003) in which derived lineages conserve their relative distributions across current elevational zones. Assuming that elevational-ranges of extant species retain phyletic information, the partition of range overlap among lineages shows contraction and expansion of ancestral elevational ranges through the entire history of the core tanagers group.

Contraction and expansion of elevational-range overlap conform to the notion that subdivision of ancestral ranges constitutes a fundamental step of speciation events (Coyne & Orr 2004). Deep valleys and mountaintops across the Andes have been shown to play an important role in facilitating subdivision of ranges between sister-species (Campgna *et al.* 2011; Sedano & Burns 2010). GEOSSE provides evidence that most tanagers diversified in lowlands, and highland diversity resulted from dispersal from the lowlands. This supports that cladogenesis is sensitive to spatial scales (Kissel & Barraclough 2010), where the larger area through landmass effect is expected to more effectively maintain isolates. The best GEOSSE model strongly suggests that the overall pattern of lineage split can be explained by changes in the rate of

speciation and extinction *within* highland and lowland regions. On the contrary, a lesser optimal model utilizing a uniform-rate of cladogenesis would likely lead to the conclusion that a larger proportion of lineage splits across the phylogeny occurs in the highlands as compared to the lowlands (Sedano & Burns 2010). Model comparison as shown in here is a powerful approach to examine the assumption that cladogenesis is sensitive to elevation-range.

The decline in cladogenesis of core tanagers may also be taken as evidence of the progressive restriction of spatial and ecological openings after the Andes final uplift. Rate-shifts in cladogenesis of core tanagers conform to the likely greater chances of speciation in larger lowland area. Spatial constraints at higher elevations could inhibit the ability for species divergence in strict isolation, thereby potentially slowing diversification (Goldberg *et al.* 2011; Pigot *et al.* 2010).

The Evolution of Body Size Disparity Across Elevation

I do not find evidence of a correlation between body size and elevation. First, OUCH and BROWNIE provides evidence that body masses evolve to a modal bird of roughly 28.5 g with unpredictable trajectories with respect to highland and lowland ranges. The phylogenetic-based analysis does not support a predicted correlation between elevation and avian mass-specific metabolic rate (McNab 2009b). This implies that abiotic factors that co-vary with elevational ranges (i.e. thermal zonation) are unlikely dominant predictors of body size in core tanagers and other passerines in general (Blackburn and Ruggiero 2001). Second, elevational-range overlap among related forms does not predict evolution of body size in core tanagers. Smaller size disparities *among* core tanagers may not be necessary to overcome spatial limits along elevation. Collectively, there is absence of evidence of direct reciprocal interactions between size and

elevation, and yet size and elevation are important controls of cladogenesis in core tanagers. Overall progressively smaller disparities in size and greater elevational range overlap strongly suggest that closely related forms are filling the ecological openings in the Tropical Andes.

CONCLUSIONS

Few studies of adaptive radiation support slowdowns of both cladogenesis and trait evolution simultaneously (Kennedy *et al.* 2012; Weir & Mursleen 2013), while few others have found evidence only for a slowdown in trait evolution (Derryberry *et al.* 2011; Slater *et al.* 2010). Here, I found evidence for slowdowns in cladogenesis and evolution of body size in tanagers, as broadly expected under adaptive radiation. Overall a decelerating pattern of size evolution, as the absolute contrasts in size values increased from the root to the tips in the Node-Height scatter plot can be taken as indicative of niche saturation. Both a decelerating pattern of size evolution and size disparity becoming progressively smaller than expected under a BM suggest that the initial burst in core tanager diversification was accompanied by diversification along different body size adaptive-trajectories. Body size and elevation are important controls of diversification in core tanagers, and yet there is no evidence of reciprocal interactions between size and elevation. More studies are in need to understanding the relationship between trait variation along elevational gradients over long-term generation time.

In contrast to the changes in the rate of cladogenesis in core tanagers, a relatively uniform-rate of cladogenesis is found in other avian families such as neotropical ovenbirds and woodcreepers (Derryberry *et al.* 2011) and antshrikes (Bravo, in prep). This discordance highlights the complexities in the processes controlling avian cladogenesis within the recent geological history of the Tropical Andes. The newly formed highland grounds, particularly

during the final mountain uplift and the formation of higher elevational grounds 2-7 mya, opened opportunities for colonization and ecological adaptation. The best GEOSSE model supports the prediction of colonization of higher elevation-ranges, major centers of diversification are at lower elevations, where the larger area through landmass effect is expected to facilitate population isolation more effectively than a smaller area at higher elevation.

Although explicit spatial considerations affect the process of cladogenesis in core tanagers, elevation as a geographic determinant does not seem to affect body size evolution. There is no phyletic evidence of larger core tanagers species at higher elevation, as expected on the basis of energetic requirements for heat conservation (Bergmann 1847; McNab 2009). Further, species elevational-range overlap is also a poor predictor of the differences in size among lineages, as the absolute contrasts in size values do not cluster with respect elevational overlap at the Node Height scatter plot. The correlation analyses between body size and elevational ranges, or range overlap suggest that ecological processes that lead to differences in size are different from the processes that limits species elevational distribution among lineages.

In this study the signature of an adaptive radiation is retained in ecological relevant traits and within the branching pattern in the phylogeny; however, the phylogeny and neontological data on species distribution also retained information on the geographic effect of elevational zonation on cladogenesis. Core tanagers represent one of the first examples of continental radiation, which diversification is likely dependent on adaptive accumulation of trait diversity and spatial constraints. I suggests that future studies of adaptive radiation in the Tropical Andes given the numerous examples of super rich avian clades with striking pattern of morphological diversity should include models of cladogenesis that allow to incorporate both morphological and spatial information.

FIGURES AND TABLES

Table 1-1. Rate heterogeneity test using FOSSILMEDUSA

Models	Parameters	-Log likelihood	Akaike Criterion (AICc)
1-Rate-Break	2	349.3	702.7
1-Rate-Break+1	5	337.6	685.5*
2-Rate-Break+1	8	331.9	680.6

Table 2-1. Rate heterogeneity tests as function of body mass and elevation

Models	Parameters	-Log likelihood	Akaike Criterion AICc
Quantitative Speciation and Extinction (QUASSE) analysis of cladogenesis dependent on body-size			
Constant Speciation & Constant Extinction	3	248.2	502.5
Modal Speciation & Constant Extinction	6	243.1	498.3*
Sigmoidal Speciation & Constant Extinction	6	248.3	508.6
Stepwise Speciation & Constant Extinction	5	246.6	503.1
Constant Speciation & Modal Extinction	6	248.3	508
Constant Speciation & Sigmoidal Extinction	6	248.3	508
Constant Speciation & Stepwise Extinction	5	246.2	506
Geographic Speciation and Extinction (GEOSSE) analysis of diversification dependent on highland and lowland regions			
Unequal speciation or extinction rates within elevational regions with additional speciation parameter between regions	7	320.4	654.8
Unequal speciation and extinction rates within elevational regions without speciation parameter between lowland and highland regions	6	320.4	652.8*
Constant speciation rate within and between lowland and highland regions	5	328.28	666.5

Table 3-1. Ornstein-Uhlenbeck approach for phylogentic-based correlation analysis (OUCH) between size and elevational ranges

Models	Parameters	-Log likelihood	Akaike Criterion AICc
Brownian motion process of size evolution	2	20.8	45.8*
OU with unknown elevational regimes at deep internal branches	6	23.8	53.9
OU single unknown optimum elevational regime across the phylogeny	3	21.0	54.9
OU rooted highland origin with three categorical regimes	5	23.2	57.0
OU rooted lowland origin with three categorical regimes	5	21.8	54.3
OU rooted lowland/highland origin with three categorical regimes	5	23.7	56.8

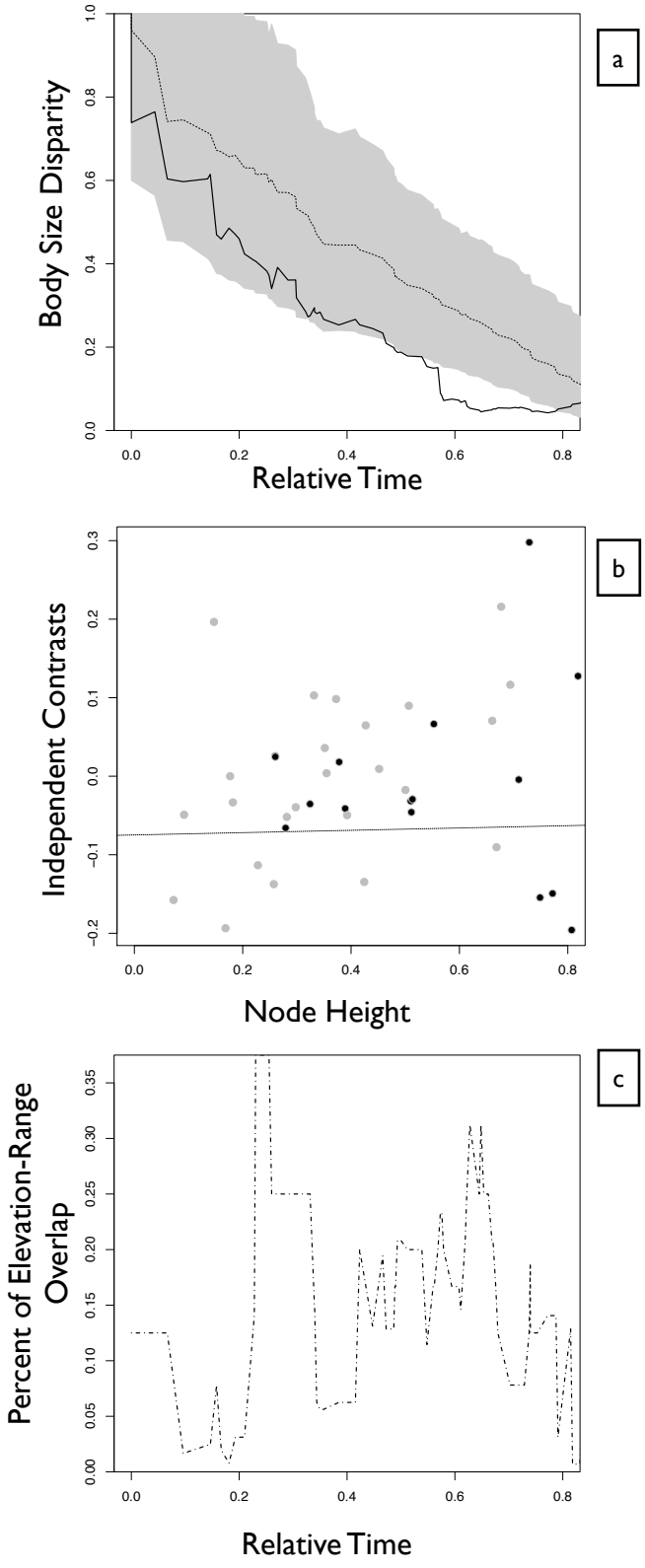
a
Body Size Disparity
Relative Time
b
Independent Contrasts
Node Height
c
Percent of Elevation-Range Overlap
Relative Time

Figure 1-1. Changes in body size disparity and elevational-range overlap between close related core tanagers. Body size disparity through time plot (a), Height-Node scatter plot (b) and partition of lineages elevational-range overlap over time (c). Partition of body-mass disparity (upper box) shows that disparity became smaller *within* subclades over time (dark line), a segment of disparity deviates out of the 95% confidence interval (grey zone) later in evolutionary history and shows a mean size disparity under a uniform-rate model (dotted line). The scatter plot of independent contrasts (IC) shows differences in body size among lineages of the corresponding node height (relative age for each node). Node height is the distance from the root to a given node, such the height of the root is zero. Best fit line (dashed line) shows a positive relationship for body size and node height ($t=2.388$, $d.f.=41$, $p=0.02$). Lower ICs values indicate that paired comparisons are relatively similar in morphology. Dark circles indicate ICs with "marginal elevational-range overlap" as < 10% elevational range intersection *within* clades and grey circles show "nontrivial elevational-range overlap" as >10% (Lynch 1989). Partition of lineages elevational-range overlap shows contraction and expansion of range among lineages (lower box). Pulses of range overlap over time are plotted as the relative mean variation in elevational-range overlap *among* subclades (dashed line). Size disparity and elevation range-overlap through time were truncated before the most recent diversification events because at the tip of the phylogeny there are fewer point estimates among and within lineages that can mislead calculated values in recent times.

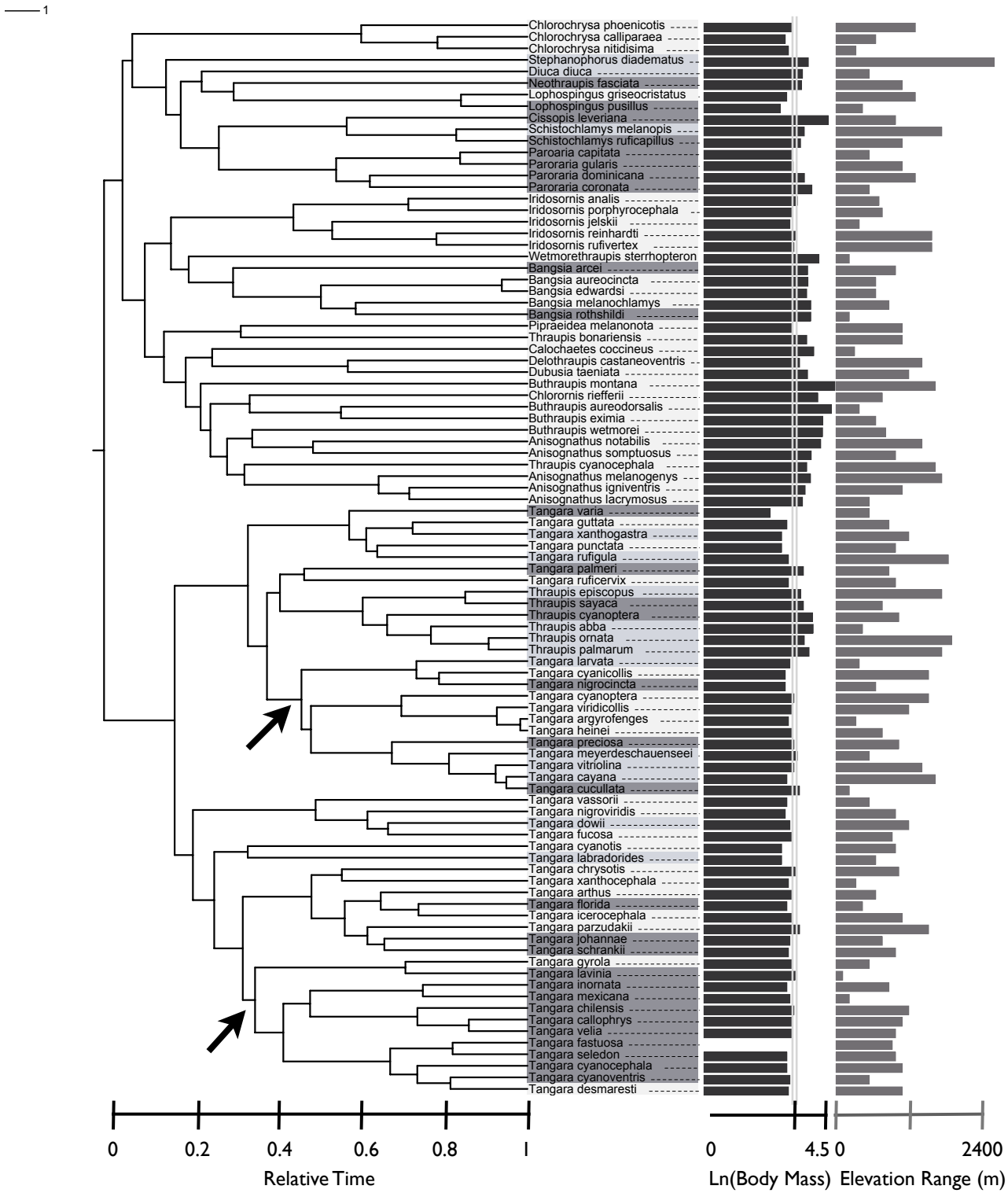
1
Chlorochrysa phoenicotis
Chlorochrysa calliparaea
Chlorochrysa nitidisima
Stephanophorus diadematus
Diuca diuca
Neothraupis fasciata
Lophospingus griseocristatus
Lophospingus pusillus
Cissopis leveriana
Schistochlamys melanopis
Schistochlamys ruficapillus
Paroaria capitata
Paroraria gularis
Paroraria dominicana
Paroraria coronata
Iridosornis analis
Iridosornis porphyrocephala
Iridosornis jelskii
Iridosornis reinhardti
Iridosornis rufivertex
Wetmorethraupis sterrhopteron
Bangsia arcei
Bangsia aureocincta
Bangsia edwardsi
Bangsia melanochlamys
Bangsia rothschildi
Pipraeidea melanonota
Thraupis bonariensis
Calochaetes coccineus
Delothraupis castaneoventris
Dubusia taeniata
Buthraupis montana
Chlorornis riefferii
Buthraupis aureodorsalis
Buthraupis eximia
Buthraupis wetmorei
Anisognathus notabilis
Anisognathus somptuosus
Thraupis cyanocephala
Anisognathus melanogenys
Anisognathus igniventris
Anisognathus lacrymosus
Tangara varia
Tangara guttata
Tangara xanthogastra
Tangara punctata
Tangara rufigula
Tangara palmeri
Tangara ruficervix
Thraupis episcopus
Thraupis sayaca
Thraupis cyanoptera
Thraupis abba
Thraupis ornata
Thraupis palmarum
Tangara larvata
Tangara cyanicollis
Tangara nigrocincta
Tangara cyanoptera
Tangara viridicollis
Tangara argyrofenges
Tangara heinei
Tangara preciosa
Tangara meyerdeschauenseei
Tangara vitriolina
Tangara cayana
Tangara cucullata
Tangara vassorii
Tangara nigroviridis
Tangara dowii
Tangara fucosa
Tangara cyanotis
Tangara labradorides
Tangara chrysotis
Tangara xanthocephala
Tangara arthus
Tangara florida
Tangara icterocephala
Tangara parzudakii
Tangara johannae
Tangara schrankii
Tangara gyrola
Tangara lavinia
Tangara inornata
Tangara mexicana
Tangara chilensis
Tangara callophrys
Tangara velia
Tangara fastuosa
Tangara seledon
Tangara cyanocephala
Tangara cyanoventris
Tangara desmaresti
0
0.2
0.4
0.6
0.8
1
Relative Time
0
4.5
Ln(Body Mass)
0
2400
Elevation Range (m)

Figure 2-2. Rate-shifts in cladogenesis of core tanagers on a time-scaled phylogeny after Sedano and Burns (2010). Arrows indicate FOSSILMEDUSA estimates of rate-shifts in the branching process of cladogenesis (see text). Categorical elevational ranges are highlighted at the tip-labels of the phylogeny. Highland (light grey), lowland (darker grey) and species widely distributed between lowland and highland (intermediate grey). Body-size for each species is shown by the horizontal bar (dark). The vertical lines indicate the approximate range of modal and maximum body mass (~28.5 grams). Elevation-range is shown for each species by the horizontal bar (grey).

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CHAPTER 2

Environmental Heterogeneity shapes Population Genetic Structure of Purplish-mantled Tanager (*Iridosornis porphyrocephalus*) in the Northern Andes

ABSTRACT

Ecological niche modeling can be useful for understanding patterns of genetic structure and processes producing adaptive variation in the wild. The fragmented distribution of bird species along narrow latitudinal ranges in the Andes offers an ideal system to examine how geographic discontinuities may influence the genetic structure among populations. To determine whether population genetic structure is explained by environmental determinants, I explore the relationship between environmental variables using a MAXENT modeling approach, and the spatial arrangement of neutral genetic variation of the Andean Purplish-Mantled Tanager (*Iridosornis porphyrocephalus*). Neutral variation of mitochondrial and microsatellites loci is also used to test for changes in demographic history that may be associated to climate change, following the predicted geographic expansion of habitat for this tanager since the Last Glacial Maxima18,000-22,000 years ago. Environmental and topographic variables, using the Generalized Distance Modeling approach, explained 72-74% of the genetic structure for mitochondrial sequence and microsatellite loci variation. This correlation strongly suggests that accumulation of mutations in mitochondrial and nuclear markers seem to be shaped by environmental factors. Environmental predictors of temporal thermal variation and habitat cover were consistently found to be important correlates with genetic structure. Present-day and LGM projections show that suitable area fluctuate with climate change and demographic analyses suggest that patterns of gene flow and isolation are greatly affected by geographical barriers on small spatial scales. However, these analyses do not support demographic expansion, as predicted by 68% expansion in potential suitable area since the Last Glacial Maxima. Although a neutral process of divergence in isolation can explain a significant proportion of spatial genetic structure of *I. porphyrocephalus*, the observed correlations with environmental variables cannot

be explained by genetic drift alone. This study provides evidence of population divergence in isolation shaped by environmental determinants along a narrow elevational zone in the northern Andes. Spatial projection of *I. porphyrocephalus* to the year 2050 predicts a shift of potential distribution across elevation, and 24.5% loss of present-day suitable area. This projection of warming conditions in the near future implies the end of the geographical expansion of the habitat for *I. porphyrocephalus* since the LGM.

INTRODUCTION

Environmental heterogeneity is a significant factor in shaping genetic structure of natural populations (Coyne & Orr 2004; Freedman *et al.* 2010; Gugger & Cavender-Bares 2013; Newman & Rissler 2011; Smith *et al.* 1997; Sork *et al.* 2010; Temunović *et al.* 2012). Examining the associations between genetic variation and environmental variables may lend insights into the processes producing genetic diversity, including gene flow, genetic drift, and natural selection (Nosil & Feder 2012; Schluter 2009; Smith *et al.* 2005). In particular, environmental characteristicss may influence the spatial scale of gene flow differently than geographic distance. Separating the relative contribution of environmental and topographic effects from their association with genetic variation is a challenge to understanding how historical genetic differences may accumulate among populations across geography (Knowles *et al.* 2007). Since species-range limits are thought to be driven in part by their topographic complexity (Graham *et al.* 2010), modeling the realized climatic niche of a given species can provide valuable information to explore into factors limiting species range (Graham & Hijmans 2006; Mohonan & Hijmans 2008; Sork *et al.* 2010; Thomassen *et al.* 2010). However, little is know to what extent changes on the geographical extension of climatic conditions to which a

species exhibit range-filling (suitable area) (Svenning & Skov 2004) contribute to the spatial genetic diversity (Sork & Waits 2010).

Estimates on the realized environmental niche of a species depend upon reliable environmental data and accurate species-range estimates. Such species distribution model (SDM) constitutes an explicit hypothesis relating the suitability of environmental conditions and geographic discontinuities within a species' range (Buermann *et al.* 2011; Buermann *et al.* 2008; Graham *et al.* 2010). The SDM for a given species can be examined in the context of available genetic data to help understand how environmental variables and evolutionary processes are related (Freedman *et al.* 2010; Gugger & Cavender-Bares 2013; Sork *et al.* 2010; Thomassen *et al.* 2010). For example, genetic differentiation between populations may be most pronounced across geographic barriers (Gugger & Cavender-Bares 2013), abrupt habitat changes (Eckert *et al.* 2010; Thomassen *et al.* 2010), or regional climate gradients (Sork *et al.* 2010). Populations with little genetic differentiation most likely have continuous gene flow and few environmental discontinuities (Slatkin 1985). In contrast, if genetic spatial differentiation among populations matches spatially isolated populations, the pattern of fragmentation may be the result of restricted gene exchange (Kozak & Weins 2006). The latter scenario may imply that fragmentation is a major factor in driving genetic structure of populations in natural populations.

Species distribution models use a discrete set of climatic variables to broadly describe ecological conditions of suitable areas. This spatial arrengment is an explicit hypothesis that may reflect the primary spatial arrengment of population genetic subdivision. The interaction between environmental conditions and processes of population divergence (the balance between genetic drift and natural selection) constitutes an important factor for the maintenance of genetic diversity, local adaptation and extinction (Frankham 2005; Slatkin 1993; Sork *et al.* 2010).

Therefore, environmental heterogeneity may encompass an important set of reliable predictors of changes in species distribution and genetic diversity.

Changes in suitable areas indicate contractions or expansions of population geographic distribution and can also indicate demographic changes in population size (Mila *et al.* 2006). Projecting past climatic conditions from models based on current climate offers the possibility of exploring spatial changes in suitable areas (Carnaval & Moritz 2008; Freedman *et al.* 2010; Hugall *et al.* 2002; Zhao *et al.* 2012). Using a historical context to investigate demographic history and population connectivity could inform conservation decisions concerning the priorities for, i.e., reserve network design (Frankham 2005; Goldberg & Waits 2010). For example, maps of past distributions had been used to identify historically stable suitable areas, and project geographic expanding range fronts (Freedman *et al.* 2010; Pease *et al.* 2009), criteria rarely considered for reserve prioritization schemes.

The objective of this paper is to examine how geographic and environmental discontinuities have influenced genetic structure of the Purplish-Mantled Tanager (*Iridosornis porphyrocephalus*), a forest-dwelling passerine, restricted to the west Andes of Colombia and northwestern Ecuador. This species' distribution range (< 16,700 km^2) is patchy and reduced within the lower montane forest to smaller and more isolated populations (BirdLife 2013). Geographic discontinuities within the species range are likely the result of its narrow elevational range at 1550-2200 m (Hilty & Brown 1986; Isler & Isler 1999; Ridgley & Tudor 1989) across latitude (1º to 7º N). The range includes rugged mountain ranges and fragmentation is likely to be much greater where environmental conditions vary widely over short distances (Graves 1988). In addition, the lower montane forest, habitat for the Purplish-Mantled Tanager was severely affected by past climatic fluctuations. The last glacial maximum (LGM) shifted-down in

elevation and compressed by 55% the lower montane forest (Hooghiemstra & van der Hammen 2004). This dramatic compression of suitable area into lower elevation where there is larger area through landmass effect would imply more dispersal restrictions between geographic isolates. Thus, I predict that such 55% compression of the forest that house the Purplish-Mantled Tanager have contributed to shape population genetic structure and demographic history in this species. I use geographic information systems (GIS) analyses and fine scale species distribution modeling to create a set of present-day, past and future environmental projections in order to quantify changes on the predicted and observed range-limits *I. porphyrocephalus*. By integrating population genetic analyses, using mitochondrial and nuclear genetic markers, this paper will examine environmental and topographic variables to address: 1) the extent to which geographic barriers correspond to population genetic structure, 2) how environmental variables correlate with neutral genetic variation, and 3) how historical changes in species potential distribution correlate with demographic history of *I. porphyrocephalus*.

METHODS

Species Distribution Modeling

In order to examine the environmental determinants of population genetic structure, I first modeled the potential geographic distribution of *I. porphyrocephalus* by using the maximum entropy (MAXENT, Version 3.1) algorithm (Phillips & Schpire 2006). The main assumption of the Maxent probabilistic framework is that only the species-occurrences data can be approximated with a probability distribution of maximum entropy (Phillips & Schpire 2006). The model of potential distribution using Maxent is subject to certain environmental constraints. This distribution approximates the species potential geographic distribution and expresses

relative probability of the presence for each grid cell as a function of the environmental variables (Phillips & Schpire 2006).

I modeled the potential distribution for *I. porphyrocephalus* with 71 georeferenced point localities selected by fieldwork crossvalidation from a vast array of data points (Biomap 2006; RNOA 2005). To model the potential distribution for *I. porphyrocephalus*, I used remote sensing data and contemporary high-resolution (1 km) environmental data set. The environmental data set from ground-based climate and remotely sensed data sources covering a diverse range of surface parameters including climatic extremes, vegetation density and seasonality, surface moisture and roughness, and topography. A series of bioclimatic metrics were obtained from WORLDCLIM version 1.4 (Hijmans *et al.* 2005) and reduced to a smaller set after controlling for covariance among the metrics (Buermann *et al*. 2008). The final climate data set included five temperature and six precipitation metrics, expressing spatial variations in annual means, seasonality and extreme or limiting climatic factors.

Remote sensing data included two MODIS archived products from the period 2000-2004: (i) the leaf area index (LAI) which provides information on net primary productivity and vegetation seasonality (Myneni *et al.* 2002), LAI is calculated as the one side projected green leaf area per unit of ground area (Knyazikhin *et al.* 1998), and (ii) the vegetation continuous field product as a measure of the percentage of tree canopy cover (Hansen *et al.* 2002). In addition I utilized radar backscatter information QuikSCAT (QSCAT) data which capture attributes related to canopy roughness and moisture, utilizing the annual mean and standard deviation based on monthly data from the year 2001 with complete data coverage (Long *et al.* 2001). The Shuttle Radar Topography Mission (SRTM) digital elevation data were included the mean elevation and ruggedness (Farr *et al.* 2007).

Monthly MODIS and QSCAT data are available at high temporal resolutions and from this region, meaningful metrics were formed which capture annual extremes (e.g., Annual Maximum LAI) and also seasonality. The identical high-resolution (~1 km) environmental data set was used in a previous study on predicting the distribution of a variety of taxa across the Andean regions (Buermann *et al.* 2008). Specific details about the various data layers and the various metrics can be found in Buermann *et al*., 2008

Last Glacial Maximum (LGM) Conditions in the Northern Andes

Predictions to past environmental conditions used the same 71 point localities but with a reduced set of only five climatic data layers (*annual mean temperature, annual mean diurnal temperature range, maximum/minimum temperature of warmest/coldest month,* and *annual mean precipitation*) aggregated to a coarser resolution (~5 km) for which reasonable spatial information regarding past climate during the last LGM (and in future climate) projections are available. The prediction at coarser scales with climate data only served to establish the present-day species-climate relationship. They were subsequently used to project onto past and future climate data in order to predict corresponding distributions of *I. porphyrocephalus*. An estimate of past species geographic distribution during the LGM is valuable for examining the changes in suitable areas relative to present-day potential distributions. The gains and losses in suitable areas can provide insight on population fragmentation to examine spatial genetic structure in the context of the LGM that range 18,000 to 22,000 years ago (Clark *et al.* 2009; Shakun & Carlson 2010), although the full last glacial period range 43,500-22,000 ya (Urrego *et al.* 2005; Zech *et al.* 2011). As a proxy of past potential geographic distribution for *I. porphyrocephalus*, I estimated the historical change in habitat of the species to the LGM. This is calculated by

subtracting the suitable area with 0.95 probability of species presence from other projections of interest as the past potential distribution.

The lower montane forest was likely the most downward shifted (800 m) along the elevational gradient and compressed vegetation habitat in the Colombian Andes during LGM (Hooghiemstra & van der Hammen 2004; van der Hammen & Hooghiemstra 2003). Because the target species is restricted to the lower montane forest and its lower record across elevation is 750 m (Isler & Isler 1999), this species is an excellent candidate to examine its spatial and demographic responses to warming trends since the LGM. Paleo-pollen records across the topographical heterogeneous mountain ranges allow reconstruction of the climatic conditions at the regional scale during the LGM (Hooghiemstra & van der Hammen 2004; van der Hammen & Hooghiemstra 2003). According to previous studies, LGM mean temperatures were about 5°C cooler and humidity levels were about 50% lower than present (Bryan & Helmens 2005; Hooghiemstra & van der Hammen 2004). Then, I created synthetic LGM climate layers by uniformly lowering the contemporary temperature metrics (annual mean and minimum/maximum of coldest/warmest month) by 5°C and the contemporary annual mean precipitation by 50%.

Estimating Future Climate Conditions in the Northern Andes

An estimate of future gains and losses in suitable areas can also provide insight into population fragmentation in the context of current warming trends in climate change. I estimated distributions for *I. porphyrocephalus* for the end of this century (2100). The projected future climate change is based on present-day climatic data using the IPCC 3rd Assessment Report (TAR) future global circulation model (GCM) projections, calibrated and statistically

downscaled using the WorldClim data for 'current' conditions (Hijmans *et al.* 2005). To provide an upper and lower bound of projected climate change for 2050, I utilized the simulations of one GCM with high climate sensitivity (HADCM3) and relatively high emission scenario (A2a) and one with low climate sensitivity (CSIRO) and lower projected emissions (B2a). Annual mean temperatures are projected to change between 1.5°C (CSIRO-B2a) and 3.5°C (HADCM3-A2a) by mid- century in the vicinity of the Northern Andes. For this epoch, both model-scenarios project annual mean rainfall to increase by up to 15%. These warming and humid patterns for the Northern Andes are also consistent with the corresponding newer IPCC Fourth Assessment Report (AR4) projections (IPCC 2007).

Genetic Analyses

Thirty-two samples for *I. porphyrocephalus* obtained through museum loans (Table A-1) were used to investigate population genetic structure. Genomic DNA was purified from blood, feathers, muscle or toe pads (Leeton *et al.* 1993; Mundy *et al.* 1997; Taberlet & Bouvet 1991) by using Wizard Genomic (Promega Corp., Madison, WI, USA) and gDNA Mini Tissue ChargeSwitch® (Invitrogen Corp., Carlsbad, California) kits. To investigate maternally inherited variation, I amplified and subsequently sequenced a mitochondrial (mtDNA) gene, the subunit 2 of NADH dehydrogenase (ND2; 1041 bp). Each 30 µL of PCR master mix contained ~100 ng/µL of DNA, 1X M/µl (10 mM Tris pH 9, 50 mM KCl), 10 µM dNTP, 10 µM of both forward and reverse primers following Sedano and Burns (2010), and 0.5 U Taq polymerase (Invitrogen Corp., Carlsbad, California) ran on PTC-100 Programmable Thermal Controller, MJ Research, Inc., Watertown, MA. Then, adding 20% polyetilenglicol and 2.5 M NaCl the PCR products were cleaned before they were sequenced using Multi–Color Capillary Electrophoresis (ABI 310

- 3100). Sequence alignment was implemented using SEQUENCHER version 6.1.0 (Gene Code Corporation, Ann Arbor, Michigan).

A total of seven microsatellites were screened. These markers were originally isolated for *Diglossa cyanea* (Bardeleben & Gray 2005). Polymorphic PCR products in *Irisodornis porphyrocephalus* were expected to be generated based on mtDNA distances (Primmer *et al.* 2005). Each 30 µL of PCR master mix contained ~30 ng/µL of DNA, 10 mM Tris pH 9, 50 mM KCl, 1.5 mM MgCl2, 200 µM of each dNTP, 0.4 µM of both forward and reverse primers and 0.5 U Taq polymerase (Invitrogen Corp., Carlsbad, California). Post-PCR products were added with ~1/5 (v/v) solution of 0.05% bromophenol blue and xylen cyanol, and 95% of formamide. Then products were denaturalized for 3 min and seeded on 4% polyacralamyde gel at ~50 °C. After two hours of vertical electrophoresis (70-80 W), alleles were stained following Basam et al. (1991) and their molecular weight was identified using UVGELSTARTMW version 11.01, and a 10 pb marker (Invitrogen Corp., Carlsbad, California).

Genetic Structure-mtDNA

The mtDNA genealogy was used to examine genetic structure across latitude within species range. The ND2 genealogy used outgroup information including species within the genus *Iridosornis* (GeneBank: EU648037 – EU648042). Gene trees were estimated by using a generalised time-reversible (GTR) plus gamma evolutionary model (Tavare 1986) in MrBayes v 3.0 (Ronquist & Huelsenbeck 2003). The Tajima's D neutrality test (Tajima 1996) and population genetics parameters were calculated in Arlequin 3.0 (Excoffier *et al.* 2005; Excoffier *et al.* 1992; Schneider *et al.* 2006). The structure of mtDNA variation was calculated using an AMOVA by regional structure (Excoffier *et al.* 1992) and genetic distances using the ϕ_{st}-statistic,

were corrected using a GTR model. The linear pattern of pairwise differentiation among six populations along latitude was examined using the ϕ_{st}- statistic and geographical distances between isolates under explicit models of local population differentiation (Wright 1943; Kimura and Weiss 1964) by using the program Isolation-by-Distance (IBD) v 1.52 (Bohonak 2002).

Genetic Structure--Microsatellites

Nuclear microsatellite genetic loci were used to examine structure across latitude within species range. First, microsatellites heterozygosity (H_E), and inbreeding coefficient (F_{IS}) were estimated using ARLEQUIN v3.11. Seven loci were tested for departure from Hardy-Weinberg equilibrium (HWE), as well as linkage disequilibrium among pairs of loci using ARLEQUIN v3.11. Genotype frequencies were calculated using POPGENE (Yeh *et al.* 1997). Microsatellite statistical power for *F*-statistics was estimated using POWSIM v4.0 (Raymann 2006). Then, population spatial structure was estimated using an AMOVA by region using sampling sites for sample grouping (Table A-1), as implemented in ARLEQUIN v3.11 (Excoffier *et al.* 1992). A model of isolation-by-distance was tested on microsatellites using IBD v1.52 (Bohonak 2002).

Geneland Delimitation of Population Structure

Spatial population structure was also assessed using GENELAND, which performs an individual assignment test that incorporates georeferenced data and different molecular markers types (Guillot *et al.* 2009). The individual assignment test used microsatellite loci, mtDNA sequence variation, as well as, a data set that combines these two markers types. The model infers which *K* population has the highest posterior probability density by several runs with K = 1 to 3, then, I calculated each *K* mean posterior probability over its runs. Because results were consistently within converging to K = 2 to 3, I test for the congruence of population subdivision

with areas of geographic discontinuity within the species range using the simplest *K*=2 model. I ran 400,000 MCMC iterations, maximum rate of Poisson process fixed to 200, uncertainty attached to georeferenced data points fixed to 0,00001, and maximum number in the Poisson-Voronoi tessellation fixed to 100. The posterior probability of individual membership for each pixel of the spatial domain was set to 175 x 175 to minimize overlap of two individuals in the same pixel. I finally check consistency of the results across five runs.

Coalescent Analyses

The coalescent model Isolation-with-Migration (Hey 2010) was used to estimate demographic parameters between populations across purported geographic barriers. This coalescent approach is an attempt to examine a non-equilibrium estimate of population differentation (unlike GENELAND) as implied from standard *F*-statistics (Templeton 1998). The Isolation-with-Migration framework was used to simultaneously estimate the following parameters scaled to the mutation rate: population sizes of effective ancestral and contemporary populations (θ), migration rates (*m*) and time since divergence between populations (*t*). Because, the objective was also to determine patterns of gene flow, I ran a paired two-population analysis for the seven microsatellites and mtDNA loci, separately. The Isolation-with-Migration model assumes that the two populations being compared are each panmictic and not exchanging genes with other populations (Hey and Nielsen 2004, Won et al 2005). The IMa2 model further assumes that loci are selectively neutral with no intralocus recombination. I defined inheritance scalars for mtDNA as 0.25 and autosomal loci as 1.0 to reflect differences in effective population sizes. I used the HKY model of mutation for mtDNA and infinite-sites model for microsatellites. Coalescence IMa2 for the mtDNA data set used initially a burn-in period of 20,000 and a run

length of 100,000 to assess whether the priors were suitable and the heating conditions were appropriate. From the results of these runs, I defined narrower upper bounds for each parameter. Using those priors, I used a burn-in of 10 million steps and recorded results every 12 h. Effective sample size for each parameter exceeded 500. I repeated analyses three times, using different random seed to verify independent runs.

For IMa2 on maternal inherited mtDNA for ND2 region, I used mutation rate (μ) of 4.8 x 10^{-8} substitutions per site per year (s/s/y) based on (Peters et al 2005) and converted *t* to real time (t) by $t = t\mu$. For IMa2 on microsatellites, I used a conservative range mutation rates (μ) of 10^{-3} - 10^{-6} (mutation/locus/generation) and applied a modest $\mu = 10^{-5}$ to all analyses in this study (Runemark *et al.* 2012). The upper limit of the prior for divergence time was set to $t = 1$. To place t = 1 in a demographic context, with a mutation rate of 10^{-3} and assuming a 2-year generation time, this value corresponds to a demographic splitting time ($T = t$ x g/u) of 200,000 years. I consider a prior distribution of 4Nμ with an upper bound of 15. The upper bounds of the migration priors were set to $m = M/\mu = 15$. To ensure adequate mixing of a Markov chain, I used Metropolis-coupling of 120 independent heated chains. Burn-in duration was set to 6 million steps and the most heated chain had a heating factor of 0.9 with other heating chains having heating values between 1 and 0.75.

Demographic History--mtDNA Genealogy

The gene tree for *I. porphyrocephalus* was used to test the null hypothesis of single-refugia for an ancestral population. This hypothesis predicts habitat fragmentation of a panmictic population from an ancestral range. In contrast, if multiple-refugia best explain the mtDNA tree topology, this would imply historical fragmentation into populations (Knowles 2001). I tested the

gene genealogy and branch lengths on population trees for these two hypotheses using the *S*-statistic as implemented in MESQUITE v 2.0 (Maddison & Maddison 2011). A null distribution of *S*-statistic was generated by simulating neutral coalescent process within the population trees (N_E = 2.5 million, and 21,000) to represent between 2.5 million to 21,000 years since divergence. I assumed a period of 21,000 years represents the maximum expected time since divergence from the late Pleistocene LGM, while a period of 2.5 million years corresponds to the early Pleistocene. Rejection of the null single-refugia hypothesis is accepted when *S*-statistic is less than 5% of values generated at random (1000 replicates). The level of deep coalescence discordance was measured when gene trees were compared within the species trees using demographic parameters. Tree depth (number of generations when the gene tree coalesces) was estimated with a process using 1000 replicates and range of effective population size (21,000 - 2.5 million) in Mesquite.

Integrating Genetic Structure with Species Distribution Modeling

I partitioned the relationships between environmental variables, latitude and genetic structure using the generalized dissimilarity model (GDM) (Ferrier *et al.* 2007). This method runs a statistical regression of matrices that can accommodate non-linear relationships between environmental and population genetic distances (Ferrier *et al.* 2007; Freedman *et al.* 2010; Thomassen *et al.* 2010). The GDM correlation uses matrices of environmental dissimilarities among sites extracted from SDM and high-resolution GIS layers; the matrix of genetic differentiation are based on F_{st} statistics among sites. The best predictors are selected using model selection techniques based on Monte Carlo permutation for forward selection as well as backward elimination of variables in stepwise procedures (Ferrier *et al.* 2007). This process

results in a spline-like function that best describes a series of non-linear relationships between environmental factors, latitude and genetic distance as dependent variables. The spline function that best describes the relationship between environmental and genetic variation can also account for linear relationships such as the one expected between geographic and genetic distances in an isolation by distance model (Bohonak 2002). This makes this model more appropriate in this study rather than methods that strictly examine linear relationships such as the distance-based redundant analysis as implemented DISTLM-forward v.5 (Anderson 2004).

RESULTS

Predicted Distributions

Present day SDMs using MAXENT (1 km^2 data precision, AUC = 0.99) resulted in a mosaic of suitable areas across its latitudinal elongated range, primarily in Colombia and Northern Ecuador (Fig. 1-2b and A-1). Nine out 11 climatic variables had the largest contribution in the MAXENT model, and as few as five climatic variables accumulate 84,5% of the environmental variation (Table 1-2). Four of this latter subset of variables provides information associated with temperature variability (Table 1-2), and the model increases and decreases the most gain of information when the maximum temperature of the warmest month and temperature seasonality are used or omitted in MAXENT respectively.

This projection of present-day potential distribution provides evidence that the area of the realized climatic niche is far larger than the area of species distribution based only on presence data (Fig. 1-2a-b). However, the model did not support suitability for of *I. porphyrocephalus* from Loja (~3° S) southeast Ecuador (Ridgely & Greefield 2001; Ridgley & Tudor 1989) (Fig. 1-2a-b). The resulting fine scale map provides evidence for geographical discontinuities in

suitable area within the species range (Fig. 1-2a-b), along latitude nearby dry valleys. These are the Patia River Valley close to the Colombian-Ecuadorian border (~1° N) and further north, the Dagua River Valley (~3° N) in the pacific versant of the Western Cordillera of Colombia. These two geographical gaps constitute putative geographical barriers to dispersal between population segments of *I. porphyrocephalus* (Fig. 1-2a-b).

Present-day and LGM projections provide evidence of historical changes in suitable area for *I. porphyrocephalus* (Fig. 1-2b, 1-2c and A-1). The projection to the LGM shows the lack of suitable environmental conditions in most areas where present-day projection tend to predict local distribution. The projection to the LGM resulted in the compression and downshift in elevation of suitable habitat. Such suitable areas and geographical discontinuities are largely concordant across latitude between past and present-day projections (Fig. 1-2b-c). Since the LGM *I. porphyrocephalus* has experience an expansion in potential distribution, a net gain of 68% in suitable area. In contrast, future climate model for 2050 predicts an upward contraction in suitable area for *I. porphyrocephalus*, and a net loss of 24.5% suitable area from present-day (Fig. A-1c). Overall a historical comparison of SDM projections shows distinct geographical discontinuities that may correspond with spatial population genetic structure. Further, the realized climatic niche may also correlate with genetic variation. These predictions that geographic and environmental variables correlate with genetic diversity are addressed in detail below.

Microsatellite and mtDNA Screening

The screening of a maternal inherited marker shows eight haplotypes within mitochondrial DNA sequences, including 13 informative sites and low nucleotide diversity

(0.003± 0.001). Sequences of mtDNA of *I. porphyrocephalus* conform to a monophyletic genealogy (p =1.0) summarized using a minimum-spanning network of absolute distances between ND2 haplotypes (Fig. A-2). Tajima's D neutrality test was not rejected; thus, selective neutrality assumption seems appropriate for mtDNA sequence evolution (D = -0.73, p = 2).

Analyses in Arlequin 3.0 for bi-parentally inherited markers show no evidence of deviation from selective neutrality. Including seven polymorphic microsatellites, the observed (Ho) and expected heterozygosity (He) varied between 0.5-0.6 for all loci and there is a high level of private alleles among localities (38%). There is evidence of inbreeding over all loci (F_{is}= 0.15) and there is no apparent departure from Hardy-Weinberg equilibrium; however, two individual loci are potentially out of HWE ($p < 0.05$). The linkage disequilibrium test was rejected between pairs of loci ($p < 0.1$).

Population Genetic Structure

The AMOVA provided evidence of strong regional structure for mtDNA variation (Φ_{st} = 0.38 $P < 0.05$). Further, the genetic structure coefficient (Φ_{st}) regressed on geographical distances among regions is marginally consistent with a pattern of isolation by distance across latitude (r = 0.445; Z = 2430.854; P = 0.049). I tested if multiple areas of suitable habitat (ancestral refugia) explain the structure of the mtDNA genealogy. The analysis using Mesquite v2.0 rejects a single-refugia model to explain the mtDNA genealogy (Slatkin's *S*-statistics = 8; $P < 0.001$) and favors a multiple-refugia hypothesis. This implies that node-split in the mtDNA genealogy correlates with multiple areas of suitable habitat rather than single ancestral refugia.

The AMOVA showed evidence for weak spatial structure for microsatellite variation (F_{st}=0.051; $P < 0.05$). The statistical power analysis supports such low level of regional

differentiation (F_{st} = 0.051; p=0.94; α = 0.043; No = 500; generations = 52 and 2000 permutations). However, the genetic structure coefficient (F_{st}) regressed on geographical distances among regions is not consistent with a pattern of isolation by distance across the latitudinal linear range of *I. porphyrocephalus* (r=-0.1374; Z= 1336.6393; P = 0.447).

Assignment Tests and Geographic Discontinuities

Individual assigment in Geneland suggests that the number of clusters is 2-3 (K = 2 to 3 population subdivisions). I decided to fix the number of populations to the minimum modal number; the selection of the ten selected runs are fairly consistency for mtDNA sequence variation (K=2, posterior probability=0.59), microsatellites loci (K=2, pp=0.8) or both markers combined in a total evidence data set (K=2, pp=0.8) (Fig. A-4). The assignment of individuals was different for each marker type by itself, the test using both microsatellites and mtDNA variation showed population breaks nearby geographic discontinuities within species range. However, the combined data set supports that most individuals of *I. porphyrocephalus* are assigned similarly to the microsatellite predictive subdivision (Fig. 2-2). The southern population was separated from the northern population by a boundary zone situated nearby the Dagua River Valley (Fig. 2-2). The geographic location of lowest probability for individual assignment is spatially congruent with the Dagua River Valley, as a salient geographic feature acting as a putative barrier to dispersal.

Environmental Variables Predict Genetic Variation

Latitude, elevation and environmental variables have great explanatory power regarding spatial structure of mtDNA and microsatellites (Table 1-2). GDM analysis (Table A-2) shows that only three variables: latitude, mean diurnal temperature, and vegetation density explains

71.6% of mtDNA variation. GDM analysis on microsatellites spatial structure also supports a correlation with the climate realized niche model for *I. porphyrocephalus.* Four variables have the highest predictive power: mean diurnal range temperature, annual mean temperature, mean elevation, and information of forest structure (Table 1-2). The environmental variable selection predicts 74.1% of microsatellite spatial structure (Table 1-2). Topography (i.e. latitudinal distance and elevation) is an important predictor of mtDNA and microsatellite spatial variation respectively. Removing geographic distances among sites from the analysis using mtDNA variation decreases explanatory power to 53.3% and invokes more climatic predictors into the GDM model (Table 1-2). By removing latitude from the analysis there is an increase in model complexity and substantial loss in explanatory power. However, by removing latitude from the GDM does not affect model prediction on microsatellite spatial variation, thus geographic distance is not as important as elevation and environmental factors in explaining microsatellite variation.

HISTORICAL DEMOGRAPHY

The Isolation-with-Migration analyses highlight expected discordance in demographic histories for microsatellites loci and mtDNA sequence variation. IMa2 for microsatellites shows evidence of demographic changes across the Dagua River Valley. The population size parameters (θ) to the north ($\theta = 0.22$; 0.07-0.77 High Posterior Density Interval) and to the south ($\theta = 0.22$; 0.07-1.17 HPDI) of the valley are fairly equivalent, but smaller as compared to their most recent common ancestor ($\theta > 49.98$). Nevertheless, the tail of the ancestral (θ) distribution did not approach zero in any replicate (Fig. 3-2). The most probable estimate for the migration rate (26.1; 10.8-57.1 HPDI) was high and did not overlap zero (Fig. 3-2). Thus, I rejected the

hypothesis of no gene flow between population segments of *I. porphyrocephalus* across the Dagua geographical discontinuity. Nevertheless, Time since divergence (*t*) between population across the Dagua River Valley peak at 0.027 (0.009-0.069 HPDI), *t* scaled to a mutation rate = 10^{-5} suggests a divergence of approximately 27,000 years before the present (9,000-69,000) that predate the LGM 18,000-22,000 ybp.

Coalescent analyses using IMa2 for mtDNA sequences show a slightly different demographic history. All parameter estimates are conditional to estimates of time since divergence (*t*), which distribution did not converge to zero at any replicate, thus the upper 95% HPDI is not reported for *t*. The population size parameters (θ) across the Dagua River Valley broadly overlap their most recent common ancestor (θ > 13.05); nevertheless, the downward tail of the ancestral θ distribution did not approach zero in any replicate. The population south the Dagua River Valley (θ=12.25; 2.95-80.65 HPDI) was slightly larger as compared to the one north of the valley (θ=2.75; 0.65-10.05 HPDI) (Fig. 4-2). The most probable estimate for the migration rate (*m*) between regions was low and its confidence interval broadly overlapped zero in both directions (0.45; 0.0-3.95 HPDI) (Fig. 4-2). Thus, I cannot reject the hypothesis of no gene flow between population segments across the Dagua River Valley.

DISCUSSION

Species Distribution Modeling

A spatial projection using georeferenced data points, fine-scale environmental GIS layers and the MAXENT algorithm predicts *I. porphyrocephalus* distribution in the northern Andes. Figure 1 shows the present-day potential geographic distribution of *I. porphyrocephalus*, this

projection provides evidence that the extension of the potential distribution is larger than the species-range based only on presence data (Ridgely & Greefield 2001; Ridgley & Tudor 1989) (Fig. 1-2a). The present-day projection is robust to MAXENT estimates using subsets of georeferenced data, 50% of point localities can predict an opposite area within the species range, and substantial environmental heterogeneity across latitude supports the absence of climatic gradients within the scale of the species-range. This projection also provides more accurate information on suitable areas and geographical discontinuities and does not support suitability for *I. porphyrocephalus* nearby Loja (~3° S) southeastern Ecuador (Fig. 1-2a-b). Overall *I. porphyrocephalus* is more geographically restricted to the western Cordillera of Colombia and northern areas in Ecuador than previously hypothesized on the basis of an uncertain record propagated in the literature (Ridgely & Greefield 2001; Ridgley & Tudor 1989).

Historical Changes in Species Range

Present-day and LGM spatial projections show substantial geographic expansion of the realized climatic niche for *I. porphyrocephalus*. This geographic expansion posits the possibility of concurrent population expansion following newly open habitat since the LGM. These two spatial projections also support changes in the geographic extension of two major discontinuities within the range of *I. porphyrocephalus*. The Dagua River Valley and the Patía River Valley are low elevational passes with drier conditions than adjacent areas, two well-known geographic discontinuities disrupting over 13 avian species-ranges in the Northern Andes (Graham *et al.* 2010). Present-day and LGM spatial projections suggests that the distribution of *I. porphyrocephalus* was likely disrupted by contraction and expansion of suitable area across

geographic discontinuities. This also suggests that such barriers and climate change have shaped population genetic structure.

Spatial Genetic Structure and Environmental Heterogeneity

There is clear evidence of population structure from analyses that do not incorporate any climatic information. First, the AMOVA provides evidence of significant spatial genetic structure for either molecular marker type. Second, the Mesquite coalescent framework shows that the mtDNA genealogy of *I. porphyrocephalus* is better explained by multiple suitable areas rather than by a single ancestral isolate. Third, the individual assignment test in GENELAND provides an accurate delimitation of population genetic subdivision. Individuals are clearly assigned into populations across the Dagua River Valley; however, GENELAND does support subdivision at the Patía River Valley. This result shows the extent to which geographic barriers correspond to population genetic structure and demonstrates the idiosyncratic nature of putative geographical discontinuities within species range. Populations isolated across the Dagua River Valley support a dominant process of IBD due to genetic drift on neutral markers (Bohonak & Roderick 2001). Below, analysis that do incorporate environmental information also provides valuable insights on the pattern of population genetic structure

Environmental and topographic factors predict suitable areas but these factors also predict mtDNA sequence and microsatellite loci spatial variation. These predictors fall within three distinct groups: 1) latitude and elevation as topographic determinants, and 2) climatic variables of temporal thermal amplitude and 3) those associated vegetation density and forest structure as information of habitat cover. Topographic, climatic and variables of habitat cover can predict a large percentage of mtDNA structure and microsatellite variation. Because these

two DNA markers are presumably neutral, their correlation with environmental determinants cannot be explained by strict differentiation in geographic isolation and genetic drift alone. Neutral markers are not expected to concentrate genetic variation that resulted from environmental processes that may affect individual survival and reproduction. The predictive power of these environmental variables on two neutral genetic markers types provides independent evidence that accumulation of mutations co-vary with environmental and topographic heterogeneity. Here, the pattern of genetic differentiation can be associated with climate change, as has been shown in an increasingly number of studies (Carstens & Knowles 2007; Davis & Shaw 2001; Hoffmann & Sgro 2011; Zhao *et al.* 2012).

Demographic History

The accumulation of mutations co-varying with environmental and topographic determinants is the result of a long-term process shaping neutral genetic variation. The different time scales implied by the two molecular marker types in this study suggest a long-term process of association between environmental and genetic variation. This is because the mutation rate is inversely proportional to θ (Avise 1998), the mitochondrion is haploid and maternal-inherited, and therefore has a four-fold smaller effective population size (θ) than any single nuclear marker (Zink & Barrowclough 2008). Inheritance properties of mtDNA make it more likely to reflect recent divergence than any single nuclear marker, while scans of microsatellites loci provide accurate information of more contemporary processes of differentiation than any single nuclear marker with a simpler mutation mechanism (Brito & Edwards 2008). Microsatellite divergence time roughly correspond to the timeframe for the last glacial period. This suggests that accumulation of mutations in this set of nuclear markers can be associated with contemporary

climate change. IMa2 divergence time estimate from mtDNA sequences alone was uninformative and its posterior probability distribution did not converge, which is not surprising given the limited information at any single DNA sequence marker.

In the case of *I. porphyrocephalus,* neither mtDNA nor microsatellites support demographic expansion in populations separated by the Dagua River Valley. This also implies that effective population size is at equilibrium to the expansion of suitable area since the LGM. Stable population sizes over time indicate the lack of demonstrable demographic response to climate change and forest expansion since the last glacial period (Hooghiemstra & van der Hammen 2004). This pattern is different from the observed in temperate and boreal fauna, for which both geographic and demographic expansion has been shown to follow warming climate change since the LGM (Davis & Shaw 2001; Mila *et al.* 2006).

Evidence of stable population sizes highlights the significance of the balance between isolation and gene flow shaping genetic structure across geographical barriers. Extensive gene flow across the Dagua River Valley is inferred from microsatellites loci variation, while restricted gene flow is inferred based on mtDNA variation. This provides evidence of a biased movement of DNA markers between populations across the Dagua River Valley. The gap isolating populations across the Dagua River Valley has changed in extension since the LGM and this suggests that climate change can also affect migration of parts of the genome.

However, demographic asymmetries, such as sex-biased dispersal can also explain the biased gene flow (Rheind & Edwards 2011). The high ratio between F-statistics ($\Phi^{mt}st/F^{nuc}st$ = 7.3) supports the possibility of female phylopatry or male-biased dispersal (Prugnolle & Meeus 2002). Male biased dispersal has been inferred in a few species (Maki-Petays *et al.* 2007), whereas, female-biased dispersal is considered to be the generalized pattern among birds (Clarke

et al. 1997). Although little is know on the dispersal abilities of both sexes in *I. porphyrocephalus*, sex-biased dispersal may have contributed to promoting associations between environmental conditions and neutral genetic variation.

CONCLUSIONS

Topographic, climatic and habitat-cover variables are important predictors of potential distribution of *I. porphyrocephalus* and population genetic structure. These three groups of variables are also known to predict spatial changes in bird species abundances across the Neotropics (Filloy & Bellocq 2013). Present-day and LGM projections of species potential distribution are a valuable tool for exploratory analyses of geographic discontinuities within species-range. These projections support changes in the geographic extension of two major discontinuities within the range of *I. porphyrocephalus*. Particularly, an area nearby the Dagua River Valley that have been shown to disrupt ranges in multiple bird species (Graham *et al.* 2010) is a landmark for asymmetrical gene flow and population genetic subdivision of *I. porphyrocephalus*. Divergence time estimates of microsatellite variation across such geographical barrier are roughly consistent with the last glacial period. The LGM and present-day projections show geographic expansion of the potential distribution of *I. porphyrocephalus*. However, non-expanding effective population sizes indicate the lack of demonstrable demographic response to climate change and forest expansion since the last glacial period (Hooghiemstra & van der Hammen 2004). The correlation between topographic, climatic and variables of habitat cover, with mtDNA sequence and microsatellite loci variation provides evidence of a process in which accumulation of mutations co-vary with changing environmental conditions. Because these two DNA markers are nearly neutral with respect survival and

reproduction, their correlation with environmental determinants is not predicted by neutral theory and cannot be explained by strict geographic isolation and genetic drift alone. This study reveals the potential for divergence in isolation driven by climatic fluctuations along latitude.

Present-day and LGM projections are also valuable tools to explore factors limiting species' future potential distribution. By the year 2050, the projection to warming conditions predicts a shift in the elevational range of *I. porphyrocephalus* as suggested for other taxa (Buermann *et al.* 2011) and 24.5% loss of present-day potential distribution. The projection to warming conditions in the near future implies smaller ranges at higher elevations and the end of geographic expansion of its potential distribution since the LGM. In this study, projections to the past and present-day conditions provide independent evidence of elevation-range limits imposed on *I. porphyrocephalus*. Elevation-range limits are important determinants of avian extinction risk under scenarios of current climate change (Buermann *et al.* 2011; Sekercioglu *et al.* 2008). Since environmental heterogeneity encompasses an important set topographic, climatic and habitat-cover predictors for species suitable habitat and neutral genetic variation it is expected a dependency of the processes shaping genetic structure on the accelerating influence of climate change (Bradley *et al.* 2006). Such dependency can potentially affect nearly 450 bird species restricted within the narrow elevational (1500-2500 m) (Kattan & Franco 2004) and patchy latitudinal range of *I. porphyrocephalus.*

FIGURES AND TABLES

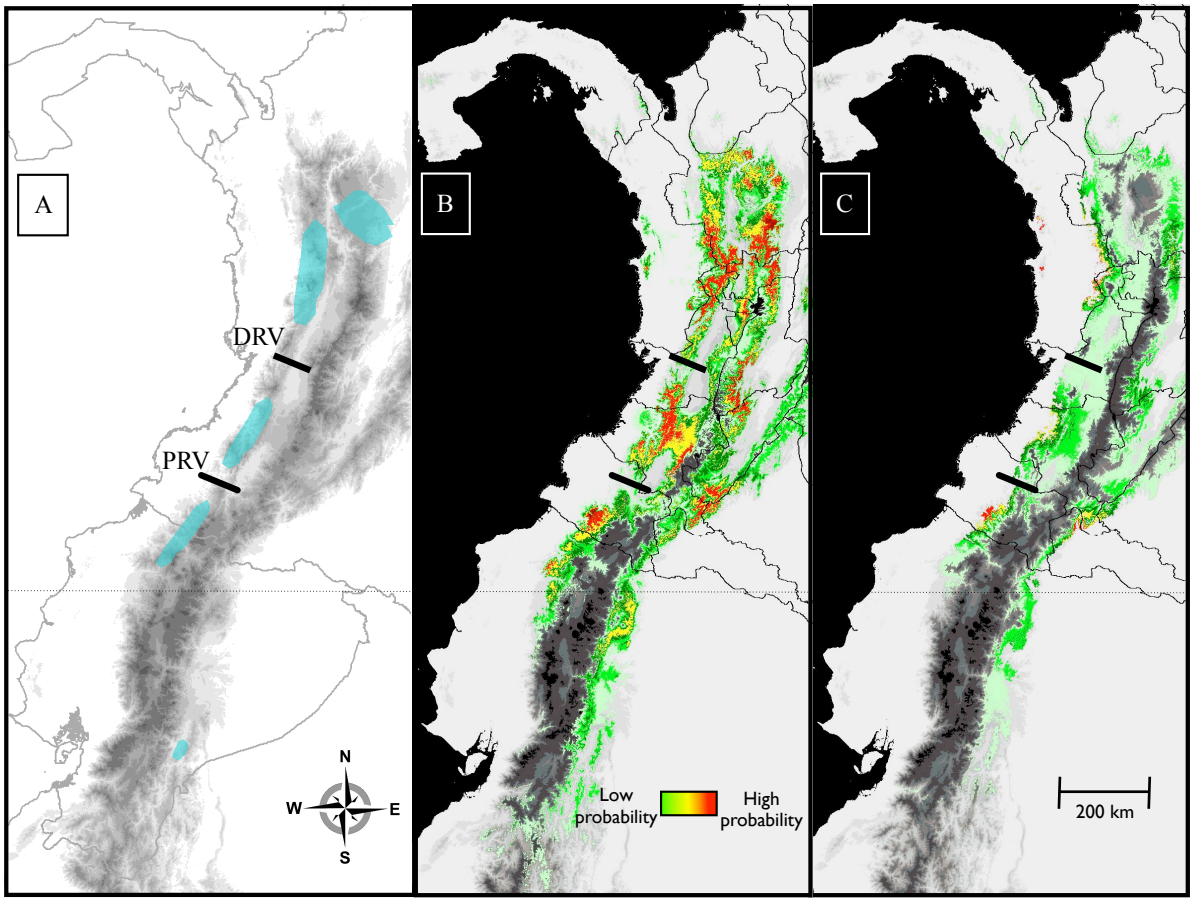

Figure 1-2. Spatial distribution models of *I. porphyrocephalus*. Panes from left to right are (A) Presence map (Ridgely et al. 2005), (B) Present-day species distribution model using MAXENT, and (C) MAXENT projection to the LGM. Geographic discontinuities within species range (dash) includes the Dagua River Valley (DRV) and the Patía River Valley (PRV) both north of equator line (dotted)

Table 1-2. Predicted response of species distribution, sequence mtDNA, and microsatellite variation to topographic and environmental variables

	MAXENT[1]	GDM[2]	
Predictors[9]	Species Distribution	mtDNA[3]	Micros[4]
Latitude	idpv	>90%	NS
Mean elevation	idpv	NS[5]	20%
Vegetation density[6]		25%	NS
Forest structure[7]		>90%	
Precipitation of Coldest Quarter (B19)	15%	NS	NS
Temperature Seasonality[8] (B04)	9%	NS	NS
Maximum temperature of the warmest month (B05)	2.2%	NS	NS
Mean diurnal temperature/range (B02)	0.2%	80%	45%
Annual mean temperature (B01)	68%	NS	10%
Additive model	91.2%	72%	74.1%
Excluding latitude	NA	53%	74.1%

[1]Analysis of variable contribution to the MAXENT model. Correlation among variables < 0.7. idpv (independent variable in MAXENT model)

[2]Predictive response of the Generalized Dissimilarity Model.

[3]Sequence mtDNA variation is a dependent variable in GDM (see Figure A-2)

[4]Variation of microsatellite loci is a dependent variable in GDM (see Figure A-2)

[5]Non-selected variables (NS) among significant predictors of DNA variation

[6]Annual maximum leaf area index for spatial distribution of vegetation density

[7]Qscat-mean provide information on spatial distribution of surface moisture and roughness of forest structure (Annual mean Radar Backscatter of year 2001)

[8]Temperature Seasonality use in the MAXENT model as the standard deviation *100

[9]Correlation among climatic variables < 0.37; however, correlation between annual mean temperature correlate and either temperature seasonality or maximum temperature of the warmest month > 0.98.

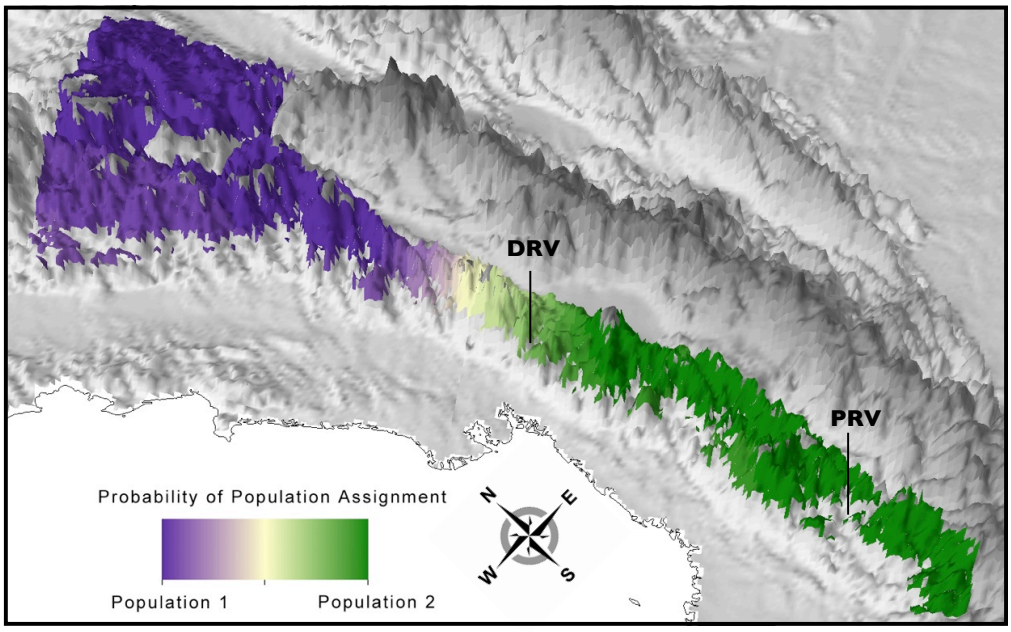

Figure 3-2. Population genetic subdivision of *I. porphyrocephalus*. Individual assignment in Geneland used a combine dataset of mtDNA sequence variation and microsatellite loci, and georeferenced information for each sample. Posterior probability is interpolated on the realized climatic niche where the species is known to occur. Low probability of assignment is spatially congruent with the Dagua River Valley (DRV) but not with the Patía River Valley (PRV), both well known geographical discontinuities within species-range.

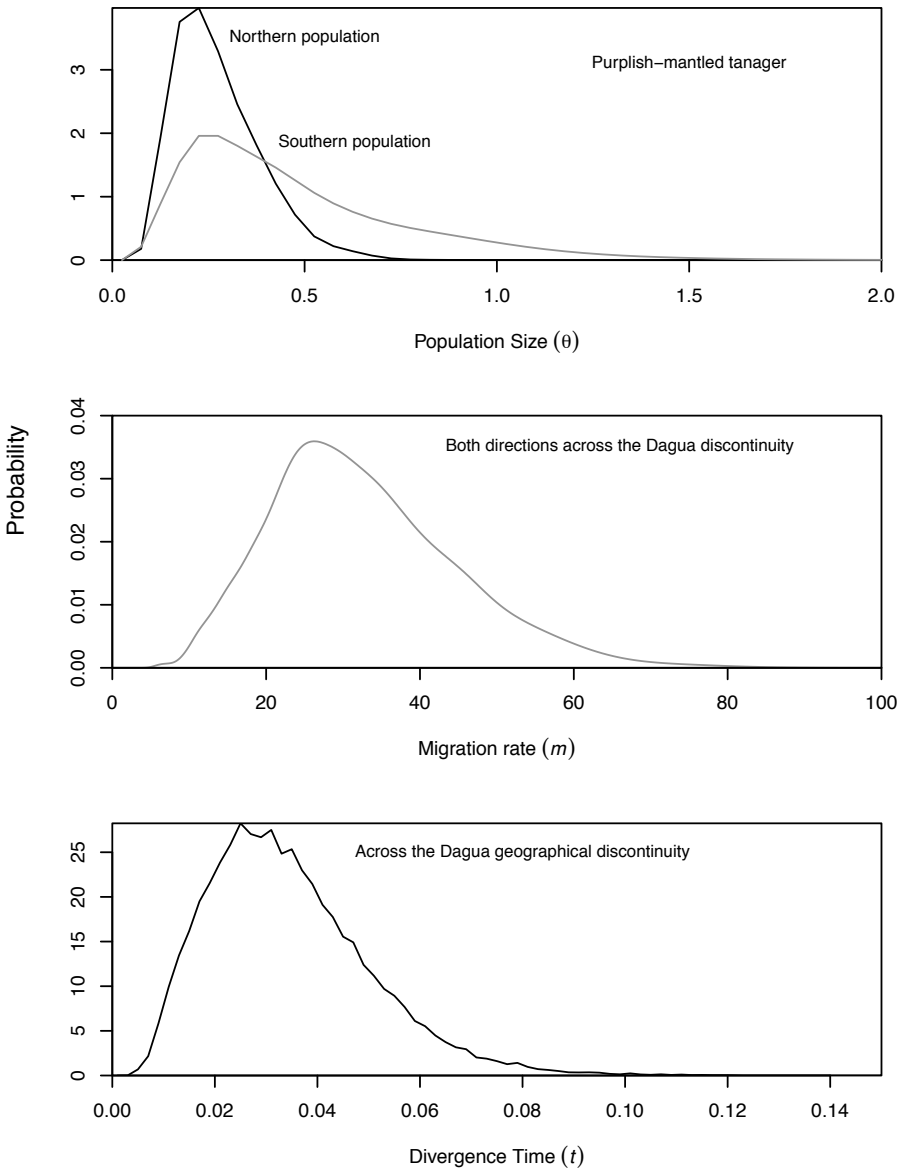

Figure 4-2. Coalescent analysis for six microsatellite loci. Posterior distribution of effective population size, θ, immigrants, *m*, and time since divergence, *t* calculated with IMa2.

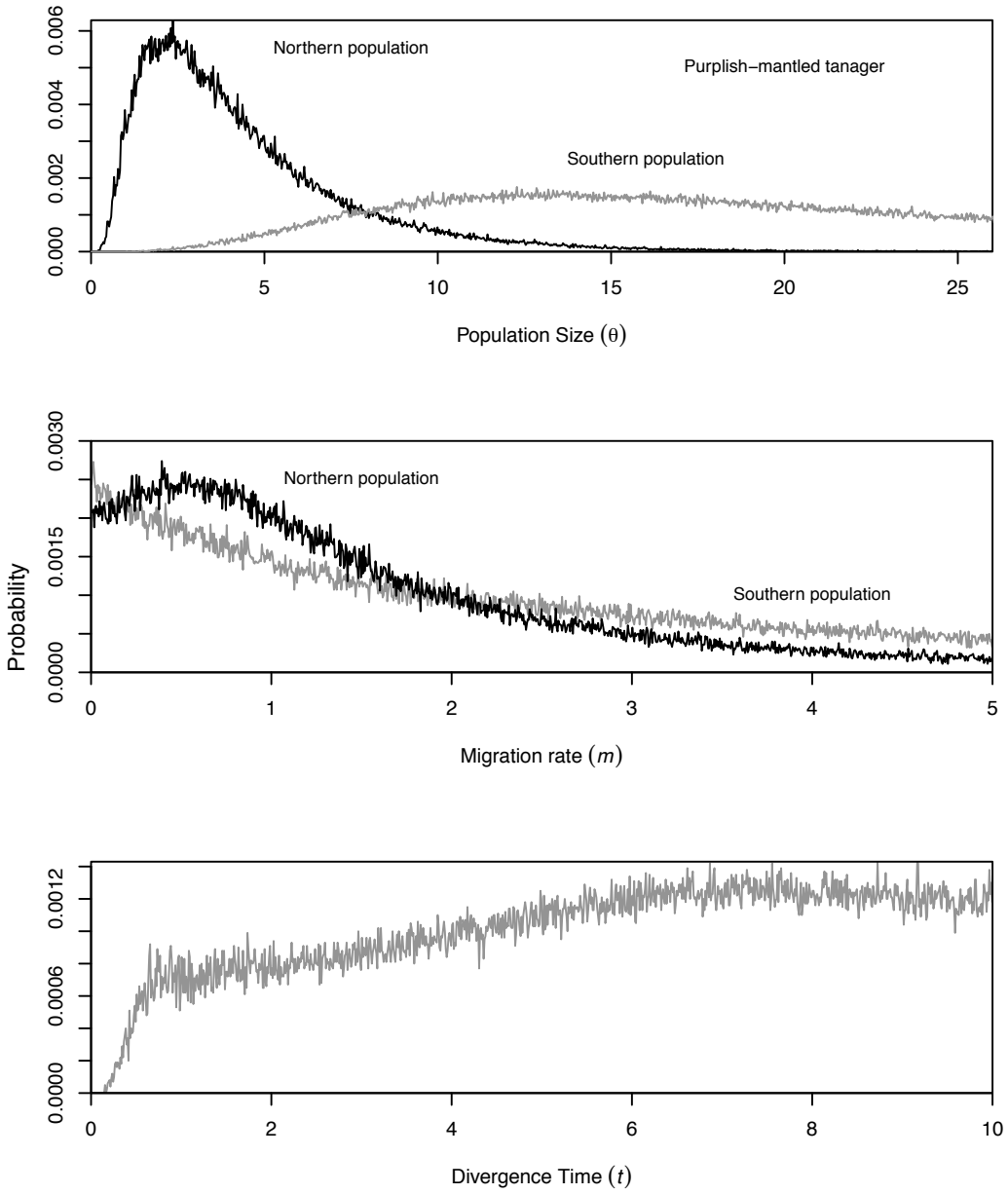

Figure 5-2. Coalescent analysis for mtDNA ND2 region. Posterior distribution of effective population size, θ, immigrats, *m*, and time since divergence, *t* calculated with IMa2.

APPENDIX

Table A-1. List of sampling material, voucher number, site, latitude and longitude

Voucher number	Site	Latitude and Longitude
ICN-34502	Antioquia, Amalfi	06 58 00 N 75 03 00 W
IAvH-CT 2163	Antioquia, Amalfi	06 58 00 N 75 03 00 W
IAvH-CT 4922	Antioquia, Amalfi	06 58 00 N 75 03 00 W
IAvH-CT 7206	Antioquia, Mesenia	05 29 00 N 75 54 00 W
IAvH-CT 7201	Antioquia, Yarumal, Alto Ventanas	07 04 00 N 75 26 00 W
IAvH-CT 7202	Antioquia, Yarumal, Alto Ventanas	07 04 00 N 75 26 00 W
IAvH-CT 7203	Antioquia, Yarumal, Alto Ventanas	07 04 00 N 75 26 00 W
IAvH-CT 3980	Risaralda, Pueblo Rico, La selva	05 09 29 N 76 01 00 W
IAvH-CT 3991	Risaralda, Pueblo Rico, La selva	05 09 29 N 76 01 00 W
ICN-31132	Risaralda, Mistrato, El empalado	05 17 58 N 75 53 15 W
ICN-31156	Risaralda, Mistrato, El empalado	05 17 58 N 75 53 15 W
UV-6382	Valle del Cauca, El Cairo, El Ingles	04 44 00 N 76 18 00 W
UV-6448	Valle del Cauca, El Cairo, El Ingles	04 44 00 N 76 18 00 W
IAvH-CT 7204	Valle del Cauca, El Cairo, El Ingles	04 44 00 N 76 18 00 W
IAvH-CT 7205	Valle del Cauca, El Cairo, El Ingles	04 44 00 N 76 18 00 W
UV 6457	Valle del Cauca, El Cairo, El Ingles	04 44 00 N 76 18 00 W
UV 6459	Valle del Cauca, El Cairo, El Ingles	04 44 00 N 76 18 00 W
UV 6452	Valle del Cauca, Paso Galápagos	04 48 00 N 76 10 00 W
FNA1	Valle del Cauca, Paso Galápagos	04 48 00 N 76 10 00 W
IAvH-CT 7200	Valle del Cauca, Paso Galápagos	04 48 00 N 76 10 00 W
IAvH-CT 2490	Valle del Cauca, La Cumbre	03 34 09 N 76 35 19 W
MHNUC-AV 04316	Cauca, El Tambo	02 27 15 N 76 49 04 W
MHNUC-AV 03333	Cauca, El Tambo	02 27 15 N 76 49 04 W
MHNUC-AV 04216	Cauca, El Tambo	02 27 15 N 76 49 04 W
FNA2	Cauca, El Tambo	02 27 15 N 76 49 04 W
FNA3	Cauca, El Tambo	02 27 15 N 76 49 04 W
FNA4	Cauca, El Tambo	02 27 15 N 76 49 04 W
ICN-32270	Nariño, Barbacoas	01 15 00 N 78 07 00 W
ICN-32269	Nariño, Barbacoas	01 15 00 N 78 07 00 W
IAvH- 6914	Nariño, Planada	01 34 60 N 77 31 00 W

ICN: Instituto de Ciencias Naturales, Univerisdad Nacional de Colombia. UV: Universidad del Valle, Cali, Colombia. MHNUC: Museo de Historia Natural, Universidad del Cauca, Colombia. IAvH: Instituto Alexander von Humboldt, Ciat, Palmira, Valle, Colombia.

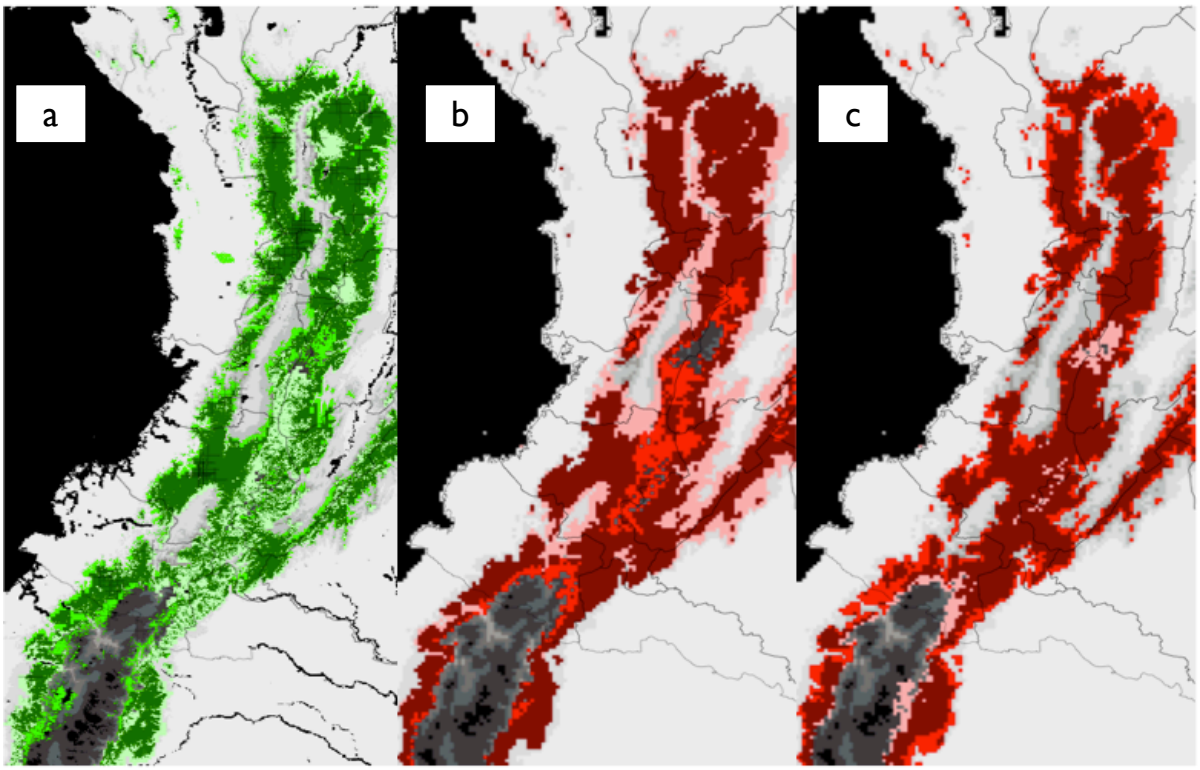

Figure A-1. Projections of species distribution using Maxent. (a) Present-day potential distribution shows overlap between 1 km^2 and 5 km^2 climatic data sets using high suitable probabilities (dark green) and poor suitable area (light green). (b) Projections to the LGM using cool and dry surfaces (see text), and overlap with the present distribution projection (dark red), habitat lost (scarlet red), and gains of suitable habitat (pink). (c) Projection of species distribution by the year 2050 using warmer surfaces for future temperature conditions. The overlap with present-day distribution (dark red), the loss of present-day suitable habitat (scarlet red), and the gain in suitable habitat on warmer conditions (pink) are illustrated on the right panelA.

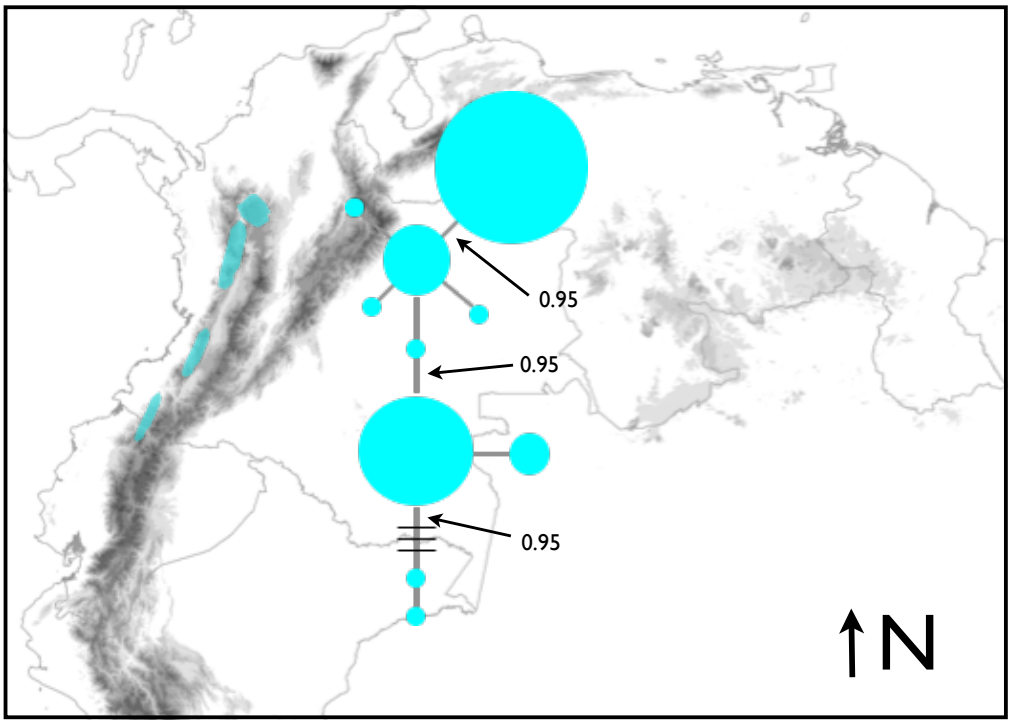

Figure A-2. Unrooted allelic network for mtDNA. The network of ND2 haplotypes is aligned along latitude with the geographic distribution of *I. porphyrocephalus*. Sizes of circles are proportional to the frequency of each haplotype observed. Dashes crossing the network indicate putative ancestral alleles not sampled. Node support with high posterior probability calculated with Mr. Bayes and imposed on this unrooted genealogy.

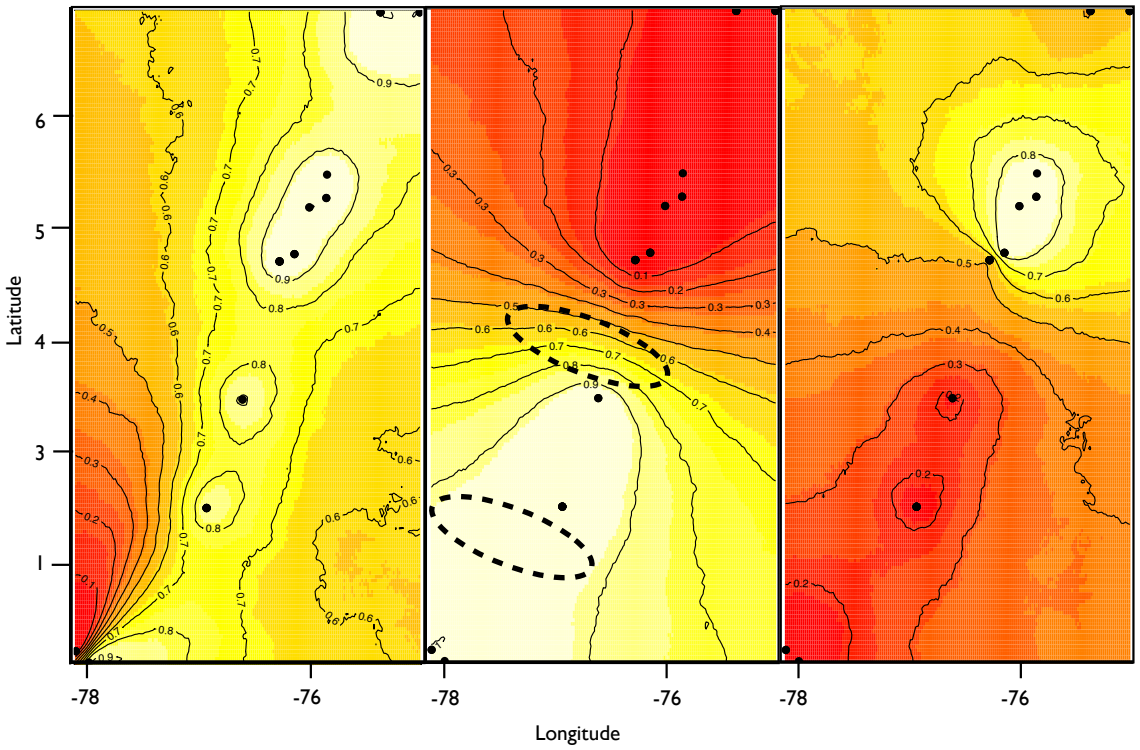

Figure A-3. Individual assignment test using Geneland, heat map shows the transition in posterior probability density (contour lines) of population membership *K*=2. Test conducted using mtDNA sequence variation of the ND2 region (left panel), microsatellite loci variation (right panel), and a data set combining mtDNA and microsatellites (central panel). Locations nearby the Dagua River Valley (dashed ellipse top) and the Patia River Valley (dashed ellipse bottom)

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CHAPTER 3

Evolutionary Differentiation Across the Andes and Along an Elevational Gradient Between the Olive-striped and the Streak-necked Flycatcher (the Montane *Mionectes*)

ABSTRACT

Lineage divergence is the process in which populations accumulate local adaptation in trajectories of evolutionary independence. Disjunctive geographic distributions and morphological differentiation between closely related species are often taken as indications of lineage divergence. To understand divergence between and within close related forms, I examined the evolutionary history of two montane passerine species, the Olive-striped (*Mionectes olivaceus*) and the Streak-necked Flycatcher (*Mionectes striaticollis*). These two species are phenotypically distinct and occupy different elevational zones. Here, I applied isolation-with-migration (IM) coalescent method to estimate divergence times, population sizes, and gene flow between these species but also between populations within the same species. I found that a restricted pattern of gene flow, equivalent population sizes and recent divergence times characterized the demographic history of these two species of montane *Mionectes*. Between populations within the same species, IM strongly supports changes in effective population sizes and restricted gene flow across the Andes. This provides evidence that the Andes acts as an effective barrier between populations within each species. This topographic discontinuity within each species range also suggests that divergence in isolation contributes to shaping spatial genetic structure. The amount of differentiation is concentrated at lower elevations, possibly because the larger area through landmass effect restricts gene flow more effectively. Congruence in divergence times between species and between populations within the same species implies rapid differentiation in neutral genetic variation. The geographical discontinuity between populations across the Andes within the same species had structured demographic histories and resulted in substantial differentiation in genetic, plumage and vocalization patterns between montane *Mionectes.*

INTRODUCTION

Montane regions in the Tropical Andes harbor striking patterns of biodiversity across highland and lowland areas. Narrow elevational zones often constitute the entire geographic range of many avian species (Graves 1988; Kattan & Franco 2004). In many instances, no obvious barriers between elevational zones separate closely-related populations, and yet, there are usually intriguing patterns of phenotypic variation (Cadena & Loiselle 2007; Caro *et al.* 2013; Cheviron & Brumfield 2009b; McCormack & Smith 2008; Mila. *et al.* 2009). These studies provide evidence of local adaptation along elevational gradients. However, the questions of how biological diversity accumulates between and within elevational zones or how rapid divergence takes place in mountain ranges are actively debated among ecologists, biogeographers, and evolutionary biologists (Altshuler *et al.* 2004; Blackburn & Ruggiero 2001; Cadena *et al.* 2011; Caro *et al.* 2013; Cheviron & Brumfield 2009b; Dingle *et al.* 2006; Guarnizo *et al.* 2009; Gutierrez-Pinto *et al.* 2012; Kattan & Franco 2004; McCormack & Berg 2010; McCormack & Smith 2008). Further research addressing accumulation of biodiversity along elevational zones is in urgent need for making more informed conservation decisions on threaten species. Elevation range-shifts of bird species are predicted given the accelerating influence of climate change (Buermann *et al.* 2011), such range-shifts can potentially increase the risk of extinction of 2700 land bird species by the late 21st Century conditions (Sekercioglu *et al.* 2008).

Phenotypically distinguishable populations constitute evidence that some divergence had taken place between populations; but the presence of a geographic discontinuity between populations also provides indirect evidence that divergence may have occurred (Gutierrez-Pinto *et al.* 2012) . The possibility that over time populations also change their geographic distribution along elevation presents a major challenge to understanding the spatial origin of lineage

divergence in mountain ranges (Caro *et al.* 2013). Whether or not population divergence occurs in isolation has important implications to understand if geographic overlap between populations is the result of secondary contact (Guarnizo *et al.* 2009; Niemiller *et al.* 2008; Smith *et al.* 2005), implying formerly allopatric conditions of divergence.

The demographic histories and spatial patterns of biological diversity are valuable for understanding the process of divergence between populations (Schluter 2000; Schluter 2009). Coalescent methods are instrumental to address how genetic diversity accumulates between or within elevational zones, concerning time since divergence, demographic changes, and gene flow (Beerli & Felsenstein 1999; Hey 2006; Hey & Nielsen 2004; Knowles & Maddison 2002). The goal of this study is to determine the relative roles of gene flow, changes in population size, and time since divergence on the differentiation of two species flycatchers in the Tropical Andes, and between populations within each of those species.

The Olive-striped (*Mionectes olivaceus*) and Streak-necked Flycatcher (*Mionectes striaticollis*) shared common ancestry, strikingly distinctive phenotypes and their particular tendency to replace each other along elevation make this sister-species group well suited for study divergence and gene flow. These two montane *Mionectes* differ in plumage, vocalization, and spatial distribution (Ridgley & Tudor 1989). Both species, the yellow and the green *Mionectes,* are more similar to one another than either is to other members of the genus *Mionectes* (Remsen *et al.* 2013). These two montane *Mionectes* occupy different elevation ranges. While *M. striaticollis* is restricted to a higher elevational zone (1300-2500 m), *M. olivaceus* is more common at lower elevations (400-1600) (Hilty & Brown 1986; Restall *et al.* 2006; Ridgely & Greefield 2001). Vocalizations between the two montane *Mionectes* are thin, twittering calls; however, little is known of the acoustic aspects that make these species two

distinguishable groups. Plumage is also an important diagnostic trait between populations within the two species (Remsen *et al.* 2013), and four distinguishable populations converge across the Andes in Ecuador and southwestern Colombia. *Mionectes striaticollis* subs. *columbianus* to the east of the Andes (*cis*-Andean) and *Mionectes striaticollis* subs. *viridiceps* to the west side of the Andes (*trans*-Andean) These populations are replaced at lower elevations by *Mionectes olivaceus* subs. *fasciaticollis* (*cis*-Andean) and *Mionectes olivaceus* subs. *hederaceus* (*trans*-Andean) (Hilty & Brown 1986; Restall *et al.* 2006; Ridgely & Greefield 2001). Despite the striking differences in plumage coloration between populations, and modest sexual dimorphism in size, males are difficult to distinguish from females by coloration in both species. The reproductive biology of these sister-species pair is largely unknown and there are no records of interbreeding events, perhaps because of the limited elevational zone where they overlap. Genetic-based studies on montane *Mionectes* can provide valuable information to understanding spatial population structure and the history of gene exchange among populations in relation with traits that are directly involved in survival and mate choice.

Here, I investigate the evolutionary history of the Olive-striped and Streak-necked Flycatcher that apparently diverged in the absence of a barrier along the elevational gradient. Divergence between species is presumably driven by environmental heterogeneity along elevation, as the replacement of species along elevational zones may reflect the primary *in situ* divergence. I expect little concordance in patterns of genetic differentiation with and without a putative geographical barrier to gene flow. To explore the process of divergence, I aim to examine whether or not the Andes acts as a barrier to gene flow and thus divergence in isolation as potential cause for speciation. If a geographical barrier does not have a significant effect on population subdivision across the Andes, and populations on either side within the same species

are genetically indistinguishable, then it is unlikely that divergence between the two montane *Mionectes* species resulted from geographical isolation. Low differentiation between populations within the same species would support a process govern by differences along elevation without regard to a geographical barrier between the two *Mionectes* (Fig 1-3a). However, if substantial genetic differentiation between populations within the same species is observed across the Andes (Fig 1-3b), geographic isolation would be an important determinant in population differentiation. But equally important, a larger amount of differentiation are expected to be concentrated at low and middle-elevations (Fig 1-3c), where the larger area through landmass effect should contribute to restrict gene flow more effectively than at higher elevations.

METHODS

Mitochondrial DNA and Nuclear Intron Sequencing and Analysis

I collected 29 *Mionectes olivaceus* and 24 *Mionectes striaticollis* from 15 localities in Ecuador and genotyped them (Fig 5-3). Total genomic DNA was extracted from blood with a Qiagen DNeasy tissue kit (Qiagen, Valencia, CA). The 1043 bp of the mtDNA NADH dehydrogenase subunit 2 (ND2) was amplified following conditions as described in previous studies for close related taxa (Miller *et al.* 2011). PCR products were Sanger sequenced for both strands with BigDye Terminator Cycle sequencing kits on an ABI3730XL sequencer (Applied Biosystems, Foster City, CA). Sequences were aligned in SEQUENCHER 4.7 (Gene Codes Corporation. Ann Arbor, MI) and variables sites reconciled visually for accuracy. Additional ND2 sequences were obtained from GENBANK for both *M. olivaceus* (EF110694-98 from Panama) and *M. striaticollis* (EF110693 from Bolivia).

I also sequenced two independent nuclear gene interspaced regions (introns), the ornithine decarboxylase (ODC1) intron six (327 bp) and the β-fibrinogen (Fib7) intron seven (935 bp) (Peters et al. 2008, McCracken et al. 2009a, Prychitco and Moore 1997). The PCR products were obtained using the Qiagen Multiplex PCR kit following previously described conditions (Batalha-Filho *et al.* 2012; Prychitko & Moore 1997) and the PCR products were subsequently sequenced by the Sanger method. Sequences that contained double peaks, indicating the presence of two alleles, were coded with IUPAC degeneracy codes and treated as polymorphisms. Insertion and/or deletions (Indels) were resolved by comparing the unambiguous 5′ ends of sequences of the 3′ ambiguous ends of forward and reverse strands (Peters *et al.* 2007). Gaps resulting in shifted peaks in the chromatogram enabled us to resolve length polymorphisms within the sequences. All the nuclear DNA sequences were aligned visually with SEQUENCHER 4.7 (Gene Codes Corporation, Ann Arbor, MI). I determined the gametic phase of each intron sequence that was heterozygous at two or more nucleotide positions. First, the diploid consensus sequences of each individual were analyzed with PHASE 2.1 (Stephens *et al.* 2001) with the default values and 0.7 as the minimum probability for a given sample to be included in downstream analyses. Multiple runs from different starting points ensured convergence of the Bayesian method for selecting the output with the best overall goodness of fit. For downstream analysis that requires sequence data with no evidence of recombination, I subtracted from the PHASE output the largest non-recombining block of each nuclear haplotype using IMgc (Woerner *et al.* 2007).

Microsatellite Diversity

To estimate the amount of population differentiation within species, 83 *Mionectes olivaceus* and 53 *Mionectes striaticollis*, samples from 15 localities in Ecuador were genotyped for microsatellites (Fig 5-3). I used eleven tetranucleotide microsatellite loci (M9, M13, M33, M104, M119, M127, M139, M153, M209, M199, M235), specific to *M. striaticollis* (Berdeleben & Gray 2005). Genotypes were obtained by PCR amplification using the Qiagen Multiplex PCR kit using a hybrid fluorescent dye labeled forward primer (M13F) (Boutin-Ganache *et al.* 2001). PCR reactions were performed on a programmable Peltier Thermal Cycler (MJ Research PTC-200) using previously defined conditions (Randall *et al.* 2010). Fragments sizes were scored using CEQTM 2000 software version 3.0 with reference to a size standard. To account for error genotyping individuals, 10% of the samples for each species were genotyped at least twice.

Genetic Diversity and Population Differentiation

Genetic diversity for microsatellite loci was measured as the observed (H_o) and expected (H_e) heterozygosity (Nei 1978) and the level of inbreeding was measured by the fixation index using ARLEQUIN version 3.0 (Excoffier *et al.* 2005). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using GENEPOP version 3.2 (Raymond & Rousset 1995) with an adjusted *P*-value corresponding to $\alpha = 0.05$. We measured genetic differentiation using the fixation index (Φ_{ST}) (Excoffier *et al.* 1992). Further, I estimated Φ_{ST} for microsatellite loci but also to mtDNA and introns sequence variation in a hierarchical analysis of molecular variance (AMOVA) between and within species using ARLEQUIN 3.0 (Excoffier *et al.* 2005).

Diversity in Vocal Traits

To understand acoustic elements that made the green and the yellow *Mionectes* also distinguishable groups in patterns of vocalizations, sound recordings of montane *Mionectes* from Ecuador and elsewhere were used to describe vocal traits in each species. Spectrograms of 16 individuals of *M. olivaceus* and 8 *M. striaticollis* were obtained from the Xenocanto Database (Planqué *et al.* 2005). Three major acoustic elements were examined in each spectrogram including, the number of notes in a phrase, the minimum and maximum phrase bandwidth, and bandwidth changes during phrasing within a bout (Table A-3).

Coalescent Analyses for Sequence Data

The isolation-with-migration (IM) coalescent model (Hey 2006) was used to estimate demographic parameters between species and populations within species so I could obtain a non-equilibrium estimate of population genetic differentiation (with respect to gene flow and isolation) as implied in the standard fixation index *F*-statistic (Bohonak & Roderick 2001; Hey & Pinho 2012). The IM framework simultaneously estimated the following parameters scaled to the mutation rate: population sizes of effective ancestral and contemporary populations (θ), migration parameter (m/μ), time since divergence between populations (t) and the splitting parameter (s), that provides an estimate of the fraction of the ancestral population in each derived population. The analyses were conducted in IM (Nielsen & Wakeley 2001) because the population size change option that allow for changes in θ is unlike IMa2 in which uniform growth rate of θ is assumed once divergence from its ancestral population took place. Paired two-population analyses were run for a combined data set of mtDNA and two introns, ODC1 and Fib7: (1) between species (Olive-striped Flycatcher vs. Streak-necked Flycatcher), (2) within the

Olive-striped Flycatcher (*cis* vs. *trans*), and (3) within the Streak-necked Flycatcher (*cis* vs. *trans*). I used between species or populations within the same species comparisons to test whether or not of gene flow occurs between partially sympatric taxa along elevation or the role of the Andes as a putative barrier separating populations across the ranges.

The Isolation-with-Migration model assumes that the two populations being compared are each panmictic and not exchanging genes with other populations (Hey & Nielsen 2004), assumptions likely to be violated by sampling error. However, IM is fairly robust to violation of this assumption (Strasburg & Rieseberg 2010). The IM model further assumes that loci are selectively neutral with no intralocus recombination; thus, I included the largest non-recombining block and truncated ODC1 and Fib7 haplotypes to 321 bp and 543 bp respectively. Inheritance scalars were defined as 0.25 for mtDNA and 1.0 for autosomal loci to reflect differences in effective population sizes, and I used the HKY model of mutation (Hasegawa *et al.* 1985) for mtDNA and Infinite-Sites model (Kimura 1969) for introns. Coalescent analysis ran in IM used initially a burn-in period of 20,000 and a run length of 100,000 to assess whether the priors were suitable and the heating conditions were appropriate.

From the results of these runs, I defined upper bounds for each parameter (θ, m and t). I consider a prior distribution of $4N\mu$ with an upper bound of 20 and upper bounds of the migration priors to $M = m/\mu = 20$. To ensure adequate mixing of a Markov chain, I used Metropolis-coupling of 120 independent heated chains, with the most heated chain had a heating factor of 0.9 with other heating chains having heating values between 1 and 0.75. Using those priors, I used a burn-in of 10 million steps and recorded results every 12 h. Effective sample size for each parameter exceeded 500. I repeated analyses three times, using different random seeds to verify independent runs. IM t parameter was converted to divergence time in year using an

estimate of mutation rate for each marker type. For maternal inherited ND2 region, I used mutation rate (μ) of 4.8×10^{-8} substitutions per site per year (s/s/y) (Peters et al 2005), 1.2×10^{-9} s/s/y for ODC1, and 1.0×10^{-9} s/s/y for Fib7 (Peters et al 2008). Further conversions used the geometric mean of substitution rates of these three DNA markers types.

Gene Flow Estimates using Microsatellite Loci

The IM model would not converge with the large microsatellite data set, so I then used MIGRATE-N 3.2.6 for estimating bidirectional gene flow as the number of migrants per generation (m/μ) across the Andes between populations within the same species. The model assumes that shared genetic variation between populations within each species is due to recent divergence from a common ancestor, such migration estimates using MIGRATE-N are fairly robust to violating the assumption of constant population size (Franchini *et al.* 2012). Each run included nine loci and 35% of randomly selected individuals of each species from the full microsatellite data set; All runs used 10 short chains of 10,000 recorded genealogies and four long chains of 100,000 genealogies with sampling increments of 200 and a burn-in of 50,000 genealogies. An unweighted pair-group method using arithmetic averages (UPGMA) starting tree and static heating with default temperatures were selected. Microsatellite loci were run assuming a Brownian motion process as an approximation of the Single Mutation Model (Ohta & Kimura 1973). Runs were replicated with different random seeds at least five times for each species to ensure convergence.

RESULTS

Genetic differentiation Between Species

Mionectes olivaceus and *M. striaticollis* surveyed are two distinct mtDNA groups with most variable sites fixed between them. The global Φ_{ST} for the mtDNA ND2 region was high, with 76.3% of the genetic diversity explained by differences between species. Both introns were also structured between species to a lesser extend as compared with mtDNA (Φ_{ST} = 0.000 - 0.089 for ODC6 and 0.030 - 0.2089 for Fib7) (Table 1-3).

Microsatellite diversity for all loci shows that the observed and expected heterozygosity varied between 0.53-0.71 for the Olive-striped Flycatcher and 0.63-0.79 for the Streak-necked Flycatcher (Table 2-3). Linkage disequilibrium (LD) test in ARLEQUIN 3.0 suggests LD between pairs of loci for each species and most loci tend to deviate from HWE. The microsatellite dataset shows that the global F_{ST} for microsatellite loci was high, with 13.7% of the genetic diversity explained by differences between species.

Population Genetics of Olive-striped Flycatcher Population Genetics

The *Mionectes olivaceus* surveyed contained 19 unique haplotypes for the mtDNA ND2 region, comprising 76 variables sites. Eleven haplotypes were shared between the two populations across the Andes. The global Φ_{ST} for mtDNA ND2 was high, with 64.6% of the genetic diversity explained by differences between populations in either side of the Andes. I found 2 ODC1 alleles with one variable site and 24 FIB7 alleles comprising 20 variables sites in autosomal intron sequences (Table 1-3), most alleles were broadly shared between populations across the Andes. Nucleotide diversity in both introns was consistently lower than in mtDNA with weak evidence of subdivision between *cis* and *trans*-populations (Φ_{ST} = 0.000-0.046 for

ODC6 and 0.000-0.100 for Fib7). Microsatellite loci provide evidence of population subdivision. Microsatellite expected heterozygosity ranged 0.60-0.72 (Table 2-3) and eight pairs of loci showed evidence of LD. Despite this result, the global F_{ST} for microsatellite loci was also high compared to other molecular marker types, with 10% of the genetic diversity explained by differences between *cis* and *trans*-Andean populations.

Streak-necked Flycatcher Population Genetics

The *Mionectes striaticollis* surveyed contained 19 unique haplotypes for the mtDNA ND2 region, comprising 30 variables sites. Ten haplotypes were shared between populations across the Andes. The global Φ_{ST} for mtDNA ND2-region was high, with 12.6% of the genetic diversity explained by differences between populations on either side of the Andes. I found 2 ODC1 alleles based on one variable site and 24 FIB7 alleles based on 11 variable sites in autosomal intron sequences. Populations on both sides of the Andes broadly share most alleles. Nucleotide diversity in introns was consistently lower than in mtDNA (Table 1-3). Both introns were structured between *cis* and *trans*-populations (Φ_{ST} = 0.000-0.038 for ODC6 and 0.000-0.135 for Fib7). Microsatellite expected heterozygosity ranged 0.75-0.78 (Table 2-3) and five pairs of loci showed significant LD. In spite of this result, the global F_{ST} for microsatellite loci was low compared to its sister species, with 3.3% of the genetic diversity explained by differences between east and west.

IM Analyses Between Species

The effective population size parameter (θ) estimates for *Mionectes olivaceus* and *M. striaticollis* were 5.3 (2-11; 95% High Posterior Density Interval) and 7.2 (2-27.5; 95% HPDI), respectively, which have broadly overlapping posterior density distributions (Fig 2-3), but

population size of the *M. olivaceus* is clearly smaller than the ancestral population size (13.3; 8.88-20.9 HDPI), suggesting demographic contraction (Fig 2-3). The most probable estimate of migration (m/μ) between the species was low (0.04-0.61 HDPI), and confidence intervals broadly overlapped zero in both directions (Fig 2-3). Thus, I could not reject the hypothesis of no gene flow into either species. Time since divergence (*t*, scaled to mutation rate) between the *M. olivaceus* and *M. striaticollis* peak at 0.22 (0.08-1.99 HPDI) (Fig 3-3), suggesting a divergence time of approximately 65,447 years before present (range 23,798-591,998). The posterior distribution of the splitting parameter, *s*, between these two species peak high to the right side of the distribution and the tail did not approach zero in any replicate; therefore, an accurate estimate could not be obtained of the percent of ancestral population that contributed to derived lineages.

IM Analyses within the Olive-striped Flycatcher

The population size parameter (θ) was higher for the *cis* population to the east of the Andes (6.23; 1.16-117.1 HDPI) as compared to the *trans* population to the west (2.73; 0.99-6.28 HPDI). These two populations are smaller than the ancestral population size (10.9; 6.01-19.9 HPDI) (Fig 2-3). This suggests demographic contraction, but posterior distributions broadly overlapped between the ancestral populations size and the estimate for the *cis* population (Fig 2-3). The most probable estimate of migration (m/μ) between populations in either side of the Andes was low (0.08; 0.00-0.54 HPDI), and confidence intervals broadly overlapped zero (Fig 2-3). Thus, I could not reject the hypothesis of no gene flow between *cis* and *trans*-Andean populations. Time since divergence (*t*, scaled to mutation rates) between both populations of *M. olivaceus* on either side of the Andes peaked at 0.47 (0.27-0.99 HPDI) (Fig 3-3), but the downward tail of the distribution did not approach zero in most replicates. The posterior density

distribution of divergence time within *M. olivaceus* is extending down (Fig 3-3), implying that this parameter estimate has a flat distribution over all probabilities. By setting the upper range estimate of *t* to the highest bound of time since divergence between the two species, I recovered a highly conservative divergence time range estimated to be between 80,321 and 591,998 years among populations within *M. olivaceus*. The posterior distribution of the splitting parameter, *s*, between populations east and west peaked at 91% (0.39-0.98%), as the percent of the ancestral population that contributed to these populations on either side of the Andes.

IM Analyses within the Streak-necked Flycatcher

The sizes of the two populations (θ) *M. striaticollis* across the Andes (*cis* 43.9; *trans* 6.17) show a broad distribution of probabilities, but the downward tail of the distribution did not converge at zero in any replicate. Thus, it is difficult to ascertain the difference between these two populations. The ancestral population size (4.30; 2.12-6.87 HPDI) suggests demographic expansion, but the posterior probability distribution of the ancestral population size did overlap with estimates for each population (Fig 2-3). The most probable estimate for migration (m/μ) between populations across the Andes was low (0.69; 0.00-3.54 HPDI), and confidence intervals broadly overlapped zero (Fig 2-3). Although there is indication of low levels of gene flow, I could not reject the hypothesis of no gene flow between the *cis* and *trans*-Andean populations. Time since divergence (*t*, scaled to mutation rate) between both populations across the Andes peaked at 0.19 (0.05-0.50 HPDI), and the tail of the distribution did approach zero (Fig 3-3). Thus, divergence traces back about 56,222 years before present (range 14,874-148,743). The posterior distribution of the splitting parameter, *s*, between these two populations peaked at

0.91% (39.9-98.0%) as the percent of the ancestral population that contributed into these populations in either side of the Andes.

MIGRATE-N Analysis of Gene Flow Between Populations Within the Same Species

For microsatellites, migration was probably higher between populations across the Andes for *Mionectes striaticollis* ($0 < m/\mu < 32$), as compared to the estimates for *Mionectes olivaceus* ($0 < m/\mu < 1$) (Fig 4-3). However, these estimates of gene exchange between populations across the Andes broadly overlapped zero in both directions (Fig 4-3). Thus, I could not reject the hypothesis of no gene flow between populations within the same species. Since IM provides evidence of subtle changes in θ, inferences of gene flow between populations within the same species rely in the IM framework for the most part.

Vocal Traits

Several acoustics elements are distinct between the green and the yellow *Mionectes*. The twittering calls between species are distinguishable in the number of basic notes within a phrase; recordings show three notes within phrases of *M. striaticollis*, while only two notes are characteristic of *M. olivaceus*. Changes in the minimum and maximum frequencies in phrases within a bout are also distinguishable between species. Every other phrase consistently alternates bandwidth among individuals of *M. striaticollis* (Table 3-3). While phrases in the case of *M. olivaceus* change bandwidth in a parabolic-like fashion. Overall individuals of *M. olivaceus* show a higher bandwidth range than individuals of *M. striaticollis* (Table 3-3) (t-test, $p < 0.01$). Bandwidth ranged 2-7.5 kHz in *M. striaticollis*. Although these individuals often range 4-7 kHz (SM2), *cis*-Andean and *trans*-Andean recordings do not substantially differ in their maximum frequency (t-test, $p = 0.38$). In the case of *M. olivaceus* bandwidth range 7-11 kHz, but often is

narrower among individuals 8.5-10 kHz (SM2) and *cis* and *trans* Andean recordings do differ in maximum frequency (t-test, $p < 0.05$). Clearly, montane *Mionectes* do not overlap in bandwidth and show marked differences in basic acoustic elements. It is difficult to ascertain the amount of differentiation in vocal traits within the same species. However, individuals within the same species in montane *Mionectes*, across broad latitudinal expenses, are more similar to one another than each of them is to other individual members of their sister-species.

DISCUSSION

Divergence Between and Within Species

The elevational replacement of *M. olivaceus* by *M. striaticollis* at higher elevations suggests the possibility that these species have primarily diverged along elevation, in the absence of a putative geographical barrier to gene flow. In this study, genetic differentiation and demographic history are concordant with divergence in isolation between *M. olivaceus* and *M. striaticollis* (Figure 1-1a). However, genetic differentiation between populations within each of the same species provides independent evidence of the Andes acting as an effective geographic barrier isolating populations in either side (Figure 1-1b). The prediction that populations across the Andes are genetically isolated for either *M. olivaceus* or *M. striaticollis* is also supported by estimates of gene flow based on microsatellites using MIGRATE-N (Figure 1-1b). However, the amount of genetic differentiation between populations of *M. olivaceus* is higher than the observed differentiation between populations of *M. striaticollis,* which is consistent in both cases by gene flow estimates using mtDNA and intron sequence data in IM. This indicates that populations separated across the Andes are genetically isolated to varying degrees. Although limited in resolution, gene flow estimates based on microsatellite using MIGRATE-N data do not

conflict results from the IM coalescent analyses. Estimates of gene flow using two different sets of DNA markers support the expected concentration of genetic differentiation between populations across a geographic barrier (Figure 1-1b and 1-1c). Little or restricted gene flow between populations of *M. striaticollis* and no gene flow between populations of *M. olivaceus* suggests that genetic differentiation is concentrated between populations at lower elevations, where the larger area through landmass effect should restrict gene flow more effectively (Figure 1-1c).

Divergence Between Species

M. olivaceus and *M. striaticollis* are distinct in plumage and vocal patterns that correspond with well-supported genetic differentiation (76.3% mtDNA, 13.7% microsatellite loci) and no evidence of gene flow. The IM method also suggests that effective population size estimates for the two species broadly overlapped and show no indication of dramatic demographic differences between them. There is clear evidence of demographic contraction only in the case of *M. olivaceus* with respect its most recent common ancestor. Further evidence of restricted gene flow between *M. olivaceus* and *M. striaticollis* suggests that for these two montane *Mionectes,* genetic drift is probably an important process shaping neutral genetic variation (Hey & Pinho 2012; Runemark *et al.* 2012). Coalescent analyses support the hypothesis that *M. olivaceus* and *M. striaticollis* have diverged in isolation. Evidence that these two montane *Mionectes* are genetically differentiated, independent with respect to gene flow, and phenotypically distinguishable in plumage and vocal patterns strongly suggest that they have reached evolutionary independence (Crandall *et al.* 2000). Divergence time estimates range between 23,798 and 591,998 years since *M. olivaceus* and *M. striaticollis* diverged from their

most recent common ancestor. Such time estimates broadly overlapped with divergence times between populations within the same species. However, it is difficult to ascertain the exact nature of the slightly older divergence time estimate (*t*) between populations of *M. olivaceus*, such discrepancy could be associated with violations to the IM model, as the possibility of different mutation rates between lineages (i.e. rates dependent upon highland and lowland zones) would affect calculations of divergence time (Tajima 1996).

Secondary Contact in Species-Ranges

The central question in this paper is that geographic distribution of *M. olivaceus* and *M. striaticollis* suggests the possibility that these species have primarily diverged in the absence of a putative geographical barrier to gene flow. I regard this scenario as less parsimonious because time since divergence and restricted gene flow between species are consistent with a clear pattern that support a uniform-process of geographic isolation between *cis* and *trans* populations within the same species. This implies that the modest geographic overlap at middle elevation between these two species is likely the result of secondary contact. However, the possibility that an ecological barrier isolating montane *Mionectes* species cannot be ruled out. Middle elevational zones where montane *Mionectes* are in contact today have experienced the largest impact due to climatic fluctuations, including dramatic changes in forest structure since the LGM (Hooghiemstra & Hammen 2004). The narrow elevational zones where montane *Mionectes* are in contact also accumulate high phenotypic turnover within bird species in the northern Andes (Caro *et al.* 2013; Thomassen *et al.* 2010), and elsewhere (McCormack & Berg 2010; McCormack & Smith 2008). Such transitional areas in terms of ecological conditions are predicted to be instrumental promoting phenotypic divergence (Niemiller *et al.* 2008; Smith *et*

al. 2005; Smith *et al.* 1997), particularly in later stages of speciation with restricted gene flow (Wu 2001).

CONCLUSIONS

Overall genetic differentiation between populations in either side of the Andes within either *M. olivaceus* or *M. striaticollis* is higher than the average intraspecific differentiation in mtDNA variation across the Andes (Mila *et al.* 2012). The unambiguous differentiation between *cis* and *trans* populations is consistent with highly restricted gene flow estimates between populations separated across the Andes (Cadena *et al.* 2007). High amounts of genetic differentiation between the green and the yellow montane *Mionectes* and restricted geneflow strongly suggests that these two species have reached evolutionary independence through a process that clearly involved divergence in isolation.

It stands to reason that the ubiquity of genetic drift in finite isolated populations has contributed to differentiation of genetic diversity, plumage and vocal patterns; such patterns are consistent with adaptive processes along the elevational gradient between species. Local adaptation along elevational gradients is been shown in an increasingly number of studies (Caro *et al.* 2013; Cheviron & Brumfield 2009a; Dingle *et al.* 2006; McCormack & Berg 2010; McCormack & Smith 2008; McCracken *et al.* 2009; Mila. *et al.* 2009). However, to reiterate in the case of montane *Mionectes*, the extensive overlap in divergence times between the two species and between *cis* and *trans* populations within the same species suggests a rapid concurrent process of genetic differentiation at different elevational zones and does not support divergence along elevation in the absence of a barrier as the primary mode of speciation.

With enough time, drift alone could lead to population differentiation and local fixation of neutral traits (Kimura & Weiss 1964; Wright 1931, 1943). Although understanding the adaptive value of plumage and vocal variation in montane *Mionectes* requires further work; vocal traits between populations within the same species are fairly equivalent across both sides of the Andes, while *cis* and *trans* populations within the same species do exhibit substantial plumage differentiation across the Andes. Because divergence times broadly overlapped between and within the same species, discordant patterns of differentiation in plumage and vocal traits between populations within the same species as compared with distinctive plumage and vocal phenotypes between species cannot be simply explained by rapid differentiation due to genetic drift alone.

FIGURES AND TABLES

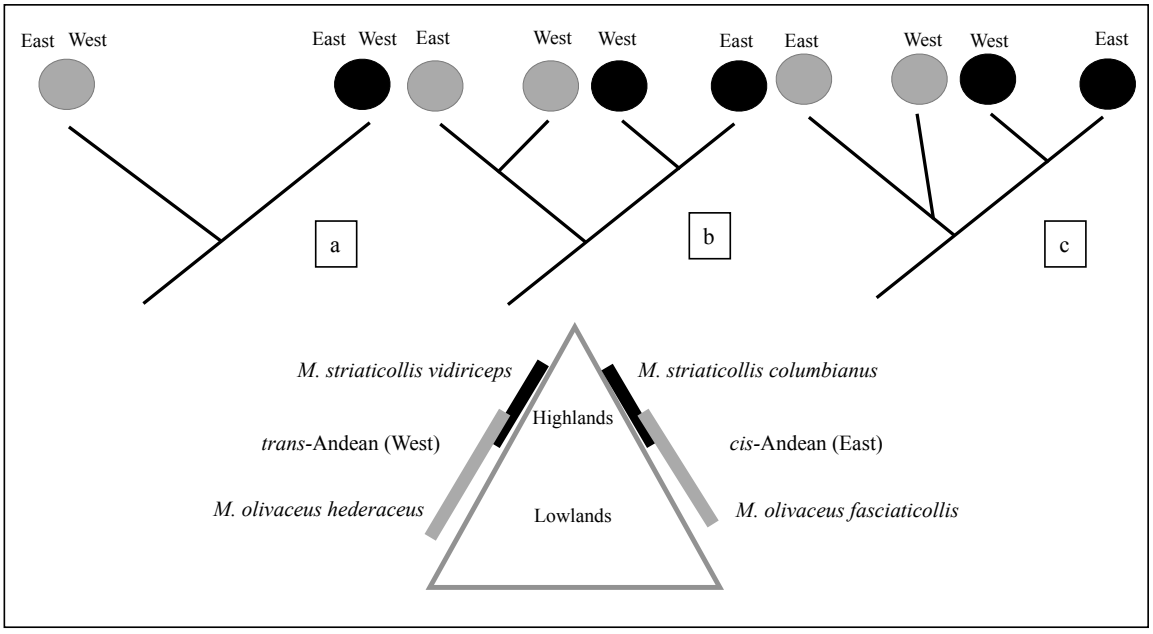

Figure 1-3. Hypotheses of population differentiation across Andean elevational zones for montane *Mionectes*. (a) Divergence between *M. olivaceus* (dark) and *M. striaticollis* (grey) resulted in different elevational distributions and phenotypically distinguishable species (see text); however, populations across the Andes within the same species might be genetically undistinguishable. (b) Genetically differentiated populations across the Andes within the same species would provide evidence that the Andes range acts as a barrier isolating populations in either side. (c) Genetic differentiation should be concentrated at lower elevations, particularly between populations of *M. olivaceus*, where the larger area through landmass effect should restrict gene flow more effectively than at higher elevations between population within *M. striaticollis*.

Table 1-3. Numbers of samples, haplotypes/alleles per population, and nucleotide diversity (π) for the mtDNA ND2 region, Fib7, and ODC1 of montane *Mionectes*

	ND2			Fib7			OD1		
	N	Haplotypes	π[1]	N	Haplotypes	π	N	Haplotypes	π
M. olivaceus (*cis*)	13	10	0.003801	12	11	0.001714	11	4	0
M. olivaceus (*trans*)	16	9	0.007493	12	12	0.002802	11	8	0.000334
M. striaticollis (*cis*)	12	11	0.005228	12	12	0.000638	8	7	0.000491
M. striaticollis (*trans*)	12	9	0.002310	12	12	0.002598	11	4	0

[1] Average differences between two sequences in a population as a proportion of sequence length

Table 2-3. Numbers of microsatellite loci, gene copies, observed (Ho) and expected (He) heterozygosity and genetic diversity between *cis* and *trans*-Andean populations of montane *Mionectes.*

Microsatellites Populations	Loci*	Gene copies (s.d.)	*Ho*, (s.d.)	*He*, (s.d.)	Average genotypic diversity over all loci, (s.d.)
M. olivaceus (*cis*)	4	89.1 (11.7)	0.45224 (0.29)	0.60596 (0.36)	0.440707 (0.28)
M. olivaceus (*trans*)	7	57.5 (3.8)	0.66113 (0.28)	0.72696 (0.29)	0.66327 (0.36)
M. striaticollis (*cis*)	5	120.6 (10.9)	0.66487 (0.15)	0.78696 (0.13)	0.790984 (0.44)
M. striaticollis (*trans*)	8	34.6 (2.4)	0.53460 (0.28)	0.75506 (0.20)	0.718254 (0.39)

- Loci with < 1% missing data

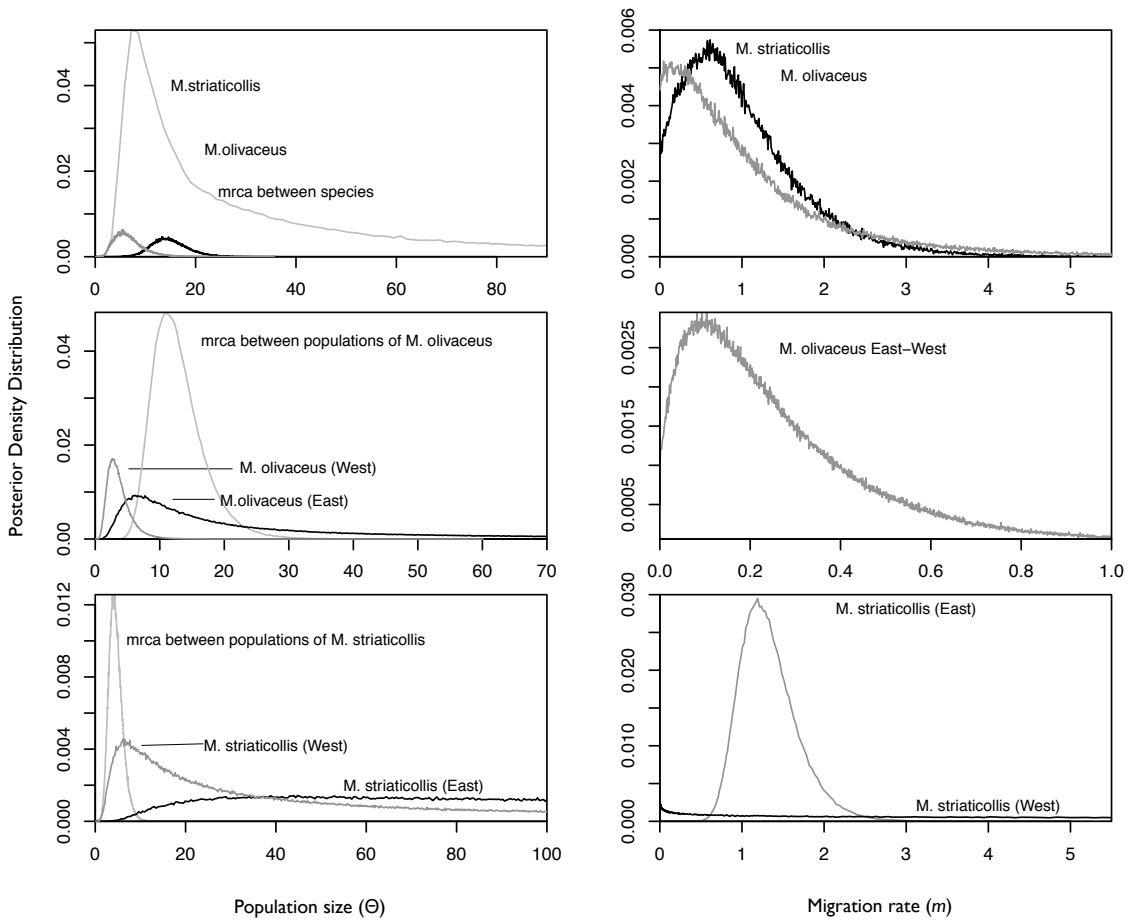

Figure 2-3. Coalescent analysis for a combined data set including mtDNA and nuclear introns ODC1 and Fib7. Posterior distribution of effective population size, θ, and migration, m/μ, calculated with IM.

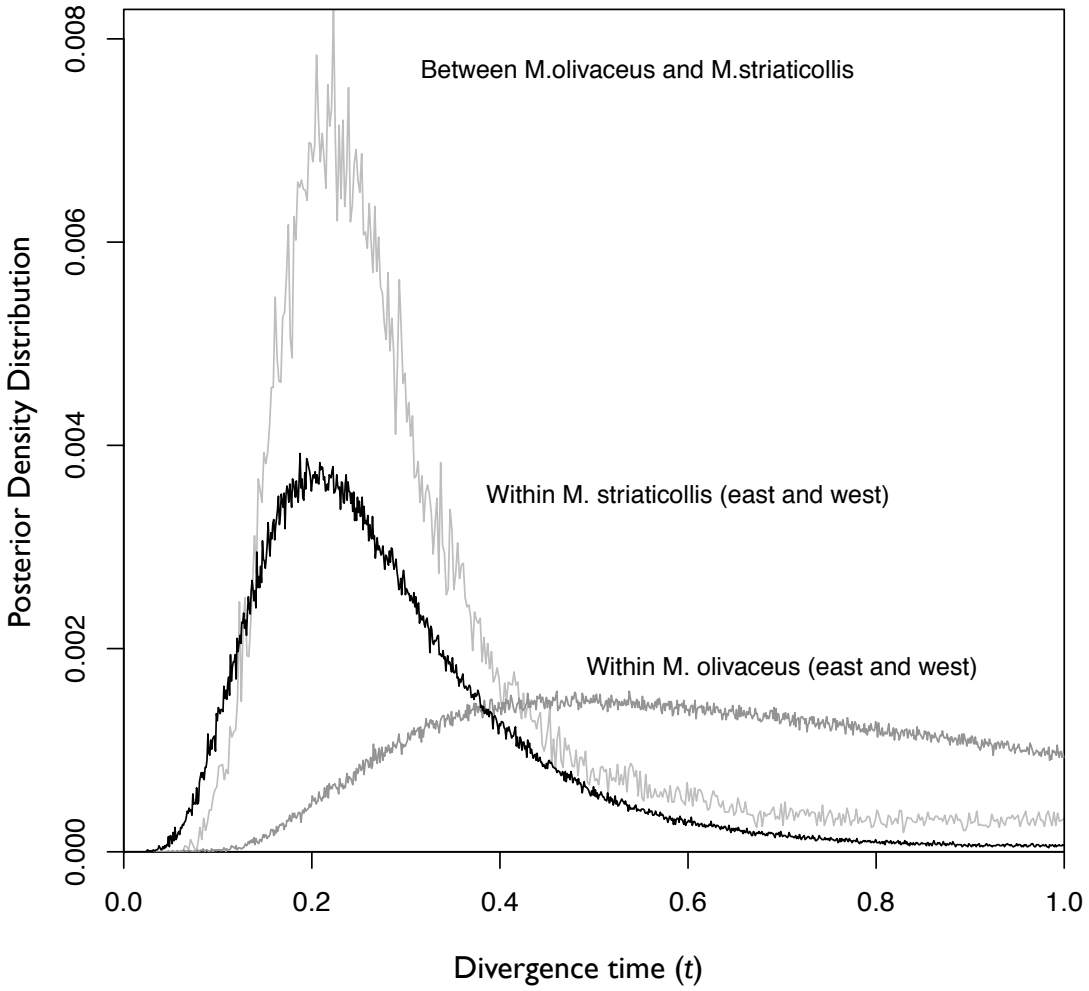

Figure 3-3. Coalescent analysis for parameter estimate of divergence time using a combined data set including mtDNA and nuclear introns ODC1 and Fib7. Posterior distribution of time since divergence, *t* was calculated with IM.

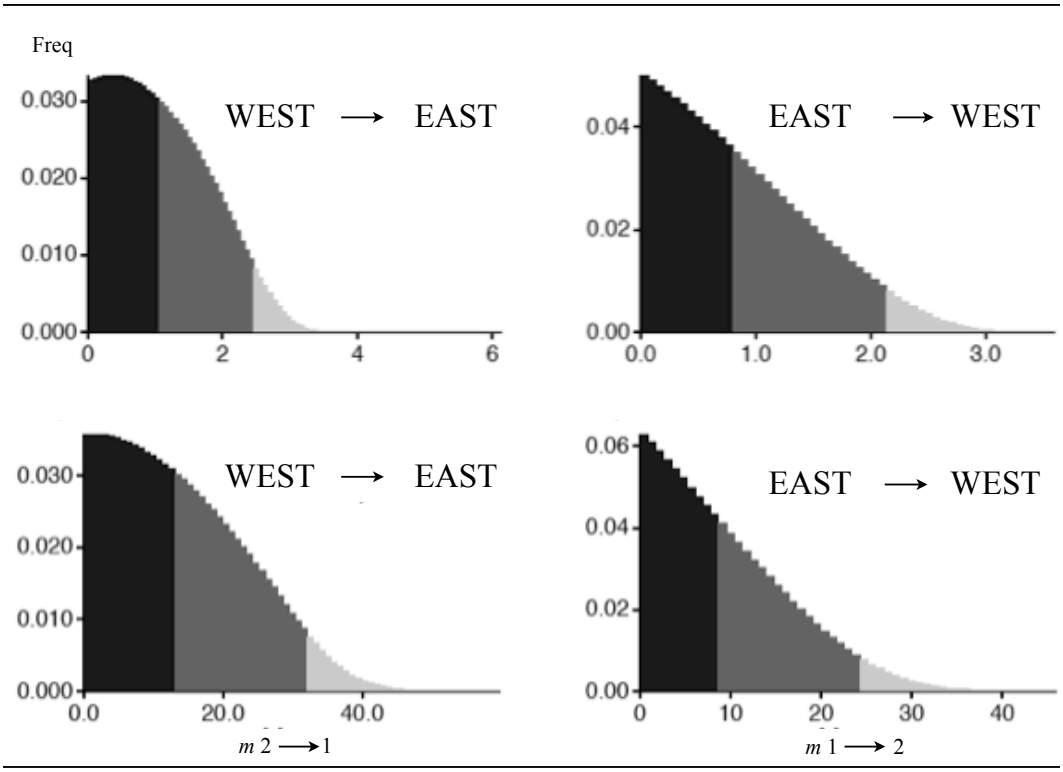

Figure 4-3. Coalescent analyses in MIGRATE-N using microsatellite loci between populations across the Andes for *M. olivaceus* and *M. striaticollis*. Posterior distribution (95% high posterior density) immigrantion, *m* stands for *m*/μ (same units as in IM analyses), calculated with migrate-n using nine microsatellite loci for either species. Top panel shows parameter estimates between populations across the Andes within *M. olivaceus* (n=30). Bottom panel shows parameter estimates between populations across the Andes within *M. striaticollis* (n=18). The arrow indicates the directionality of gene flow estimates between populations in either side of the Andes, *trans*-Andean (1) and *cis*-Andean (2).

APPENDIX

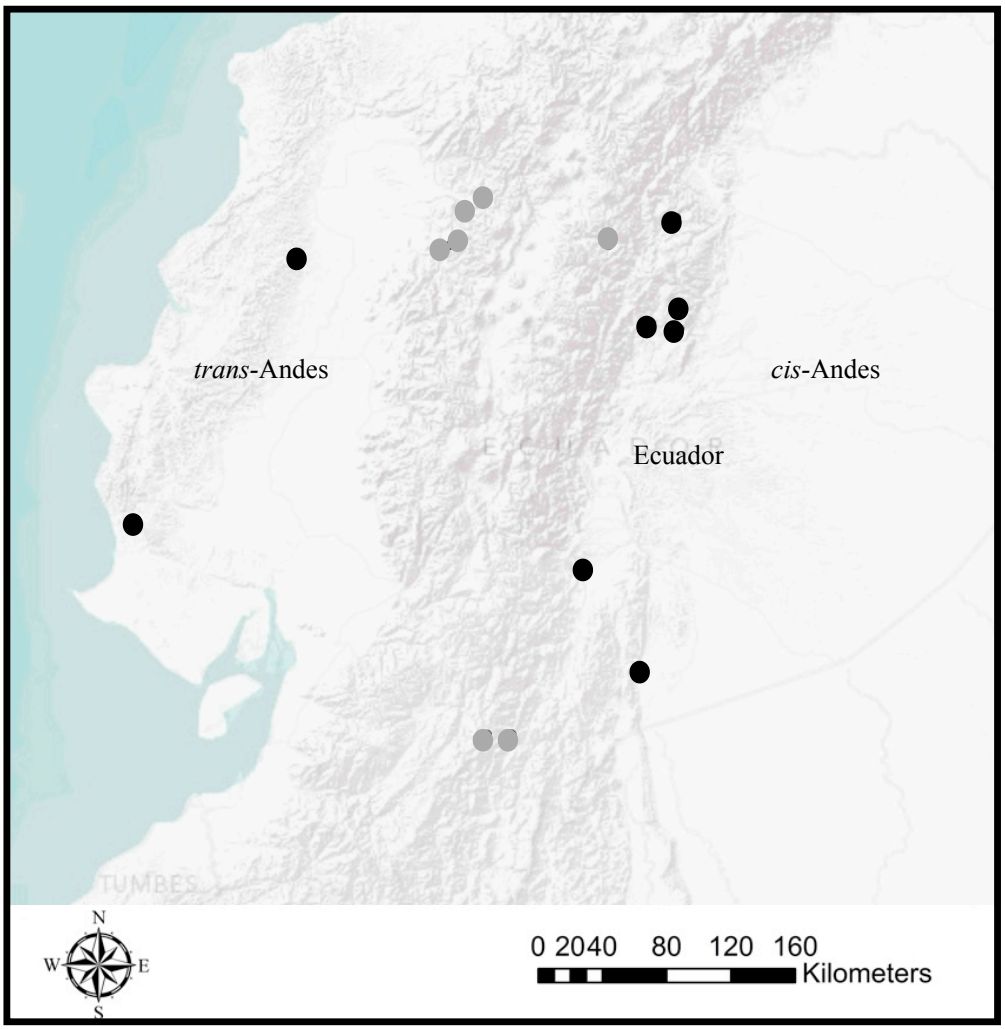

Figure B-1. Sites where samples were obtained for DNA genotyping. *M. olivaceus* (dark) and *M. striaticollis* (grey)

Table B-1. Range of acoustic frequencies within vocal sounds of montane *Mionectes*.

Species	ID	Freq (kHz)	Distribution	Country	Location	Altitude	Recorder
M. olivaceus	XC130168	8.2-10	Andean	Colombia	"Parque Regional Barbas-Bremen, Filandia, Quindio. Sector Barbas"	1800	Oscar Humberto Marin-Gomez
M. olivaceus	XC130160	8.1-10	Andean	Colombia	"Parque Regional Barbas-Bremen, Filandia, Quindio. Sector Barbas"	1800	Oscar Humberto Marin-Gomez
M. olivaceus	XC130472	8-10	Andean	Colombia	"Parque Regional Barbas-Bremen, Filandia, Quindio. Sector Barbas"	1800	Oscar Humberto Marin-Gomez
M. olivaceus	XC98248	7-10	*cis*-Andean	Ecuador	"Wildsumaco, 5km NW Guagua Sumaco, Napo"	1450	Tayler Brooks
M. olivaceus	XC98105	7-10.5	*cis*-Andean	Ecuador	"Wildsumaco, 5km NW Guagua Sumaco, Napo"	1450	Tayler Brooks
M. olivaceus	XC14823	8-10.1	*cis*-Andean	Venezuela	"San Isidro Tunnel Road, Barinas"	NA	Andrew Spencer
M. olivaceus	XC8043	7-10	*cis*-Andean	Ecuador	"Loreto Road, Napo"	1300	Nick Athanas
M. olivaceus	XC83483	7-10	*cis*-Andean	Peru	"Limonchayoc, Cusco"	950	David Geale
M. olivaceus	XC108603	8-10.5	*trans*-Andean	Ecuador	"Milpe, Pichincha"	1100	Andrew Spencer
M. olivaceus	XC128322	9-11	*trans*-Andean	Panama	"Cerro Gaital, Anton, Cocle"	850	Thore Noernberg
M. olivaceus	XC97454	9-11	*trans*-Andean	Costa Rica	Tapanti Wildlife Refuge	1250	Richard C. Hoyer
M. olivaceus	XC6646	9-11	*trans*-Andean	Ecuador	"Finca Cuatro Rios, San Miguel de Los Bancos, Pichincha"	800	Nick Athanas
M. olivaceus	XC2970	8-10.2	*trans*-Andean	Panama	"Cerro Chucanti, Darien Province"	700	David Bradley
M. olivaceus	XC16056	9-10.8	*trans*-Andean	Panama	"El Valle, Cocle Province"	650	Ken Allaire
M. olivaceus	XC8351	9-10	*trans*-Andean	Panama	"Altos del Maria, Panama"	800	Ken Allaire
M. olivaceus	XC13093	8.5-11	*trans*-Andean	Ecuador	"Mindo, Pichincha"	1400	Roger Ahlman
M. striaticollis	XC17358	6-7	*cis*-Andean	Ecuador	"Caba√±as San Isidro, Cosanga, Napo"	2100	Andrew Spencer
M. striaticollis	XC17357	5.5-7.2	*cis*-Andean	Ecuador	"Caba√±as San Isidro, Cosanga, Napo"	2100	Andrew Spencer
M. striaticollis	XC4741	4.5-6	*cis*-Andean	Bolivia	"Tunquini Biological Station, Cotapata NP, La Paz"	1500 - 2000	Sebastian K. Herzog
M. striaticollis	XC89354	2.2-7.5	*cis*-Andean	Colombia	FARALLON DE MEDINA	2000	Oswaldo Cortes
M. striaticollis	XC96243	2-7.5	*cis*-Andean	Colombia	Municipio El Calvario (Meta)	2100	Oswaldo Cortes

M. striaticollis	XC17356	6-7.5	*trans*-Andean	Ecuador	"Reserva Bosque Hanne, Utuana, Loja"	2400	Andrew Spencer
M. striaticollis	XC3823	4-6.2	*trans*-Andean	Ecuador	"Tandayapa Bird Lodge, Tandayapa Valley, Pichincha"	1830	Nick Athanas
M. striaticollis	XC13233	4-6	*trans*-Andean	Ecuador	"Tandayapa Bird Lodge, Tandayapa Valley, Pichincha"	1600	Roger Ahlman

XC, Xenocanto (http://www.xeno-canto.org/) last date 04/21/2013

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