IMPLICATIONS OF GENETIC DIFFERENTIATION IN NEOTROPICAL MONTANE FOREST BIRDS[1]

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ABSTRACT

The complex geography of the Neotropical montane system is a natural laboratory for population divergence. Understanding which geographic barriers (lowland barriers, arid river valleys, and montane barriers above the tree line separate these regions of endemism) are instrumental in promoting and maintaining population divergence is an important step in preserving genetic diversity and endemism within the region. Here, I analyze patterns of genetic differentiation between 16 predefined regions of endemism for 43 co-distributed zoogeographic species complexes of Neotropical montane forest birds. The analysis shows that lowland barriers generate the highest levels of genetic differentiation, while barriers above the tree line in the Andes show the least. Within the Andes, arid river valleys promote population divergence to varying degrees. The Río Marañón shows the greatest effect, but the Río Apurímac and Río Quinimari are also associated with extensive genetic differentiation, while genetic divergence across other river valleys is generally weak. Most barriers are associated with a wide span of divergence times, supporting a protracted history of dispersal postdating barrier formation. If the goal is to maintain genetic diversity, preservation of populations within each region of endemism would help to ensure the continued survival of evolutionarily distinct lineages within species. Considering the alarming rate of deforestation in Neotropical montane regions, preservation of suitable tracts of montane forest is needed within each region of endemism, with special emphasis placed on endemism regions separated by lowland barriers and by deep intermontane river valleys.

Key words: Andes, birds, endemism, Neotropical, phylogeography, river barriers.

The Neotropics possess the world's most species-diverse floras and faunas (Rosenzweig, 1995; Newton, 2003; Kreft & Jetz, 2007). In birds, ca. 3000 continental species occur (Newton, 2003), far more than in any other biogeographic realm. Almost half of Neotropical avian species occur in a complex chain of highland regions extending from Mexico to Argentina. The chief components of the Neotropical highland system are the Andes, extending the entire length of South America from Tierra del Fuego in the south to Venezuela in the north. A chain of isolated highland regions extends northward through Central America to Mexico, and additional isolated highland regions occur in northern South America (Fig. 1). This highland system is the largest and geographically most complex at tropical latitudes, and its ongoing uplift has promoted extensive diversification opportunities (Weir, 2006; Ribas et al., 2007).

Over the past 10 million years, extensive orogeny occurred throughout the Neotropics (Gregory-Wodzicki, 2000; Grafe et al., 2002; Audemard, 2003; Dhont et al., 2005). Diversification occurred in Neotropical highland birds throughout this period, with little indication of a slowdown in diversification rates (Weir, 2006). However, Neotropical lowland faunas experienced a slowdown in diversification rates over the same time period (Weir, 2006). The contrast suggests that highland regions are key cradles of diversity in the Neotropical region and their preservation is of utmost importance.

Unlike lowland wet forest species, which generally occupy large geographic ranges, a high degree of local endemism has resulted because of the rugged terrain and geographic complexity of the Neotropical highland system (Gentry, 1992; Stattersfield et al., 1998; Valencia et al., 2000). Co-distributed species complexes often exhibit similar patterns of endemism. This finding has led several authors to define common patterns of endemism in Neotropical montane birds shared across a wide diversity of taxa (Cracraft, 1985; Stotz et al., 1996; Stattersfield et al., 1998). The boundaries of many highland regions of endemism coincide with known or suspected geographic barriers believed by most authors to reduce or prevent gene flow (Vuilleumier, 1969; O'Neill, 1992). These boundaries include intervening lowland regions

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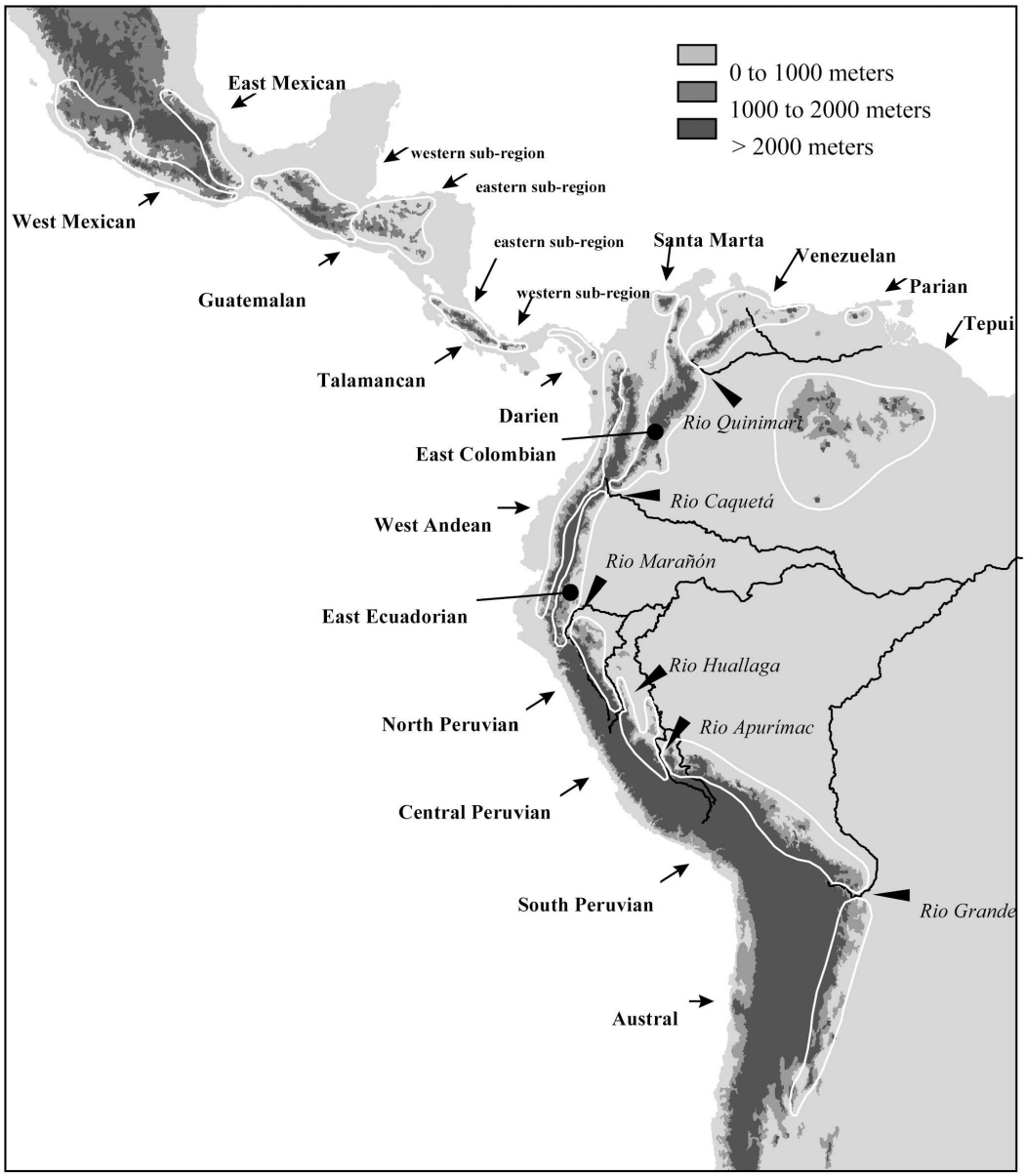

Figure 1. Geographic ranges of regions of endemism are analyzed. River valleys and lowland isthmuses separating Andean regions of endemism are illustrated. See text for other non-riverine barriers separating regions of endemism.

(which support a variety of habitat types that differ floristically from montane regions), arid inter-Andean river valleys, and high-elevation barriers above the tree line. However, the exact role of these barriers in promoting population divergence and speciation is debatable. Some authors suggested that montane forest diversification was primarily associated with ecologically stable regions that survived intact during recent glacial episodes, rather than as a consequence of geologic barriers (Fjeldså, 1995). While speciation in a select group of highland taxa appears to have occurred on opposite sides of these barriers, giving rise to the observed patterns of endemism, many other species are distributed across barriers and occupy multiple regions of endemism. Thus, it remains unknown to what extent these barriers promote population divergence in general.

Here, I analyzed phylogenetic patterns in a set of 43 Neotropical humid montane forest species complexes in order to understand how proposed barriers

promote and maintain genetic differentiation between regions of endemism. I compared genetic differentiation in mitochondrial DNA between adjacent highland regions of endemism for both species-level taxa and intraspecific populations in order to assess the general usefulness of treating regions of endemism as distinct conservation regions.

Materials and Methods

Taxon Sampling

I used zoogeographic species complexes (Mayr & Short, 1970; Fjeldså & Krabbe, 1990; Mayr & Diamond, 2001) to analyze phylogenetic patterns within Neotropical highland regions. Zoogeographic species, as used in this study, include monophyletic (at least in mitochondrial DNA phylogenies) clades composed of one or more allopatric or parapatric taxa, but do not contain taxa with sympatric distributions (other than highly local sympatry along contact zones). In a phylogenetic context, zoogeographic species represent the largest inclusive clades of taxa within genera that lack sympatric overlap. Because taxa within zoogeographic species lack sympatry, they retain the geographic signature of the diversification process and are ideal units to analyze the role of dispersal barriers in promoting diversification. In most cases, zoogeographic species include very closely related taxa that are considered to form superspecies complexes and are likely capable of at least limited interbreeding, should migration occur between them.

All zoogeographic species occupying Neotropical montane forest were included (Table 1), for which mitochondrial protein-coding DNA sequences representing two or more highland regions (Fig. 1) were available. The sample included both zoogeographic species composed of a single and multiple allopatric species and spanned a wide taxonomic diversity (Table 1). Sampling within zoogeographic species was not random geographically, with the greatest effort occurring in Bolivia, Peru, Ecuador, Panama, and Costa Rica. Samples from Colombia, Venezuela, and the tepuis were available for only a few zoogeographic species (Table 1).

Endemism Regions

Areas of highland endemism have been defined for Neotropical birds by Cracraft (1985) for South America and in greater detail by Stattersfield et al. (1998) and Stotz et al. (1996) for the entire Neotropical region. The regions of endemism defined by the three studies correlate closely in most cases, although Stattersfield et al. (1998) split a number of Cracraft's regions more finely. I studied differentiation across a modified classification of these regions for montane humid forest (Fig. 1). I did not include some regions for which sequence data were unavailable, and several regions of endemism were split more finely into subregions of endemism (see Fig. 1).

Montane humid forest (also referred to as montane evergreen forest) ranges ca. 1000–1500 m (locally lower, especially along the Pacific slope of the north Andes and in the Darién Highlands; Gentry, 1986) to the tree line, which at tropical latitudes generally varies from 3000–3500 m. Humid montane forest is characterized by a profusion of moss and other epiphytes, which liberally blanket branches and trunks of trees (Stotz et al., 1996). Humid montane forest is strongly bisected in the Neotropical region by a series of lowland barriers, arid river valleys, and highland barriers above the tree line. Adjacent regions of endemism are almost always separated by one of these barriers.

Phylogenetic Analysis

Mitochondrial protein-coding DNA sequences for Neotropical montane forest birds were obtained from previously published phylogenetic or phylogeographic studies or sequenced for this project (Tables 1, 2) using standard protocols (Weir et al., 2008). Model-corrected genetic distances were compared between adjacent highland regions. Parameters of the general time reversible (GTR)-Γ model of sequence evolution were estimated using maximum likelihood in PAUP 4.0b10 (Swofford, 2002) from a neighboring tree rooted with *Struthio* L. Parameters were estimated separately for each gene. Maximum likelihood parameter estimates were used to obtain GTR-Γ distances between sampled individuals.

Barriers may promote genetic differentiation between populations in several ways. Barrier formation may result in the vicariant fragmentation of widespread species into populations on either side of a barrier. If gene flow is prevented or greatly reduced, then the populations will begin to differentiate in mitochondrial DNA markers on opposite sides of the barrier. In such cases, mitochondrial sequence divergence can be transformed into time estimates of barrier formation using an appropriate molecular clock. For species that did not span a barrier when it formed, dispersal across a barrier might result in the formation of founder populations any time after barrier formation. Again, provided that gene flow is prevented or greatly reduced by the barrier, genetic differentiation will occur on either side of the barrier and sequence divergence can be converted into a time estimate of the founder event using a molecular clock. Caveats arise when leaky barriers allow limited amounts of gene flow, which may

prevent mitochondrial differentiation after vicariance or founder events. The degree of genetic differentiation, however, provides an approximate timescale for the cessation of gene flow and may postdate the initial vicariant or founder events.

Based on 84 avian calibrations supported by cross validation, an average molecular rate of close to 2% was strongly supported (Weir & Schluter, 2008) for model (GTR-Γ)-corrected distances of the mitochondrial cytochrome b gene. The 2% rate applies to divergence dates spanning the past 12 million years (Weir & Schluter, 2008) and is used throughout this study to convert model-corrected sequence divergence (using the same model that is used in the clock calibrations) into approximate time estimates of when populations began to differentiate in protein-coding mitochondrial DNA markers. DNA from cytochrome b was used in all but eight of the 44 zoogeographic species complexes analyzed here. The remaining eight used either ATPase 6 and 8 (*Henicorhina* P. L. Sclater & Salvin) or ND2 (*Hemispingus* Cabanis, *Ochthoeca* Cabanis, *Troglodytes* Vieillot), two protein-coding mitochondrial DNA genes that evolve at similar rates to cytochrome b (e.g., Lovette, 2004; Weir et al., 2008).

Sequence divergence actually measures coalescent times, the point at which haplotypes begin to diverge. Coalescent times and population splitting times will be equivalent if no standing variation occurred at the time of population splitting. If ancestral polymorphism in DNA haplotypes was present at population splitting, daughter populations will inherit a degree of genetic variation. If daughter populations randomly fix different alleles, then the date at which the fixed haplotypes coalesce will predate the time of population splitting. The discrepancy between coalescent and population splitting dates can be estimated by assuming that current and past levels of standing genetic variation within populations are the same. In Neotropical highland birds, protein-coding mitochondrial DNA haplotype divergence averages 0.4%, suggesting that coalescent times predate population splitting times by only 0.2 million years (Ma) (Weir, 2006). Given that the discrepancy is slight, uncorrected coalescent dates are reported here.

Two methods were used to construct consensus area cladograms of highland regions. The first used standard approaches for constructing supertrees (Baum, 1992; Bininda-Emonds, 2004). Supertrees are generally constructed from different gene phylogenies for a common set of taxa, although each phylogeny may not contain all taxa. Here, I used phylogenies of co-distributed zoogeographic species to generate a supertree for a common set of geographic regions (supertree area cladogram [SAC]). Input phylogenies in the SAC method may show discordant topologies just as they do in the regular supertree method when using topologies from different genes. The SAC method finds the topology with the fewest steps required under parsimony criteria. This topology should be viewed as a compromise that finds the strongest overriding phylogenetic signal in the data set.

Maximum likelihood or Bayesian molecular phylogenies of montane forest taxa were obtained from published molecular phylogenies or were generated from unpublished data sets using standard methods (Weir, 2006). Trees were included for all zoogeographic species with samples from three or more highland regions. Next, I converted phylogenies manually to matrix representation (Bininda-Emonds & Bryant, 1998). A fully bifurcating tree with n tips is converted to a matrix with n rows and n-1 columns (polytomies can also be coded but result in n-1-p columns, where p is the number of nodes collapsed into polytomies). Each row represents a geographic region and each column a node on the phylogeny. All descendent regions for a given node are coded as 1, and all outgroup regions to that node are coded as 0. Geographic regions not sampled are coded either as missing if the zoogeographic species occupies the region but was not sampled, or as 0 if the zoogeographic species is absent from the region in question.

The SAC method may perform poorly by giving equal weight to each column in the supermatrix irrespective of whether nodes in input phylogenies received strong support. One option is to weight each column (i.e., input node) in the supermatrix based on its nodal support. This option requires that the same nodal support metric be used consistently across input trees. In this analysis, most input topologies were derived from published maximum likelihood and Bayesian phylogenies, which use different measures of support, and no weighting was implemented.

Matrices for each zoogeographic species were concatenated, and parsimony analysis was performed on the combined matrix. Several types of parsimony analysis have traditionally been performed in supertree analysis. These analyses generally perform similarly (Bininda-Emonds & Sanderson, 2001; Bininda-Emonds, 2004). However, when adapting supertree methods to generate consensus area cladograms, reversible parsimony methods are preferable because they make no assumptions regarding extinction. I performed standard parsimony (standard matrix representation parsimony; Baum, 1992), which allows reversals and local extinction. A heuristic search of tree space was performed in PAUP 4.0b10 (Swofford, 2002) with stepwise addition, random sequence addition (25 replicates), tree bisection-reconnection swapping, and a maximum of 100 trees stored at any time. Strict consensus cladograms were constructed from all equally

Table 1. Geographic distributions of Neotropical montane forest zoogeographic species complexes included in this study. Regions of endemism occupied by a zoogeographic species but not sampled in this study are represented by 0; those that were sampled are represented by 1. Regions of endemism codes as used in Figure 4 are shown for Mexico and Middle America (C1 to C7), the Andes (A1 to A8), and isolated South American regions (S1 to S3).

Zoogeographic species complex	West Mexican (C1)	East Mexican (C2)	Guatemalan West (C3)	Guatemalan East (C4)	Talamanca West (C5)	Talamanca East (C6)	Darién (C7)	Tepui (S1)	Parian (S2)	Santa Marta (S3)	Venezuelan (A1)	East Colombian (A2)	East Ecuadorian (A3)	West Andean (A4)	North Peruvian (A5)	Central Peruvian (A6)	South Peruvian (A7)	Austral (A8)	Phylogenetic reference
Three or more regions sampled																			
Arremon brunneinucha Lafresnaye (chestnut-capped brush-finch)	1	1	1	1	1	1	1				1	1	1	1	1	1	1		Cadena et al., 2007
A. torquatus Lafresnaye & d'Orbigny (stripe-headed brush-finch)					1		1	0	1	1	1	1	1	1	1	1	1	1	Cadena et al., 2007
Andigena Gould complex[1]													0	1	0	1	1		Nahum et al., 2003; Moyle, 2004; Weckstein, 2005
Cacicus chrysonotus Lafresnaye & d'Orbigny (mountain cacique)											0	0	1	0	1	0	1		Price & Lanyon, 2004
Campylorhamphus pusillus P. L. Sclater (brown-billed scythebill)					1	0	1					0	1	1	0				This study
Chlorospingus canigularis Lafresnaye (ashy-throated bush-tanager)					1							0	1	1	0	0	0		Weir et al., 2008
C. flavigularis P. L. Sclater (yellow-throated bush-tanager)						1	0					0	0	1	0	0	1		Weir et al., 2008
Chlorospingus Cabanis complex[2]	1	1	1	1	1	1	1				1	0	1	1	1	1	1	1	Weir et al., 2008
Cinclus Borkhausen complex[3]	0	0	0	0	0	0	0			0	0	0	0	1	0	1	0	1	Voelker, 2002
Cranioleuca Reichenbach complex A[4]					0	0	1	1	0	0	0	1	1	1	0	1	1	1	This study; Garcia-Moreno et al., 1999

Table 1. Continued.

Zoogeographic species complex	West Mexican (C1)	East Mexican (C2)	Guatemalan West (C3)	Guatemalan East (C4)	Talamanca West (C5)	Talamanca East (C6)	Darién (C7)	Tepui (S1)	Parian (S2)	Santa Marta (S3)	Venezuelan (A1)	East Colombian (A2)	East Ecuadorian (A3)	West Andean (A4)	North Peruvian (A5)	Central Peruvian (A6)	South Peruvian (A7)	Austral (A8)	Phylogenetic reference
Diglossa Wagler complex[5]	1	0	0	0	1	1	0		0	0	0	0	0	0	1	0	0	0	Hackett, 1995
Hemispingus Cabanis complex A[6]												0	0	1	0	1	1	0	Garcia-Moreno et al., 2001
Hemispingus complex B[7]												0	1	0	1	1	0	0	Garcia-Moreno et al., 2001
Henicorhina leucophrys Tschudi (gray-breasted wood-wren)	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	Dingle et al., 2006
Lampornis amethystinus Swainson (amethyst-throated hummingbird)	1	1	1	1															Garcia-Moreno et al., 2006
Lampornis Swainson complex[8]			1	1	1	1													Garcia-Moreno et al., 2006
Metallura tyrianthina Loddiges (Tyrian metaltail)										0	0	0	1	1	0	1	1		Garcia-Moreno et al., 1999
Myadestes Swainson complex[9]					1	1	1				0	0	1	1	0	1	1	0	Miller et al., 2007
M. occidentalis Stejneger (brown-backed solitaire)	1	1	1	0															Miller et al., 2007
Myioborus S. F. Baird complex[10]					1	0		1	1	1	1	1	1	1	1	1	1	1	Perez-Eman, 2005
M. miniatus Swainson (slate-throated redstart)	1	0	1	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	Perez-Eman, 2005
Myrmotherula schisticolor Lawrence (slaty antwren)			0	0	1	0	1		0	0	0	0	0	1	0	0	1	0	Weir, unpublished data
Ochthoeca cinnamomeiventris Lafresnaye (slaty-backed chat-tyrant)											0	0	1	1	0	0	1		Garcia-Moreno et al., 1998
O. frontalis Lafresnaye (crowned chat-tyrant)												0	1	1	0	1	1		Garcia-Moreno et al., 1998
O. fumicolor P. L. Sclater (brown-backed chat-tyrant)											0	0	1	1	0	0	1		Garcia-Moreno et al., 1998
Ochthoeca Cabanis complex[11]										0	0	0	1	0	1	1	1		Garcia-Moreno et al., 1998

Table 1. Continued.

Zoogeographic species complex	West Mexican (C1)	East Mexican (C2)	Guatemalan West (C3)	Guatemalan East (C4)	Talamanca West (C5)	Talamanca East (C6)	Darién (C7)	Tepui (S1)	Parian (S2)	Santa Marta (S3)	Venezuelan (A1)	East Colombian (A2)	East Ecuadorian (A3)	West Andean (A4)	North Peruvian (A5)	Central Peruvian (A6)	South Peruvian (A7)	Austral (A8)	Phylogenetic reference
Pionus sordidus Linnaeus (red-billed parrot)									1	1	1	1	0	1	1	0	0		Ribas et al., 2007
Tangara Brisson complex A[12]					1	1	1			0	0	0	0	1	0	1	0		This study; Burns & Naoki, 2004
T. florida P. L. Sclater & Salvin (emerald tanager)					1	1	1							1					This study; Burns & Naoki, 2004
T. guttata Cabanis (speckled tanager)					0	1	1	1	0		0	0	0	0					This study; Burns & Naoki, 2004
Thamnistes anabatinus P. L. Sclater & Salvin (russet antshrike)			0	0	1	0	1					0	1	1	1	0	0		This study; Irestedt et al., 2004
Troglodytes Vieillot complex[13]			1	0	1	0	0	1		0	0	0	0	0	0	1	0	0	Rice et al., 1999
Xiphocolaptes promeropirhynchus Lesson (strong-billed woodcreeper)	0	1	0	1	0	0		0	0	0	0	0	1	0	0	0	0	0	Weir, unpublished; Aleixo, 2002; Irestedt et al., 2004
Xiphorhynchus erythropygius P. L. Sclater (spotted woodcreeper)	0	0	0	0	1	0	1							1					This study; Aleixo, 2002
Haplospiza rustica Tschudi (slaty finch)	0	0	0	0	1	1		1		0	0	0	0	1	0	0	0		This study
Two adjacent regions sampled																			
Catharus aurantiirostris Hartlaub (orange-billed nightingale-thrush)	1	0	0	1	0	0			0	0	0	0	0	0					Outlaw et al., 2003; Winker & Pruett, 2006
Chrysothlypis chrysomelas P. L. Sclater & Salvin (black-and-yellow tanager)					0	1	1												This study; Burns, 1997
Cranioleuca complex B[14]												0	1	1	0	0	0		Garcia-Moreno et al., 1999

Table 1. Continued.

Zoogeographic species complex	West Mexican (C1)	East Mexican (C2)	Guatemalan West (C3)	Guatemalan East (C4)	Talamanca West (C5)	Talamanca East (C6)	Darién (C7)	Tepui (S1)	Parian (S2)	Santa Marta (S3)	Venezuelan (A1)	East Colombian (A2)	East Ecuadorian (A3)	West Andean (A4)	North Peruvian (A5)	Central Peruvian (A6)	South Peruvian (A7)	Austral (A8)	Phylogenetic reference
Pselliophorus Ridgway complex[15]					1	1													This study; Klicka & Spellman, 2007
Semnornis Richmond complex[16]					1	0								1					Barker & Lanyon, 2000
Tangara complex B[17]									0	0	0	0	1	1	0	0	0		Burns & Naoki, 2004
Turdus Linnaeus complex[18]	0	0	1	0	1														Voelker et al., 2007

[1] *Andigena laminirostris* Gould (plate-billed mountain-toucan), *A. hypoglauca* Gould (gray-breasted mountain-toucan), *A. cucullata* Gould (hooded mountain-toucan).

[2] *Chlorospingus ophthalmicus* Du Bus de Gisignies (common bush-tanager), *C. semifuscus* P. L. Sclater & Salvin (dusky bush-tanager), *C. tacarcunae* Griscom (Tacarcuna bush-tanager), *C. inornatus* Nelson (Pirre bush-tanager).

[3] *Cinclus mexicanus* Swainson (American dipper), *C. leucocephalus* Tschudi (white-capped dipper), *C. schulzi* Cabanis (rufous-throated dipper).

[4] *Cranioleuca marcapatae* Zimmer (Marcapata spinetail), *C. albiceps* d'Orbigny & Lafresnaye (light-crowned spinetail), *C. vulpina* Pelzeln (rusty-backed spinetail, lowland species), *C. obsoleta* Reichenbach (olive spinetail, lowland species), *C. henricae* Maijer & Fjeldså (Bolivian spinetail), *C. pyrrhophia* Vieillot (stripe-crowned spinetail), *C. albicapilla* Cabanis (creamy-crested spinetail), *C. demissa* Salvin & Godman (tepui spinetail), *C. erythrops* P. L. Sclater (red-faced spinetail).

[5] *Diglossa baritula* Wagler (cinnamon-bellied flowerpiercer), *D. plumbea* Cabanis (slaty flowerpiercer), *D. sittoides* d'Orbigny & Lafresnaye (rusty flowerpiercer).

[6] *Hemispingus atropileus* Lafresnaye (black-capped Hemispingus), *H. calophrys* P. L. Sclater & Salvin (orange-browed Hemispingus).

[7] *Hemispingus verticalis* Lafresnaye (black-headed Hemispingus), *H. rufosuperciliaris* Blake & Hocking (rufous-browed Hemispingus).

[8] *Lampornis castaneoventris* Gould (white-throated mountain-gem), *L. viridipallens* Bourcier & Mulsant (green-throated mountain-gem), *L. sybillae* Salvin & Godman (green-breasted mountain-gem), *L. calolaemus* Salvin (purple-throated mountain-gem), *L. cinereicauda* Lawrence (gray-tailed mountain-gem).

[9] *Myadestes ralloides* d'Orbigny (Andean solitaire), *M. coloratus* Nelson (varied solitaire), *M. melanops* Salvin (black-faced solitaire).

[10] *Myioborus torquatus* S. F. Baird (collared redstart), *M. brunniceps* Lafresnaye & d'Orbigny (brown-capped redstart), *M. albifrons* P. L. Sclater & Salvin (white-fronted redstart), *M. melanocephalus* Tschudi (spectacled redstart), *M. ornatus* Boissonneau (golden-fronted redstart), *M. flavivertex* Salvin (yellow-crowned redstart), *M. pariae* Phelps & Phelps (Paria redstart), *M. albifacies* Phelps & Phelps (white-faced redstart), *M. castaneocapillus* Cabanis (tepui redstart), *M. cardonai* Zimmer & Phelps (saffron-breasted redstart).

[11] *Ochthoeca pulchella* P. L. Sclater & Salvin (golden-browed chat-tyrant), *O. diadema* Hartlaub (yellow-bellied chat-tyrant).

[12] *Tangara dowii* Salvin (spangle-cheeked tanager), *T. fucosa* Nelson (green-naped tanager), *T. nigroviridis* Lafresnaye (beryl-spangled tanager).

[13] *Troglodytes rufociliatus* Sharpe (rufous-browed wren), *T. ochraceus* Ridgway (ochraceous wren), *T. monticola* Bangs (Santa Marta wren), *T. solstitialis* P. L. Sclater (mountain wren), *T. rufulus* Cabanis (tepui wren).

[14] *Cranioleuca curtata* P. L. Sclater (ash-browed spinetail), *C. antisiensis* P. L. Sclater (line-cheeked spinetail).

[15] *Pselliophorus luteoviridis* Griscom (yellow-green finch), *P. tibialis* Lawrence (yellow-thighed finch).

[16] *Semnornis ramphastinus* Jardine (toucan barbet), *S. frantzii* P. L. Sclater (prong-billed barbet).

[17] *Tangara heinei* Cabanis (black-capped tanager), *T. argyrofenges* P. L. Sclater & Salvin (green-throated tanager).

[18] *Turdus infuscatus* Lafresnaye (black thrush), *T. nigrescens* Cabanis (sooty thrush).

Table 2. Collecting localities and GenBank accession numbers of samples sequenced for this project.

Taxon	Museum (tissue number)	Locality	Accession number
Campylorhamphus pusillus	LSUMZ B33822	Peru, Cajamarca, Cordillera del Condor, Picorama	FJ222626
C. pusillus	LSUMZ B11879	Ecuador, Esmeraldas, El Placer	FJ222627
C. pusillus	LSUMZ B1411	Panama, Darién, Cerro Pire, 9 km NW of Cana	FJ222628
C. pusillus	STRI JTW094	Panama, Bocas del Toro, Chiriquí to Chiriquí Grande Rd. at continental divide	FJ222629
Chlorospingus canigularis	LSUMZ B34949	Ecuador, Napo	FJ222652
C. canigularis	LSUMZ B34918	Ecuador, Pichincha	FJ222653
C. canigularis	LSUMZ B35820	Costa Rica, Cartago	FJ222654
C. flavigularis	FMNH 430078	Peru, Cusco, Paucartambo	FJ222655
C. flavigularis	STRI JTW 018	Panama, Veraguas, Santa Fe	FJ222656
C. flavigularis	LSUMZ B11936	Ecuador, Esmeraldas, El Placer	FJ222657
Chrysothlypis chrysomelas	STRI JTW016	Panama, Veraguas, Santa Fe	FJ222631
Cranioleuca erythrops	LSUMZ B2144	Panama, Darién	FJ222630
Haplospiza rustica	STRI EC-HRU-514	Ecuador, Carchi	FJ222647
H. rustica	LSUMZ B16173	Costa Rica, San José, La Georgina	FJ222648
H. rustica	LSUMZ B7451	Venezuela, Amazonas, Cerro de la Neblina	FJ222649
Myrmotherula schisticolor	LSUMZ B16048	Costa Rica, Heredia, 4 km SE of Virgen del Socorro	FJ222632
M. schisticolor	LSUMZ B2124	Panama, Darién, 6 km NW of Cana	FJ222633
M. schisticolor	FMNH 429987	Peru, Cusco, Paucartambo	FJ222634
M. schisticolor	LSUMZ B11979	Ecuador, Esmeraldas, El Placer	FJ222635
Pselliophorus luteoviridis	STRI JTW 533	Panama, Chiriquí, Cerro Colorado	FJ222659
Tangara florida	STRI JTW 169	Panama, Bocas del Toro, Chiriquí to Chiriquí Grande Rd. at continental divide	FJ222636
T. florida	STRI PA-TAL-1014	Panama, Veraguas, Santa Fe	FJ222637
T. guttata	STRI JTW 013	Panama, Veraguas, Santa Fe	FJ222638
T. guttata	AMNH 8807	Venezuela, Amazonas, Tamacuari	FJ222639
Thamnistes anabatinus	LSUMZ B6152	Ecuador, Morona-Santiago, W slope Cordillera del Cutucu	FJ222640
T. anabatinus	LSUMZ B2154	Panama, Darién, 6 km NW of Cana	FJ222641
T. anabatinus	FMNH JTW179	Panama, Bocas del Toro, Chiriquí to Chiriquí Grande Rd. at continental divide	FJ222642
Xiphocolaptes promeropirhynchus	FMNH 394013	Mexico, Hidalgo, 5 km E of Tlanchinol	FJ222643
X. promeropirhynchus	UWBM 56169	Nicaragua, 10 km N of Matagalpa	FJ222644
Xiphorhynchus erythropygius	FMNH JTW596	Panama, El Copé, El Copé National Park	FJ222645
X. erythropygius	FMNH JTW669	Panama, Darién, Puerto Pina	FJ222646
Pselliophorus tibialis	LSUMZ B9941	Costa Rica, San José	FJ222659

AMNH, American Museum of Natural History; FMNH, Field Museum of Natural History; LSUMZ, Louisiana Museum of Natural History; STRI, Smithsonian Tropical Research Institute (collected by author); UWBM, University of Washington Burke Museum of Natural History and Culture.

most-parsimonious trees. A bootstrap analysis of nodal support with 1000 bootstrap replicates was performed on the entire matrix following Purvis (1995). However, because nodes for each tree in matrix form are stored as nonindependent pseudocharacters, the assumption of character independence is not met. Furthermore, zoogeographic species occupying many geographic regions will be represented in the matrix by a greater number of nodes and will thus contribute more to bootstrap analysis. Given the current lack of more robust statistics of nodal support for supertrees, I use bootstrap analysis to provide a rough measure of nodal support. Regions of the tree with poor support are interpreted as reflecting conflicting topologies in input trees.

I constructed a second class of area cladograms directly from the sequence data for each zoogeographic species. Following Weir and Schluter (2004), sequences for each highland region were concatenated across all zoogeographic species complexes (sequence concatenation area cladogram [SCAC]). This was done by combining a DNA sequence from a single individual for each zoogeographic species within a highland region into a composite sequence. When a zoogeographic species was absent from a particular highland region or if a sequence was not available for that region, a series of *n*'s of appropriate length was inserted to represent the missing sequence. This method allows both the relationships between geographic regions and the

average branch lengths connecting geographic regions to be estimated directly from the sequence data and in this sense is more robust than supertree methods. A difficulty arises in rooting the analysis without selecting any geographic region to be an outgroup. Maximum-likelihood methods that use a clock or relaxed clock assumption automatically root the analysis without the aid of a predefined outgroup.

A Bayesian cladogram was estimated in Bayesian Evolutionary Analysis Sampling Trees (BEAST) v1.4.3 (Drummond & Rambaut, 2007) using the log-normal relaxed clock model with a Yule-prior for branch lengths. The coefficient of variation in rates (σ; standard deviation in rates across lineages divided by mean rate) was significantly greater than zero in this analysis but less than one (mean = 0.52, 95%; highest posterior density = 0.38–0.66), indicating significant but minor rate variation across lineages. The analysis was run for 10 million generations and a sample saved every 1000 generations. The first 2.5 million generations were deleted as the burn-in period, and a consensus phylogeny with mean branch lengths was generated in FigTree (Rambaut, 2007).

A caveat of the SCAC method is that sequences from older zoogeographic species complexes will be more divergent than younger zoogeographic complexes. As a result, sequences from older taxa will contain more phylogenetically informative characters and will contribute more to the phylogenetic signal. One possibility is to weight each character for a given zoogeographic complex by the inverse of the number of phylogenetically informative characters in that complex. Weighting each character would force each zoogeographic complex to contribute evenly to the phylogenetic signal. However, weighting characters will interfere with branch length estimation and is not implemented here.

The same zoogeographic species were included in the SAC and SCAC analyses except that *Cinclus* Borkhausen was not included in the SCAC analysis because DNA sequences from a published phylogenetic analysis of *Cinclus* (Voelker, 2002) were not deposited in GenBank.

Results

Genetic distance analysis

GTR-Γ distances between adjacent highland regions are shown in Figures 2 and 3. Genetic distances are plotted separately for specifically distinct taxa versus conspecific populations.

Isthmus of Tehuantepec. Genetic distances between Mexican and Guatemalan regions (Fig. 2A) were available for six zoogeographic species complexes, all but one considered conspecific by current taxonomic treatment. Genetic divergence ranged 0.6%–10% (or 0.3–5 Ma using the standard 2% clock) across the intervening Isthmus of Tehuantepec, suggesting that this lowland barrier has promoted extensive population differentiation in a number of zoogeographic species. Interestingly, the least diverged complex was the only one considered to comprise distinct species. By contrast, populations of *Arremon brunneinucha* Lafresnaye and *Chlorospingus ophthalmicus* Du Bus de Gisignies separated for approximately 5 and 2.6 Ma, respectively, are currently considered conspecific (American Ornithologists' Union, 1998).

Lowland isthmuses in lower Central America. The Guatemalan region is isolated from the Talamancan region by extensive lowland barriers in Nicaragua. Genetic differentiation across this lowland gap ranged 1%–9% (Fig. 2B) and had a similar distribution of divergence dates to the Isthmus of Tehuantepec. The three taxa with the greatest genetic differentiation across this gap are recognized as specifically distinct. Genetic differentiation in conspecific taxa ranged 1%–4.5%, again demonstrating substantial population divergence within some species. Genetic differentiation across the lowland gaps that separate Talamanca, Darién, and the west Andes ranged 0.7%–11.6% for taxa considered specifically distinct in these regions and 4.9%–11.7% for taxa considered conspecific (Figs. 2C, 3A, B).

South American lowland barriers. The Santa Marta, Parian, and tepui regions are isolated from each other and the Andes by lowland barriers. The Santa Marta in northern Colombia possesses a large number of endemic species (n = 18) and subspecies (n = 55) (Strewe et al., 2006), few of which have sequence data in GenBank. In the samples available, genetic divergence between the Santa Marta and east Colombian Andes ranged 2.6%–9.4% for conspecific taxa and 5.8%–11.3% for specifically distinct taxa (Fig. 3C). The Parian region represents an isolated highland region in the Parian Peninsula of eastern Venezuela. It is isolated by expansive lowland barriers from the closest highland region, the Venezuelan Andes. Across this gap, genetic distances for four zoogeographic species complexes ranged 0.4%–6.5% (Fig. 3D). Only one of these is considered specifically distinct (*Myioborus* S. F. Baird; 6.2%). The tepuis, a series of ancient highland regions of the Guayana Shield, are isolated from the Andes and the Parian region by the extensive Amazon and Orinoco basins. Many Andean zoogeographic species complexes do not

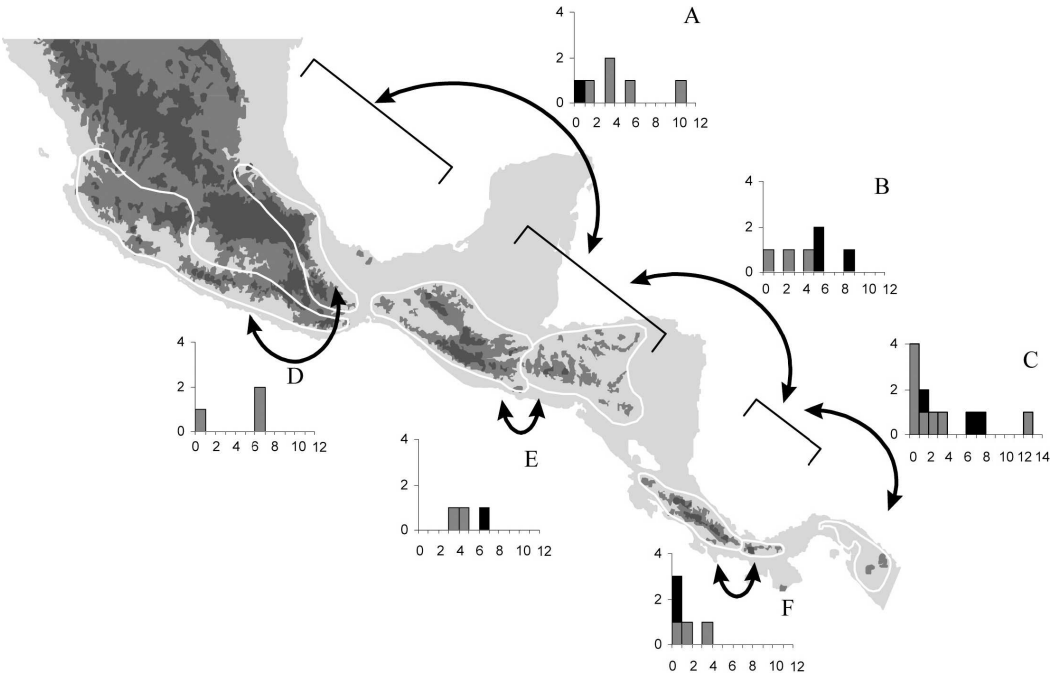

Figure 2. Histograms of genetic distances (x-axis = GTR-Γ corrected distances) between Mexican and Middle American regions of endemism derived from protein-coding mitochondrial DNA sequences. Distances between species-level taxa are shown in black and between conspecific populations in gray. The y-axis is the number of zoogeographic complexes with a given level of genetic differentiation. Comparisons of genetic differentiation between regions of endemism are as follows: (A) east Mexican and west Mexican versus Guatemalan, (B) Guatemalan versus Talamancan, (C) Talamancan versus Darién, (D) west Mexican versus east Mexican, (E) western versus eastern subregions within the Guatemalan region, and (F) western versus eastern subregions within the Talamancan region.

reach the tepuis. Of those that do, a large proportion of taxa represent endemic species or subspecies. Unfortunately, DNA sequences were available for only one zoogeographic complex (*Myioborus*), which is differentiated by 4%–5% from its relatives in the Parian (Fig. 3E), Venezuelan, and east Colombian regions. This complex is specifically distinct in each of these regions, and within the tepuis multiple species are described, suggesting that the tepuis may contain subregions of multiple endemism. Additional data are necessary to determine the magnitude of genetic differentiation of tepui populations from other highland regions of endemism.

Divergence across intermontane river valleys. A series of deep river valleys bisect montane forest patches along the eastern slope of the Andes and form barriers between adjacent regions of endemism. Each river valley promoted genetic differentiation, but to different extents. The Río Quinimari and its tributaries, which carved out the deep and arid Tachira Depression, separate the east Colombian and Venezuelan regions (Fig. 1). Genetic differentiation across this depression in four zoogeographic species ranged 0%–6.2% (Fig. 3F), suggesting that this barrier has effectively promoted divergence in at least some species complexes. The Eastern Cordillera of the Colombian Andes and the Mérida Andes of Venezuela were uplifted rapidly, primarily between 5 and 3 Ma (Gregory-Wodzicki, 2000; Audemard, 2003; Dhont et al., 2005). The oldest divergence suggests that population divergence between the east Colombian and adjacent regions of endemism began around the time of when uplift was completed 3 Ma.

Either the Río Caquetá or a series of low-elevation montane passes may form the barrier between the east Colombian and east Ecuadorian regions. However, only limited genetic differentiation occurred in the two zoogeographic species complexes that span these potential barriers (Fig. 3G). One of these complexes has endemic species on either side of these barriers, yet showed only low levels of divergence. Additional sampling from the east Colombian region is necessary, but the two complexes available suggest that genetic differentiation is not great between these regions.

The North Peruvian Low and Río Marañón represent a barrier that fully bisects the Eastern

Cordillera of the Andes. This region is believed to form one of the most important barriers for Andean birds and other groups (Vuilleumier, 1969, 1980), because a number of sister species occur north and south of this region. Genetic differentiation across this barrier ranged as high as 6.1% (11% when the north and central Peruvian regions are analyzed together) in seven zoogeographic species, confirming its importance in promoting population divergence (Fig. 3I, J).

In contrast to the Río Marañón, five of the six species that span the Río Huallaga in Peru showed only limited genetic differentiation (Fig. 3K). However, *Chlorospingus ophthalmicus* showed 6% divergence across this river barrier, even though it showed no differentiation across the Río Marañón (Weir et al., 2008). Although Cracraft (1985) stated the importance of the Río Huallaga valley in delimiting the ranges of a small number of Peruvian endemics, he did not officially recognize the separation of the north and central Peruvian regions. The available evidence suggests that these regions were important in promoting population divergence in relatively few species. Further sampling is necessary.

Genetic differentiation across the Río Apurimac, which separates the central and south Peruvian regions, ranged 0.2%–14.3% (Fig. 3L). Like the Río Marañón, this river barrier has played a key role in promoting and maintaining population divergence and speciation in the Andes. Still, a substantial number of zoogeographic species showed limited or no divergence across this barrier, implying recent range expansions or ongoing gene flow across this valley.

Finally, the Río Grande and its tributaries have carved out an arid valley in Bolivia, isolating montane forest in the south Peruvian and Austral regions. Only three zoogeographic complexes spanned this barrier, and they showed moderate to large genetic differentiation ranging 1.3%–6% (Fig. 3M).

Divergence across nonforested montane barriers. Montane forest is distributed along both the eastern and western slopes of the Andes of Colombia and Ecuador south to the North Peruvian Low. High-elevation barriers above the tree line bisect montane forest along each slope. Genetic differentiation between the eastern and western slopes (Fig. 3H) ranged 0%–5.3% with nine of 12 taxa differentiated by less than 1%. The lack of strong genetic differentiation in most complexes suggests that at least prior to the mid and late Pleistocene glacial cycles, the high Andes did not form a strong barrier to dispersal. The severe glacial episodes of the mid and late Pleistocene directly glaciated high elevations of the Andes, lowering montane forest zones (Bennett, 1990; Hooghiemstra et al., 1993; Van't Veer & Hooghiemstra, 2000; Hooghiemstra & van der Hammen, 2004).

North of the Isthmus of Tehuantepec in Mexico, humid montane forest is distributed along the Sierra Madre Oriental in the east and the Sierra Madre Occidental and Sierra Madre Sur in the west (Fig. 1). These forests are separated by an arid high-elevation plateau. Population divergence between the west and east Mexican regions was available for only three zoogeographic species complexes, each considered conspecific by current taxonomic treatment. Genetic divergence between these regions was low for *Lampornis amethystinus* Swainson (0.6%), but was between 6% and 7% for *Arremon torquatus* Lafresnaye & d'Orbigny and *Chlorospingus ophthalmicus* (Fig. 2D), suggesting that populations of the latter two species have been separated for more than 3 million years.

Other barriers. I compared genetic differentiation between eastern and western subregions within both the Talamancan and Guatemalan regions. Three zoogeographic species complexes (*Pselliophorus* Ridgway, *Lampornis* Swainson, *Selasphorus* Swainson) have endemic species in each of the Talamancan subregions, two of which are included here (*Pselliophorus* and *Lampornis*). Populations in five zoogeographic species complexes exhibited minor to moderate genetic differentiation between the eastern and western subregions of the Talamanca (0.3%–3.8%; Fig. 2F). Two complexes contain endemic species, but in each case, these endemic species are separated by divergence values less than 1%. In addition, in *Lampornis*, samples from multiple individuals demonstrate that populations are not reciprocally monophyletic (Garcia-Moreno et al., 1999).

Three zoogeographic species complexes spanned the two Guatemalan subregions, including one with endemic species in each subregion (*Lampornis*). In contrast to the Talamancan subregions, genetic distances between the Guatemalan subregions were high, ranging 3.7%–6% and suggesting divergence dates of 1.6–3 Ma (Fig. 2E).

AREA CLADOGRAM ANALYSIS

Input topologies of zoogeographic species complexes used in the SAC analysis are shown in Figure 4.

Supertree area cladograms. The supermatrix obtained from phylogenies for 35 zoogeographic species had 124 columns. Standard matrix representation parsimony resulted in a single most-parsimonious tree, requiring 196 steps (Figs. 5A, 6A).

Sequence concatenation area cladograms. The topology uncovered in the relaxed-clock Bayesian phylogeny (SCAC) varied in important ways from the SAC topology

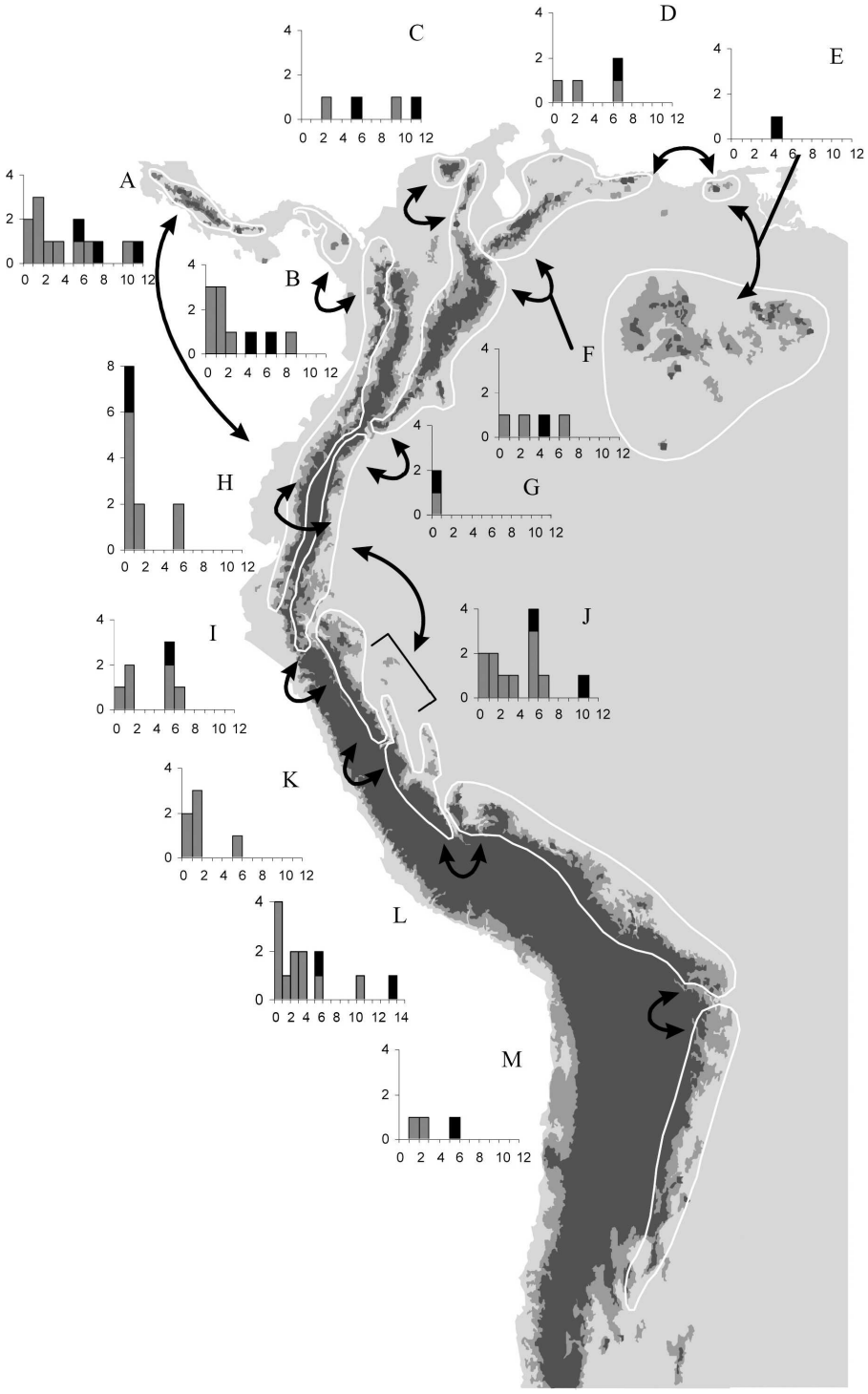

Figure 3. Histograms of genetic distances (x-axis = GTR-Γ corrected distances) between South American regions of endemism derived from protein-coding mitochondrial DNA sequences. Distances between species-level taxa are shown in black and between conspecific populations in gray. The y-axis is the number of zoogeographic complexes with a given level of genetic differentiation. Comparisons of genetic differentiation between regions of endemism are as follows: (A) Talamancan versus west Andean, (B) Darién versus west Andean, (C) Santa Marta versus east Colombian, (D) Venezuelan versus Parian,

(Figs. 5, 6). The most striking difference was the grouping of the Talamanca and Darién regions with the Guatemalan and Mexican regions to produce monophyletic South American, Middle American, and Mexican clades, although posterior probability was low for these groupings. In the SAC topology, Talamanca and Darién were nested within the South American radiation.

Topology in those parts of the SCAC and SAC trees with nodal support greater than 0.9 posterior probability (SCAC) and 70% bootstrap support (SAC) generally agreed (Fig. 6). The main differences were the retention of a monophyletic Andean clade in the SCAC analysis but not in the SAC analysis.

Poor support for many nodes in both the SCAC and SAC analyses highlights the general discordance in input phylogenies (Fig. 4) between many regions of endemism. Because of this conflict, it is not straightforward how to interpret branch-length information in the SCAC analysis, even for those parts of the cladogram that have high nodal support.

Discussion

The role of lowland barriers

Populations within zoogeographic species complexes of Neotropical montane forest birds exhibited a wide range of genetic divergence between geographically adjacent regions of endemism (range, 0%–14%; Figs. 2, 3, 7, 8). Populations of zoogeographic species spanning lowland barriers (Fig. 7) exhibited the greatest genetic divergence (median = 3.4%), while populations spanning river valley barriers (median = 2.0%) and highland barriers (median = 0.6%) were less diverged; differences were significant only between the lowland and highland barriers (Mann-Whitney U test, $W = 451$; $P = 0.0139$). These differences are not surprising given that lowland barriers are generally wider geographically than other barrier types.

The lowland Isthmus of Panama distributed between the Talamanca and Darién highlands was inundated until 3–4 Ma when the final formation stage of the Central American land bridge was completed (Coates et al., 1992; Coates & Obando, 1996). Land bridge completion initiated full faunal interchange between North and South America in birds (Weir, unpublished data), mammals (Simpson, 1980; Webb, 1985), and other groups (Stehli & Webb, 1985), an event known as the Great American Biotic Interchange. A burst of interchange between the Talamanca and Darién and between the Talamanca and the west Andean regions at or shortly after the land bridge completion is evident for highland birds (6%–8% sequence divergence; Figs. 2C, 3A).

A more recent burst during the last 1 million years (0%–2% sequence divergence) coincides with the severe glaciations of the Andes (Bennett, 1990; Hooghiemstra et al., 1993; Hooghiemstra & van der Hammen, 2004), Talamanca (Lachniet & Seltzer, 2002), Guatemalan highlands (Anderson, 1969), and Mexican highlands (White & Valastro, 1984; Heine, 1988) when montane forest was repeatedly lowered by as much as 1000 m in elevation (Van't Veer & Hooghiemstra, 2000; Hooghiemstra & van der Hammen, 2004). This lowering allowed some montane forest elements to expand into intervening lowland regions and may have increased the probability of dispersal of montane species across the isthmus (Hooghiemstra & Cleff, 1995).

Montane interchange during the past 1 million years was also observed across other lowland barriers (Figs. 2, 3), but none of them exhibited a distinct interchange burst during this time as observed between the Talamanca, Darién, and west Andes. This finding may simply reflect the small sample sizes across other lowland barriers, and additional sampling may reveal that interchange at this time was more extensive than the current data suggest. Alternatively, the mid to late Pleistocene elevational lowering of montane forests may have provided the first opportunity for most Andean species to disperse into the recently formed Talamanca and Darién highland regions following the land bridge completion. Late Pleistocene dispersal into other highland regions may have been hindered by competition with the species-diverse faunas that already inhabited those regions.

The role of arid river valleys

The role of arid river valleys in promoting population differentiation is controversial. These barriers may directly promote differentiation by preventing or minimizing gene flow (Vuilleumier, 1969). Alternatively, Fjeldså and his coauthors (Fjeldså, 1995; Fjeldså & Lovett, 1997; Fjeldså et al., 1997, 1999) proposed that river valleys were not responsible for promoting diversification events despite the fact that they demarcate adjacent regions of endemism and the range limits of a large proportion

←

(E) Parian versus tepui, (F) east Colombian versus Venezuelan, (G) east Colombian versus east Ecuadorian, (H) east Ecuadorian versus west Ecuadorian, (I) east Ecuadorian versus north Peruvian, (J) east Ecuadorian versus north Peruvian and central Peruvian, (K) north versus central Peruvian, (L) central versus south Peruvian, and (M) south Peruvian versus Austral.

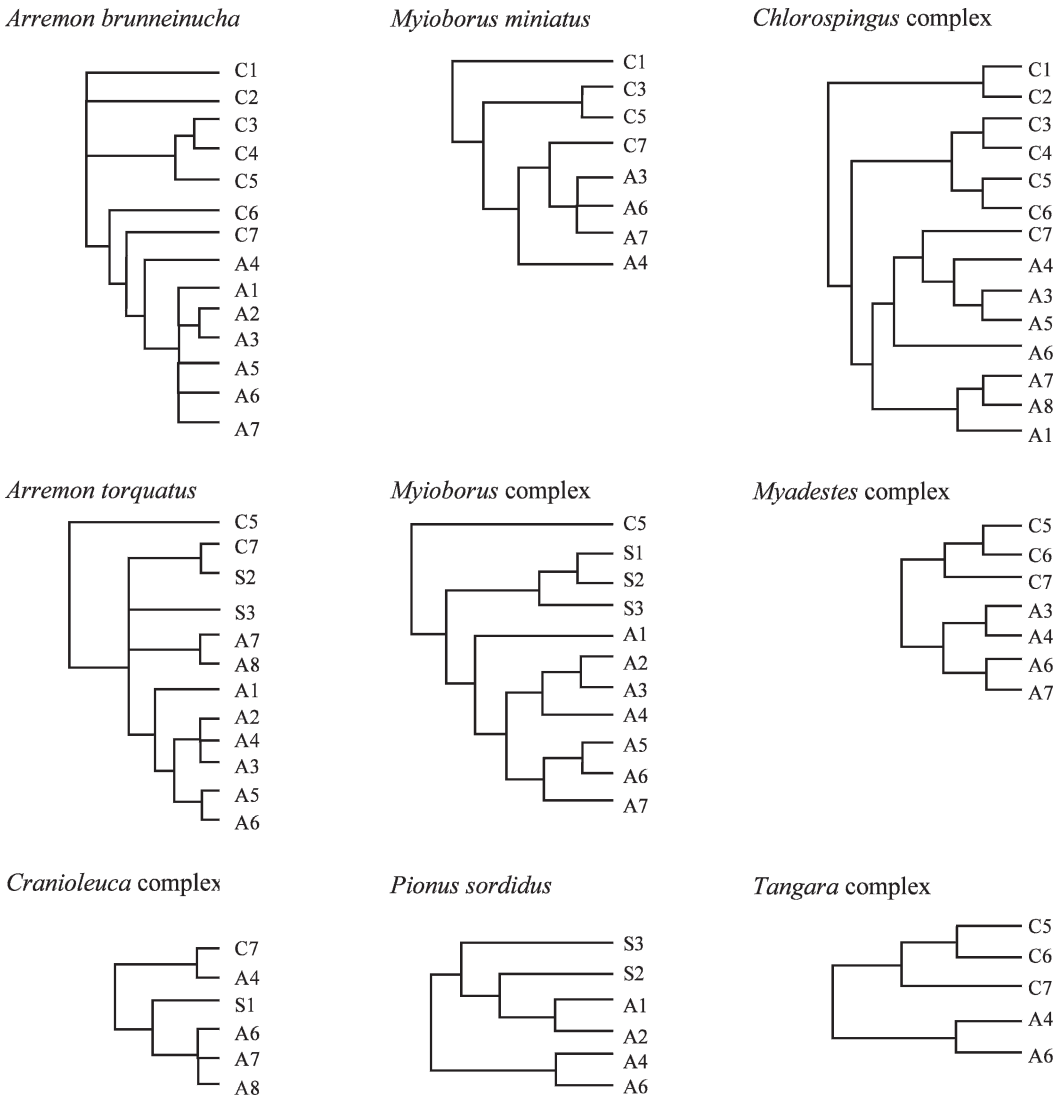

Figure 4. Area cladograms of Neotropical montane zoogeographic species complexes based on Bayesian or maximum likelihood topologies. Regions of endemism are labeled following codes in Table 1.

of Andean birds. In their view, regions of endemism along the Andes are associated with areas of climatic and ecologic stability that persisted over long time spans and throughout the Pleistocene climatic cycles. These areas of stability are seen to promote parapatric or sympatric speciation, without the need for geographic barriers to gene flow. While the model may apply commonly to taxa with parapatric or sympatric speciation, it is unlikely to apply commonly in birds for which sympatric speciation is rare and allopatric speciation is the predominant mode of diversification (Coyne & Price, 2000; Phillimore et al., 2008). While ecologically stable areas may help maintain species diversity in birds, they are unlikely to have commonly promoted speciation without the aid of intervening geologic barriers to gene flow.

Rather than forming opposing models, ecologically stable areas and arid river valleys may have worked in concert to promote population divergence between regions of endemism. During periods of intense glaciation, montane forests were lowered by 1000 m or more in altitude (Van't Veer & Hooghiemstra, 2000; Hooghiemstra & van der Hammen, 2004), potentially forming a continuous forest belt across river valleys, or at least increasing the possibility of dispersal across them. During interglacial periods, montane forest returned to high altitudes, becoming once again fragmented by intervening river valleys.

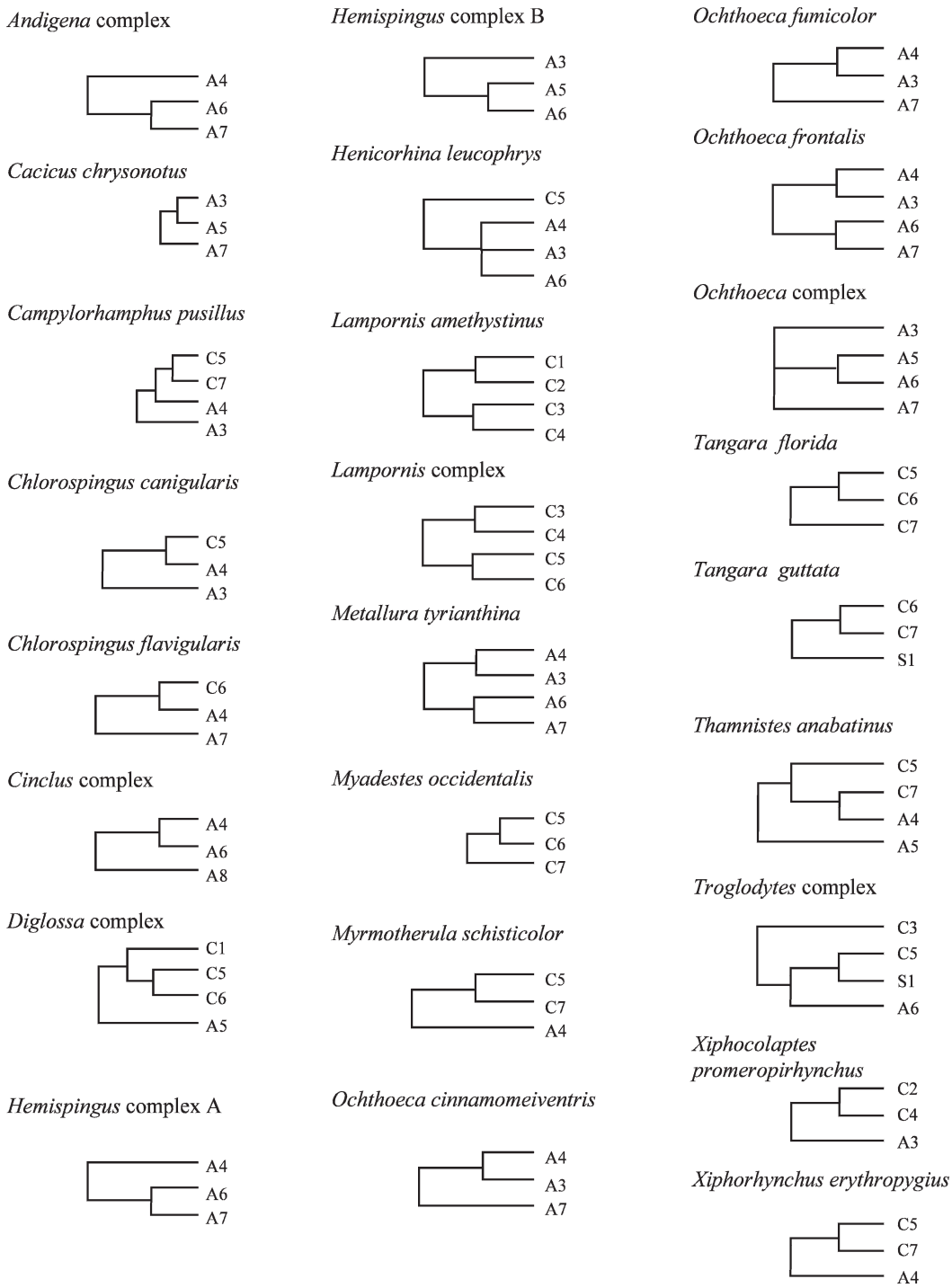

Figure 4. Continued.

Ecologically stable areas are represented by the intervening slopes between river valleys that remained forested throughout glacial and interglacial cycles.

Almost 50% (16 of 34) of populations from adjacent endemism regions separated by river valleys date to the last 1 million years (Fig. 7), suggesting that the intense glacial cycles during the mid and late Pleistocene may have resulted in repeated episodes of range expansion across river valley barriers, followed by isolation by those barriers. However, a large proportion (11 of 36) of

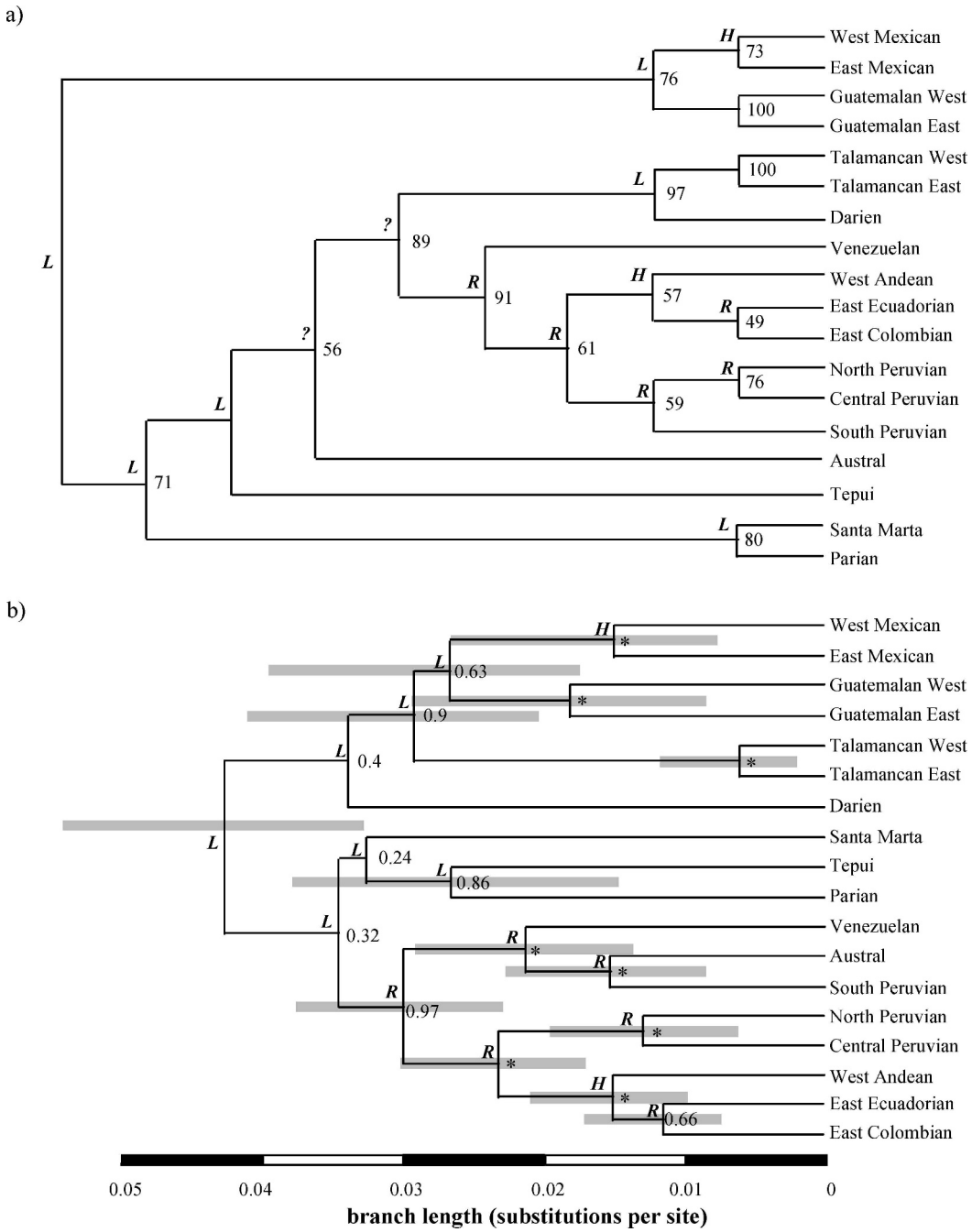

Figure 5. Supertree area cladogram (A) and sequence concatenation area cladogram (B) for Neotropical montane forest zoogeographic species. Support indices are shown to the right of nodes. Bootstrap values are shown for the supertree area cladogram and posterior probabilities for the sequence concatenation area cladogram. Posterior probability of 1.0 shown by asterisk (*). Ninety-five percent confidence intervals for node ages shown only for the sequence concatenation area cladogram. Lowland barriers indicated by *L*, highland barriers by *H*, and river valley barriers by *R*.

divergence events across river valley barriers predate the late Pliocene and Pleistocene glacial cycles altogether (2.4 Ma to recent; Fig. 7), demonstrating that glacial cycles alone were not responsible for all divergence events across river valleys. These results are consistent with the long-term role played by arid river valleys in promoting differentiation between regions of endemism.

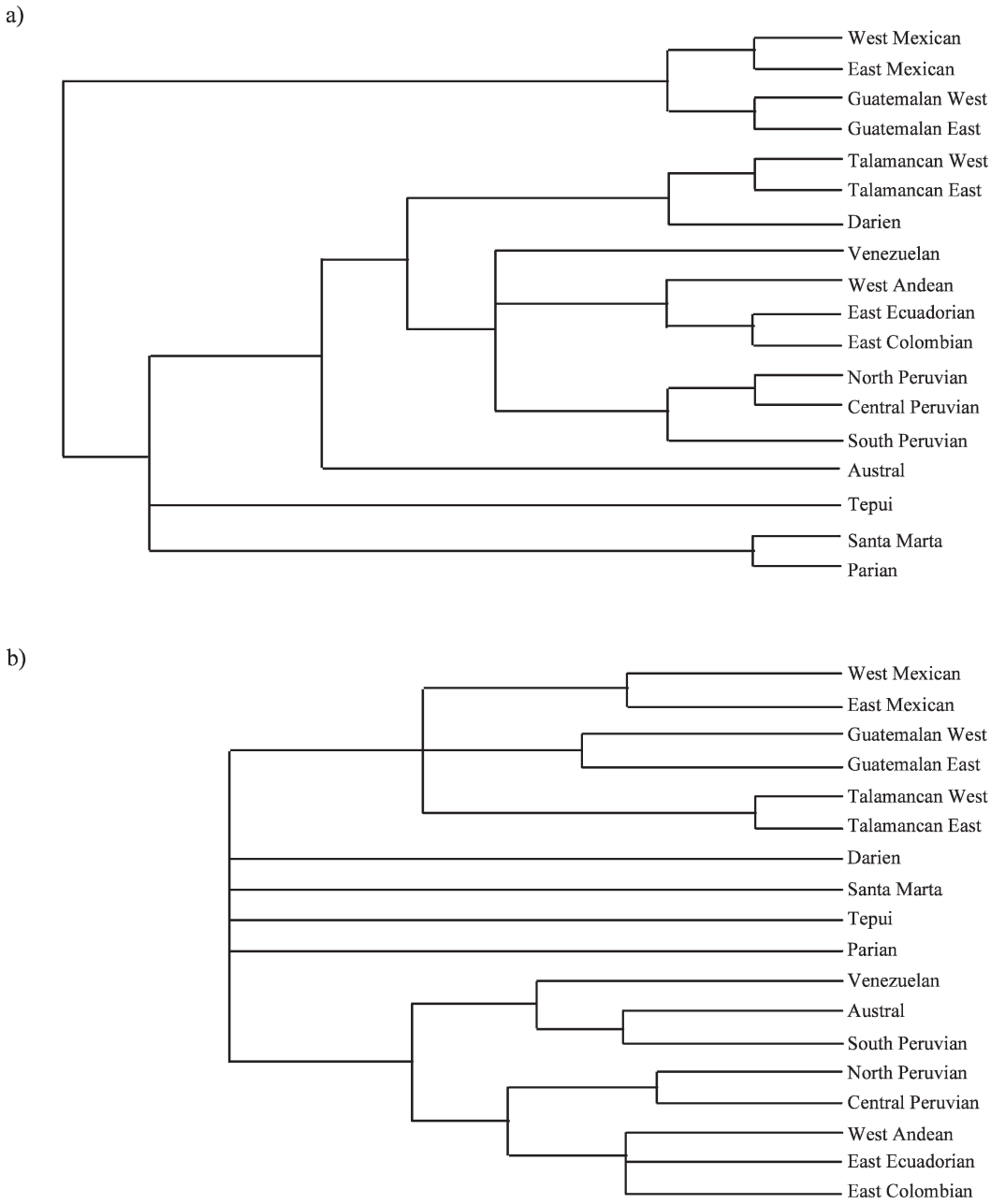

Figure 6. Cladograms showing only nodes with greater than 70% bootstrap support for the supertree area cladogram (A) and greater than 0.95 posterior probability for the sequence concatenation area cladogram (B).

THE ROLE OF DISPERSAL AND VICARIANCE

The role of dispersal and vicariance in promoting the formation of patterns of endemism is widely debated (e.g., Zink et al., 2000). A number of highland species or species complexes are derived from lowland source faunas. This has most often been interpreted as speciation following dispersal from lowland to highland regions (i.e., Weir, 2006; Brumfield & Edwards, 2007). However, Ribas et al. (2007) recently suggested that highland populations were originally distributed at low elevations, but were gradually uplifted and vicariantly isolated from adjacent lowland populations during orogeny of the Eastern Cordillera of the Andes. Although such a vicariant scenario may have played a role in the origin

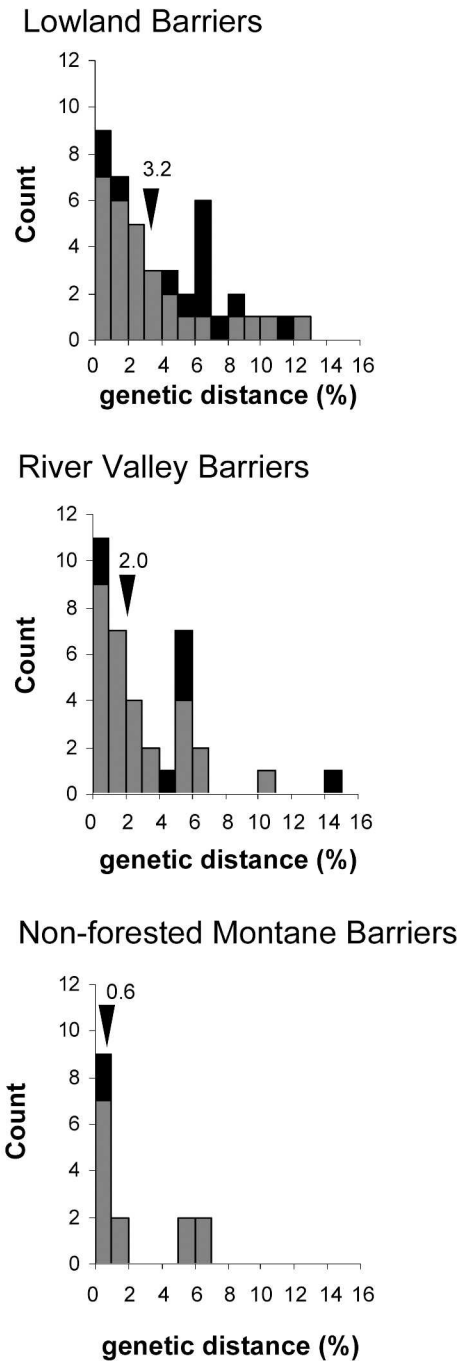

Figure 7. Genetic distances between adjacent regions of endemism separated by lowland, river valley, and nonforested montane barriers. Median values shown by arrows. Distances between species-level taxa are shown in black and between conspecific populations in gray.

of certain highland clades, most highland zoogeographic species complexes lack lowland components, suggesting they have diversified solely within Neotropical highland regions. Vicariant isolation from lowland ancestors is highly unlikely for divergence events within the zoogeographic species complexes included in this data set.

Other possible modes of vicariance include the development of river valleys or uplift of highland regions above the tree line. Formation of such barriers in the Andes may have simultaneously fragmented the ranges of co-distributed species, resulting in populations with similar ages along either side of the barrier. A burst of divergence events should date to the time of barrier formation. For taxa isolated on one side of a barrier, dispersal across the barrier could produce founder populations at any time following barrier formation. The combination of initial vicariance and subsequent founder events would result in a burst of events dating to the time of barrier formation followed by a series of subsequent events up to the present.

Unfortunately, the geologic literature does not provide specific dates for the formation of Andean river valleys. Given that extensive uplift of highland regions along the eastern edge of the Andes occurred throughout the past 10 million years in Ecuador and Peru and the past 5 million years in Colombia (Gregory-Wodzicki, 2000) and Venezuela (Audemard, 2003; Dhont et al., 2005), river valleys in these regions almost certainly date to these time periods. The wide span of divergence dates for most river valley barriers suggests that a protracted history of dispersal was the predominant mode by which the current fauna in highland regions of endemism originated. Sample sizes are too small to detect bursts of divergence dates associated with formation of most river barriers, but this may be possible as other species are investigated. However, divergence across the Río Marañón might conform to such a pattern (Fig. 3I, J). The Río Marañón is characterized by a burst of divergence events around 3 Ma followed by a smaller number of divergence events up to the present.

In contrast to the wide range of divergence dates associated with most river valley barriers, almost all populations isolated on either slope of the northern Andes date to the past 1 million years. This recent burst suggests either some ongoing gene flow or very recent isolation between these slopes. The latter seems likely, as Pleistocene glacial cycles were severe in the Andes only during the past 0.9 million years, and glaciation at high altitudes likely provided a hard barrier to gene flow, vicariantly separating populations along the eastern and western slopes (Weir, 2006).

CONSENSUS AREA CLADOGRAMS

The SAC and SCAC methods produced different topologies (Figs. 5, 6), and interpretation of the patterns uncovered is not straightforward. Still,

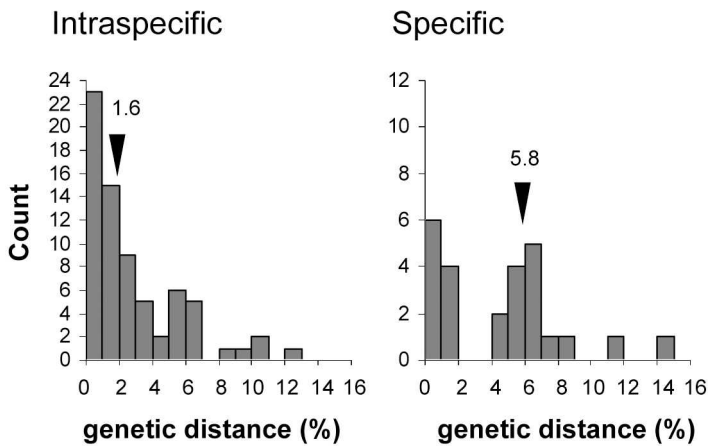

Figure 8. Genetic distances between adjacent regions of endemism for taxa differentiated at the species level and intraspecific taxa. Median values are shown by arrows. Distances between species-level taxa are shown in black and between conspecific populations in gray.

branching patterns shared in common by the two methods highlight some key points. First, both methods suggest that the basal divergence event in montane forest taxa occurred somewhere between the north Andes and Nicaragua. In the SAC topology, this break occurs north and south of the Nicaraguan lowlands, while in the SCAC topology it occurs across the lowland barrier that separates the Darién from the Andes. This basal break suggests the presence of two or more distinct montane forest source faunas for the Neotropics, one in North America (i.e., Mexico, Middle America) and one or more in South America.

The biogeographic placement of this basal break just north (SAC) or just south (SCAC) of the Isthmus of Panama suggests that the narrow isthmuses that separate the North and South American continents formed an important barrier for highland birds. This basal (or near basal) split is also reflected in the topologies of a large proportion of individual zoogeographic species complexes (Fig. 4). A few of these complexes dispersed between North and South America before land bridge completion, when marine channels still bisected these isthmuses, while most dispersed at or after its final uplift (Figs. 2C, 3A). In source trees, Darién and Talamancan highland regions share affinities with highland regions to the north in some zoogeographic species and highland regions to the south in others (Fig. 4). In the two area cladogram methods, the conflicting placement of the Talamanca and Darién highlands in either the North or South American clades likely reflects this mixed ancestry for zoogeographic species complexes invading these recently uplifted highland regions along the land bridge.

In both analyses, the Parian, Santa Marta, and tepui regions—isolated from each other and the Andes by lowland regions—are basal within the predominantly South American clade. Within the Andes, relationships between regions of endemism were poorly resolved in the SAC method but well resolved in the SCAC method (Fig. 6). The SCAC topology supports a monophyletic Andean clade but suggests a curious connection between the Venezuelan region and the Austral and south Peruvian regions despite the fact that four geographically intervening regions separate these regions. This unusual relationship is likely driven by the *Chlorospingus ophthalmicus* complex, whose Venezuelan representative shares high sequence similarity to representatives from the Austral region despite a series of genetically differentiated and geographically intermediate populations (Fig. 4; Weir et al., 2008).

In the SCAC topology, the west Andean, east Ecuadorian, and east Colombian regions formed a well-supported clade in the north Andes. This clade was sister to a clade containing the north and central Peruvian regions with the Río Marañón forming the barrier between them. Basal to these was the clade containing the south Peruvian, Austral, and Venezuelan regions. Ignoring the unusual placement of the Venezuelan region, the basal placement of the southernmost Andean regions is consistent with a biogeographic history of northward expansion. Beginning with a source fauna in the southern Andes, northward expansion may have occurred in a stepwise fashion, first across the Río Apurímac, and then across the Río Marañón. Given the geologically younger ages for uplift of many highland regions in the north Andes, with uplift occurring in some regions (i.e., the east Colombian region) as recently as 3 Ma, expansion into these regions from a South Andean

source fauna is not unreasonable. Alternatively, a vicariant history may explain this pattern with formation of the Río Apurímac forming the initial vicariant event followed by subsequent formation of the Río Marañón. While the burst of divergence events across the Río Marañón near 3 Ma (Fig. 3I, J) may suggest a vicariant event, a clear burst is not detected for the Río Apurímac.

Relationships in Middle America and Mexico are not well resolved for deeper nodes, but do strongly suggest that subregions within each highland block formed monophyletic assemblages. Lowland barriers separate these monophyletic clades within highland regions from each other.

Nodes represented by lowland barriers generally occurred deepest in the SAC and SCAC phylogenies (Fig. 5), with nodes spanning river and highland barriers occurring in more derived positions. These results further demonstrate that lowland barriers have played the most important role in diversification of Neotropical highland birds.

CONSERVATION IMPLICATIONS

Highland montane forest regions of endemism were defined on the basis of endemic species in each of these regions (Cracraft, 1985; Stotz et al., 1996; Stattersfield et al., 1998). This study demonstrates that a large proportion of populations within widespread species possess genetically differentiated populations in many of these regions of endemism (Fig. 8), emphasizing the value of preserving tracts of highland forest in each. Genetic differentiation was greatest between highland regions separated by lowland barriers and by deep, arid river valleys. Regions of endemism on either side of these barriers have a high conservation priority. With the sole exception of the division between the east Colombian and east Ecuadorian regions, all geographically proximate regions of endemism had at least one divergence date greater than 2 Ma (assuming a standard 2% clock), and most had intraspecific splits greater than 2 Ma. Dates for intraspecific splits between adjacent highland regions ranged as high as 6.5 Ma. It is important to note that almost half (32 of 70) of all intraspecific divergence events between adjacent highland regions (Fig. 8) occurred more than 1 Ma and one third (23 of 70) occurred more than 2 Ma, demonstrating substantial isolation of populations in these regions.

The extensive genetic differentiation between conspecific populations in highland regions of endemism demonstrates that currently defined species boundaries do not adequately represent all evolutionarily distinct lineages. Under the biologic species concept (Price, 2007), this is not an issue because biologic species may contain multiple genetically differentiated taxa provided those populations are not reproductively isolated. Under the biologic species concept, genetically differentiated populations should be managed as separate evolutionary significant units (Vogler & DeSalle, 1994). Under the phylogenetic species concept (Price, 2007), these results suggest that many currently defined highland species contain multiple, unrecognized species. As the biologic species concept is currently favored (although species definitions are arbitrary for many allopatric populations) by the South American Classification Committee (Remsen et al., 2007) for South American birds and the American Ornithologists' Union (American Ornithologists' Union, 1998) for North and Central American birds, it is important that governments treat evolutionarily significant units rather than species-level taxa as the basic units of conservation.

Given the narrow altitudinal distributions of many montane species, it may be necessary to preserve large tracts of intact forest in order to maintain substantial population sizes. However, extensive deforestation threatens the continuity of forest tracts within montane regions. Continued development of road systems and slash-and-burn agricultural practices are primarily responsible. Ironically, government control of illicit crops accelerates montane deforestation as drug growers are forced to clear additional land along higher, more inaccessible slopes (Fjeldså et al., 2005). Existing forest tracts are rapidly disappearing from many montane regions of endemism. In the Andean region as a whole, less than 10% of montane forest is estimated to be intact (Henderson et al., 1991). The percentage is lower in some regions of endemism, with less than 4% of forest remaining intact in the west Andean region (Dodson & Gentry, 1991). The key finding reported here, that many widespread montane forest species are composed of multiple genetically differentiated populations in geographically localized areas of endemism, shows that a failure to adequately preserve remaining montane forest tracts within each region of endemism will result in the loss of much greater diversity than predicted from a straightforward taxonomic analysis.

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