ECOLOGICAL EVOLUTIONARY GENETICS
OF SOME NEOTROPICAL BIRDS

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EVOLUTIONARY ECOLOGICAL GENETICS OF SOME NEOTROPICAL BIRDS

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Abstract

Most of the current models to explain the diversification of Neotropical birds focus on physical barriers to gene flow. However, for any species the geographic structuring of populations is caused by an interaction between physical barriers to gene flow and a species' propensity to overcome those barriers. The three chapters presented in this dissertation provide three perspectives on this interaction and how it has shaped the diversification of some Neotropical birds. First, the widespread Neotropical lowland forest flycatcher *Mionectes oleagineus* had three phylogeographic splits across the Andes, resulting in four geographically structured lineages west of the Andes. At least two of these splits post-date Andean uplift, and therefore represent dispersal across the Andes. Coalescent estimates suggest that gene flow occurred with some regularity after the third colonization event several hundred thousand years ago. Secondly, I found that within-population genetic variation in nine codistributed Neotropical landbirds fit a humped distribution, whereby mid-range populations had higher genetic diversity than range-edge populations. This finding is not consistent with a model of increasing genetic diversity with decreasing latitude. Thirdly, I examined variation in genetic differentiation between two populations in 60 codistributed Neotropical landbirds. All species were sampled in southern Belize and central Panama, and I found that the net nucleotide divergence (D_A) spanned two orders of magnitude (0.00% – 0.085%). Species of frugivores and nectivores had significantly lower D_A values than species of insectivores, and in a subsample of 19 species with population-level sampling I found that populations of frugivores and nectivores were significantly

more likely to show genetic signals of population expansion than populations of insectivores. These results suggest that foraging ecology plays a fundamentally important role in determining diversification patterns of Neotropical birds. These three results should provide important baseline data and new insights into the processes that have led to the Neotropical region having the highest avian diversity of all the Earth's biomes.

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PREFACE

This theme of this thesis was developed by thesis advisor Kevin Winker (UAF Biology and Wildlife, UA Museum [UAM]) and me. For the first chapter, I defined the question, designed the study, conducted all molecular and statistical analyses, and wrote the original draft of the manuscript. Co-authors John Klicka (Marjorie Barick Museum), Jason Weir (University of British Columbia), Fabio Raposo do Amaral (Universidade de São Paulo), and Patricia Escalante (Universidad Nacional Autónoma de México) provided indispensable specimens and valuable contributions to the final manuscript, as did co-authors Winker and Eldredge Bermingham (Smithsonian Tropical Research Institute [STRI]).

For the second chapter, I defined the question, designed the study, conducted all molecular and statistical analyses, and wrote the original draft of the manuscript. Co-author John Klicka (Marjorie Barrick Museum) provide indispensable specimens from Honduras, and co-authors Winker and Bermingham made valuable contributions to the final manuscript.

For the third chapter, Winker devised a general concept of studying evolutionary ecological genetic variation between co-distributed Middle American resident bird species sampled in Belize and Panama. He and I defined the particular question to address, designed the study. I did all molecular and statistical analyses and wrote the original draft of the manuscript. Co-authors Winker and Bermingham made valuable contributions to the final manuscript.

Over 1200 different bird specimens were sequenced for the three chapters included in this thesis; over 95% of these represent vouchered museum specimens. Thus, untold numbers of hours were spent in the field collecting and preparing specimens, and in the lab preparing, archiving and preserving them. Andy Johnson (UAM) deserves special credit for collecting and preparing many of the specimens from Belize that are included in this thesis. Joshua Bacon, Peggy Guitton Mayerma, Michael Lelevier, James Maley, and Kevin Winker (all UAM) provided considerable help to me during field expeditions to Panama, as did the STRI. While I collected most of the sequence data, Mersee-Madison Villar (UAM), Michael Lelevier (UAM), Peggy Guitton Mayerma (STRI), and Melida Núñez (STRI) all sequenced a considerable portion of the birds in this thesis. The overwhelming majority of specimens in this thesis come from the University of Alaska Museum. The Academy of Natural Sciences of Philadelphia, the American Museum of Natural History, the Colección Nacional de Aves, México, the Louisiana State University Museum of Zoology, the National Museum of Natural History, the Marjorie Barrick Museum, and the Museu de Zoologia da Universidade de São Paulo each loaned tissues from specimens in their care that were critical to the completion of this research.

A substantial portion of my PhD career was spent in the Bermingham lab of STRI. I am particularly grateful to Oris Sanjur (STRI) for her unwavering logistical and emotional support during my tenure at STRI. Chris Dick (STRI) and Andrew Crawford (STRI) were valued colleagues and role models. STRI's administrative staff

provided critical logistical support ranging from help with immigration visas to collecting permits and the use of research vehicle and firearms.

During my graduate career I was financially supported principally by the UA Museum. Additional financial support came from an Angus Gavin Memorial Bird Research Grant, an EPSCoR graduate fellowship, several Frank M. Chapman Memorial Fund awards, a UAF graduate school fellowship, and a STRI pre-doctoral fellowship. My parents Michael and Dawn Miller have never wavered in their support of my academic dreams for over two decades; thank you.

Finally, I would like to give the my greatest thanks to my wife, Peggy Guitton Mayerma. Peggy was my number one field assistant, having been at my side on nearly every field trip in Panama and Peru, including during her eighth month of pregnancy. She's tireless with mistnets, careful with field notes, and cheerful in the face of setbacks customary to field expeditions. She has slept in improvised medical clinics in the Amazon basin, ramshackle huts in the middle of cow pastures in Caribbean Panama, bed-bug infested rooms in downtown Panama City, and countless nights in tents during the heavy rains so customary for jungles in Latin America. For years, Peggy was literally at my side daily in the lab, often until after midnight. Amazingly, she accepts a seven-day work week, the hijacking of nearly every social gathering we attend by science talk, a relationship with my laptop that borders on infidelity, and that most of our vacations somehow involve killing birds. Despite all that, she remains my greatest cheerleader and my best friend. I dedicate this thesis to her and to the light of my life, our daughter, Gaia Denali.

General Introduction

The Neotropical region is home to about one in three of all bird species, making this region far and away the most diverse (in terms of birds) on Earth (Stotz *et al.* 1996; Orme *et al.* 2006). Nearly every model of the speciation process requires reduction or elimination of gene flow between the nascent species (Mayr 1963; Endler 1977; Dieckmann & Doebeli 1999; Schluter 2000), and perhaps the most common way this occurs is by geography (Coyne & Orr 2004). Thus, for most species, the initial step in biological diversification is geographic structuring of genetic variation; in fact, Price (2008) posited that more than 99% of all bird speciation events begin with differences arising in geographically separated populations. For any species, the geographic structuring of populations is caused by an interaction between physical barriers to gene flow and a species' propensity to overcome those barriers. For some species, distance alone inhibits gene flow, whereas others maintain gene flow over immense physical barriers such as oceans and mountain ranges. Finding consistent patterns at work in the interplay of barriers and different species' responses to them would provide greater insight into the origins of spectacular species assemblages such as the Neotropical avifauna.

Most of the current models used to explain the diversification of Neotropical birds focus on the physical barriers to gene flow. The incredible richness of Neotropical birds has been explained variously by the Amazon River (e.g., Sick 1967), Andean uplift (e.g., Chapman 1917) or Pleistocene forest refugia (e.g., Haffer 1969),

what I like to refer to as "rivers, rocks, and refugia". Although precise dates for the speciation events of most Neotropical birds are unknown, given the general observation that higher species richness in the tropics predates the Amazon River, the Andes, or purported Pleistocene forest refugia (Jablonski 1993; Rosenzweig 1995), there must be more to the story. My dissertation uses phylogeographic and population genetic approaches to attempt to synthesize the relationship between barriers to gene flow and the ecological propensity of Neotropical birds to overcome them.

The first chapter is a phylogeographic study of a widespread Neotropical flycatcher, *Mionectes oleagineus* (Ochre-bellied Flycatcher). Previously, it was believed that Andean uplift isolated Neotropical lowland plants and animals into lineages to the west and east, setting them on independent evolutionary trajectories (Chapman 1917; Cracraft 1985; Cracraft & Prum 1988; Prum 1988). Using mitochondrial DNA sequences, this study recovered five well-supported clades within *M. oleagineus*. Even more surprisingly, most of these lineages are related to dispersal events across the Andes after the uplift of this montane barrier. This phylogenetic reconstruction demonstrates that this species repeatedly dispersed over or around the Andes and colonized the lowlands of northwestern South America and Middle America on three separate occasions, resulting in four geographically structured lineages west of the Andes. The last of these four clades spans the Andes, and coalescent-based population genetic analyses provided evidence that gene flow occurred with some regularity after the third colonization event several hundred thousand years ago. Interestingly, these findings suggest that for this species the

Andes provided the catalyst for generating genetic diversity by serving as a filter barrier, rather than an absolute barrier to gene flow.

The second chapter expands on the approach of the *Mionectes* study, narrowing the geographic focus to the Neotropical lowlands west of the Andes but expanding the number of species examined to nine. Under typical conditions, how might genetic variation be partitioned across a species' range? Some models predict that genetic variation should be maximized in the center of a species' range and diminish towards the range edges (central-marginal model: da Cunha *et al.* 1950; Brussard 1984; Hewitt 2000; Eckert *et al.* 2008). Other models predict that genetic variation could be related to underlying gradients within a species' range. A frequently cited example posits that, as a result of poleward range expansions following the retreat of Pleistocene glaciers, the greatest neutral genetic variation should occur at the equatorial limits of a species' range and decrease with increasing latitude (Hewitt 1996; Vellend 2003). Another model predicts that the MacArthur-Wilson dynamics leading to an equilibrium of species richness in a community should also regulate the relative diversity of neutral genetic variants within that community (Vellend 2005). A latitudinal gradient in genetic diversity is commonly reported for many species (Eckert *et al.* 2008), although the majority of examples come from the north temperate zone, and no study to date has looked at exclusively tropical taxa. Chapter Two explores the variation in within-population mitochondrial DNA diversity along a latitudinal transect for nine species of resident Neotropical landbirds. Within-population genetic diversity was not inversely related to latitude. Instead, it showed a humped

distribution, wherein all nine species showed the highest genetic diversity occurring in mid-latitude populations rather than in latitudinally extreme populations. These results were too consistent to be explained by chance, and therefore suggest that for tropical species the central-marginal model may be more common than a latitudinal gradient in genetic variation.

Whereas Chapter Two examined *within*-population genetic variation, Chapter Three focused on the degree of genetic variation *between* geographically separated populations. The two extremes of geographic structuring of genetic variation between populations are panmixia, in which all variation is shared equally across a species' range, and complete geographic structuring, in which each genetic variant is unique to a particular population. All species are somewhere in the middle of this continuum, but the combination of physical barriers to gene flow and that species' ability to overcome those barriers are key factors affecting a species' position on this continuum. A common approach in comparative phylogeography is to ask whether a purported physical barrier was an important factor affecting the diversification of a region's biota by looking for similar patterns of geographic structure across the space containing the barrier among many species. The third chapter turns this approach on its head by asking whether we can gain information about ecological factors that might affect the geographic structuring of genetic variation by examining patterns among many species over a shared geography. This study compared the degree of genetic differentiation between southern Belize and central Panama for 60 species of codistributed resident Neotropical landbirds, which represent about 40% of all species

of such birds that are more or less continuously distributed between northern and southern Middle America. Considerable variation occurs in the degree of genetic structure, ranging from some species that shared most variation between Belize and Panama to others in which the two sites varied by greater than 8% mtDNA sequence divergence. Foraging ecology was significantly correlated with the degree of differentiation: as a group, insectivorous species were highly differentiated, while frugivorous and nectivorous species showed low levels of differentiation between the two sites.

Because few species shared identical DNA sequences between Belize and Panama, these results show that most species have been genetically isolated between northern and southern Middle America for some time. However, the amount of time that populations have been isolated varies, and, on average, it is greater for insectivores than for frugivores and nectivores. Detailed population sampling of 19 of these species indicated that a significantly greater proportion of the frugivore and nectivore populations showed signs of recent demographic expansion relative to insectivore populations. Together these results suggest that foraging ecology plays a fundamentally important role in determining diversification patterns of Neotropical birds.

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CHAPTER 1: OUT OF AMAZONIA AGAIN AND AGAIN: EPISODIC CROSSING OF THE ANDES PROMOTES DIVERSIFICATION IN A LOWLAND FOREST FLYCATCHER[1]

ABSTRACT.– Most Neotropical lowland forest taxa occur exclusively on one side of the Andes despite the availability of appropriate habitat on both sides. Almost all molecular phylogenies and phylogenetic analyses of species assemblages (i.e., area cladograms) have supported the hypothesis that Andean uplift during the late Pliocene created a vicariant barrier affecting lowland lineages in the region. However, a few widespread plant and animal species occurring in lowland forests on both sides of the Andes challenge the generality of this hypothesis. To understand the role of the Andes in the history of such organisms, we reconstructed the phylogeographic history of a widespread Neotropical flycatcher (*Mionectes oleagineus*) in the context of the other four species in the genus. A molecular phylogeny based on nuclear and mitochondrial sequences unambiguously showed an early basal spit between montane and lowland *Mionectes*. Phylogeographic reconstruction of lowland taxa revealed a complex history, with multiple cases in which geographically-proximate populations do not represent sister lineages. Specifically, three populations of *M. oleagineus* west of the Andes do not comprise a monophyletic clade; instead, each represents an independent lineage with origins east of the Andes. Divergence time estimates suggest that at least two cross-Andean dispersal events post-date Andean uplift.

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1.1 INTRODUCTION

The high passes and montane habitats of the Andean cordilleras present a formidable ecological interruption of the Amazonian lowland moist tropical forests and similar habitats found in northwestern South America and most of Middle America. Thus, it is not surprising that when lowland organisms from this region have been analyzed in a phylogenetic framework, most researchers have found a basal split between the lowlands east and west of the Andes (arachnids: Zeh *et al.* 2003; birds: Cracraft & Prum 1988; Brumfield & Capparella 1996; Cheviron *et al.* 2005; Eberhard & Bermingham 2004; Eberhard & Bermingham 2005; primates: Cortes-Ortiz *et al.* 2003; reptiles: Zamudio & Greene 1997; trees: Dick *et al.* 2003). Likewise, when geographic relationships among entire faunal assemblages have been evaluated either phenetically (Silva & Oren 1996; Bates *et al.* 1998) or cladistically (Prum 1988; Ron 2000), similar results were obtained. One obvious explanation for these results is that for many widespread species the final uplift of the northern Andes in the late Pliocene (~ 2.7 Myr ago, Gregory-Wodzicki 2000) split the distributions of organisms found in the lowland forests of the region, an hypothesis advanced nearly a century ago by Chapman (1917). Even in birds, which must be among the most vagile of lowland Neotropical organisms, distributional patterns suggest that the rise of the Andes restricted gene flow and dispersal: of the approximately 3800 bird species found in the Neotropics, only 178 (<5%) are encountered in lowland forests both east and west of the Andes (Haffer 1967).

Several observations point to the role that the Andes may play in limiting dispersal of lowland forest birds over or around them. First, even the lowest passes in the northern Andes reach nearly 2000 m higher in elevation than the surrounding lowland forests (Haffer 1967). At these elevations, Andean montane habitats present novel physiological (Janzen 1967) and competitive (Terborgh & Weske 1975) challenges to birds typically found in lowland forest habitats (Terborgh 1971). Second, the northern extent of the forests of the northwestern Amazon basin is bordered by the large *llanos* savannah, which itself is bounded by the eastern Andean cordillera, extending northeastward into the Caribbean ocean and terminating with the island of Trinidad. Under current climatic conditions, the shortest low-elevation route around the Andes is interrupted by extensive stretches of ocean, *llanos*, and arid scrublands in the Caribbean lowlands north and east of the Andes (Eva *et al.* 2002).

Thus, for species with populations occurring in lowland forests on both sides of the Andes, three possibilities exist: 1) populations have been isolated too recently for speciation to occur; 2) gene flow across presumably significant barriers occurs with sufficient regularity to inhibit speciation; or 3) phenotypic evolution is sufficiently conservative that we fail to recognize species-level differences. We investigated these hypotheses by reconstructing the evolutionary history of *Mionectes oleagineus* (Ochre-bellied Flycatcher), which is widespread in lowland forests both east and west of the Andes. Furthermore, we placed our phylogeographic analysis of *M. oleagineus* within the phylogenetic context of the remaining species in the genus. *Mionectes* consists of a pair of montane flycatchers found in the Andes and southern

Middle America and three lowland species, including our focal species. *M. oleagineus* is found exclusively in the understory of lowland tropical forests and woodlands and is replaced by congeners at higher elevations, suggesting that dispersal across the Andes should be unlikely in this species. Furthermore, because morphological evolution is very conservative among *Mionectes* species (Capparella & Lanyon 1985), it is possible that cross-Andean populations have been isolated since before Andean uplift yet remain sufficiently similar phenotypically to be classified as conspecific.

1.2 METHODS

The genus *Mionectes* consists of five species of drab, principally frugivorous flycatchers found in the understory of most Neotropical forests. Two species are found in montane forests: *Mionectes olivaceus* inhabits premontane and lower montane forests in the Andes and southern Middle America (north to Costa Rica); in higher elevations in the Andes this species is replaced by *M. striaticollis*. There are three lowland species in the genus. The most widespread, *M oleagineus*, ranges throughout tropical Middle America, Amazonia, and the lowland forests of the Guiana Shield and also includes two disjunct populations in western Ecuador and the Atlantic Forest of Brazil (figure 1.1b). In the field, it is often difficult to separate *M. oleagineus* from the two other lowland *Mionectes* species (*M. macconnelli* and *M rufiventris*), both of which are partially sympatric with *M. oleagineus*. *M. macconnelli* has a disjunct distribution in southwestern Amazonia and in the Guiana Shield (figure 1.1b). In both regions it is almost entirely sympatric with *M. oleagineus*. *M. rufiventris* is restricted

to forest and woodland habitats in coastal southeastern South America, where it narrowly overlaps with *M. oleagineus* (figure 1.1b).

(a) phylogenetic tree reconstruction

We generated three different molecular datasets to establish phylogenetic relationships among *Mionectes* species and populations. Because earlier classifications (e.g., Todd 1921; Meyer de Schaunsee 1970) placed lowland *Mionectes* in their own genus (*Pipromorpha*) we wanted to confirm the sister relationship between montane and lowland *Mionectes* and to place a root for the latter. To do this we generated a dataset using a portion of the cytochrome-*b* mitochondrial gene (999 basepairs [bp]) and fragments of two nuclear, single-copy, protein-coding genes: RAG-1 (930 bp) and *c-myc* (477 bp). We sequenced a single individual of both montane and all three lowland *Mionectes* species; for outgroups, we used several taxa available from GenBank (Johansson *et al.* 2002). We generated phylogenetic trees from this dataset using two methods: Bayesian inference (implemented in MrBayes v3.1.2; Ronquist & Huelsenbeck 2003) and branch-and-bound maximum likelihood phylogeny (implemented in PAUP* 4.0b10; Swofford 2002). To further resolve phylogenetic and phylogeographic variation within lowland *Mionectes*, we obtained the entire mitochondrial ND2 gene for 153 additional lowland *Mionectes* and five additional montane *Mionectes* from widespread geographic origins within their respective ranges, focusing on the widespread *Mionectes oleagineus* (see Appendix 1.2 for details about locality and other voucher specimen data.) Similar to the first dataset, for this second dataset we generated a Bayesian inference phylogeny using MrBayes.

Although this analysis showed strong support for *M. oleagineus* nodes near the tips of the phylogeny, some interior nodes were not strongly supported. To test the validity of these nodes, we selected one individual from each major lowland *Mionectes* clade recovered in the second phylogenetic tree (n=14) as well as one each of the two montane species and sequenced the entire cytochrome-*b* mitochondrial gene to create a new mtDNA dataset that combined this gene with the ND2 sequence from the previous analysis. For the clade comprising individuals from eastern Panama and northern South America we included one individual from each side of the Andes. We generated a Bayesian inference phylogeny using MrBayes from this new dataset as well. Details of laboratory sequencing techniques and phylogenetic tree reconstruction can be found in Appendix 1.1.

(b) ancestral area analysis and molecular clock techniques

Using the consensus phylogram from the combined ND2 and cyt-*b* dataset, we reconstructed the ancestral areas of lowland *Mionectes* using maximum parsimony and maximum likelihood ancestral state simulations in Mesquite v1.06 (Maddison & Maddison 2005) with the default maximum likelihood model for character state reconstruction. Terminal taxa were coded as either west or east of the Andes. A likelihood ratio test failed to reject the assumption of a molecular clock ($-2\Delta \ln L = 9.37$, d.f. = 12, $p = 0.67$), so we modified the consensus topology to conform to a molecular clock as implemented in PAUP*.

Because the widely-used 2% Myr^{-1} mtDNA molecular clock rate calibration has not been critically examined in suboscines, following Ribas *et al.* (2007) we

calibrated a relaxed molecular clock (nonparametric rate smoothing [NPRS]:Sanderson 1997) topology for a dataset consisting of the *Mionectes* RAG1 sequences and a variety of RAG1 sequences obtained from GenBank. This provided an independent estimate for the age of the split between montane and lowland *Mionectes* and thus an alternative calibration for the clock-enforced cyt-*b*/ND2 tree. Uncertainty in this alternative calibration was evaluated by bootstrapping the expanded RAG1 data matrix. The NPRS molecular dating analysis is described in further detail in Appendix 1.1.

(c) cross-Andes gene flow

The lack of reciprocal monophyly found between *M. oleagineus* populations in eastern Panama and northern South America, which are bisected by the Andes (figures 1.2 & 1.4), can be due to incomplete lineage sorting or to continued gene flow. To estimate the extent of post-separation gene flow between populations, we fitted a population genetic model of divergence with gene flow using Metropolis-coupled Markov Chain Monte Carlo simulations of the coalescent in IM (Hey & Nielsen 2004). This analysis determined whether the more complex model including post-separation gene flow was a better fit to the data than a model without gene flow, as evaluated by a likelihood ratio test (*per* Vollmer & Palumbi 2002). Several trial runs assuming unrealistic priors helped determine the range of priors for final runs. Final run conditions included an HKY model of molecular evolution, Metropolis coupling involving geometric heating along 10 chains with 10 chain-swap attempts per step, a burn-in of 500,000 steps, and symmetric gene flow between the two populations,

because initial runs showed broad overlap between the 95% highest posterior densities (HPD) for directional migration estimates. We ran the program four times with unique starting seeds to ensure proper convergence of parameter estimates; all runs lasted over 30×10^6 steps, which ensured that lowest effective sample sizes for all parameter estimates were at least an order of magnitude larger that the value (500) suggested by the authors (Hey & Nielsen 2004). We obtained estimates for θ_E and θ_W, which are equal to two times the effective size of females scaled to the mutation rate (e.g., $2N_{ef}\mu$) for the populations east and west of Andes respectively, and m_E and m_W, which represent the migration rate per generation into the respective population. Following Peters *et al.* (2005), we calculated the number of females moving across the Andes per generation as: $N_f = (\theta_E + \theta_W) \times (m_E + m_W)/2$. Because the results from all four runs were similar, we present parameter estimates obtained from the longest run. To visualize relationships among this clade of birds that span the Andes (the YELLOW clade using the nomenclature presented in the figures), we used a haplotype network obtained by statistical parsimony using TCS 1.21 (Clement *et al.* 2000). The resulting network was redrawn by hand.

1.3 RESULTS

Our multi-locus phylogeny recovered all five *Mionectes* species as a monophyletic clade with 100% posterior probability (figure 1.1a). The branch-and-bound ML search recovered an identical topology (not shown) with 100% bootstrap support for a monophyletic *Mionectes*, as did an unpartitioned MrBayes search (not shown). Among

the species sampled, *Leptopogon* and *Corythopis* were the closest outgroups for *Mionectes*. However, these taxa are only distantly related to *Mionectes*: average cyt-*b* pairwise model-corrected distance between these two genera and *Mionectes* was 35.9%. Adopting the commonly-used avian mitochondrial clock of 2% sequence divergence Myr^{-1} or related approximations thereof (Fleischer *et al.* 1998; Weir & Schluter 2004) places the origin of *Mionectes* in the mid-Miocene. Within *Mionectes*, two clades were recovered with 100% posterior probability (100% ML bootstrap), corresponding to the lowland and montane *Mionectes* clades, respectively (figure 1.1a). This split is old: average model-corrected cyt-*b* distance between the montane and lowland *Mionectes* clades was 14.3%, dating to approximately 7 Myr ago.

In the montane species *M. olivaceus*, ND2 sequences revealed two phylogroups in Panama corresponding to an eastern-central clade (including the Darien highlands) and a western clade (Talamanca highlands). The average model-corrected distance between these two clades was 2.0%. Due to a lack of widespread geographic sampling in *M. striaticollis* we have no phylogeographic results for this montane species.

Our broad geographic sampling of ND2 sequences from birds collected throughout the range of the three lowland *Mionectes* species identified a series of strongly supported clades (figure 1.2) with posterior probability nodal support greater than 95% (figure 1.2). *Mionectes rufiventris* was represented by a single mtDNA haplotype clade, whereas the other two, more widespread, lowland taxa showed phylogeographic complexity. *Mionectes macconnelli* was represented by two clades,

corresponding to geographically disjunct populations in southwestern Amazonia and the Guiana Shield. Within *M. oleagineus* we recovered five clades: three exclusively west of the Andes (BLUE, RED, and GREEN clades, figure 1.2), one found east and west of the Andes (YELLOW, figure 1.2), and one exclusively east of the Andes (ORANGE, figure 1.2). For heuristic purposes we refer to each clade by its color in figure 1.2, because mtDNA clades do not correlate well with currently recognized subspecific limits (see below). West of the Andes, the BLUE clade ranged from southeastern Mexico to the northwestern corner of Panama. The RED clade occupied points throughout central Panama, and the GREEN clade was found in the Pacific lowlands of western Ecuador. West of the Andes, the YELLOW clade was found only in eastern Panama, whereas east of the Andes it had a broad distribution north of the Amazon River (Ecuador, Venezuela, Guyana, Trinidad, northern Brazil). The ORANGE clade was the only *M. oleagineus* clade found exclusively east of the Andes, where it was widespread: southwestern Amazonia, Guyana, and the Atlantic Forest of southeastern Brazil.

For *M. oleagineus,* current subspecies do not correlate well with the recovered mtDNA clades. Based on a recent revision of *oleagineus* subspecies (Fitzpatrick 2004), our clades represent the following subspecies: BLUE: *assimilis*, RED: *parcus*, YELLOW: *parcus, abdominalis*, *pallidiventris*, and *oleagineus*, GREEN: *pacificus*, ORANGE: *oleagineus*. Furthermore, in two instances, sampling locations included individuals from more than a single clade. In Panama province (central Panama) we recovered five RED haplotypes and one YELLOW haplotype, while in Iwokarma

Reserve (Guyana) we recovered three ORANGE haplotypes and one YELLOW haplotype (figure 1.2). This broad sampling of ND2 sequences from *M. oleagineus* did not resolve sister relationships among clades in every instance (figure 1.2).

The addition of cyt-*b* sequences to a subsample of birds provided a phylogeny with greatly improved nodal support throughout the tree (figure 1.3), with all bifurcations supported by at least 95% posterior probabilities. Based on this phylogeny, geographically proximate clades were not one another's closest phylogenetic neighbor, and several sister relationships among clades were bisected by the Andes. All of the lineages west of the Andes had a sister lineage found to the east. Both maximum likelihood and maximum parsimony analyses indicated that the ancestral area for lowland *Mionectes* taxa was east of the Andes, requiring a minimum of three cross-Andean biogeographic events. In the clock-enforced maximum likelihood tree, the earliest divergence across the Andes occurred at node A (figure 1.3), roughly 1.9 Myr ago assuming a 2% pairwise divergence rate (Fleischer *et al.* 1998; Weir & Schluter 2004). The other two nodes corresponding to cross-Andean events date to 1.0 Myr ago and 0.2 Myr ago, respectively. For either of these latter events to be coincident with the final uplift of the Andes, the single-lineage rate of mtDNA evolution in *Mionectes* for node B (the second crossing of the Andes) would have to be less than 0.38% Myr^{-1}, and for node C (the third crossing) slower than 0.06% Myr^{-1}. The former is slower than any reported rate for birds and less than half of the typical result for passerines such as *Mionectes* (Lovette 2004), while the latter is nearly an order of magnitude slower than the reported rate of mtDNA evolution for

any vertebrate. Dates for these nodes obtained using NPRS and a RAG1 calibration (see Methods and the electronic supplement) were similar (node A, 1.5 ± 0.4 Myr ago; node B, 0.8 ± 0.2 Myr ago; and node C 0.5 ± 0.1 Myr ago) and give support to the 2% Myr^{-1} mtDNA calibration henceforth used in this paper.

Individuals from the YELLOW clade were found on both sides of the Andes and were not reciprocally monophyletic with respect to the mountains (figure 1.4). Parameter estimates for θ east and west of the Andes and the average migration rate since separation of the eastern Panama and northern South American populations (i.e., θ_E, θ_W, and m) were highly unimodal and similar in all four runs. Posterior distributions peaked at 2.0 (95% HPD: 0.5 – 6.6) for θ_E and 50.2 (95% HPD: 16.4 – 265.2) for θ_W, whereas the posterior distribution of estimates of the scaled migration parameter (m) peaked at 0.5 (95% HPD: 0.1 – 1.7). These parameters yielded a peak value of 6.2 females per generation (N_f) migrating across the Andes, with a range of 0.3 – 115.3 assuming extreme 95% HPD values. Our model, which included cross-Andean migration, was a significantly better fit to the data than a model without post-divergence gene flow across the Andes ($-2\Delta \ln L = 8.65$, d.f. = 1, $p = 0.003$).

1.4 DISCUSSION

Evidence from nuclear and mitochondrial DNA supported the monophyly of the five flycatcher species currently placed in the genus *Mionectes* relative to allied genera (figure 1.1a), consistent with recent classifications (Sibley & Monroe 1990; American Ornithologists' Union 1998; Fitzpatrick 2004; Remsen *et al.* 2007). Genetic distances

between these taxa and putative outgroups is considerable, again in agreement with earlier studies of genetic relationships among *Mionectes* and its allies (Sibley & Monroe 1990; Bates & Zink 1994; Chesser 2004). Within the genus, both mitochondrial and nuclear gene sequences identified a basal phylogenetic split between montane and lowland *Mionectes* species, providing support for earlier classifications that placed the three lowland species in the genus *Pipromorpha* (e.g., Traylor 1977). The model-corrected cyt-*b* mtDNA distance between montane *Mionectes* and lowland *Mionectes* was 14.3%, dating the split between these forms to the late Miocene, or approximately 7 Myr ago.

The montane *Mionectes* group consists of two species that inhabit higher-elevation habitats in South America and southern Middle America: *M olivaceus* can be found in premontane and montane forests, and in the Andes it is replaced at even higher elevations by *M. striaticollis*. Our evidence indicates that the two montane species last shared a common ancestor in the late Miocene or early Pliocene.

Despite only modest geographic sampling of montane *Mionectes* (table 1 found in electronic supplement), some comparisons to phylogeographic patterns in other Neotropical montane bird taxa are possible. The model-corrected ND2 distance between the Darien (eastern Panama) and Talamanca (western Panama) clades of *M. olivaceus* was 2.0%. Across this same geographic span, *Myadestes* solitaires showed identical mtDNA divergence (Miller *et al.* 2007). If we assume a constant rate of mtDNA divergence of approximately 2.0% Myr^{-1}, then both montane *Mionectes* and *Myadestes* in southern Middle America began to differentiate across the Isthmus of

Panama approximately 1.0 Myr ago, well after its Pliocene formation. However, in the *Chlorospingus* bush-tanagers species complex, average pairwise distance between Darien and Talamanca mtDNA clades was nearly 3 times that of montane *Mionectes* and *Myadestes* (approximately 5 – 6%; Weir *et al.* 2008). These comparisons identify central Panama as an important barrier to gene flow of montane Neotropical birds but also suggest that avian lineages have responded differently to regional changes in the Pliocene and Pleistocene landscapes of lower Middle America as the Isthmus of Panama developed (see also Bermingham & Martin 1998).

The lowland and montane *Mionectes* clades are elevational replacements, and where they meet the zones of overlap are narrow. It is worth noting that despite roughly 7 Myr of independent evolution, the montane clade has not diversified to exploit lowland habitats, nor has the lowland clade diversified to exploit montane habitats. We posit that this long history of habitat segregation between montane and lowland *Mionectes* likely arises from ecological interactions between individuals of the two clades. Our hypothesis is supported by the observation that in the Pacific lowlands of Colombia and Ecuador, where lowland *M. oleagineus* is absent, *M. olivaceus,* one of the montane species, ranges down to sea level. Likewise, in areas such as Bolivia and southern Venezuela, where montane *Mionectes* are absent, lowland *M. macconnelli* populations reach elevations above 2000 m (Ridgely & Tudor 1994).

Lowland *Mionectes* are currently classified as three species. However, our mtDNA phylogeny suggests that evolutionary relationships among populations of

these three species are more complex than predicted by current taxonomy (figure 1.3). *Mionectes macconnelli*, which has a disjunct distribution in southwestern Amazonia and the Guiana Shield (figure 1.1b), is polyphyletic: specimens from southern Amazonia form a clade that is sister to all other lowland *Mionectes*, including *M macconnelli* specimens from the Guiana Shield and the Atlantic Forest endemic, *M. rufiventris* (figure 1.3). Also, *M. oleagineus* was recovered as a monophyletic clade with pronounced phylogeographic structure among mtDNA haplotypes (figure 1.3).

The geographic pattern of diversification in lowland *Mionectes* differs from previously published area cladograms for the region and other studies of the diversification of widespread Neotropical organisms (references given in Introduction). Most strikingly, the overwhelming majority of these studies found a basal split across the Andes, whereas lowland *Mionectes* show three cross-Andean divergences near the tips of the phylogeny. When only areas east of the Andes are considered, most studies have found that the deepest divergences split the Atlantic Forest from the Amazon Basin and the Guiana Shield (e.g., Ron 2000). In contrast, the basal split among lowland *Mionectes* separates the southern Amazonian *M. macconnelli* from the rest of the region including the Atlantic forest (figure 1.3), a pattern most similar to that observed for howler monkeys (*Alouatta* spp.; Cortes-Ortiz *et al.* 2003). Finally, nearly all previous studies have shown a sister relationship between northern and southern clades in western Amazonia (e.g., Cracraft & Prum 1988; Ron 2000). This was not the case in lowland *Mionectes* for either *M. macconnelli* or *M oleagineus* (figure 1.3).

The *Mionectes* mtDNA phylogeny (figure 1.3) provides strong inference that *M. oleagineus* has diversified across the Andes at least three times over the course of its evolutionary history. The earliest separation of *M. oleagineus* populations on either side of the Andes (node A, figure 1.3) might represent vicariance associated with the final uplift of the northern Andes. Assuming typical rates of passerine mtDNA evolution, these populations split approximately 1.9 Myr ago, about the same time the northern Andes reached their current elevation (Gregory-Wodzicki 2000). The other two splits within *M. oleagineus* occurred at more recent nodes on the clock-enforced phylogram (nodes B & C, figure 1.3). Forcing the date of the splits represented by nodes B & C to be coincident with the northern Andean uplift would imply unreasonably slow rates of mtDNA evolution (see Results). Thus, the two later splits between *M. oleagineus* populations on either side of the Andes must necessarily represent dispersal over or around the mountains.

Haffer (1967) proposed two alternative mechanisms for gene flow across the Andes following their final uplift. The first was via dispersal over low passes in the northern Andes (first suggested by Chapman 1917), and the second was through ephemeral forest corridors during Quaternary interglacials along the northern coast of South America. These hypothetical forest corridors passed through regions currently characterized by grassland and savannah ecosystems and might have facilitated the dispersal of forest-dwelling organisms between lowland populations east and west of the Andes. Although our mtDNA phylogenies cannot rule out either scenario, several

observations suggest dispersal over Andean passes rather than around the northern cordilleras for the splits represented by nodes B & C (figure 1.3).

As noted, the upper elevational limit for lowland *Mionectes* in the Andes may be due to competition with montane *Mionectes* rather than to physiological limits. Where highland congeners are absent, lowland *M. oleagineus* reach over 2000 m elevation, which is nearly the elevation of the lowest Andean passes. In the split at node B (figure 1.3), ancestral area analysis suggests that birds from southwestern Amazonia or the Guiana Shield colonized lowlands west of the Andes (figure 1.3). One possible route for this colonization is through the Marañon Valley in northern Peru, which is the lowest Andean pass between Venezuela and Bolivia (2140 m), and which was previously suggested as a dispersal corridor for many Amazonian taxa into a semi-humid area of endemism west of the Andes in northern Peru (Chapman 1917). While this would be the most direct route between southwestern Amazonia and the lowlands west of the Andes, this hypothesis requires the RED clade to have moved through regions along the Pacific slope of South America that are currently occupied by representatives of the GREEN clade (figure 1.2). In the most recent split (node C, figure 1.3), it is more difficult to determine whether *M. oleagineus* dispersed around or over the Andes. Tissues from northern Colombia and northwestern Venezuela were unavailable for this study, but the subspecies there is *M. o. parcus*, the same that occurs in eastern Panama (Fitzpatrick 2004). This alone provides little evidence to discern between the two routes, because the ranges of many bird species extend from Panama into this region without occurring in the Amazon basin (Chapman 1917).

Furthermore, individuals from northwestern Amazonia are genetically more similar to birds from eastern Panama than to those from the coast of north-central Venezuela and Trinidad (figure 1.4). Finally, the shortest dispersal route between northwestern Amazonia and eastern Panama is the Andalucia Pass into central Colombia (Chapman 1917), providing additional evidence that the most recent dispersal event also occurred over rather than around the Andes.

However, several observations suggest that dispersal around the Andes is a reasonable alternative. Under current climatic conditions, the shortest low-elevation route around the Andes is interrupted by extensive stretches of ocean, *llanos*, and arid scrublands in the Caribbean lowlands north and east of the Andes (Eva *et al.* 2002). But habitats during the Pleistocene in northern South America probably differed from current conditions. Conditions in the South American lowlands east of the Andes during the Pleistocene were generally cooler (Colinvaux *et al.* 2000) and wetter (Baker *et al.* 2001) than at present. Pollen records from the Colombian *llanos* suggest that savannah persisted as far back as the last glacial maximum (LGM), but no earlier data exist (Behling & Hooghiemstra 1999). However, pollen evidence from the Gran Sabana, a grassland east of the Colombian *llanos*, indicates that trees typical of contemporary premontane cloud forests were replaced by expanding savannah coincident with the onset of the Holocene (Rull 2007). If mesic forest occurred in currently arid areas, dispersal around the tip of the northern Andes would be facilitated by relatively low passes in the northern Cordillera.

Our coalescent simulations indicate that gene flow between the most recently separated populations of *M. oleagineus* in eastern Panama and northern South America may be ongoing or episodic. Estimates indicate that the rate of female dispersal across the Andes between these populations is at least 0.3 individuals per generation (95% highest posterior density: 0.3 – 115 females/generation). Furthermore, a coalescent model including post-dispersal gene flow across the Andes was a significantly better fit to the data than a model without migration. Because no lowland forest corridor currently connects Amazonia and Middle America, the coalescent simulations argue for some gene flow across the Andes.

How common is cross-Andean dispersal? Several studies of lowland birds have provided phylogenetic hypotheses discounting its importance (Brumfield & Capparella 1996; Bates *et al.* 1998; Cracraft & Prum 1988; Prum 1988; Ron 2000; Brumfield *et al.* 2001). An exception occurs in the lowland forest woodcreeper *Glyphorynchus spirurus*, in which Middle American populations nest phylogeographically within a northern Amazonian clade, perhaps due to Quaternary dispersal around the Andes (Marks *et al.* 2002). Two studies of bats have also shown lack of reciprocal monophyly in DNA lineages on either side of the Andes, which the authors attributed to post-uplift gene flow across the Andes (Ditchfield 2000; Hoffman & Baker 2003). Finally, Dick *et al.* (2004) reported phylogenetic evidence of recent cross-Andean dispersal in two groups of Euglossine bees. In sum, these studies indicate that cross-Andean movement by lowland species may be more frequent than

previously assumed. However, *M. oleagineus* stands out in the repeated role that the Andes have played in its phylogeographic differentiation.

The evolutionary history of *M. oleagineus* is also striking in the geographic pattern of populations west of the Andes. Descendants of the first cross-Andean split (figure 1.2 & 1.3, the BLUE and GREEN clades) show the broadest distribution, extending from southeastern Mexico to western Panama and western Ecuador. The second cross-Andean split, which must be a dispersal event, is evident in a population that is currently found only in central and parts of western Panama (the RED clade), where it abuts the range of the BLUE clade (figure 1.2). Whether the RED clade has displaced the BLUE clade or has simply colonized a region unoccupied by BLUE clade conspecifics cannot be discerned from our data. One presumes that the ancestor of the BLUE and GREEN clades was once continuously distributed in the lowlands west of the Andes, but the level of phylogeographic divergence between the western Ecuador (GREEN) and northern Middle America (BLUE) haplotypes suggests their separation, and perhaps local extinction on the Isthmus of Panama might have predated colonization by the RED clade. The most recent colonization episode by *M. oleagineus* west of the Andes ushered in the YELLOW mtDNA clade, which has the narrowest trans-Andean distribution of the three western clades, being restricted to eastern Panama (and probably part of northern Colombia).

In both eastern and western Panama, our data suggest relatively narrow zones of transition between mtDNA lineages. About 125 km separate our eight specimens (100% RED haplotypes) from Santa Fe, Veraguas and our 22 specimens (100% BLUE

haplotypes) from Bocas del Toro. Likewise, less than 250 km separate our sampling sites from eastern Darien province (18 individuals, 100% YELLOW haplotypes) and our easternmost site in central Panama (five of six specimens had RED haplotypes). We found no evidence of mixing of BLUE and RED mtDNA haploytpes, despite the fact that the numbers of *M. oleagineus* collected near the zone of contact (22 and 11 individuals respectively) between the two mtDNA haplotype clades was sufficient to provide an 82% probability of observing mixing occurring at a frequency of 5% or greater ($p = 1 - [0.05^{(22+11)}] = 0.82$). However, On the other hand, we did collect one YELLOW clade bird near at the eastern edge of the range of RED haplotypes. It is worth noting that the Caribbean slope of Panama in the region of both of these putative contact zones is continuously forested.

The apparently parapatric distributions of three mtDNA clades of *M. oleagineus* in Panama evoke several unanswered questions: What explains the lack of geographic overlap? Is secondary contact recent, or has demographic inertia retarded replacement of one clade by another (Reeves & Bermingham 2006)? Is Haldane's rule operating to retard female-mediated gene flow (females are the heterogametic sex in birds)? Finally, are the mtDNA clades cryptic species, with parapatry enforced through competitive exclusion? Only further study will resolve these issues.

The phylogeographic relationships in *M. oleagineus* provide an alternative model for the role of the Andes in the biogeography of lowland Neotropical animals. The area-cladogram approach to Neotropical biogeography has suggested that the Andes was an early barrier to lowland taxa, and rarely, if ever, transgressed by

descendants on either side. Our data showing episodic dispersal across (or around) the Andes suggests that these mountains can play a more persistent role in Neotropical biogeography and diversification.

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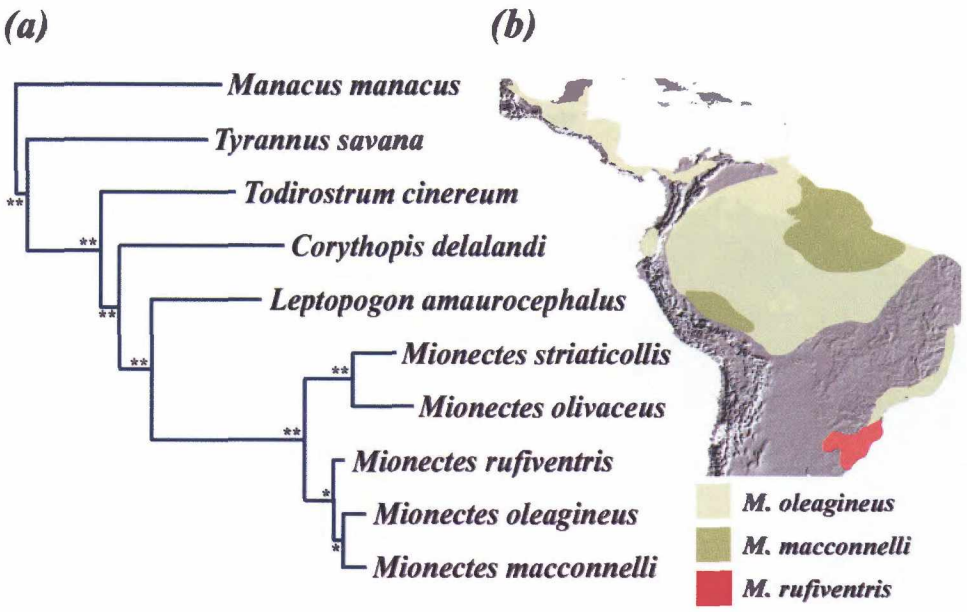

Figure 1.1. a) Relationship of *Mionectes* and related genera determined by Bayesian inference using partial sequences of the mtDNA cytochrome-*b* gene (999 bp) and the nuclear exons RAG-1 (930 bp) and *c-myc* (477 bp). The monophyly of *Mionectes* and a basal split between montane and lowland clades are strongly supported. b) Distribution map for the three lowland *Mionectes* species; two additional species, *M. olivaceus* and *M. striaticollis*, are found in montane habitats in the Andes and southern Middle America and are not depicted.

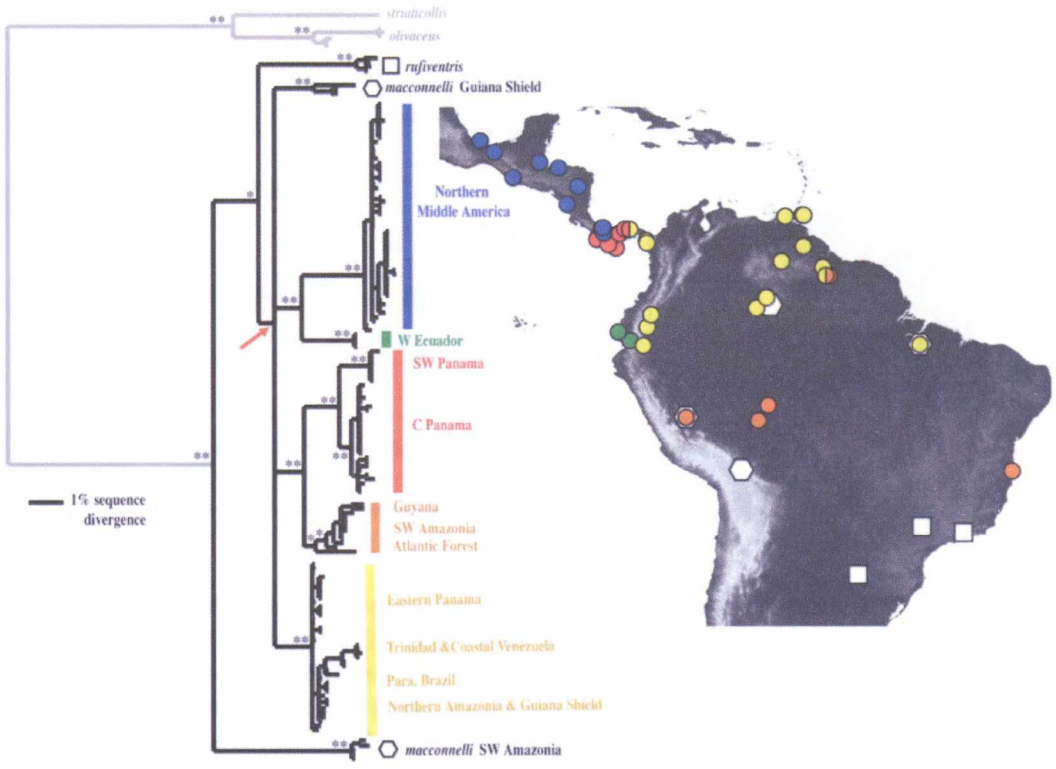

Figure 1.2. Bayesian phylogeny for 163 *Mionectes* flycatchers (156 lowland and 7 highland birds) based on complete ND2 sequences. Posterior probabilities for bifurcations indicated at node: double asterisk: 100%, single asterisk: > 95% (omitted from most terminal nodes for clarity). Internal nodes with less than 95% posterior probabilities were collapsed, but terminal nodes with less than 95% pp support were retained. The red arrow shows an unresolved polytomy (see text). The map shows localities for 156 lowland *Mionectes* color-coded to correspond to the major clades at left. Two sites (central Panama and Guyana) are bicolored to indicate two mtDNA clades at these locations. Circles are *M. oleagineus*, white hexagons *M. macconnelli*, and white squares *M. rufiventris*. Four *M. oleagineus* clades occur west of the Andes: the BLUE clade (northern Middle America), the GREEN clade (western Ecuador), the RED clade (central and southwestern Panama), and the YELLOW clade (eastern Panama). The YELLOW clade also occurs east of the Andes across northern South America. The ORANGE clade occurs exclusively east of the Andes. Within the YELLOW clade there is no reciprocal monophyly between samples from either side of the Andes (see figure 1.4).

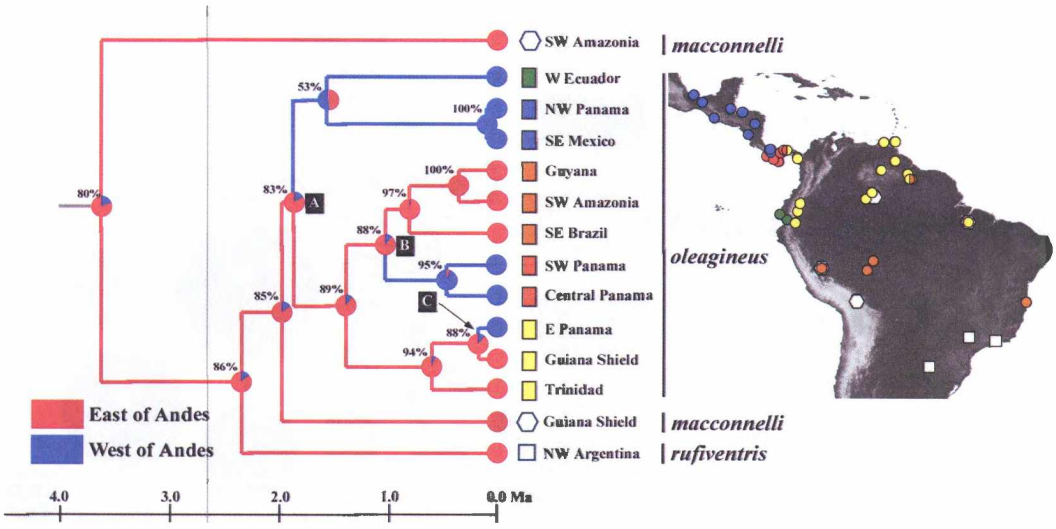

Figure 1.3. Ancestral area reconstruction for lowland *Mionectes* flycatchers. The phylogenetic tree represents the consensus Bayesian inference topology obtained from cytochrome-*b* and ND2 sequences (2184 bp) modified to conform to an enforced molecular clock (see text). Posterior probabilities of all nodes were 100% except node A (98%). Branch color reflects the most parsimonious state (east or west of the Andes) for that branch, while colored circles at nodes represent relative likelihoods of each state. For *M. oleagineus*, color coding follows figure 1.2 (see also inset map). Both parsimony and likelihood reconstructions indicate three cross-Andean biogeographic events at nodes A, B, and C. Scale bar represents millions of years before present assuming a rate of mtDNA diversification of 2.0% Myr^{-1} (Fleischer *et al.* 1998). The vertical grey line at 2.7 Myr before present indicates completion of uplift in the northern Andes (Gregory-Wodzicki 2000).

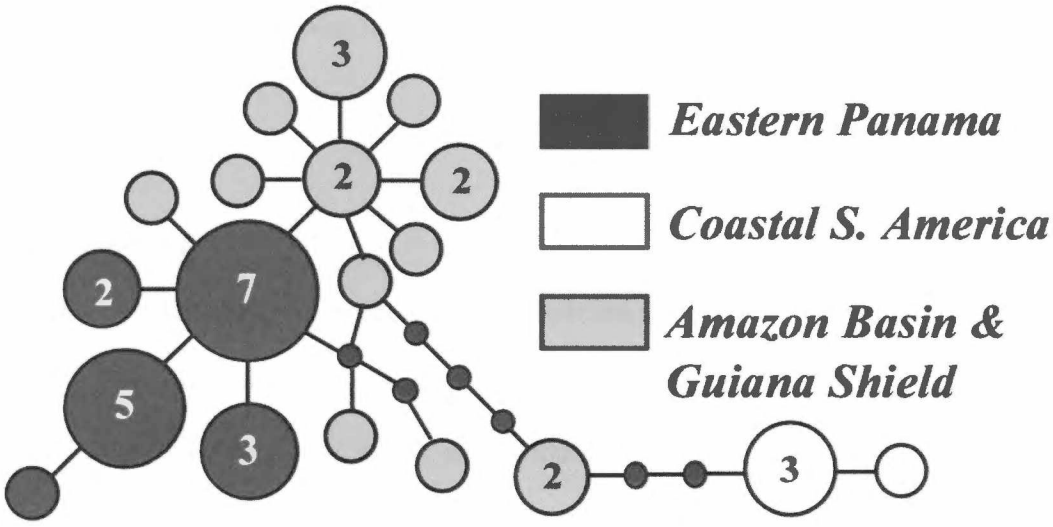

Figure 1.4. Statistical parsimony-based haplotype network for the YELLOW clade (see figure 1.2) of *M. oleagineus* showing incomplete lineage sorting between populations east and west of the Andes. Black dots (smallest circles) indicate unobserved haplotypes; larger circle sizes indicate haplotype frequencies. Birds from eastern Panama (west of the Andes) are more closely related to birds from the Amazon basin and the Guiana Shield than from coastal South America, which provides some evidence for dispersal over rather than around the Andes.

APPENDIX 1.1. ADDITIONAL DETAILS ON LABORATORY AND PHYLOGENETIC METHODS.

(a) laboratory protocols:

For all three datasets gene products were amplified and sequenced from total genomic DNA as described in previous studies: RAG-1 and *c-myc* (Irestedt *et al.* 2001) and ND2 and cyt-*b* (Miller *et al.* 2007), except that MION-525 TTCTCATCCATCTCCCATCTWGG was used as an internal sequencing primer for ND2. Sequences were aligned using Sequencher v4.1– 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA) without the presence of insertions or deletions; putatively heterozygotic sites in nuclear sequences were coded with the appropriate ambiguity code.

Dataset 1:

We took advantage of the fact that Johansson *et al.* (2002) had sequenced the mitochondrial cytochrome-*b* gene (cyt-*b,* 999 basepairs [bp]) and partial fragments of two nuclear, single-copy, protein-coding genes (RAG-1, 930 bp; *c-myc* 477 bp) for five outgroup species (Johansson *et al.* 2002): *Manacus manacus* (GenBank accession numbers: AF453787, AF453801, AF453818), *Tyrannus savanna* (AF295182, AF295203, AF453813), *Corythopis delalandi* (AF453779, AF453792, AF453805), *Todirostrum cinereum* (AF453782, AF453796, AF453809), and *Leptopogon amaurocephalus* (AF453781, AF453795, AF453808). We sequenced a single individual of the five currently recognized *Mionectes* species for these same genes.

For each phylogenetic analysis we identified the best-fitting likelihood model for the given dataset using likelihood scores from PAUP* v.4.0b10 (Swofford 2002) in ModelTest 3.7 (Posada & Crandall 1998), implementing the Akaike Information Criterion (AIC) to compare models. In each case, the best model was compared to more complex partitioned models using AIC. For all three analyses, we used Bayesian inference of phylogeny as implemented in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003), although in specific cases additional analyses were done using PAUP* v4.10b (Swofford 2002). Details are provided below.

For the phylogeny of *Mionectes* and outgroups using the combined nuclear and mitochondrial gene dataset (mtDNA cyt-*b* and nuclear RAG-1 and *c-myc*) a model with a separate partition and cyt-*b* partitioned by codon position was a better fit to the data than either the best-fitting unpartitioned model selected by ModelTest (GTR+I+ Γ) or a model without cyt-*b* codon-site partitions (AIC: 13248.4 vs. 14265.7 and 13701.9, Akaike weight of best model > 0.999). Following partition-specific results from ModelTest, all partitions had six-substitution parameters with a proportion of sites invariable; the three cyt-*b* parameters also had partition-specific gamma shape parameters. A Bayesian search with the above parameter settings was implemented in MrBayes v3.1 (Ronquist & Huelsenbeck 2003) and run twice for 2×10^6 generations, sampling every 200 generations. The burn-in was determined to be the first 8000 generations; at the termination of the run, the standard deviation of split frequencies (σ_{SF}) < 0.01. We confirmed the results of this analysis with a branch and bound maximum likelihood (ML) search executed in PAUP* with 100 bootstraps replicates

(performed using a heuristic search) and an unpartitioned MrBayes search. For molecular clock estimates, cyt-*b* GTR+I+Γ model-corrected distances were calculated in PAUP*.

Dataset 2:

To further resolve phylogenetic and phylogeographic variation within lowland *Mionectes*, we added to the ND2 sequences from figure 1.1 sequenced the entire mitochondrial ND2 gene for additional birds from widespread geographic origins within their respective ranges: two more *M. rufiventris*, seven more *M macconnelli*, and 142 more *M oleagineus*. To this dataset we added one GenBank sequence each from *M. rufiventris* and *M. oleagineus* (Tello and Bates 2007). For an outgroup, we sequenced five additional montane *Mionectes* to add to the single individual of *M. olivaceus* and *M. striaticollis* from dataset 1 (see figure 1.2 and Appendix 1.2). For this dataset, a site-specific model was a better fit to the data than the best model selected by ModelTest (AIC: 7344.7 vs. 7486.8, Akaike weight of best model > 0.999). We ran MrBayes twice for 8×10^6 generations using six chains with sampling every 1000 generations, and the burn-in period was determined to be the first 60,000 generations ($\sigma_{SF} < <0.001$).

Dataset 3:

Although the previous analysis showed strong support for nodes near the tips of the phylogeny, some interior nodes were not strongly supported. To test the validity of these nodes, we selected one individual from each major clade identified and sequenced the entire cyt-*b* mitochondrial gene to create a new mtDNA dataset that

combined this gene with the ND2 sequence from the previous analysis. For the clade comprising individuals from eastern Panama and northern South America we included one individual from each side of the Andes. As for the previous dataset we ran MrBayes on this new, two-gene data set. We found that a site-specific rate-variation model was a better fit than the best unpartitioned model (GTR+Γ; AIC: 9708.0 vs. 9993.8, Akaike weight of best model > 0.999). The partitioned dataset was run twice on MrBayes for 4×10^6 generations, with sampling every 1000 generations, and the burn-in was determined to be the first 10,000 generations ($\sigma_{SF} << 0.001$). Using the resulting consensus phylogram, we reconstructed the ancestral areas of lowland *Mionectes* using maximum parsimony and maximum likelihood ancestral state simulations in Mesquite v1.06 (Maddison & Maddison 2005) with the default maximum likelihood model for character state reconstruction. A likelihood ratio test failed to reject the assumption of a molecular clock ($-2\Delta \ln L = 9.37$, d.f. = 12, $p = 0.67$), so we modified the consensus topology to conform to a molecular clock as implemented in PAUP* (Swofford 2002). The terminal taxa for this analysis represented the eight geographically structured populations recovered in the phylogeographic analysis (see results) and were coded as either west or east of the Andes.

(b) non-parametric rate-smoothing:

We began by creating a RAG1 dataset from published passerine sequences as well as the 10 previously used *Mionectes* and outgroup sequences. New taxa added (with GenBank accession numbers) were:

Acanthisitta chloris (AY056975)
Eurylaimus ochromalus (DQ320622)
Pitta guajana (DQ320611)
Calyptomena whiteheadi (DQ320607)
Sapayoa aenigma (DQ320609)
Smithornis capensis (DQ320608)
Cercomacra melanaria (AY065752)
Conopophaga ardesiaca (AY443271)
Dendrocincla fuliginosa (AY065742)
Myiarchus tyrannulus (AF453798)
Tityra semifasciata (AY443337)
Phainoptila melanoxantha (AY307204)
Entomodestes leucotis (AY307190)
Regulus calendula (AY057028)

Acanthisitta chloris was fixed as a monophyletic outgroup to the rest of the taxa, because it was identified as the basal lineage within the passerine radiation.

The GTR+I+Γ model was identified using ModelTest v3.7 (Posada & Crandall 1998) as the best fitting model to this dataset using Akaike Information Criterion. However, a model partitioned by codon was a better fit to the data (AIC: 6665.2 vs. 6765.0). We completed a Bayesian search using the partitioned dataset and the above likelihood setting in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003); the search was run twice for 4 X 10^6 generations with sampling every 1000 generations. The first 3000 generations were discarded as burn-in, and a consensus topology was created with the remaining sampled generations; standard deviation of split frequencies was > 0.005.

We used non-parametric rate smoothing (Sanderson 1997) to transform the consensus topology into an ultrametric tree (i.e., all tip equally distant from the root node) as implemented in TreeEdit v1.0a10 (Rambaut & Charleston 2002), with the rate smoothing across all nodes with a mean. Following several previous studies (Barker *et al.* 2004; Ribas *et al.* 2007), we calibrated this tree by dating the split between *Acanthisitta* and the remaining taxa at 82 Myr ago, which provided us with an estimate for the split between lowland and montane *Mionectes*. We calibrated our cyt-

b/ND2 ultrametric topology (figure 1.3) using this date. Subsequently, we had estimated dates for all nodes in question.

In order to assess the confidence in these estimates, we bootstrapped the data matrix in PAUP* (Swofford 2002) while enforcing the consensus topology as a topological constraint and turning off the swapping function. This allows us to use resampling to explore changes in branch length while maintaining the topology fixed. The data were bootstrapped 100 times, and the 82 Myr calibration was undertaken on each replicate, with similar rescaling of the *Mionectes* cyt-*b*/ND2 splits.

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APPENDIX 1.2. SPECIMENS AND TISSUE SAMPLES USED IN THIS STUDY, WITH CORRESPONDING GENBANK ACCESSION NUMBERS.

Some geographic coordinates were estimated from specimen label information. All individuals represent museum vouchers except those indicated with an asterisk; specimens indicated with § were obtained from Tello & Bates (2007). AMNH: American Museum of Natural History (New York, USA); ANSP: Academy of Natural Sciences (Philadelphia, USA); CNAV: Colección Nacional de Aves, Instituto de Biología, Universidad Nacional Autónoma de México (Mexico City, Mexico); CU: Cornell Museum of Vertebrates (Ithaca, USA); FMNH: Field Museum of Natural History (Chicago, USA); LGEMA: Laboratório de Genética e Evolução Molecular de Aves, Universidade de São Paulo (Sao Paulo, Brazil); LSUMZ: Louisiana State University Museum of Zoology (Baton Rouge, USA); MBM: Marjorie Barrick Museum (Las Vegas, USA); NMNH: National Museum of Natural History; Smithsonian Tropical Research Institute (Balboa, Panama); UAM: University of Alaska Museum (Fairbanks, USA); MUSM: Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (Lima, Peru) (tissues available at UAM); MZUSP: Museu de Zoologia da Universidade de São Paulo (Sao Paulo, Brazil).

Specimen	Location	ND2	Cyt-*b*	RAG-1	c-myc
Mionectes olivaceus (6)					
UAM 20307	Panama: Coclé, Molejón (8°47'19"N 80°32'28"W)	EF110694	EF110844	EF110866	EF110861
MBM JK06-227	Panama: Bocas del Toro, Palo Seco I (8°47'36"N 82°11'20"W)	EF110695			
MBM JK06-265	Panama: Bocas del Toro, Palo Seco II (8°47'N 82°13'W)	EF110696			
UAM 24014	Panama: Chiriqui, Alto Chiquero (8°51'06"N 82°29'48"W)	EF110697			
NMNH B17566	Panama: Darien, Rancho Frio (8°01'12"N 77°43'54"W)	EF110699			
LSUMZ B46628	" " " " "	EF110698			
Mionectes striaticollis (1)					
AMNH CJV 27	Bolivia: La Paz, Nor Yungas (16°13'28"S 67°48'03"W)	EF110693	EF110843	EF110865	EF110860
Mionectes macconnelli (8)					
AMNH PEP1975	Venezuela: Amazonas, Tamaquari (1°13'N, 64°42'W)	EF110703	EF110845	EF110869	EF110864
LGEMA P1249	Brazil: Pará, Tailândia (2°57'S, 48°57'W)	EF110704			
LGEMA P1247	" " " " "	EF110705			
AMNH RIS 62	Bolivia: La Paz, Nor Yungas, (16°13'28"S 67°48'03"W)	EF110706			
AMNH MV15	" " " " "	EF110707			
MUSM 26534	Peru: Ucayali, Centro Pucani (10°40'23"S, 73°34'38"W)	EF110709	EF110846		
UAM 21799	" " " " "	EF110708			
UAM 22103	" " " " "	EF110710			
Mionectes oleagineus (133)					
CNAV PEP2609	Mexico: Veracruz, Los Tuxtlas Biol. St. (18°35'N 95°05'W)	EF110711			
UAM 21109	" " " " "	EF110712	EF110849		
CNAV PEP2905	" " " " "	EF110713			
CNAV 24310	" " " " "	EF110714			
CNAV PEP2799	" " " " "	EF110715			
CNAV PEP2313	" " " " "	EF110716			
CNAV 24309	" " " " "	EF110717			
CNAV 24311	" " " " "	EF110718			
UAM 20867	" " " " "	EF110719			
CNAV 24308	" " " " "	EF110720			
CNAV 24989	Mexico: Tabasco, Huimanguillo, (17°20'N, 96°36'W)	EF110721			
CNAV 24990	" " " " "	EF110722			
CNAV 24991	" " " " "	EF110723			
UAM 7908	Belize: Toledo, Big Falls (16°15'N 88°52'W)	EF110724			
UAM 7911	" " " " "	EF110732			
UAM 7912	" " " " "	EF110733			
UAM 7933	" " " " "	EF110725			
UAM 9573	" " " " "	EF110726			
UAM 10266	" " " " "	EF110727			
UAM 14310	" " " " "	EF110731			
UAM 14328	" " " " "	EF110728			
UAM 14494	" " " " "	EF110730			
UAM 15426	" " " " "	EF110729			
MBM 10464	Guatemala: Quetzaltenango (14°39.8'N, 91°36.6'W)	EF110734			
MBM 10465	" " " " "	EF110735			

Specimen	Location	ND2	Cyt-*b*	RAG-1	c-myc
STRI HA-MOL27*	Honduras: Atlántida, La Ceiba (15°41'21"N, 86°54'00"W)	EF110736			
STRI HA-MOL47*	" " " " "	EF110737			
STRI HA-MOL75*	" " " " "	EF110738			
STRI-HA-MOL85*	" " " " "	EF110739			
STRI-HA-MOL90*	" " " " "	EF110740			
MBM 4464	Nicaragua: Atlántico Norte, Rio Uli (13°42.1'N, 84°51.1'W)	EF110741			
MBM 4460	Nicaragua: Granada, (11°50.46'N, 85° 59.69'W)	EF110742			
LSUMZ B58126	Panama: Bocas del Toro, Almirante (9°18'21"N, 82°25'13"W)	EF110748			
LSUMZ B58103	" " " " "	EF110750			
STRI IJL 067	Panama: Bocas del Toro, Chalite (8°51'31"N, 82°13'38"W)	EF110749			
STRI IJL 089	" " " " "	EF110743			
STRI IJL 090	" " " " "	EF110747			
STRI IJL 091	" " " " "	EF110756			
CU 51281	Panama: Bocas del Toro, La Gloria (8°59'18"N, 82°13'00'W)	EF110744			
STRI JTW 204	Panama: Bocas del Toro, Risco (9°13'20"N, 82°24'40"W)	EF110746			
STRI JTW 210	" " " " "	EF110745	EF110850		
CU 51263	" " " " "	EF110751			
STRI JTW 236	" " " " "	EF110752			
MBM JK06-147	Panama: Bocas del Toro, Palo Seco I (8°47'36"N 82°11'20"W)	EF110753			
MBM JK06-158	" " " " "	EF110754			
MBM JK06-179	" " " " "	EF110755			
MBM JK06-154	" " " " "	EF110757			
MBM GMS2009	" " " " "	XXXXXX			
MBM JMD771	" " " " "	XXXXXX			
MBM JMD773	" " " " "	XXXXXX			
UAM 24000	Panama: Bocas del Toro, Isla Colón (9°21'07"N, 82°15'22"W)	EF110758			
UAM 22790	" " " " "	EF110759			
UAM 24001	" " " " "	EF110760			
UAM 24004	" " " " "	EF110761			
UAM 22792	Panama: Chiriquí, Burica (8°06'22"N, 82°53'13"W)	EF110764			
UAM 22793	" " " " "	EF110762	EF110854		
STRI JTW 070	" " " " "	EF110763			
MBM 16698	Panama: Veraguas, Restingue (7°14'30"N 80°54'20")	EF110765			
MBM 16699	" " " " "	EF110766			
MBM JMD 163	" " " " "	EF110767			
MBM 14977	Panama: Veraguas, Santa Fe (8°34'38"N 81°06'59"W)	EF110768			
MBM 14978	" " " " "	EF110769			
MBM 15707	" " " " "	EF110770			
MBM 15591	" " " " "	EF110771			
MBM 15609	" " " " "	EF110772			
UAM 22794	" " " " "	EF110775			
UAM 22795	" " " " "	EF110773			
UAM MJM 2330	" " " " "	EF110774			
LSUMZ B46651	Panama, Veraguas, Isla Coiba (7°36'00"N 81°43'24"W)	EF110776			
UAM 20378	Panama: Coclé, Molejón (8°47'19"N 80°32'28")	EF110780			
UAM 20447	Panama: Coclé, Cerro Moreno (8°45'N 80°32'W)	EF110777			
UAM 20458	" " " " "	EF110785			
UAM 20468	" " " " "	EF110781			
UAM 20467	" " " " "	EF110783			
UAM 19457	Panama: Panamá, Cerro Azul (9°12'"00N 79°29'36")	EF110782	EF110851		
UAM 20494	" " " " "	EF110779			
UAM 21777	" " " " "	EF110784	EF110855		
UAM 19456	" " " " "	EF110778			
UAM MJM 437	" " " " "	EF110786			
UAM JMM 904	" " " " "	EF110788			
STRI MOL-PA24*	Panama: Colon, Old Gamboa Road (9°05'46"N 79°40'52")	EF110787			
UAM 24011	" " " " "	EU433851			
UAM 24015	" " " " "	EU433852			
MBM 15827	Panama: Colon, Achiote Road (9° 12.37'N, 79° 59.56'W)	EU433853			
MBM 15484	" " " " "	EU433854			
MBM 15485	" " " " "	EU433855			
MBM 15486	" " " " "	EU433856			
MBM 15826	" " " " "	EU433857			
MBM 16111	" " " " "	EU433858			
NMNH B17538	Panama: Darien, Rancho Frio (8°01'12"N 77°43'54"W)	EF110789	EF110856		

Specimen	Location	ND2	Cyt-*b*	RAG-1	c-myc
NMNH B17544	" " " " "	EF110790			
LSUMZ B46565	" " " " "	EF110791			
LSUMZ B46546	" " " " "	EF110792			
LSUMZ B46588	" " " " "	EF110793			
LSUMZ B46589	" " " " "	EF110794			
LSUMZ B46598	" " " " "	EF110795			
UAM JMM 945	Panama: Darien, Cana (7°45'11"N 77°40'32"W)	EF110797			
UAM 24012	" " " " "	EF110798			
UAM 24009	" " " " "	EF110804			
UAM 22800	" " " " "	EF110796			
UAM 22802	" " " " "	EF110800			
UAM 22801	" " " " "	EF110801			
UAM 22797	" " " " "	EF110799			
UAM 22798	" " " " "	EF110803			
UAM 24010	" " " " "	EF110806			
UAM 22799	" " " " "	EF110802			
UAM 22796	" " " " "	EF110805			
STRI TR-MOL12*	Trinidad: Simla Research Station (10°42'13"N 61°21'31"W)	EF110807	EF110858		
STRI TR-MOL13*	Trinidad: Hollis Reservoir (10°41'30"N 61°11'19"W)	EF110808			
STRI TR-MOL14*	" " " " "	EF110809			
STRI VE-MOL1*	Venezuela: Sucre, Guaraunos (10°33'48"N 63°7'29"W)	EF110816			
AMNH SC 811	Venezuela: Amazonas, Cerro La Neblina (00°55'N 66°10'W)	EF110817			
AMNH GFB 2231	Venezuela: Amazonas, Mavaca Base Camp (2°2'N 65°7'W)	EF110818			
AMNH GFB 2227	" " " " "	EF110819			
AMNH ROP 250	Venezuela: Bolivar, 40 km E Tumarenco (7°23'N 61°13'W)	EF110820	EF110857		
AMNH ROP 213	" " " " "	EF110822			
AMNH CJW 56	Venezuela: Bolivar, Río Carapo, (5°49'N, 63°32'W)	EF110821			
ANSP 8571	Guyana: Iwokrama, Siparuni River (5°12'N 59°10'W)	EF110823	EF110853		
ANSP 7904	Guyana: Iwokrama, Essequibo River (4°17'N 58°31'W)	EF110824			
ANSP 7742	Guyana: Iwokrama, (4°20'N, 58°51'W)	EF110825			
ANSP 7652	" " " " "	EF110826			
STRI EC-MOL1*	Ecuador: Napo, Jatun Sacha (1°04'33"S 77°39'15"W)	EF110827			
STRI EC-MOL2*	" " " " "	EF110828			
STRI EC-MOL3*	" " " " "	EF110829			
ANSP 5904	Ecuador: Sucumbios, 20 km NE Lumbaqui (0°15'N 77°15'W)	EF110830			
ANSP 5870	" " " " "	EF110831			
ANSP 1378	Ecuador: Morona-Santiago, Santiago (3°03'S, 78°03'W)	EF110832			
ANSP 1406	" " " " "	EF110834			
ANSP 1436	" " " " "	EF110833			
ANSP 3615	Ecuador: Cañar, Manta Real, Zhucay (2°30'S 79°25'W)	EF110812			
ANSP 3111	Ecuador: Manabí, Machililla (1°35'S 80°40'W)	EF110813			
ANSP 3151	" " " " "	EF110814	EF110848		
ANSP 3114	" " " " "	EF110815			
MUSM 25356	Peru: Ucayali, Centro Pucani (10°40'23"S, 73°34'38"W)	EF110835	EF110852	EF110868	EF110863
MUSM 25395	" " " " "	EF110836			
MUSM 26533	" " " " "	EF110838			
MUSM 26532	" " " " "	EF110840			
UAM 21796	" " " " "	EF110837			
UAM 23946	" " " " "	EF110839			
FMNH 39117[§]	Bolivia: Beni, Hacienda Los Angeles, (11°00'S, 66°00'W)	DQ294553			
LGEMA P1244	Brazil: Rondônia, E.B. A. Mujica Nava (9°24'S, 64 °56'W)	EF110841			
LGEMA P1248	Brazil: Pará, Tailândia (2°57'S, 48°57'W)	EF110810			
LGEMA P1250	" " " " "	EF110811			
MZUSP 76289	Brazil: Bahia, Porto Seguro, E. Veracruz. (16°20'S, 39°10'W)	EF110842	EF110859		
Mionectes rufiventris (4)					
AMNH RTC 327	Argentina: Misiones, San Ignacio (27°16'S, 55°32'W)	EF110701	EF110847	EF110867	EF110862
FMNH 395477[§]	Brazil: São Paulo, Boracéia (22°11'35"S, 48°46'44"W)	DQ294555			
LGEMA P1245	Brazil: São Paulo, Bananal (22°48'S, 44°22'W)	EF110700			
LGEMA P1246	" " " " "	EF110702			

CHAPTER 2: NEOTROPICAL BIRDS SHOW A HUMPED DISTRIBUTION OF GENETIC DIVERSITY ALONG A LATITUDINAL TRANSECT[1]

ABSTRACT.– Recent ecological genetic theory predicts that the species richness of a community and the within-population genetic diversity of members of that community should be correlated. Empirical evidence for this model, the 'species-genetic diversity correlation' (SGDC; Vellend 2005), comes from several studies showing that within-population genetic diversity increases with decreasing latitude. However, these results might be due instead to the genetic consequences of postglacial range expansion, or may better reflect the central-peripheral model, which posits that genetic diversity should diminish from the center of a species' range toward its edges. Patterns of within-population genetic diversity in tropical taxa could help distinguish between these hypotheses. To better understand the distribution of genetic diversity in tropical taxa, we surveyed within-population mitochondrial (mt) DNA variation in nine resident landbird species along the relatively narrow corridor of Neotropical lowlands from southern Mexico to western Ecuador, an ideal natural laboratory for evaluating these competing models. Species richness of resident landbirds increases with decreasing latitude along this latitudinal transect. However, we found no evidence for an inverse relationship between within-population genetic diversity and latitude, invalidating both a latitudinal gradient in genetic diversity and the SGDC model for these birds. Instead, we found that the distribution of estimated nucleotide diversity

[1] M.J. Miller, E. Bermingham, J. Klicka, P. Escalante, K. Winker (in preparation). Neotropical birds show a humped distribution of genetic diversity along a latitudinal transect.

($\hat{\pi}$) was humped, wherein the highest values for $\hat{\pi}$ were more frequently observed in mid-range populations than would be expected by chance. This pattern may be due to demographic factors such as increased population size variation and/or reduced gene flow into range edge populations, or it may simply reflect geographic constraints on haplotype distributions. Our findings have implications for theories of genetic variation across a species' range, for conservation planning, and for understanding how biological diversity scales up from genes to communities.

INTRODUCTION

Biogeography, community ecology, and population genetics all attempt to describe how biological diversity is spatially distributed, albeit at different scales of geographic and biological organization. Therefore, it is not surprising that researchers from these disciplines seek common patterns in how diversity is distributed (Magurran 2006). Vellend and colleagues (Vellend 2003; Vellend 2005; Vellend & Geber 2005) have made compelling arguments that species richness and genetic variation should be correlated. When considering neutral genetic variation, they have pointed out that biogeographic attributes that promote demographic conditions favorable to high species richness within a community (i.e., high immigration rates and low extinction rates) should promote high genetic diversity within the species that are members of that community. Empirical support for this model has come from forest tree communities (Wehenkel *et al.* 2006), butterflies (Cleary *et al.* 2006), alpine herbs (Schonswetter *et al.* 2005), and over half of the archipelago species surveyed in a

meta-analysis (Vellend 2003). Vellend (2003) termed this positive relationship between species richness and genetic diversity the species-genetic diversity correlation (SGDC). However, it remains to be evaluated whether the SGDC model is extendable to well-established species richness gradients.

One of the oldest and likely most famous patterns of biodiversity is the latitudinal gradient of species richness (Rosenzweig 1995). For most taxa, the number of species occurring in an area increases towards the Equator. The popularity of the latitudinal gradient in species richness is due to its ubiquity; this pattern holds at both small and large latitudinal spans, for both plants and animals, for both terrestrial and marine organisms, and for taxonomic richness not only among species, but also among genera and families, and it can be found in existing communities and fossil assemblages (Willig *et al.* 2003). The SGDC model predicts that the magnitude of within-population genetic variation should co-vary with latitudinal variation in species richness.

Several studies have reported latitudinal differences in genetic variation, including two important meta-analyses (Martin & McKay 2004; Hughes & Hughes 2007). However, care must be taken as to what is being compared, because both genetic and species diversity can be measured at several geographic scales. Species diversity is often defined on three scales (Whitaker 1972): alpha diversity (local species richness), beta diversity (variation in species composition across geographic space), and gamma diversity (the total number of species found at continental scales). The latitudinal gradient in species richness is typically measured in terms of alpha

diversity (Rosenzweig 1995), although it occasionally refers to gamma diversity (Willig *et al.* 2003). Martin and McKay (2004) found that population differentiation (i.e., F_{ST} or D_{xy}) was greater below a species' mid-range latitude than above. This measure is probably more congruent with beta diversity, which has a less clear relationship with latitude than alpha diversity (Koleff *et al.* 2003; Rodriguez & T. Arita 2004; McKnight *et al.* 2007; Qian & Ricklefs 2007). Similarly, two studies (Chek *et al.* 2003; Hughes & Hughes 2007) have addressed genetic variation between the tropical and temperate regions across entire species' ranges, finding greater genetic diversity at lower latitudes. This result is most congruent with comparisons of gamma diversity rather than alpha diversity. Instead, following Vellend (2003), alpha diversity in species richness is probably most akin to within-population genetic variation.

A variety of studies have shown that within-population genetic variation decreases with increasing latitude. Examples include Nearctic and Palearctic fishes (Bernatchez & Wilson 1998), Palearctic mammals (Jaarola & Tegelstrom 1995; Fedorov & Stenseth 2001), Palearctic frogs (Johansson *et al.* 2006), Nearctic and Palearctic birds (Merila *et al.* 1997), and South African corals (Ridgway *et al.* 2008). However, as Eckert *et al.* (2008) noted, single-species studies of geographic variation in within-population genetic diversity are disproportionately focused on taxa at their northern limits in the north temperate zone. Although the majority of these studies have shown decreasing genetic diversity with increasing latitude, the potential that postglacial expansion is the cause of this pattern (Hewitt 2000) inhibits our ability to generalize from these examples.

One of the most intriguing aspects of the latitudinal gradient in species richness is that it can be found within exclusively tropical samples (Willig *et al.* 2003). Thus, the SGDC should extend to tropical latitudinal gradients. However, Eckert *et al.* (2008) were unable to find a single study for their meta-analysis examining clinal variation in within-population genetic variation that focused exclusively on tropical taxa. To date, no adequate test has been made of the SGDC across a tropical latitudinal gradient.

An alternative to a latitudinal gradient in genetic diversity (LGGD) is the central-peripheral model, an important general model for the distribution of abundance and variation across a species' range. This model has been most frequently applied in macroecology, where it predicts that a species' abundance peaks in the center of its range and diminishes towards the range edges (Brown 1984), but it has also been extended to genetic diversity (da Cunha *et al.* 1950). This pattern is believed to be caused by diminishing ecological suitability of habitats at range edges, resulting in greater population fluctuations in edge populations than in central ones (Brown *et al.* 1995). Reduced abundance and greater fluctuations in abundance should increase genetic drift, thus reducing within-population genetic variation (Vucetich & Waite 2003). Furthermore, geometry predicts that central populations should have higher immigration rates than edge populations (Eckert *et al.* 2008), which should ameliorate the diversity-reducing effects of genetic drift in central populations. Therefore, the combined consequences of reduced effective population size and immigration rates in edge populations should cause a reduction in genetic diversity relative to central

populations. As Eckert *et al.* (2008) pointed out, the strong theoretical support for this notion is hampered by a relative lack of empirical evidence. One alternative is that edge populations may instead be demographic sinks (Curnutt *et al.* 1996), and if immigration rates and number of source populations are high, then they may actually have greater genetic variation than central source populations (Gaggiotti & Smouse 1996). This would decouple the relationship between population abundance and genetic variation. Another alternative is that many species may not reach their greatest abundance at the center of their range (Sagarin & Gaines 2002). A model of random variation in the magnitude of within-population genetic diversity across a tropical latitudinal gradient is thus a reasonable null hypothesis.

Here we evaluate within-population genetic diversity along a latitudinal gradient, contrasting central-peripheral models against a null model of no relationship between population genetic diversity and latitude. Our empirical data come from nine resident Neotropical land bird species, sampled more or less coincidently across their ranges through Middle America to the Pacific lowlands of northwestern South America. Lowland tropical forest occurs in a narrow band of lowlands from southern Mexico to western Ecuador, a transect over which avian resident species richness rises with decreasing latitude (figure 2.1), making this a natural laboratory for observing how within-species genetic diversity varies along a latitudinal and species richness gradient.

METHODS

Tropical evergreen forest is more or less continuously distributed from southern Mexico south through Central America and along the Pacific lowlands of South America until western Ecuador, where a strong moisture gradient results in a relatively abrupt transition to tropical dry forest (Leemans 1990). Blocked by the continental divide along Central America, this narrow band of forest is restricted to the lowlands of the Middle American Caribbean slope until eastern Panama, where a lower continental ridge and increased Pacific rainfall permit this band to cross the continental divide and continue south along a narrow lowland strip of the Pacific coast of Colombia and northwestern Ecuador. Many tropical forest species have a more or less continuous distribution along this transect: 42% of the resident landbirds from the Los Tuxtlas Biological Station in Veracruz, Mexico (Coates-Estrada & Estrada 1985) can be found in Bilsa Biological Station in Esmeraldas, Ecuador (Hornbuckle *et al.* 1996). This transect spans over 18 degrees of latitude separating Veracruz and Esmeraldas, and in most places it is less than 200 km wide.

We were able to develop and assemble sufficient sample sizes for nine species (table 2.1) of resident Neotropical landbirds from various locations along this transect. In most cases, we sampled these species at six sites along the transect: Veracruz, Mexico (~18.5° N), Toledo District, Belize (~16.5° N), northern Honduras (~15.5° N), Bocas del Toro, Panama (~9.0° N), Darién, Panama (~7.8° N), and western Ecuador (~0.0° N). One species, *Euphonia gouldi*, only occurs from southern Mexico to Bocas del Toro, and another, *Glyphorynchus spirurus*, occurs in our samples northward only

to Belize, while *Myrmeciza exsul* only occurs in our samples from northeastern Costa Rica (~10.4° N). to western Ecuador.

As our metric of within-population genetic diversity, we chose nucleotide diversity (π) of the complete ND2 mitochondrial gene. Nucleotide diversity equals the average pairwise distance between all sequences, and it is a standard measure of DNA polymorphism (Nei 1987). We amplified and sequenced the complete ND2 gene using the L5215 (Hackett 1996) and H6313 (Sorenson *et al.* 1999) primers. Amplification PCR was run for 35 cycles with the first five at 50° C and the remaining 30 at 56° C. In most cases DNA was extracted from mitochondrially-rich muscle tissue from vouchered frozen tissue samples; however, a few western Ecuador samples were from feathers. Estimated nucleotide diversity ($\hat{\pi}$) was calculated in DnaSP 4.2 (Rozas *et al.* 2003).

Sample sizes at each of the above-mentioned sites varied due to the vagaries of field success (table 2.1) but were augmented in a few instances by GenBank sequences (Appendix). To evaluate the effect of small sample sizes on $\hat{\pi}$, we plotted the ranked order (within that species) of $\hat{\pi}$ for each population by the sample size.

The most basic null model for the distribution of genetic diversity along a cline is uniformity, i.e., where observed differences are due to stochastic effects or sampling error. In contrast, the LGGD and SGDC models predict an increase in genetic diversity with decreasing latitude. We tested for such an increase in π for our nine Neotropical bird species by calculating the value of the expression: $\hat{\pi}_i - \hat{\pi}_{i+1}$, where i refers to a given population and $i+1$ is the next population found at a lower latitude.

The LGGD/SGDC models predict that this difference should be positive more frequently than negative. The frequency of observed versus expected positive values was compared to a null hypothesis of equal frequency of positive and negative values using an exact binomial test.

An alternative to the LGGD/SGDC models, as discussed above, is the central-peripheral model, which would produce a humped distribution, wherein the largest value of π is found in mid-range relative to edge populations. A null hypothesis for this model is that, within a species, the largest value of $\hat{\pi}$ is equally likely to be observed in any of the sampled populations. We tested for a humped distribution of π by evaluating whether the frequency of a species' highest value for $\hat{\pi}$ occurred in the northernmost or southernmost sampling point (i.e., edge populations) at a lower frequency than predicted by the null hypothesis. Specifically, each of our nine species has two edge populations and 2-4 mid-range populations, so the probability by chance that the maximum observed value of $\hat{\pi}$ occurs in a mid-range population varies from 0.50–0.66. We calculated the probability that the observed number of species with maximum $\hat{\pi}$ in an edge population was due to chance by computing the joint probabilities of all combinations of observations that equaled or were less frequent than found in our empirical data.

To visualize the collective pattern of the standing crop of genetic diversity among our nine species along this transect, we summed the average $\hat{\pi}$ values for each species across their latitudinal ranges from SE Mexico to W Ecuador. For six of the nine species, this included their entire range; the remaining three have disjunct ranges

east of the Andes in South America. However, we expect gene flow to be non-existent or greatly diminished across the Andes (Cracraft 1985; Brumfield & Capparella 1996; Miller *et al.* 2008). Because each species has a unique range, we divided the latitudinal range of each species (in Middle America and northwestern South America) into four quartiles. We averaged $\hat{\pi}$ for each quartile for each species and summed it across all nine species. When a quartile had no sampled population, we used as an extrapolated value the average of the most adjacent population to the north and south.

RESULTS

We sampled 47 populations (430 individuals) of the nine species in our study; all were sequenced for the complete ND2 mtDNA sequence (1041 bp). Among these populations, $\hat{\pi}$ varied from 0.0000 – 0.0866 and had a median value of 0.0011. Values for $\hat{\pi}$ fit an exponential distribution (figure 2.2); values less than 0.001 were most frequent (45%). Only 6% of the populations had $\hat{\pi}$ values greater than 0.005. We found no significant relationship between sample size and the rank of $\hat{\pi}$ values within a species (figure 2.3: $r^2 = 0.02$, $p = 0.31$).

In our comparison of the model of a relationship between genetic diversity and latitude with a null model of uniformity, there were 38 opportunities to evaluate the expression: $\hat{\pi}_{i+1} - \hat{\pi}_i$. Of these, 21 were non-negative, a ratio of 0.55, which is not significantly different from an expected ratio of 0.5 predicted by the null model (exact test: $p = 0.31$; table 2.1).

In our comparison of a humped distribution against a null model, we found that zero of the nine species had a maximum $\hat{\pi}$ value in an edge population (see Methods). The *p*-value of this result can be calculated analytically as the joint probability of the probability of a mid-range maximum $\hat{\pi}$ value for all nine species given a random spatial distribution of maximum values. That result is significant even after a Bonferroni correction to take into account our previous test of an inverse relationship between latitude and within-population diversity ($\alpha = 0.025$; $p = 0.01$). We thus reject the null model in favor of a humped distribution model.

Examination of the standing crop of genetic variation among this nine-member species assemblage along our transect (the summed average quartile values of $\hat{\pi}$) also showed a humped distribution, with the two intermediate latitudinal quartiles having nearly twice the summed $\hat{\pi}$ as the two range-edge quartiles (figure 2.4).

DISCUSSION

Among the nine species of resident Neotropical landbirds included in this study, a model of increasing mitochondrial DNA nucleotide diversity (π) with decreasing latitude did not fit the data better than a null model of uniformly distributed $\hat{\pi}$. However, we rejected a random distribution of maximum $\hat{\pi}$ among mid-range and edge populations in favor of a humped distribution model in which the highest $\hat{\pi}$ for a species was found in mid-range populations for all nine species examined. Figure 2.4 provides a heuristic, among-species visualization of this humped pattern across our transect.

While relatively few studies have reported within-population π from mtDNA in Neotropical birds, our results appear consistent with values found by others (Brumfield 2005; Cheviron *et al.* 2005; Aleixo 2006). Although our population sample sizes were modest and varied, sample size did not appear to bias our results; among our 47 populations, sample size was not significantly correlated with variation in $\hat{\pi}$-value ranking (figure 2.3).

Our findings caused us to reject a model of increase in π with decreasing latitude. This result conflicts with the SGDC model's prediction of a direct relationship between variation in genetic diversity across a species-richness gradient (Vellend 2003; Vellend 2005; Vellend & Geber 2005). However, variation in within-population genetic diversity and species richness may be highly correlated across many landscapes due to historical factors. In studies demonstrating a latitudinal gradient in genetic diversity, most have claimed that the pattern was due to a history of postglacial colonization of high-latitude regions (e.g., Jaarola & Tegelstrom 1995; Merila *et al.* 1997; Bernatchez & Wilson 1998; Mila *et al.* 2000; Fedorov & Stenseth 2001). However, the high-latitude populations in these studies were also relatively near the high-latitude edge of those species' ranges. Thus, latitudinal variation in the demographic conditions between central and peripheral populations may be contributing to reduced high-latitude within-population genetic diversity (Vucetich & Waite 2003). Johansson *et al.* (2006) found a strong latitudinal component to differences in within-population genetic variation among *Rana temporania* populations. However, even after controlling for latitude, a significant effect of

population size on genetic diversity remained, suggesting that demographic patterns in edge populations were principally responsible for their findings.

In our study, evidence that edge populations may be less abundant than mid-range populations is mixed: of the 18 edge populations, we found abundance estimates for 16 in area checklists. Of these, 11 (69%) were classified as abundant, very common, common, or fairly common (Coates-Estrada & Estrada 1985; Stiles & Levey 1994; Hornbuckle *et al.* 1996; Jones & Vallely 2001); the other five were classified as uncommon. Thus, whereas in some of our study species relative abundance may be reduced in edge populations, it may not usually be the case. This is consistent with meta-analyses showing that a majority of species do not show a simple pattern of high abundance at mid-range populations and low abundance at range edges (Sagarin & Gaines 2002), and that a majority of species whose ranges have contracted persist at range edges (Channell & Lomolino 2000).

What other factors could cause the humped relationship between genetic diversity and latitude? One obvious factor is the geographic context of potential gene flow. Immigration counters the loss of genetic diversity caused by genetic drift. For populations that have relatively one-dimensional distributions, such as the birds in this study, populations at the range edges have functionally half the potential source populations from which to receive immigrants as mid-range populations. Thus, mid-range versus edge variation in immigration rates might be responsible for the observed pattern.

In addition, the limits of tropical habitats in northern Middle America have shifted northward since the Pleistocene. Studies show that northern Middle America lacked forest and was instead covered with arid habitats (Leyden 1984; Leyden *et al.* 1993; Hillesheim *et al.* 2005). A Holocene regeneration of this forest has been documented (Leyden 1984). In contrast, Caribbean lower Middle America remained continuously forested throughout the late Pleistocene (Bush & Colinvaux 1990; Bush *et al.* 1992; Colinvaux 1996). Due to founder effect dynamics, northern populations of forest-inhabiting birds are likely to have a relative impoverishment of genetic variation as a consequence of tracking this northward-colonizing forest (Hewitt 1996). However, this cannot explain the low genetic diversity found at the southern edges of the nine species we examined.

Finally, geometric constraints (Colwell & Hurtt 1994; Colwell & Lees 2000; Jetz & Rahbek 2001) may explain some portion of the central-peripheral pattern. Most haplotypes are likely to have a more-or-less continuous distribution within a species' range. However, because these distributions are ultimately bounded by the edges of species' ranges, it is more likely that the majority of haplotypes will overlap in the center portion of the range. This phenomenon has been coined the "mid-domain effect" (Colwell & Hurtt 1994; Colwell & Lees 2000). While controversial (Zapata *et al.* 2003; Colwell *et al.* 2004), proponents of the mid-domain effect argue that it is at least partially responsible for other cases in which the geographic distribution of biological diversity is humped, such as latitudinal and altitudinal species richness gradients.

Without greater sampling to accurately determine the distribution of ND2 haplotypes in the species we examined, it is impossible to determine to what extent geographic constraints are responsible for the humped mtDNA genetic diversity pattern that we observed. We note, however, that six of the nine species examined had a mid-range population comprised of individuals from two clades, one otherwise northward and the second otherwise southward (not shown), consistent with expectations of the mid-domain model. Geometric constraints refer both to the case of secondary contact of two lineages, such as a northern and southern clade occurring in some species in our study, or a case in which a single mtDNA lineage is found throughout a species' range, which also occurs in our study. If variants (i.e., mtDNA haplotypes) have relatively continuous distributions and are bound to a discrete area (i.e., a species' range), the greatest number of variants should be found in the middle, rather than the edges, of that area, regardless of any particular geographic co-association of variants (i.e., geographic structure).

The concept of the "stable tropics" (Janzen 1967; Orians 1969; MacArthur 1972) still persists, despite a variety of evidence that tropical populations undergo substantial fluctuations over both contemporary and Quaternary time scales (Karr & Freemark 1983; Leyden 1984; Loiselle & Blake 1992; Phillips *et al.* 1994; Behling & Lichte 1997; Rull 2006). Recent reviews continue to posit that effective population sizes of tropical taxa are generally expected to be more stable than those of temperate taxa (e.g., Mallet *et al.* 2005), although the limited genetic evidence for historical stability of tropical populations is mixed (e.g., Schneider & Moritz 1999; Crawford

2003; Lessa *et al.* 2003; Anthony *et al.* 2007; Francisco *et al.* 2007). In contrast, our results suggest that the effective population size (as measured by mtDNA polymorphism) of tropical species is geographically context-dependent: ranges centers have more genetic diversity than range edges. Because effective population size is a proportional to the harmonic mean of the census population size, our results suggest that populations of tropical species near the range center may have been relatively stable, but that populations on the range edges appear to have been less so.

This finding has implications for both evolutionary biology and the management of biodiversity. Debates over the relationship among population size, genetic variation, and evolutionary change have persisted for nearly 50 years (Mayr 1963; Barton & Charlesworth 1984; Gavrilets *et al.* 2000). Given our results, further study is needed to determine whether the humped pattern present in mtDNA is also found in potentially adaptive genetic variation. That relationship will be important for conservation and management, because, with respect to the maintenance of genetic diversity, we can conclude that the consequences of anthropogenic habitat fragmentation and population isolation will likely have differential effects depending on where in a species' range these phenomena occur.

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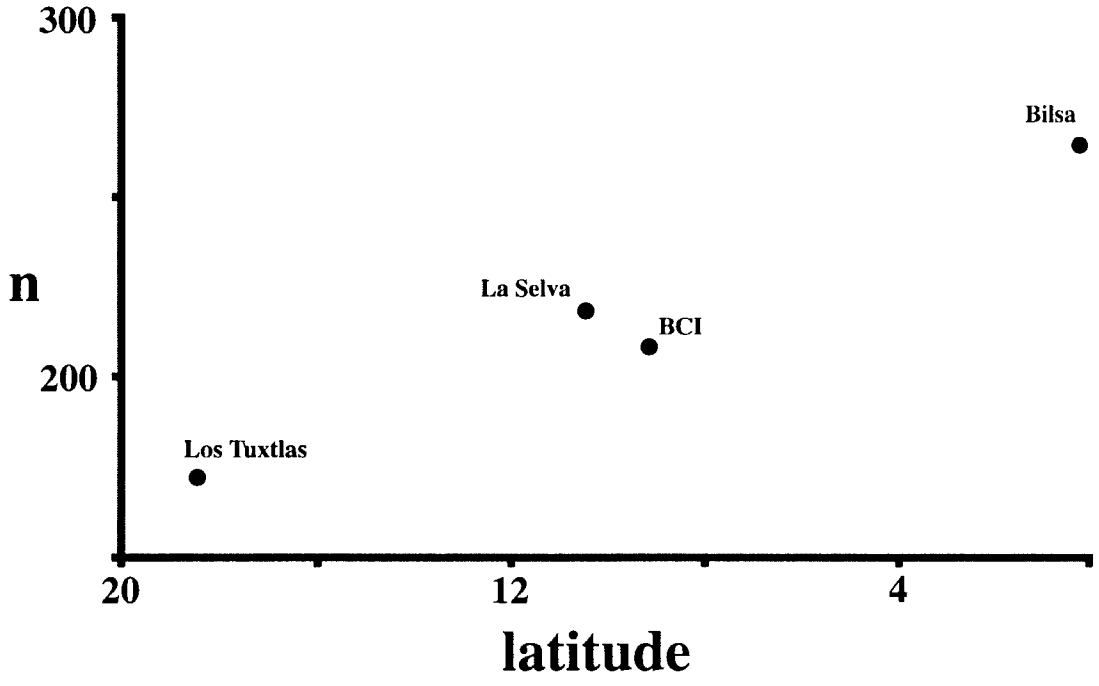

Figure 2.1. Number of breeding landbirds recorded at four research stations in the Neotropical lowlands from Mexico to Ecuador: 1) Los Tuxtlas (Veracruz, Mexico ~ 18.5°N: Coates-Estrada & Estrada 1985); 2) La Selva (Heredia, Costa Rica ~ 10.5°N: Stiles & Levey 1994); 3) Barro Colorado Island (BCI: Colon, Panama ~ 9.2°N: Willis & Eisenmann 1979); and 4) Bilsa (Esmeraldas, Ecuador ~ 0.25°N: Hornbuckle *et al.* 1996; Tobias & Seddon 2002).

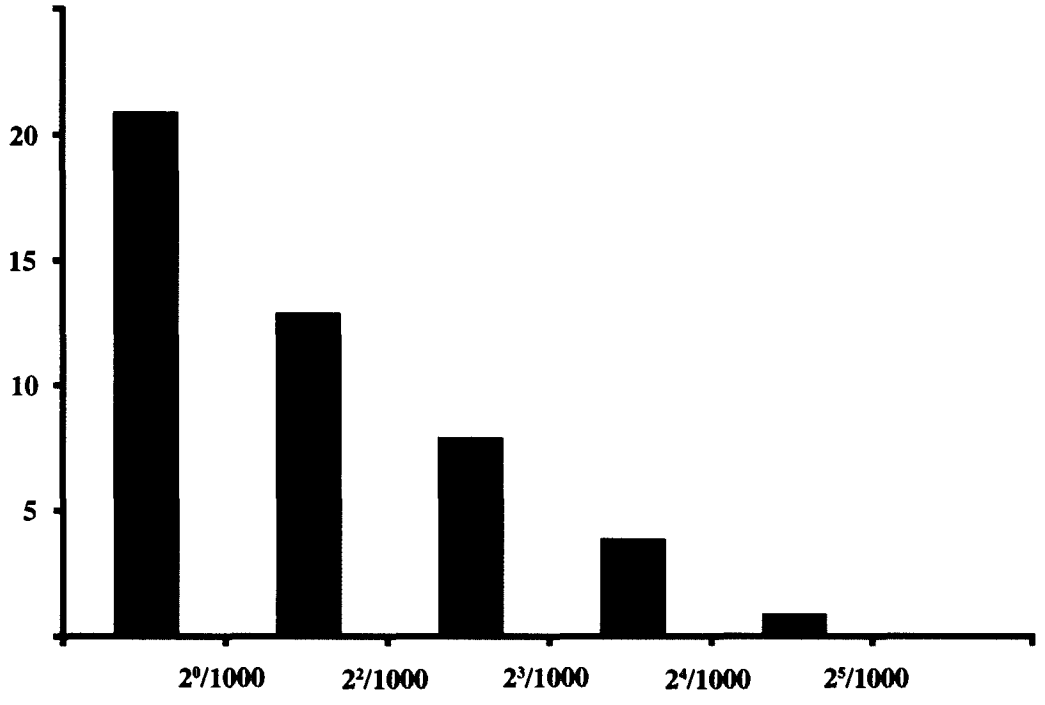

Figure 2.2. Histogram of estimated nucleotide diversity ($\hat{\pi}$) from 47 populations (nine species) of Neotropical landbirds. The data fit an exponential distribution (p = 0.08).

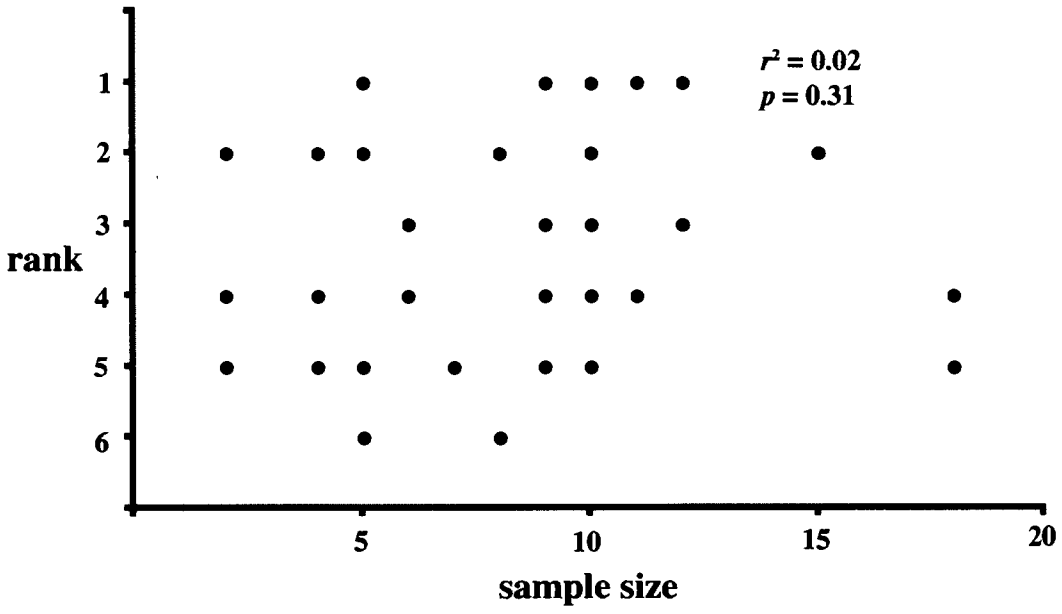

Figure 2.3. Rank of estimated nucleotide diversity ($\hat{\pi}$) relative to sample size (among populations within species, from largest to smallest) indicating that sample size and $\hat{\pi}$ have a non-significant relationship. Note inverted y-axis.

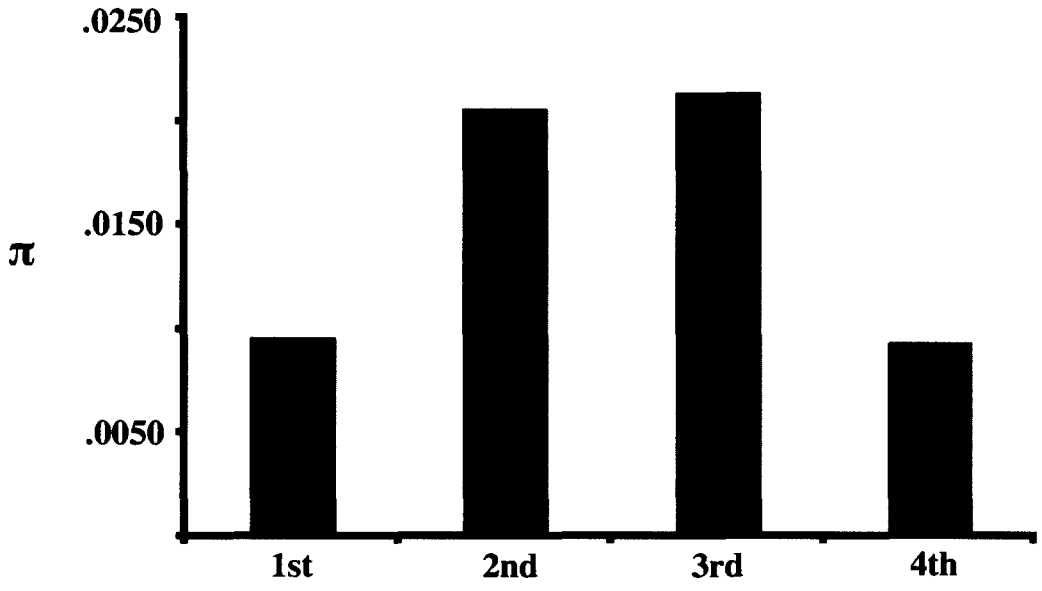

Figure 2.4. Summed latitudinal-quartile estimates of nucleotide diversity ($\hat{\pi}$) provide a range-standardized, heuristic demonstration of $\hat{\pi}$ variation over the range of nine Neotropical resident birds (between SE Mexico and W Ecuador). Quartiles are ranked from northernmost to southernmost (see Methods).

Table 2.1. Estimated nucleotide diversity for nine species (47 populations) of Neotropical landbirds ranging from SE Mexico to W Ecuador. *n* = number of individuals sampled; Num Hap: number of haplotypes; $\hat{H}$: estimated haplotype diversity; $\hat{\pi}$: estimated nucleotide diversity; $\partial\hat{\pi} = \hat{\pi}_{i+1} - \hat{\pi}_i$. Maximum $\hat{\pi}$ (per species) outlined with a box. Veracruz, Mexico: (~ 18.5° N, 95.0° W); Toledo, Belize: (~16.0° N, 89.0° W); N Honduras (Copán & Atlántida): (~15.5° N, 87.5° W); Heredia, Costa Rica: (10.5° N, 84.0° W); Bocas del Toro, Panama: (~9.0° N, 82.5° W); Darién, Panama: (~ 7.5° N, 78.0° W); W Ecuador (Esmeraldas & Manabí): (~ 0.0° N, 79.5° W).

Scientific Name	*n*	Num Hap	$\hat{H}$	$\hat{\pi}$	$\partial\hat{\pi}$
Phaethornis longirostris					
Veracruz, Mexico	10	1	0.000	0.0000	
Toledo, Belize	10	3	0.511	0.0005	0.0005
N Honduras	10	2	0.467	0.0004	-0.0001
Bocas del Toro, Panama	11	4	0.691	0.0016	0.0012
Darién, Panama	12	4	0.455	0.0005	-0.0011
W Ecuador	5	1	0.000	0.0000	-0.0005
Phaethornis striigularis					
Veracruz, Mexico	7	1	0.000	0.0000	
Toledo, Belize	10	5	0.756	0.0014	0.0014
N Honduras	6	4	0.867	0.0018	0.0004
Bocas de Toro, Panama	9	5	0.889	0.0033	0.0015
Darién, Panama	10	8	0.956	0.0022	-0.0011
W Ecuador	3	1	0.000	0.0000	-0.0022
Amazilia tzacatl					
Veracruz, Mexico	4	3	0.833	0.0010	
Toledo, Belize	10	5	0.667	0.0010	0.0000
n Honduras	4	4	1.000	0.0048	0.0038
Bocas del Toro, Panama	9	6	0.917	0.0021	-0.0027
Darién, Panama	5	2	0.600	0.0087	0.0066
W Ecuador	2	2	1.000	0.0010	-0.0077
Glyphorynchus spirurus					
Toledo, Belize	10	2	0.200	0.0002	
N Honduras	9	2	0.389	0.0004	0.0002
Bocas del Toro, Panama	10	7	0.933	0.0061	0.0057
Darién, Panama	10	3	0.378	0.0004	-0.0057
W Ecuador	8	3	0.679	0.0024	0.0020
Myrmeciza exsul					
Heredia, Costa Rica	10	3	0.600	0.0008	
Bocas, del Toro, Panama	12	6	0.818	0.0048	0.0040
Darién, Panama	11	3	0.564	0.0005	-0.0043
W Ecuador	15	8	0.838	0.0015	0.0010

Table 2.1 continued

Pipra mentalis					
Veracruz, Mexico	4	1	0.000	0.0000	
Toledo, Belize	12	4	0.561	0.0009	0.0009
N Honduras	10	6	0.889	0.0014	0.0005
Bocas del Toro, Panama	9	3	0.417	0.0004	-0.0010
W Ecuador	2	2	0.100	0.0010	0.0006
Mionectes oleagineus					
Veracruz, Mexico	10	5	0.667	0.0014	
Toledo, Belize	10	5	0.822	0.0011	-0.0003
N Honduras	10	5	0.844	0.0015	0.0004
Bocas del Toro, Panama	18	6	0.627	0.0010	-0.0005
Darién, Panama	18	5	0.771	0.0010	0.0000
W Ecuador	5	1	0.000	0.0000	-0.0010
Henicorhina leucosticta					
Veracruz, Mexico	5	5	1.000	0.0023	
Toledo, Belize	10	8	0.933	0.0023	0.0000
N Honduras	9	4	0.583	0.0015	-0.0008
Bocas del Toro, Panama	11	6	0.855	0.0015	0.0000
Darién, Panama	12	5	0.833	0.0029	0.0014
W Ecuador	8	3	0.464	0.0007	-0.0022
Euphonia gouldi					
Veracruz, Mexico	6	1	0.000	0.0000	
Toledo, Belize	9	4	0.806	0.0011	0.0011
N Honduras	10	6	0.889	0.0050	0.0039
Bocas del Toro, Panama	10	8	0.933	0.0029	-0.0021

Appendix 2.1. Specimens and tissue samples used in this study, with corresponding GenBank accession numbers.

ANSP: Academy of Natural Sciences of Philadelphia (Philadelphia, USA); CNAV: Colección Nacional de Aves, Instituto de Biología, Universidad Nacional Autónoma de México (Mexico City, Mexico); CU: Cornell Museum of Vertebrates (Ithaca, USA); FMNH: Field Museum of Natural History (Chicago, USA); LSUMZ: Louisiana State University Museum of Zoology (Baton Rouge, USA); NMNH: National Museum of Natural History; MBM: Marjorie Barrick Museum (Las Vegas, USA); NMNH: National Museum of Natural History; STRI: Smithsonian Tropical Research Institute (Balboa, Panama); UAM: University of Alaska Museum (Fairbanks, USA).

Phaethornis longirostris

Veracruz, Mexico	
UAM 20926	FJ231527
CNAV TUX1035	FJ231528
CNAV MNF36	FJ231529
CNAV PEP2806	FJ231530
UAM 20761	FJ231531
UAM 20926	FJ231532
UAM 21621	FJ231533
CNAV PEP2590	FJ231534
UAM 20753	FJ231535
UAM 21228	FJ231536

Toledo, Belize	
UAM 7928	FJ175629
UAM 9253	FJ175630
UAM 10248	FJ175631
UAM 14444	FJ175632
UAM 14484	FJ175633
UAM 7938	FJ175634
UAM 9493	FJ175635
UAM 9566	FJ175636
UAM 7939	FJ175637
UAM 8058	FJ175638

N Honduras	
STRI HA-PSU46	FJ231537
STRI HA-PSU73	FJ231538
STRI HA-PSU09	FJ231539
STRI HA-PSU56	FJ231540
STRI HA-PSU26	FJ231541
STRI HA-PSU57	FJ231542
STRI HA-PSU10	FJ231543
STRI HA-PSU19	FJ231544
MBM 7838	FJ231545
MBM 7839	FJ231546

Bocas del Toro, Panama	
CU 51048	FJ231547
STRI IJL04-050	FJ231548
STRI JTW212	FJ231549
STRI IJL04-049	FJ231550
LSUMZ B58310	FJ231551
STRI IJL04-150	FJ231552
LSUMZ B58096	FJ231553
LSUMZ B58063	FJ231554
LSUMZ B58092	FJ231555
LSUMZ B58131	FJ231556
LSUMZ B58132	FJ231557

Darien, Panama	
UAM 22655	FJ231558
UAM 22658	FJ231559
UAM MJM2044	FJ231560
UAM JMM924	FJ231561
UAM MJM1952	FJ231562
UAM JMM985	FJ231563
LSUMZ B46544	FJ231564
USNM B17542	FJ231565
LSUMZ B46561	FJ231566
UAM MJM1991	FJ231567
UAM 22662	FJ231568
LSUMZ B46543	FJ231569

W Ecuador	
ANSP 3110	FJ231570
ANSP 3458	FJ231571
ANSP 3135	FJ231572
ANSP 4680	FJ231573
ANSP 18113	FJ231574

Phaethornis striigularis

Veracruz, Mexico	
CNAM PEP2387	FJ231575
UAM 21124	FJ231576
UAM 18878	FJ231577
UAM 21278	FJ231578
UAM 21573	FJ231579
UAM 18062	FJ231580
UAM GLS38	FJ231581

Toledo, Belize	
UAM 8035	FJ175751
UAM 15274	FJ175752
UAM 10290	FJ175753
UAM 8034	FJ175754
UAM 24379	FJ175755
UAM 7920	FJ175756
UAM 24318	FJ175757
UAM 24317	FJ175758
UAM 24371	FJ175759
UAM ABJ1205	FJ175760

N Honduras	
STRI HA-PLO40	FJ231582
STRI-HA-PLO39	FJ231583
MBM JK01-090	FJ231584
MBM GAV061	FJ231585
MBM JK00-071	FJ231586
MBM JK01-079	FJ231587

Bocas del Toro, Panama	
STRI JTW223	FJ231588
STRI JTW233	FJ231589
UAM MJM1197	FJ231590
LSUMZ B58077	FJ231591
LSUMZ B58078	FJ231592
LSUMZ B58084	FJ231593
CU 51042	FJ231594
LSUMZ B58079	FJ231595
LSUMZ B58085	FJ231596

Darien, Panama	
UAM 24504	FJ231597
UAM MJM2101	FJ231598
UAM 24506	FJ231599
UAM JMM1096	FJ231600
UAM 24406	FJ231601
UAM MJM1965	FJ231602
UAM 24503	FJ231603
UAM 24407	FJ231604
UAM JMM1071	FJ231605
UAM 24505	FJ231606

W Ecuador	
ANSP 3648	XXXXXX
ANSP 4635	XXXXXX
ANSP 114808	XXXXXX

Amazilia tzacatl

Veracruz, Mexico	
UAM TUX1120	EU983301
UAM PEP2504	EU983302
UAM PEP2505	EU983303
UAM PEP2512	EU983304

Toledo, Belize	
UAM 8037	EU983311
UAM 14312	EU983312
UAM 14322	EU983313
UAM 14313	EU983314
UAM 14461	EU983315
UAM 14513	EU983316
UAM 7963	EU983317
UAM 9079	EU983318
UAM 9203	EU983319
UAM 9237	EU983320

N Honduras	
MBM JK01-122	EU983321
MBM GAV2089	EU983322
MBM JK01-081	EU983323
MBM GAV2148	XXXXXX

Bocas del Toro, Panama	
STRI JTW248	XXXXXX
CU 51234	XXXXXX
MBM GMS1994	EU983372
MBM JK06-222	EU983375

MBM JK06-138	EU983376
MBM JK06-143	EU983377
MBM JK06-217	EU983378
MBM JMD758	EU983379
MBM JMD766	EU983380
Darien, Panama	
STRI JTW610	EU983370
STRI JTW721	EU983371
UAM 22691	EU983367
UAM 24255	EU983368
UAM 22690	EU983369
W Ecuador	
ANSP 3638	EU983386
ANSP 3333	EU983387

Glyphorynchus spirurus

Toledo, Belize	
UAM 24470	FJ175828
UAM 24324	FJ175829
UAM 24349	FJ175830
UAM 24350	FJ175831
UAM ABJ419	FJ175832
UAM 24513	FJ175833
UAM 24516	FJ175834
UAM 18313	FJ175835
UAM 18312	FJ175836
UAM 24320	FJ175837
N Honduras	
STRI HA-11	FJ231607
STRI HA-33	FJ231608
STRI HA-06	FJ231609
STRI HA-54	FJ231610
MBM GAV2019	FJ231611
MBM GAV2003	FJ231612
MBM GAV2018	FJ231613
MBM GMS162	FJ231614
MBM GAV1991	FJ231615
Bocas del Toro, Panama	
STRI IJL-071	FJ231616
STRI JTW109	FJ231617
STRI IJL-088	FJ231618
STRI IJL04-024	FJ231619
CU 50837	FJ231620
CU 50869	FJ231621
CU 44208	FJ231622
CU 51261	FJ231623
CU 51690	FJ231624
CU 44209	FJ231625
Darien, Panama	
UAM 24465	FJ231626
UAM MJM983	FJ231627
UAM MJM893	FJ231628
STRI JTW632	FJ231629
LSUMZ B46537	FJ231630
UAM MJM2052	FJ231631
UAM JMM968	FJ231632
UAM MJM2006	FJ231633
NMNH B17588	FJ231634
UAM 24467	FJ231635
W Ecuador	
UAM MJM1563	FJ231636
UAM MJM1719	FJ231637
UAM MJM1720	FJ231638
UAM MJM1721	FJ231639
UAM MJM1558	FJ231640
UAM MJM1561	FJ231641
UAM MJM1690	FJ231642
UAM MJM1724	FJ231643

Myrmeciza exsul

Heredia, CR	
SW T001	FJ229369
SW T002	FJ229370
SW T003	FJ229371
SW T004	FJ229372
SW T005	FJ229373
SW L007b	FJ229374
SW L009b	FJ229375
SW L016b	FJ229376
SW L017b	FJ229377
SW L022b	FJ229378
Bocas del Toro, Panama	
STRI IJL04-010	FJ229391
CU 50916	FJ229392
CU 44211	FJ229393
STRI IJL04-033	FJ229394
CU 50834	FJ229395
STRI IJL04-012	FJ229396
UAM 23991	FJ229397
UAM 23993	FJ229398
STRI JTW309	FJ229399
STRI JTW258	FJ229400
STRI JTW289	FJ229401
STRI JTW287	FJ229402
Darien, Panama	
UAM KSW4791	FJ229433
UAM KSW4790	FJ229434
UAM JMM1018	FJ229435
UAM MJM2023	FJ229436
UAM 24473	FJ229437
LSUMZ B46542	FJ229438
LSUMZ B46551	FJ229439
LSUMZ B46593	FJ229440
UAM MJM985	FJ229441
UAM 23992	FJ229442
UAM 23994	FJ229443
W Ecuador	
UAM MJM1470	FJ229444
UAM MJM1471	FJ229445
UAM MJM1474	FJ229446
UAM MJM1480	FJ229447
UAM MJM1481	FJ229448
UAM MJM1482	FJ229449
UAM MJM1583	FJ229450
UAM MJM1584	FJ229451
UAM MJM1475	FJ229452
UAM MJM1476	FJ229453
UAM MJM1477	FJ229454
UAM MJM1478	FJ229455
UAM MJM1479	FJ229456
UAM MJM1473	FJ229457
UAM MJM1656	FJ229458

Mionectes oleagineus

Veracruz, Mexico	
CNAV PEP2609	EF110711
UAM 21109	EF110712
CNAV PEP2905	EF110713
UAM GLS286	EF110714
CNAV PEP2799	EF110715
CNAV PEP2313	EF110716
UAM GLS277	EF110717
UAM GLS287	EF110718
UAM 20867	EF110719
UAM GLS 264	EF110720
Toledo, Belize	
UAM 7908	EF110724
UAM 7911	EF110732
UAM 7912	EF110733
UAM 7933	EF110725
UAM 9573	EF110726
UAM 10266	EF110727
UAM 14310	EF110731
UAM 14328	EF110728
UAM 14494	EF110730
UAM 15426	EF110729
N Honduras	
STRI HA-MOL27	EF110736
STRI HA-MOL47	EF110737
STRI HA-MOL75	EF110738
STRI-HA-MOL85	EF110739
STRI-HA-MOL90	EF110740
MBM DHB3716	FJ231706
MBM GAV2090	FJ231707
MBM DHB3857	FJ231708
MBM DHB3856	FJ231708
MBM JK01-250	FJ231710
Bocas del Toro, Panama	
LSUMZ B58126	EF110748
LSUMZ B58103	EF110750
STRI IJL067	EF110749
STRI IJL089	EF110743
STRI IJL090	EF110747
STRI IJL091	EF110756
CU 51281	EF110744
STRI JTW204	EF110746
STRI JTW210	EF110745
CU 51263	EF110751
STRI JTW236	EF110752
MBM JK06-147	EF110753
MBM JK06-158	EF110754
MBM JK06-179	EF110755
MBM JK06-154	EF110757
MBM GMS 2009	XXXXXX
MBM JMD 771	XXXXXX
MBM JMD 773	XXXXXX
Darien, Panama	
NMNH B17538	EF110789
NMNH B17544	EF110790
LSUMZ B46565	EF110791
LSUMZ B46546	EF110792
LSUMZ B46588	EF110793

LSUMZ B46589	EF110794
LSUMZ B46598	EF110795
UAM JMM945	EF110797
UAM 24017	EF110798
UAM 24009	EF110804
UAM 22800	EF110796
UAM 22802	EF110800
UAM 22801	EF110801
UAM 22797	EF110799
UAM 22798	EF110803
UAM 24010	EF110806
UAM MJM2011	EF110802
UAM MJM2008	EF110805
W Ecuador	
ANSP 3615	EF110812
ANSP 3111	EF110813
ANSP 3151	EF110814
ANSP 3114	EF110815
UAM MJM1608	FJ231711

Pipra mentalis

Veracruz, Mexico	
UAM 20903	FJ231644
UAM 21613	FJ231645
UAM MGL115	FJ231646
UAM PEP2802	FJ231647
UAM 20914	FJ231648
UAM 21187	FJ231649
LSUMZ B18078	DQ294535
Toledo, Belize	
UAM 24372	FJ175991
UAM 24512	FJ175992
UAM 24514	FJ175993
UAM 24515	FJ175994
UAM 24517	FJ175995
UAM 24518	FJ175996
UAM 24519	FJ175997
UAM 24520	FJ175998
UAM 8006	FJ175999
UAM 9068	FJ176000
UAM 9507	FJ176001
UAM 9577	FJ176002
N Honduras	
MBM JK01-099	FJ231650
MBM JK01-055	FJ231651
MBM GAV1975	FJ231652
MBM GAV2015	FJ231653
MBM JK01-071	FJ231654
MBM GAV2039	FJ231655
MBM JK01-078	FJ231656
MBM GAV1974	FJ231657
STRI HA-PME72	FJ231658
STRI HA-PME22	FJ231659
Bocas del Toro, Panama	
STRI JTW240	FJ231660
STRI JTW227	FJ231661
STRI JTW247	FJ231662
STRI IJL069	FJ231663
STRI JTW267	FJ231664
UAM MJM1181	FJ231665
STRI JTW243	FJ231666
STRI IJL087	FJ231667
STRI IJL084	FJ231668
CU 44247	FJ231669

Henicorhina leucosticta

Veracruz, Mexico	
CNAV GLS278	EU983455
CNAV MGL78	EU983456
UAM 20910	EU983457
CNAV TUX230	EU983458
CNAV PEP2506	EU983460
Toledo, Belize	
UAM 24662	EU983473
UAM 9233	EU983474
UAM 9232	EU983475
UAM 9069	EU983476
UAM 22763	EU983477
UAM 24323	EU983478
UAM 14319	EU983479
UAM 14318	EU983480
UAM 22731	EU983481
UAM 24659	EU983482
N Honduras	
MBM GMS169	EU983490
MBM GAV1744	EU983483
MBM GAV1743	EU983484
MBM GMS197	EU983485
MBM GAV1457	EU983486
MBM GAV1742	EU983487
MBM JK99-081	EU983488
MBM GAV1745	EU983489
MBM GMS112	EU983491
Bocas del Toro, Panama	
STRI JTW280	EU983493
CU 50230	EU983494
STRI JTW319	EU983495
STRI JTW089	EU983496
STRI JTW090	EU983497
MBM GMS2007	EU983498
MBM JK06-125	EU983499
MBM JK06-130	EU983500
MBM JMD754	EU983501
MBM GMS2006	EU983502
MBM JK06-124	EU983503
Darien, Panama	
STRI JTW728	EU983517
STRI JTW641	EU983518
UAM 22768	EU983519
UAM 22770	EU983520
UAM 22762	EU983521
UAM 22767	EU983522
UAM 22766	EU983523
UAM 22761	EU983524
UAM 22769	EU983525
UAM 24008	FJ231670
W Ecuador	
UAM MJM023	EU983529
LSUMZ B11739	EU983530
LSUMZ B11756	EU983531
LSUMZ B11867	EU983532
LSUMZ B12005	EU983533
LSUMZ B11738	EU983534
LSUMZ B11868	EU983535
LSUMZ B11869	EU983536

Euphonia gouldi

Veracruz, Mexico	
UAM TUX100	FJ231671
UAM TUX93	FJ231672
UAM TUX104	FJ231673
UAM 21269	FJ231674
UAM TUX99	FJ231675
UAM 20756	FJ231676
Toledo, Belize	
UAM 24509	FJ231677
UAM 24510	FJ231678
UAM 24511	FJ231679
UAM 7996	FJ231680
UAM 8046	FJ231681
UAM 8053	FJ231682
UAM 8079	FJ231683
UAM 9555	FJ231684
UAM 14506	FJ231685
N Honduras	
STRI HA-EGO25	FJ231686
STRI HA-EGO31	FJ231687
STRI HA-EGO32	FJ231688
MBM JK01-244	FJ231689
MBM DHB3716	FJ231690
MBM GAV2149	FJ231691
MBM GAV2034	FJ231692
MBM JK01-240	FJ231693
MBM GAV2145	FJ231694
MBM GAV2146	FJ231695
Bocas del Toro, Panama	
CU 51038	FJ231696
UAM MJM1185	FJ231697
CU 44255	FJ231698
UAM MJM1184	FJ231699
CU 51300	FJ231700
UAM MJM1201	FJ231701
NMNH B1228	FJ231702
NMNH B491	FJ231702
NMNH B391	FJ231703
NMNH B1231	FJ231704

CHAPTER 3: FORAGING ECOLOGY INFLUENCES POPULATION GENETIC DIFFERENTIATION IN SIXTY CODISTRIBUTED NEOTROPICAL BIRD SPECIES

ABSTRACT.– The Neotropical lowlands harbor the world's greatest bird species richness. Most of these species are widespread, which presents a paradox because among widespread species gene flow is expected to retard the formation of geographic isolates, the first stage in speciation. Explanations for the unparalleled Neotropical avian richness have focused on extrinsic barriers to gene flow, such as rivers, mountains, or habitat fragmentation caused by climatic fluctuations. However, differences in habitat preferences and/or foraging ecology affect movements and thus might also affect the genetic cohesion of populations across landscapes. We sampled populations of 60 codistributed landbird species in the lowlands of Belize and Panama (~1300 km apart) and found considerable variation in levels of genetic divergence (mitochondrial DNA differentiation of 0 – 8.4%). We found no difference in genetic divergence between birds of forest versus open or edge habitats. However, as a group, principally insectivorous birds showed significantly greater genetic divergence than birds that were principally nectivores or frugivores. Our data suggest that over relatively large geographic distances, few Neotropical insectivorous bird species maintain regular gene flow, and instead tend to become isolated. However, the demographic and dispersal characteristics of frugivores and nectivores may cause

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normal or episodic genetic cohesion between geographically distant populations. We conclude that foraging ecology plays a fundamentally important role in regulating diversification patterns of Neotropical birds, and that this intrinsic factor should be considered in tandem with extrinsic barriers to gene flow in the processes that generate avian diversity.

3.1 INTRODUCTION

The Neotropical lowlands harbor the world's greatest bird species richness: nearly one in every three bird species breeds in the Neotropics (Newton & Dale 2001). Of these, nearly two-thirds can be found in the lowlands (Stotz *et al.* 1996), making this the ecoregion with the greatest avian diversity on Earth (Orme *et al.* 2006). Most of these species are relatively widespread (Stotz *et al.* 1996; Orme *et al.* 2006), which presents something of a paradox, because among widespread taxa gene flow is expected to retard the formation of geographic isolates, the first step in the speciation process (Mayr 1963; Coyne & Orr 2004; Price 2008).

Although it is widely recognized that both intrinsic and extrinsic factors may promote or inhibit geographic expansion and/or geographic isolation of a given species (Avise 2000), the relative attention paid to each set of factors has varied based on the system being studied. Terrestrial phylogeographic studies tend to focus on barriers such as rivers, mountains, and unsuitable habitats. Also, many studies of codistributed terrestrial animals have demonstrated that phylogeographic structure

varies with habitat preference (e.g., Rocha *et al.* 2002; Marko 2004; Crawford *et al.* 2007). However, few studies of codistributed animals have identified life-history traits associated with differences in phylogeographic patterns. The exceptions come from some studies of marine organisms, taxa that lack obvious physical barriers to gene flow (e.g., Palumbi 1994; Shulman 1998; Ayre & Hughes 2000; Collin 2001), which raises the question of whether intrinsic factors might also be important for population differentiation among terrestrial organisms (Irwin 2002).

In the case of Neotropical birds, proposed mechanisms for reducing gene flow have focused almost exclusively on extrinsic, non-biological barriers to gene flow such as rivers (Sick 1967), Andean tectonics (Chapman 1917; Weir 2006), and/or forest refugia caused by climatic fluctuations (Haffer 1969), largely ignoring any role that intrinsic ecological characteristics might play in regulating gene flow. However, ecological characteristics are correlated with well-known differences in the demography and dispersal abilities of Neotropical birds. Levey and Stiles (1992) found that seasonal movements typically occurred among species found in open habitats and those that primarily feed on nectar and fruit. Similarly, large between-year fluctuations have been demonstrated for local populations of frugivores (Blake & Loiselle 1991) and nectivores (Stiles 1992), whereas insectivores tend to be more sedentary and have populations with more stable dynamics (Willis 1974; Greenberg & Gradwohl 1986; Sekercioglu *et al.* 2002). We chose to investigate the role of habitat and foraging ecology in the phylogeographic structuring of a suite of Neotropical birds. By focusing on codistributed species whose populations have been sampled at

two relatively distant points across a shared landscape, we test the hypothesis that ecological differences will be correlated with genetic differentiation between populations.

3.2 METHODS

Fully 390 species of landbirds reside in the Caribbean lowlands between southern Belize and central Panama (Jones & Vallely 2001; Angehr 2006). Of these, 38% are widespread, in that they breed more or less continuously throughout this region. We sequenced the complete ND2 mitochondrial gene (1041 base pairs [bp]) from 60 of these species from specimens collected in southern Belize and central Panama. Belize and Panama were chosen for logistical reasons as relatively distant points from which we could obtain vouchered specimens of a substantial proportion of shared, codistributed landbirds. For each species, the Belize population comprises specimens collected in Toledo District, southern Belize (~16.5° N, 89.0° W), and the Panama population comprises specimens collected in the Caribbean drainage of the Panama Canal watershed (~9.2° N, 80.0° W). The 60 species included in our study represent 19 avian families and exhibit a wide diversity of ecological traits, including habitat type and foraging strategy. Details on specimens and collecting locations can be found in the Appendix.

For 19 of the 60 species, we were able to obtain and sequence at least eight

individuals per species from both the Belizean and Panamanian populations. For the other 41 species, we sequenced 1-3 individuals per population, depending on specimen availability (Appendix). DNA extraction, gene amplification, automated sequencing protocols, and alignment procedures were described in Miller *et al.* (2008). For each of the 60 species, we calculated the net nucleotide difference (D_A) between Belize and Panama, which is the average number of substitutions per site between populations (D_{XY}) minus the average number of substitutions per site within populations ($[D_X + D_Y]/2$; Nei 1987).

Species were classified into foraging and habitat guilds. Each species was classified as either primarily forest or open habitat/forest edge-inhabiting and either principally nectivorous/frugivorous (including species such as sparrows that principally consume seeds) or principally insectivorous using information from specimen labels and the literature (Coates-Estrada & Estrada 1985; Ridgely & Gwynne 1989; Stotz *et al.* 1996; Robinson *et al.* 2000). *Chloroceryle aenea*, which preys on small fish and insects in forest streams, was grouped with the insectivores for all analyses. Because the distribution of D_A values was highly right-skewed, we tested for differences in D_A by habitat and foraging guild using a Mann-Whitney *U*-test. D_A values could be biased by differences in substitution rate and population size. Several physiological and life history traits have been shown to affect substitution rates, but all these traits covary with body size (Dobson 1990; Martin & Palumbi 1993); therefore we tested for differences in mass between species of insectivores and species of frugivores and nectivores in our study using a Mann-Whitney *U*-test, and we also

tested for a relationship between body mass and D_A using linear regression. Body mass information was obtained from specimen labels, averaged by species for all species in the study. We also tested for differences in average population size between the frugivores and nectivores and insectivores in our study using a Mann-Whitney *U*-test. Because population size can be estimated by the average pairwise distance among individuals in a population (Nei 1987), we calculated average population size as the total nucleotide divergence (D_{XY}) minus the net nucleotide difference (D_A).

For the 19 species with population-level sample sizes, we estimated genetic differentiation using F_{ST}, incorporating genetic distance between haplotypes following Excoffier *et al.* (1992). Calculation of F_{ST} and its statistical significance, determined by permutation tests (3000 replicates), was performed in Arlequin 3.11 (Excoffier *et al.* 2005).

Several statistics have been proposed to test for genetic signal consistent with recent population expansion. Ramos-Onsins and Rozas (2002) demonstrated that their R_2 statistic had greater statistical power for small sample sizes (i.e., $n < 15$). For the 19 species with population sample sizes of 8–10, we calculated R_2 and determined its significance by coalescent simulations (10,000 replicates) using DnaSP v4.20 (Rozas *et al.* 2003) for each population separately ($n = 38$). We tested for differences in the proportion of populations showing a significant R_2 statistic based on foraging guild using Fisher's exact test.

3.3 RESULTS

We calculated net mtDNA genetic divergence between populations (D_A; Nei 1987) for 60 Middle American resident landbird species, representing 41% of the widespread resident landbird species that occur between Belize and Panama. Among these species, levels of D_A ranged from -0.0006 to 0.0853 (table 3.1), with a median value of 0.0048; the distribution of D_A values was highly right-skewed (figure 3.1). Included in our sampling were 31 species classified as forest-inhabiting and 29 species classified as primarily inhabiting open or edge habitats. Similarly, these 60 species were divided into 27 species classified as primarily frugivores and nectivores and 33 species classified as primarily insectivores (table 3.1). D_A values were not significantly different between open/edge species and forest-inhabiting species (Mann-Whitney *U*-test, $p = 0.14$; figure 3.2a). However, D_A values were significantly different between frugivorous/nectivorous species and insectivorous species (Mann-Whitney *U*-test, $p = 0.0026$; figure 3.2b).

Similarly, 15 of the 60 species shared haplotypes between Belize and Panama. These were the 15 species with the lowest D_A values (-0.0006 – 0.0003), and frugivores/nectivores were significantly more likely than insectivores to share haplotypes between Belize and Panama (12 of 15 cases; exact binomial test: $p = 0.017$). 19 species had population-level sampling. For these species, F_{ST} values between Belize and Panama ranged from 0.005 to 0.981, with 18 species showing significantly higher F_{ST} values than expected if individuals were randomly assigned to

two populations. Furthermore, only two species (1 nectivore, 1 frugivore) had F_{ST} values < 0.1, and 14 species had values > 0.5 (table 3.2). Fifteen species showed reciprocal monophyly (assuming a mid-point root) between individuals from Belize and Panama. Of the remaining four species, two shared haplotypes between the two populations (*Phaethornis longirostris* and *Cyanerpes cyaneus*), while two other species (*Phaethornis striigularis* and *Saltator maximus*) shared no haplotypes yet were paraphyletic with respect to Belize and Panama (figure 3.3). All four of these species are frugivores or nectivores.

Data from the 19 species with population-level sampling provided 38 populations for which we could evaluate historical demographic signals in the distribution of within-population variation. 15 of the 38 populations had significantly lower R_2 values than could be expected under a model of no population expansion. A significantly greater proportion of these 15 populations were frugivores or nectivores compared to insectivores (Fisher's exact test, $p = 0.04$; table 3.3; figure 3.4). This suggests that as a group, frugivores and nectivores are more likely to show a signal of recent population expansion than insectivores.

Among our 60 species, body mass was not significantly lower for frugivores and nectivores than for insectivores (median average body mass for frugivore and nectivore species = 18 g, median average body mass for insectivore species = 24.5 g, Mann-Whitney *U*-test, $p = 0.99$), suggesting that differences in D_A between frugivores/nectivores and insectivores were unlikely to be caused by different

substitution rates. Furthermore, a linear regression of D_A against body mass found no relationship ($r^2 = 0.00005$, $p = 0.96$). Likewise, D_A values might be affected by population size; however we found no difference between frugivore/nectivore and insectivore species in the average population size as measured by average within-population pairwise difference among all 60 species (i.e., $D_{XY} - D_A$; Mann-Whitney U-test, $p = 0.93$).

3.4 DISCUSSION

Among 60 codistributed, resident Neotropical landbirds, the degree of genetic differentiation (D_A) between sampling points in Belize and Panama differed by two orders of magnitude. Fully 40% of the species studied had D_A values greater than 1%, and more than 28% had values above 2%, suggesting that a substantial proportion of widespread resident lowland birds are genetically isolated between northern and southern Middle America (a distance of ~1300 km.).

As a group, frugivorous/nectivorous species showed lower levels of differentiation and were more likely to share haplotypes than insectivorous species (figure 3.2, table 3.1). Lower D_A values likely indicate more recent time-since-isolation; however, care must be taken because D_A values could also be lowered by a slower rate of nucleotide substitution. The rate of mitochondrial DNA substitution is believed to be relatively uniform among birds (Lovette 2004). However, substitution

rates could vary with body size, lower metabolic rates, or longer generation time; evidence suggests that higher body mass is positively correlated with lower metabolic rates and longer generation time (Martin & Palumbi 1993). In contrast with expectations, given their lower D_A values, body mass in our study was slight lower for frugivores and nectivores than for insectivores, but the relationship was insignificant, and a linear regression of D_A against body mass showed no relationship. Similarly, while D_A values might be affected by population size, we found no difference between species groups in the average population size as estimated by average within-population pairwise differences. Therefore, we conclude that frugivorous and nectivorous species systematically show shorter times since divergence between Belize and Panama than codistributed insectivorous species.

How might such a pattern arise? There are three possibilities. One possibility is that the majority of widespread frugivorous and nectivorous species expanded across Middle America more recently than most insectivores. Our sampling of such a large proportion of the widespread avifauna, the taxonomic diversity of our sample, and the evidence that, as a group, frugivores and nectivores have greater dispersal tendencies than insectivores make this hypothesis unlikely.

A second possibility is that frugivores and nectivores might maintain gene flow across Middle America with greater frequency than insectivores. The exchange of only 10 females per generation would be sufficient to effectively render Belize and Panama as a single population (Teshima & Tajima 2002). Contemporary gene flow

estimates are unavailable for the species in our study, but it is reasonable to infer that gene flow is only possible for those species that share haplotypes between Belize and Panama. Species of frugivores and insectivores were more likely than species of insectivores to show shared haplotypes in our dataset. The presence of shared haplotypes could also indicate recent isolation without contemporary gene flow. However, the fact that F_{ST} values are above 0.2 in 17 of 19 well-sampled species suggests that contemporary gene flow, if it occurs, is not sufficiently great enough to have much effect on differences in D_A values between frugivores/nectivores and insectivores.

The third possibility is that genetic isolation between Belize and Panama is the tendency for most species regardless of foraging ecology. However, episodic population expansion driven by the boom-bust demography of Neotropical frugivorous and nectivorous species might cause sufficient movement between Belize and Panama to effectively re-unite formerly isolated populations. Such episodic reunification would have the effect of "resetting the clock" of time since isolation (figure 3.5), causing D_A values to be lower for frugivores and nectivores as a group than insectivores, because the latter are less likely to experience population reunification across relatively large geographic distances. Consistent with this hypothesis, among our 38 population samples (of 19 species), frugivores and nectivores were significantly more likely to show signs of recent demographic expansion than insectivores (figure 3.4, table 3.3).

Whether through ongoing or episodic gene flow, the lower levels of differentiation found among frugivores and nectivores stand in stark contrast to the deeper levels occurring in insectivores, indicating that the local demographic and dispersal differences associated with foraging guilds of Neotropical birds have consequences that scale up to large geographic areas and to evolutionary timescales. Thus, foraging ecology appears to have fundamental consequences for the patterns of differentiation of Neotropical landbirds and should be considered alongside models that focus on the role of extrinsic, physical barriers to gene flow in generating biological diversity.

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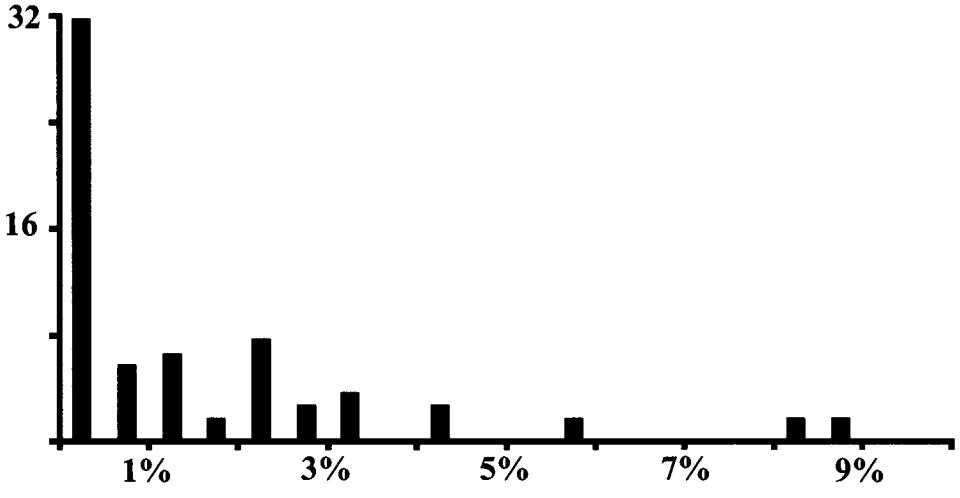

Figure 3.1. Levels of net mtDNA differentiation (D_A) between Belize & Panama for 60 species of resident Neotropical landbirds.

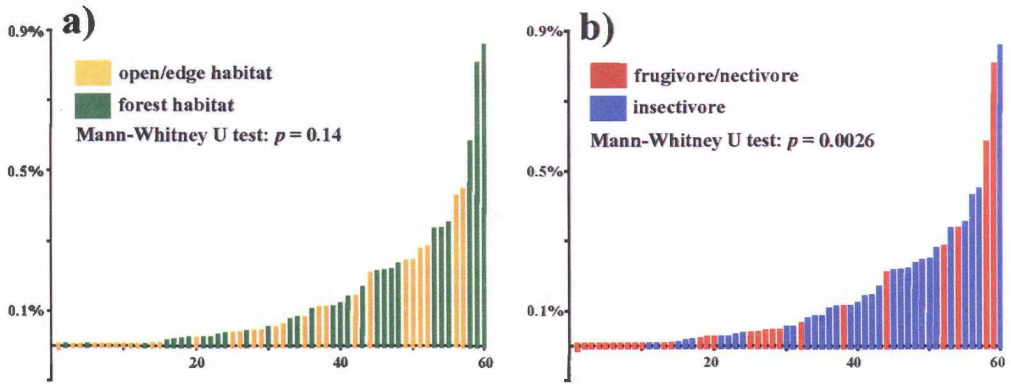

Figure 3.2. Rank order of D_A for species by habitat and foraging ecology. Among 60 species of resident Neotropical landbirds, net nucleotide divergence (D_A) between Panama and Belize were not significantly different among species preferring forest and open/edge habitats and those preferring forest (a); however, D_A was significantly lower for species of frugivores and nectivores compared with insectivores.

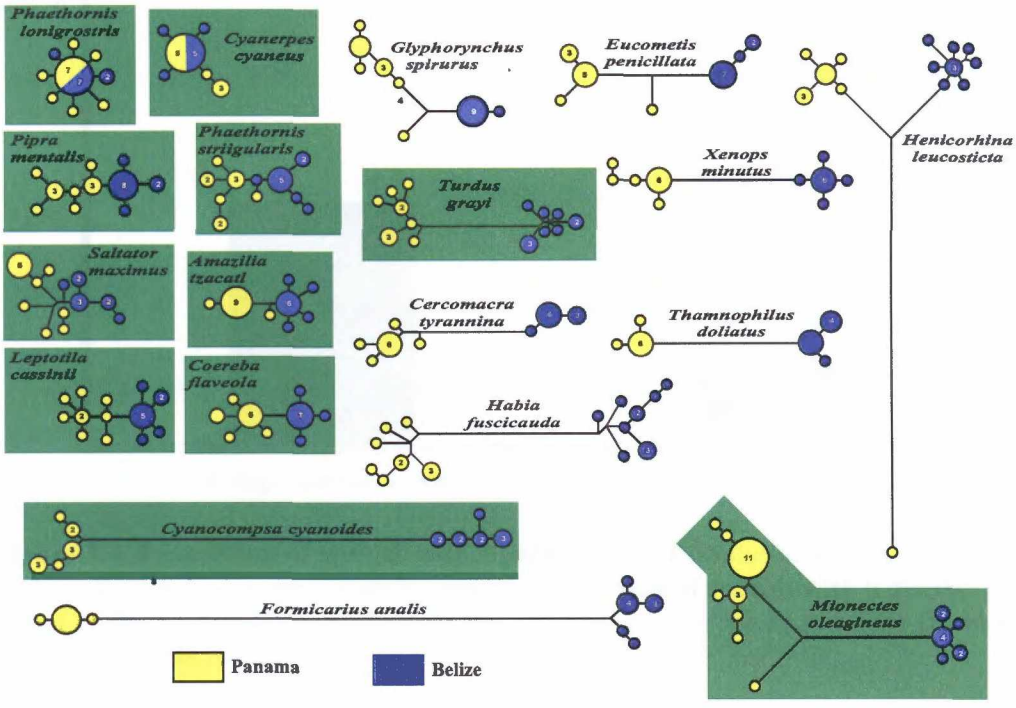

Figure 3.3. Minimum spanning trees for 19 species of resident Neotropical landbirds sampled in Belize and Panama. Among all 60 species studied, frugivores and nectivores (here framed in green) had lower levels of inter-population differentiation and were more likely to share haplotypes than insectivores; these tendencies are apparent among the 19 species shown here.

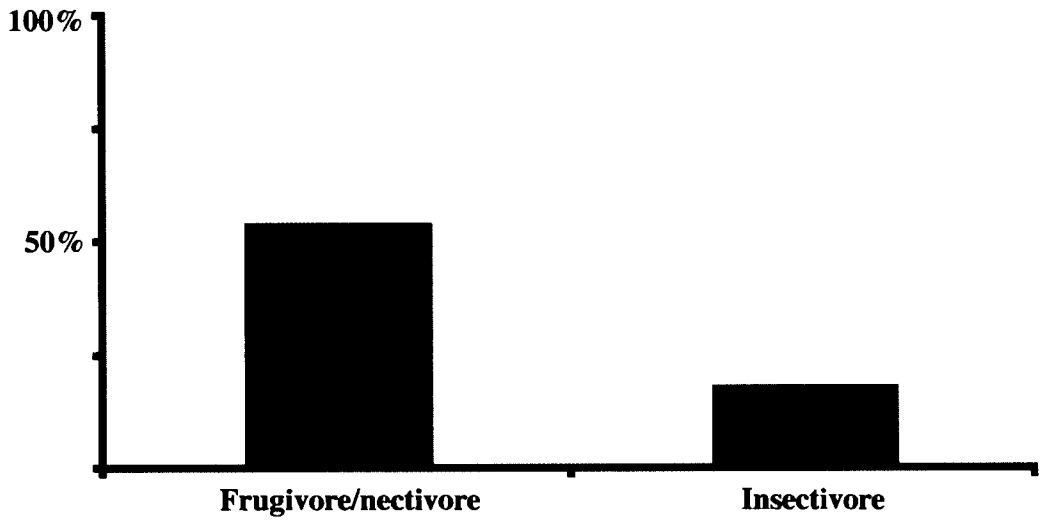

Figure 3.4. Proportion of species with significant R_2. Populations of frugivores and nectivores had a significantly greater proportion of species with a genetic signal of recent demographic expansion, as measured by the R_2 stastistic (Ramos-Onsins & Rozas 2002).

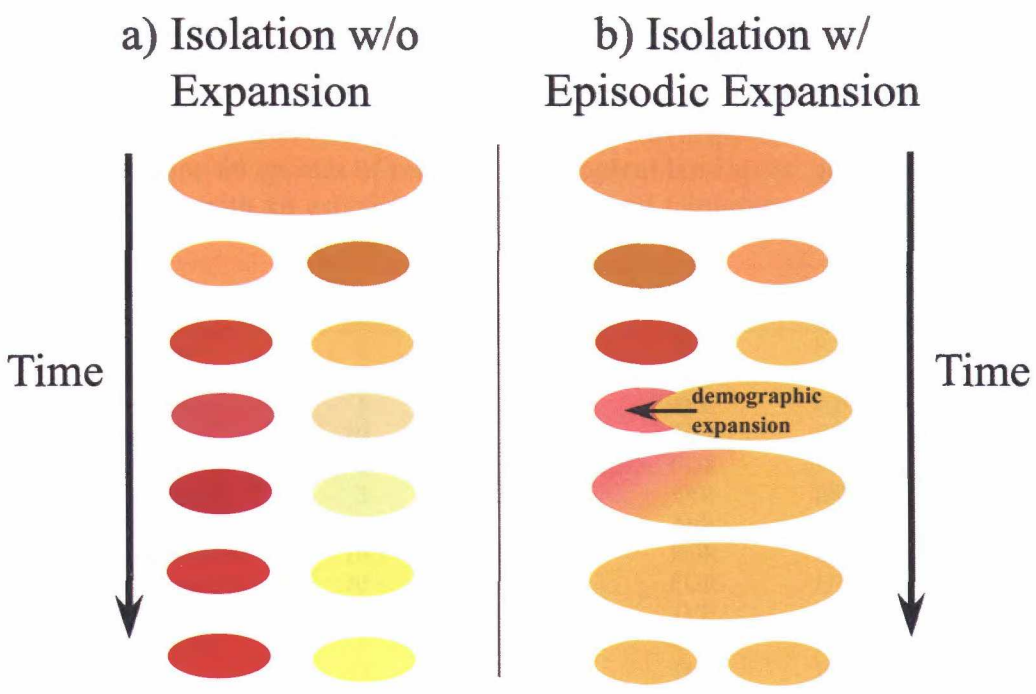

Figure 3.5. Alternative models of genetic differentiation over time between two populations. a) Without periodic re-unification, populations become more differentiated over time. b) Episodic expansion and reuniting of the two populations obscure earlier differences, causing them to appear to be more recently isolated when compared to a).

Table 3.1. Scientific name and ecological characteristics of 60 species. Scientific name, sample size in Belize and Panama (n), habitat (O/E; open and edge habitat; FOR: forest) and foraging guild classifications (F/N: frugivores or nectivores; INS: insectivore), and net nucleotide divergence (D_A) between Belize and Panama for 60 species of resident Neotropical landbirds. Species with D_A values marked with an asterisk (*) showed shared haplotypes between Belize and Panama.

scientific name	n_{BELIZE}	n_{PANAMA}	habitat	foraging guild	D_A
Columbina talpacoti	3	3	O/E	F/N	0.0000*
Claravis pretiosa	2	1	O/E	F/N	0.0000*
Leptotila verreauxi	1	3	O/E	F/N	0.0279
Leptotila cassinii	10	9	FOR	F/N	0.0037
Geotrygon montana	1	3	FOR	F/N	0.0000*
Nyctidromus albicollis	3	1	O/E	INS	0.0272
Threnetes ruckeri	2	3	O/E	F/N	0.0000*
Phaethornis longirostris	10	13	FOR	F/N	0.0000*
Phaethornis striigularis	10	10	FOR	F/N	0.0019
Florisuga mellivora	3	2	O/E	F/N	0.0000*
Thalurania columbica	1	2	O/E	F/N	0.0106
Amazilia tzacatl	10	11	O/E	F/N	0.0033
Chloroceryle aenea	3	3	FOR	INS	0.0003*
Notharchus macrorhynchos	2	1	O/E	INS	0.0048
Pteroglossus torquatus	3	3	O/E	F/N	0.0000*
Melanerpes pucherani	1	3	FOR	INS	0.0115
Xenops minutus	10	10	FOR	INS	0.0209
Sclerurus guatemalensis	1	3	FOR	INS	0.0231
Dendrocincla homochroa	3	3	FOR	INS	0.0135
Glyphorynchus spirurus	10	11	FOR	INS	0.0107
Dendrocolaptes sanctithomae	2	1	FOR	INS	0.0019
Taraba major	3	2	O/E	INS	0.0138
Thamnophilus doliatus	10	8	O/E	INS	0.0241
Thamnophilus atrinucha	3	3	FOR	INS	0.0010
Microrhopias quixensis	2	3	FOR	INS	0.0013
Cercomacra tyrannina	10	10	FOR	INS	0.0210
Gymnocichla nudiceps	3	2	O/E	INS	0.0423
Formicarius analis	10	10	FOR	INS	0.0853
Myiopagis viridicata	1	1	FOR	INS	0.0346
Mionectes oleagineus	10	20	FOR	F/N	0.0329
Poecilotriccus sylvia	5	1	O/E	INS	0.0077
Todirostrum cinereum	2	2	O/E	INS	0.0000*
Onychorhynchus coronatus	3	4	FOR	INS	0.0048
Terenotriccus erythrurus	3	3	FOR	INS	0.0030
Myiobius sulphureipygius	3	2	FOR	INS	0.0077
Attila spadiceus	3	3	FOR	INS	0.0099
Myiarchus tuberculifer	3	2	FOR	INS	0.0019
Megarhynchus pitangua	1	2	O/E	INS	0.0000*
Myiozetetes similis	2	2	O/E	INS	0.0442
Schiffornis turdina	3	3	FOR	F/N	0. 0801
Tityra semifasciata	2	2	O/E	F/N	0.0000*
Pipra mentalis	12	11	FOR	F/N	0.0015

Table 3.1 continued

Hylophilus decurtatus	3	3	O/E	INS	0.0239
Henicorhina leucosticta	10	11	FOR	INS	0.0214
Ramphocaenus melanurus	2	3	FOR	INS	0.0070
Polioptila plumbea	1	3	FOR	INS	0.0026
Turdus grayi	10	10	O/E	F/N	0.0203
Coereba flaveola	10	10	O/E	F/N	0.0039
Eucometis penicillata	10	10	FOR	INS	0.0163
Habia fuscicauda	10	11	FOR	INS	0.0329
Thraupis episcopus	2	3	O/E	F/N	-0.0006*
Tangara larvata	2	2	O/E	F/N	0.0000*
Chlorophanes spizea	2	2	O/E	F/N	0.0019
Cyanerpes cyaneus	8	9	O/E	F/N	0.0002*
Volatinia jacarina	3	3	O/E	F/N	0.0000*
Sporophila americana	3	3	O/E	INS	0.0105
Oryzoborus angolensis	2	2	O/E	F/N	0.0038
Tiaris olivaceus	2	3	O/E	F/N	0.0058
Cyanocompsa cyanoides	10	10	FOR	F/N	0.0576
Saltator maximus	9	11	O/E	F/N	0.0032

Table 3.2. Degree of population structure (F_{ST}) and significance values for 19 species of Middle American resident landbirds between Belizean and Panamanian populations. For all species except *Phaethornis longirostris*, F_{ST} values were significant and relatively high, consistent with the expectation of no significant ongoing gene flow between Belize and Panama.

scientific name	n_{BELIZE}	n_{PANAMA}	F_{ST}	p-value
Leptotila cassinii	10	9	0.656	< 0.001
Phaethornis longirostris	10	13	0.005	0.400
Phaethornis striigularis	10	10	0.471	< 0.001
Amazilia tzacatl	10	11	0.784	< 0.001
Xenops minutus	10	10	0.949	< 0.001
Glyphorynchus spirurus	10	11	0.842	< 0.001
Thamnophilus doliatus	10	8	0.976	< 0.001
Cercomacra tyrannina	10	10	0.943	< 0.001
Formicarius analis	10	10	0.981	< 0.001
Pipra mentalis	12	11	0.514	< 0.001
Mionectes oleagineus	10	20	0.918	< 0.001
Henicorhina leucosticta	10	11	0.695	< 0.001
Turdus grayi	10	10	0.863	< 0.001
Coereba flaveola	10	10	0.044	< 0.001
Eucometis penicillata	10	10	0.860	< 0.001
Habia fuscicauda	10	11	0.860	< 0.001
Cyanerpes cyaneus	8	9	0.213	0.037
Saltator maximus	9	11	0.450	< 0.001
Cyanocompsa cyanoides	10	10	0.969	< 0.001

Table 3.3: R_2 summary statistics and significance values for populations of 19 species of Middle American resident landbirds. R_2 measures the ratio of singletons to the overall number of segregating sites in a population; small values are expected for populations that have recently expanded (Ramos-Onsins & Rozas 2002). Significance (p-value) was determined non-parametrically by comparing the observed R_2 to those obtained from 10,000 coalescent simulations assuming no population expansion (performed in DnaSP 4.20; Rozas *et al.* 2003). Populations with significant p-values are in bold.

scientific name	population	R_2	p-value
FRUGIVORES/NECTIVORES:			
Leptotila cassinii	**Belize**	**0.137**	**0.04**
Leptotila cassinii	**Panama**	**0.087**	**< 0.01**
Phaethornis longirostris	Belize	0.017	0.14
Phaethornis longirostris	Panama	0.120	0.07
Phaethornis striigularis	**Belize**	**0.133**	**0.04**
Phaethornis striigularis	Panama	0.178	0.53
Amazilia tzacatl	**Belize**	**0.134**	**0.03**
Amazilia tzacatl	Panama	0.241	0.75
Mionectes oleagineus	**Belize**	**0.134**	**0.02**
Mionectes oleagineus	Panama	0.162	0.80
Pipra mentalis	**Belize**	**0.120**	**0.02**
Pipra mentalis	**Panama**	**0.118**	**0.03**
Turdus grayi	**Belize**	**0.118**	**< 0.05**
Turdus grayi	**Panama**	**0.119**	**0.04**
Coereba flaveola	**Belize**	**0.153**	**0.04**
Coereba flaveola	**Panama**	**0.137**	**0.04**
Cyanerpes cyaneus	**Belize**	**0.161**	**0.03**
Cyanerpes cyaneus	Panama	0.264	0.80
Cyanocompsa cyanoides	Belize	0.181	0.45
Cyanocompsa cyanoides	Panama	0.202	0.19
Saltator maximus	Belize	0.169	0.30
Saltator maximus	Panama	0.130	0.17
INSECTIVORES:			
Xenops minutus	**Belize**	**0.122**	**< 0.01**
Xenops minutus	Panama	0.192	0.52
Glyphorynchus spirurus	Belize	0.300	0.70
Glyphorynchus spirurus	Panama	0.200	0.83
Thamnophilus doliatus	Belize	0.201	0.44
Thamnophilus doliatus	Panama	0.217	0.37
Cercomacra tyrannina	Belize	0.190	0.32
Cercomacra tyrannina	Panama	0.140	0.10
Formicarius analis	Belize	0.151	0.25
Formicarius analis	Panama	0.200	0.32
Henicorhina leucosticta	**Belize**	**0.101**	**< 0.01**
Henicorhina leucosticta	Panama	0.267	1.00
Eucometis penicillata	Belize	0.206	0.53
Eucometis penicillata	Panama	0.240	0.96
Habia fuscicauda	**Belize**	**0.111**	**0.04**
Habia fuscicauda	Panama	0.115	0.08

Appendix 3.1. Specimens and tissue samples used in this study, with corresponding GenBank accession numbers.

ANSP: Academy of Natural Sciences of Philadelphia (Philadelphia, USA); LSUMZ: Louisiana State University Museum of Zoology (Baton Rouge, USA); MBM: Marjorie Barrick Museum (Las Vegas, USA); STRI: Smithsonian Tropical Research Institute (Balboa, Panama); UAM: University of Alaska Museum (Fairbanks, USA)

Columbina talpacoti

Belize (3)	
UAM 18185	EU713836
UAM 18186	EU713837
UAM 18187	EU713838
Panama (4)	
UAM MJM1404	EU713839
STRI PA-CTA87	EU713840
STRI MJM4662	EU713841
STRI MJM4461	EU713842

Claravis pretiosa

Belize (2)	
UAM ABJ436	FJ175690
UAM 18183	FJ175691
Panama (1)	
UAM 20362	FJ175692

Leptotila verreauxi

Belize (1)	
UAM 18178	FJ175693
Panama (3)	
UAM 20361	FJ175694
UAM 21700	FJ175695
UAM 20418	FJ175696

Leptotila cassinii

Belize (10)	
UAM 18173	FJ175697
UAM 15269	FJ175698
UAM 14341	FJ175699
UAM 18176	FJ175600
UAM 14500	FJ175601
UAM 8005	FJ175602
UAM 18174	FJ175603
UAM 18177	FJ175604
UAM 18172	FJ175605
UAM 18175	FJ175606
Panama (9)	
UAM 20256	FJ175607
UAM 20360	FJ175608
STRI PA-LCA70	FJ175609
MBM 14834	FJ175610
MBM 15128	FJ175611
UAM 20257	FJ175612
UAM 21703	FJ175613
STRI MJM3013	FJ175614
STRI MJM4623	FJ175615

Geotrygon montana

Belize (1)	
UAM 24370	FJ175616
Panama (3)	
UWBM 76880	FJ175617
UWBM 76970	FJ175618
STRI PA-GMO27	FJ175619

Nyctidromus albicollis

Belize (3)	
UAM 24365	FJ175620
UAM 18219	FJ175621
UAM 9478	FJ175622
Panama (1)	
MBM JMD734	FJ175623

Threnetes ruckeri

Belize (2)	
UAM 24529	FJ175624
UAM 18197	FJ175625
Panama (3)	
UAM MJM1465	FJ175626
UAM MJM629	FJ175627
UAM 20351	FJ175628

Phaethornis longirostris

Belize (10)	
UAM 7928	FJ175629
UAM 9253	FJ175630
UAM 10248	FJ175631
UAM 14444	FJ175632
UAM 14484	FJ175633
UAM 7938	FJ175634
UAM 9493	FJ175635
UAM 9566	FJ175636
UAM 7939	FJ175637
UAM 8058	FJ175638
Panama (13)	
UAM 20457	FJ175639
UAM 20465	FJ175640
UAM 20582	FJ175641
UAM MJM490	FJ175642
UAM 19207	FJ175643
UAM 20441	FJ175644
UAM 20287	FJ175645
UAM 20500	FJ175646
UAM 20430	FJ175647
UAM MJM548	FJ175648
UAM JMM604	FJ175649
UAM JMM588	FJ175650
LSUMZ B-28503	EU042579

Phaethornis striigularis

Belize (10)	
UAM 8035	FJ175751
UAM 15274	FJ175752
UAM 10290	FJ175753
UAM 8034	FJ175754
UAM 24379	FJ175755
UAM 7920	FJ175756
UAM 24318	FJ175757
UAM 24317	FJ175758
UAM 24371	FJ175759
UAM ABJ1205	FJ175760
Panama (10)	
UAM 19800	FJ175761
UAM 20464	FJ175762
UAM 20255	FJ175763
UAM 20253	FJ175764
UAM 20248	FJ175765
UAM MJM1453	FJ175766
UAM 20246	FJ175767
MBM 15668	FJ175768
MBM 15667	FJ175769
ANSP 189143	FJ175770

Florisuga mellivora

Belize (3)	
UAM 24528	FJ175771
UAM 10246	FJ175772
UAM 18198	FJ175773
Panama (2)	
UAM MJM1036	FJ175774
UAM KSW4404	FJ175775

Thalurania columbica

Belize (1)	
UAM ABJ617	FJ175776
Panama (2)	
UWBM 76938	FJ175777
UWBM 76922	FJ175778

Amazilia tzacatl

Belize (10)	
UAM 8037	XXXXXX
UAM 14312	XXXXXX
UAM 14322	XXXXXX
UAM 14323	XXXXXX
UAM 14461	XXXXXX
UAM 14513	XXXXXX
UAM 7963	XXXXXX
UAM 9079	XXXXXX
UAM 9203	XXXXXX
UAM 9237	XXXXXX
Panama (10)	
UAM 20618	XXXXXX
UAM 20629	XXXXXX
UAM 20372	XXXXXX
UAM 19208	XXXXXX

UAM 24462	XXXXXX
UAM 22692	XXXXXX
UAM 20299	XXXXXX
UAM 24403	XXXXXX
UAM KSW4380	SKINNED
MBM 15822	XXXXXX
LSUMZ B-16538	EU042524

Chloroceryle aenea

Belize (3)	
UAM 24364	FJ175779
UAM 24369	FJ175780
UAM 24385	FJ175781

Panama (3)	
UAM MJM1464	FJ175782
UAM JMM572	FJ175783
UAM JMM825	FJ175784

Notharchus macrorhynchos

Belize (2)	
UAM 18215	FJ175785
UAM 18214	FJ175786

Panama (1)	
MVUP JMM533	FJ175787

Pteroglossus torquatus

Belize (3)	
UAM 9087	FJ175788
UAM 24521	FJ175789
UAM ABJ1568	FJ175790

Panama (3)	
UAM 20345	FJ175791
UAM KSW4393	FJ175792
ANSP 189170	FJ175793

Melanerpes pucherani

Belize (1)	
UAM 8072	FJ175794

Panama (3)	
UAM 20349	FJ175795
UWBM 76898	FJ175796
STRI MJM1171	FJ175797

Xenops minutus

Belize (10)	
UAM 24522	FJ175798
UAM ABJ428	FJ175799
UAM ABJ622	FJ175800
UAM ABJ607	FJ175801
UAM 24355	FJ175802
UAM 24347	FJ175803
UAM 14330	FJ175804
UAM 24348	FJ175805
UAM 24357	FJ175806
UAM 24356	FJ175807

Panama (10)	
UAM 20338	FJ175808
UAM 20495	FJ175809
UAM KSW4392	FJ175810
UAM 20326	FJ175811
UAM 20492	FJ175812
UAM 20350	FJ175813
UAM MJM1462	FJ175814
UAM 22105	FJ175815
UAM 22110	FJ175816
UAM MJM1461	FJ175817

Sclerurus guatemalensis

Belize (1)	
UAM ABJ1580	FJ175818

Panama (3)	
MBM 15326	FJ175819
UAM 20498	FJ175820
STRI PA-SGU23	FJ175821

Dendrocincla homochroa

Belize (3)	
UAM 24534	FJ175822
UAM ABJ1215	FJ175823
UAM ABJ1264	FJ175824

Panama (3)	
UAM MJM655	FJ175825
UAM MJM671	FJ175826
UAM 20615	FJ175827

Glyphorynchus spirurus

Belize (10)	
UAM 24470	FJ175828
UAM 24324	FJ175829
UAM 24349	FJ175830
UAM 24350	FJ175831
UAM ABJ412	FJ175832
UAM 24513	FJ175833
UAM 24516	FJ175834
UAM 18313	FJ175835
UAM 18312	FJ175836
UAM 24320	FJ175837

Panama (10)	
UAM 20246	FJ175838
UAM 20269	FJ175839
UAM 20421	FJ175840
UAM 20429	FJ175841
UAM 20304	FJ175842
UAM 20428	FJ175843
UAM 20329	FJ175844
UAM 20245	FJ175845
UAM 20254	FJ175846
MBM 15324	FJ175847
STRI MJM2596	FJ175848

Dendrocolaptes sanctithomae

Belize (2)	
UAM ABJ627	FJ175849
UAM ABJ1528	FJ175850

Panama (1)	
MJM1414	FJ175851

Taraba major

Belize (3)	
UAM 18276	FJ175852
UAM 7995	FJ175853
UAM 7997	FJ175854

Panama (2)	
UAM 20416	FJ175855
UAM 20454	FJ175856

Thamnophilus doliatus

Belize (10)	
UAM 8070	FJ175857
UAM 24352	FJ175858
UAM 24390	FJ175859
UAM 24394	FJ175860
UAM ABJ600	FJ175861
UAM 9072	FJ175862
UAM 24362	FJ175863
UAM 15470	FJ175864
UAM 8042	FJ175865
UAM 18367	FJ175866

Panama (8)	
UAM 20595	FJ175867
UAM 22838	FJ175868
UAM MJM663	FJ175869
UAM 22837	FJ175870
UAM 22836	FJ175871
UAM MJM1425	FJ175872
STRI PA-THD29	FJ175873
STRI PA-THD120	FJ175874

Thamnophilus atrinucha

Belize (3)	
UAM ABJ1216	FJ175875
UAM ABJ1239	FJ175876
UAM ABJ1240	FJ175877

Panama (3)	
UAM 20567	FJ175878
UAM 20613	FJ175879
UAM 20572	FJ175880

Microrhopias quixensis

Belize (2)	
UAM ABJ596	FJ175881
UAM ABJ610	FJ175882

Panama (5)	
UAM 20400	FJ175883
MBM 16115	FJ175884
MBM 14841	FJ175885
MBM GMS1169	FJ175886
MBM 14842	FJ175887

Cercomacra tyrannina

Belize (10)	
UAM 18283	FJ175888
UAM 18281	FJ175889
UAM 18284	FJ175890
UAM 18277	FJ175891
UAM 18279	FJ175892
UAM 18282	FJ175893
UAM 24523	FJ175894
UAM 10260	FJ175895
UAM 9582	FJ175896
UAM 10275	FJ175897

Panama (10)	
UAM 20333	FJ175898
UAM 20352	FJ175899

UAM 20292	FJ175900
UAM 20355	FJ175901
MBM 15463	FJ175902
MBM 527	FJ175903
MBM 16159	FJ175904
UAM 20469	FJ175905
MBM 15833	FJ175906
MBM 16160	FJ175907

Gymnocichla nudiceps

Belize (3)	
UAM 9500	FJ175908
UAM 8036	FJ175909
UAM KSW4246	FJ175910
Panama (2)	
MBM 14845	FJ175911
STRI PA-GNU34	FJ175912

Formicarius analis

Belize (10)	
UAM 18289	FJ175913
UAM 18251	FJ175914
UAM 15468	FJ175915
UAM 18285	FJ175916
UAM 24374	FJ175917
UAM 18287	FJ175918
UAM ABJ958	FJ175919
UAM 24354	FJ175920
UAM 9508	FJ175921
UAM 14463	FJ175922
Panama (10)	
UAM 20437	FJ175923
UAM 20270	FJ175924
UAM 20463	FJ175925
UAM 20403	FJ175926
UAM 20239	FJ175927
MBM 14846	FJ175928
UAM JMM859	FJ175929
STRI PA-FAN56	FJ175930
STRI GS-PC20	FJ175931
UAM 20259	FJ175932

Myiopagis viridicata

Belize (1)	
UAM ABJ1538	FJ175933
Panama (1)	
UAM JMM819	FJ175934

Mionectes oleagineus

Belize (10)	
UAM 7908	EF110724
UAM 7911	EF110732
UAM 7912	EF110733
UAM 7933	EF110725
UAM 9573	EF110726
UAM 10266	EF110727
UAM 14310	EF110731
UAM 14328	EF110728
UAM 14494	EF110730
UAM 15426	EF110729
Panama (20)	
UAM 20378	EF110780
UAM 20447	EF110777
UAM 20458	EF110785
UAM 20468	EF110781
UAM 20467	EF110783
UAM 19457	EF110782
UAM 20494	EF110779
UAM 21777	EF110784
UAM 19456	EF110778
UAM MJM437	EF110786
UAM JMM904	EF110788
STRI MOL-PA24	EF110787
UAM 24011	EU433851
UAM 24015	EU433852
MBM 15827	EU433853
MBM 15484	EU433854
MBM 15485	EU433855
MBM 15486	EU433856
MBM 15826	EU433857
MBM 16111	EU433858

Poecilotriccus sylvia

Belize (5)	
UAM 24321	FJ175935
UAM 24322	FJ175936
UAM 18253	FJ175937
UAM 24361	FJ175938
UAM 24373	FJ175939
Panama (1)	
STRI PA-TSY117	FJ175940

Todirostrum cinereum

Belize (2)	
UAM ABJ671	FJ175941
UAM ABJ693	FJ175942
Panama (2)	
UAM 20346	FJ175943
STRI MJM2584	FJ175944

Onychorhynchus coronatus

Belize (3)	
UAM 24378	FJ175945
UAM 18235	FJ175946
UAM ABJ1543	FJ175947
Panama (3)	
UAM JMM855	FJ175948
STRI PA-OCO23	FJ175949
MBM 15261	FJ175950

Terenotriccus erythrurus

Belize (3)	
UAM ABJ514	FJ175951
UAM ABJ1567	FJ175952
UAM ABJ1560	FJ175953
Panama (3)	
UAM 20278	FJ175954
STRI PA-TER3774	FJ175955
UAM JMM691	FJ175956

Myiobius sulphureipygius

Belize (3)	
UAM 24366	FJ175957
UAM 24386	FJ175958
UAM KSW4236	FJ175959
Panama (3)	
UAM KSW4417	FJ175960
MBM 16121	FJ175961
MBM 16122	FJ175962

Attila spadiceus

Belize (3)	
UAM 24358	FJ175963
UAM 24395	FJ175964
UAM 24535	FJ175965
Panama (3)	
MBM 15341	FJ175966
MBM 15255	FJ175967
STRI PA-ASP113	FJ175968

Myiarchus tuberculifer

Belize (3)	
UAM 24388	FJ175969
UAM KSW4234	FJ175970
UAM 18234	FJ175971
Panama (2)	
UAM MJM702	FJ175972
MBM 15474	FJ175973

Megarhynchus pitangua

Belize (1)	
UAM 24521	FJ175974
Panama (2)	
UAM MJM513	FJ175975
MBM 14853	FJ175976

Myiozetetes similis

Belize (2)	
UAM 24389	FJ175977
UAM 24377	FJ175978
Panama (2)	
UAM 20298	FJ175979
UAM MJM688	FJ175980

Schiffornis turdina

Belize (3)	
UAM 24387	FJ175981
UAM 24525	FJ175982
UAM ABJ441	FJ175983
Panama (3)	
UAM 20633	FJ175984
UAM 22814	FJ175985
UAM 22813	FJ175986

Tityra semifasciata

Belize (2)	
UAM ABJ601	FJ175987
UAM 24538	FJ175988
Panama (2)	
MVUP MJM610	FJ175989
UWBM 76942	FJ175990

Pipra mentalis

Belize (12)

UAM 24372	FJ175991
UAM 24512	FJ175992
UAM 24514	FJ175993
UAM 24515	FJ175994
UAM 24517	FJ175995
UAM ABJ575	FJ175996
UAM ABJ626	FJ175997
UAM ABJ631	FJ175998
UAM 8006	FJ175999
UAM 9068	FJ176000
UAM 9507	FJ176001
UAM 9577	FJ176002

Panama (11)

UAM 20252	FJ176003
UAM 20277	FJ176004
UAM 20308	FJ176005
UAM MJM523	FJ176006
UAM 20431	FJ176007
UAM 20432	FJ176008
UAM 20408	FJ176009
UAM 19206	FJ176010
UAM 20607	FJ176011
UAM 20442	FJ176012
STRI MJM2603	FJ176013

Hylophilus decurtatus

Belize (3)

UAM 24527	FJ176014
UAM 24533	FJ176015
UAM ABJ574	FJ176016

Panama (3)

MBM 15795	FJ176017
UAM JMM910	FJ176018
MBM JK06-093	FJ176019

Henicorhina leucosticta

Belize (10)

UAM KSW4232	XXXXXX
UAM 9233	XXXXXX
UAM 9232	XXXXXX
UAM 9069	XXXXXX
UAM ABJ1226	XXXXXX
UAM 24323	XXXXXX
UAM 14319	XXXXXX
UAM 14318	XXXXXX
UAM 22731	XXXXXX
UAM ABJ1248	XXXXXX

Panama (11)

UAM 20578	XXXXXX
UAM 20625	XXXXXX
UAM MJM684	XXXXXX
UAM MJM1044	XXXXXX
UAM 20396	XXXXXX
UAM 22728	XXXXXX
UAM 22726	XXXXXX
UAM JMM907	XXXXXX
UAM MJM1420	XXXXXX
STRI MJM2588	XXXXXX
STRI MJM2400	XXXXXX

Ramphocaenus melanurus

Belize (2)

UAM 24532	FJ176020
UAM ABJ350	FJ176021

Panama (3)

UAM JMM883	FJ176022
UAM JMM884	FJ176023
STRI PA-RME116	FJ176024

Polioptila plumbea

Belize (1)

UAM ABJ594	FJ176025

Panama (3)

MBM 14679	FJ176026
MBM JK06-097	FJ176027
MBM JMD743	FJ176028

Turdus grayi

Belize (10)

UAM 9236	FJ176029
UAM 14268	FJ176030
UAM 14314	FJ176031
UAM 9197	FJ176032
UAM 9074	FJ176033
UAM 9533	FJ176034
UAM 9489	FJ176035
UAM KSW4197	FJ176036
UAM 8011	FJ176037
UAM 14335	FJ176038

Panama (10)

UAM 20407	FJ176039
UAM 20415	FJ176040
UAM 20583	FJ176041
UAM 19455	FJ176042
UAM 20319	FJ176043
UAM 20566	FJ176044
UAM 20367	FJ176045
UAM 20357	FJ176046
UAM 20339	FJ176047
UAM MJM466	FJ176048

Coereba flaveola

Belize (10)

UAM 9206	FJ176049
UAM 24391	FJ176050
UAM 24380	FJ176051
UAM 24393	FJ176052
UAM 24315	FJ176053
UAM 15296	FJ176054
UAM 18361	FJ176055
UAM 24382	FJ176056
UAM 24381	FJ176057
UAM 24392	FJ176058

Panama (10)

UAM 20453	FJ176059
UAM MJM356	FJ176060
UAM 20490	FJ176061
UAM 22843	FJ176062
UAM 20280	FJ176063
UAM 20358	FJ176064
UAM 20452	FJ176065
UAM 22841	FJ176066
STRI MJM2397	FJ176067
STRI MJM4660	FJ176068

Eucometis penicillata

Belize (10)

UAM 8001	FJ176069
UAM 7980	FJ176070
UAM 8029	FJ176071
UAM 14300	FJ176072
UAM 14492	FJ176073
UAM 15250	FJ176074
UAM 15279	FJ176075
UAM 15280	FJ176076
UAM 15249	FJ176077
UAM 15275	FJ176078

Panama (10)

UAM 24005	FJ176079
UAM 24007	FJ176080
UAM MJM1417	FJ176081
STRI PA-EPE71	FJ176082
MBM 14831	FJ176083
MBM 565	FJ176084
MBM 15490	FJ176085
UAM JMM582	FJ176086
UAM JMM583	FJ176087
UAM JMM603	FJ176088

Habia fuscicauda

Belize (10)

UAM 8007	FJ176089
UAM 8033	FJ176090
UAM 8048	FJ176091
UAM 8075	FJ176092
UAM 9082	FJ176093
UAM 9222	FJ176094
UAM 9224	FJ176095
UAM 9083	FJ176096
UAM 18370	FJ176097
UAM 18369	FJ176098

Panama (10)

UAM 20291	FJ176099
UAM 20296	FJ176100
UAM 20324	FJ176101
UAM 20456	FJ176102
UAM 20455	FJ176103
UAM 22773	FJ176104
UAM 22774	FJ176105
UAM 20302	FJ176106
UAM 20301	FJ176107
UAM 20379	FJ176108
STRI MJM2602	FJ176109

Thraupis episcopus

Belize (2)

UAM 24383	FJ176110
UAM 18358	FJ176111

Panama (3)

UAM MJM1804	FJ176112
UAM JMM821	FJ176113
UAM JMM877	FJ176114

Tangara larvata

Belize (2)

UAM 24319	FJ176115
UAM 24359	FJ176116

Panama (2)	
UAM MJM568	FJ176117
UAM 22840	FJ176118

Chlorophanes spiza

Belize (2)	
UAM ABJ576	FJ176119
UAM ABJ602	FJ176120

Panama (2)	
UAM MJM637	FJ176121
UAM 19144	FJ176122

Cyanerpes cyaneus

Belize (8)	
UAM 24536	FJ176123
UAM KSW4257	FJ176124
UAM KSW4259	FJ176125
UAM 18364	FJ176126
UAM ABJ49	FJ176127
UAM 18366	FJ176128
UAM 18365	FJ176129
UAM 18367	FJ176130

Panama (9)	
UAM 22844	FJ176131
UAM MJM1412	FJ176132
UAM KSW4403	FJ176133
UAM 22847	FJ176134
UAM 22845	FJ176135
UAM 22839	FJ176136
UAM KSW4782	FJ176137
UAM JMM902	FJ176138
UAM 22846	FJ176139

Volatinia jacarina

Belize (3)	
UAM 24363	FJ176140
UAM KSW4202	FJ176141
UAM 24531	FJ176142

Panama (3)	
MBM 14839	FJ176143
MBM 16164	FJ176144
STRI MJM4545	FJ176145

Sporophila americana

Belize (3)	
UAM 24353	FJ176146
UAM 24384	FJ176147
UAM 24537	FJ176148

Panama (3)	
UAM 19793	FJ176149
STRI PA-SAM25	FJ176150
STRI PA-SAM8	FJ176151

Oryzoborus angolensis

Belize (2)	
UAM 8013	FJ176152
UAM 9077	FJ176153

Panama (2)	
UAM JMM885	FJ176154
STRI PA-OAN97	FJ176155

Tiaris olivaceus

Belize (2)	
UAM 24360	FJ176156
UAM 24376	FJ176157

Panama (3)	
UAM MJM488	FJ176158
UAM KSW4783	FJ176159
UAM JMM912	FJ176160

Saltator maximus

Belize (9)	
UAM KSW4255	FJ176161
UAM 18168	FJ176162
UAM 18167	FJ176163
UAM ABJ1269	FJ176164
UAM 8024	FJ176165
UAM 18170	FJ176166
UAM 7947	FJ176167
UAM 18171	FJ176168
UAM ABJ1569	FJ176169

Panama (11)	
UAM 20472	FJ176170
UAM 14745	FJ176171
UWBM 76997	FJ176172
MBM 14747	FJ176173
UAM 20619	FJ176174
UAM JMM592	FJ176175
UAM JMM595	FJ176176
UAM MJM1449	FJ176177
UAM 14746	FJ176178
UAM 19432	FJ176179
STRI MJM2405	FJ176180

Cyanocompsa cyanoides

Belize (10)	
UAM 24530	FJ176181
UAM 24314	FJ176182
UAM 3490	FJ176183
UAM 15286	FJ176184
UAM 9553	FJ176185
UAM 14259	FJ176186
UAM 14279	FJ176187
UAM 24351	FJ176188
UAM 24313	FJ176189
UAM ABJ1579	FJ176190

Panama (10)	
UAM 20622	FJ176191
UAM 20553	FJ176192
UAM 2-594	FJ176193
UAM 20251	FJ176194
UAM MJM707	FJ176195
UAM 20309	FJ176196
UAM 19200	FJ176197
UAM 20602	FJ176198
UAM 20374	FJ176199
UAM 20491	FJ176200

CONCLUSIONS

I used molecular genetic markers to research how gene pools of Neotropical species are spatially distributed. These questions are of fundamental importance, because the geographic structuring of genetic variation is believed to be the first step in the speciation process, and because genetic variation is usually considered important for population persistence over time. The three studies provide three perspectives on these issues. In the first study, I found that *Mionectes oleagineus* had three phylogenetic splits over the Andes. Dating these splits using molecular clock approaches indicates that at least two of these splits post-dated the Andean uplift, and indicate that *M. oleagineus* dispersed from the Amazonian lowlands over or around the Andes to colonize the lowlands west of the Andes. The four lineages found west of the Andes are apparently parapatrically distributed, resulting in a greater number of mitochondrial DNA lineages west of the Andes than to the east, despite the fact that there is considerably more land area to the east. This finding challenges the role of the Andes as a vicariant barrier, and instead suggests that episodic overcoming of barriers such as the Andes may promote the formation of phylogeographic lineages.

In the second chapter I found that within-population genetic variation in nine resident Neotropical landbirds had a humped distribution along a latitudinal gradient in Middle America. This result is important because no previous study had measured how within-population genetic diversity varied over the range of species that are exclusively tropical. Furthermore, our result agrees with classical population genetic models that predict the greatest within-population genetic variation in mid-range

populations. While our findings are limited to mitochondrial DNA sequence variation, which is believed to be neutral, they provide an important null hypothesis upon which to base future studies of the geographic distribution of adaptive variation across the range of tropical species.

In the third chapter, I examined genetic differentiation between Belize and Panama among 60 species of codistributed resident Neotropical landbirds. I found that foraging ecology was significantly correlated with the degree of genetic differentiation. Species that are frugivores and nectivores had lower levels of differentiation than species of insectivores. Furthermore, detailed population sampling of 19 populations of these species indicated that a significantly greater proportion of the frugivore and nectivore populations showed signs of recent demographic expansion relative to insectivore populations. Together, these results suggest that foraging ecology plays a fundamentally important role in determining diversification patterns of Neotropical birds. This result is important because it demonstrates that for a substantial portion of a Neotropical avian community intrinsic factors such as foraging ecology need to be considered along with external factors such as geographical barriers to gene flow to understand the history of avian diversification.