

Out of Amazonia again and again: episodic crossing of the Andes promotes diversification in a lowland forest flycatcher

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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 split between montane and lowland *Mionectes*. The 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.

Keywords: Andes; dispersal; area cladograms; ancestral area reconstruction; Neotropical phylogeography

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 analysed 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, Eberhard & Bermingham 2004, 2005, Cheviron *et al.* 2005; primates: Cortes-Ortiz *et al.* 2003; reptiles: Zamudio & Greene 1997; trees: Dick *et al.* 2003). Likewise, when geographical 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 (*ca.* 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 (less than 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

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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: (i) populations have been isolated too recently for speciation to occur, (ii) gene flow across presumably significant barriers occurs with sufficient regularity to inhibit speciation, or (iii) 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 understorey 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.

2. MATERIAL AND METHODS

The genus *Mionectes* consists of five species of drab, principally frugivorous flycatchers found in the understorey 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 *Mionectes 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 the western Ecuador and the Atlantic Forest of Brazil (figure 1b). In the field, it is often difficult to separate *M. oleagineus* from the two other lowland *Mionectes* species (*Mionectes macconnelli* and *Mionectes rufiventris*), both of which are partially sympatric with *M. oleagineus*. *M. macconnelli* has a disjunct distribution in southwestern Amazonia and the Guiana Shield (figure 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 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 Schauensee 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 out-groups, 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 v. 3.1.1.2; Ronquist & Huelsenbeck 2003) and branch-and-bound maximum-likelihood phylogeny (implemented in PAUP* v. 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 5 additional montane *Mionectes* from widespread geographical origins within their respective ranges, focusing on the widespread *M. oleagineus* (see table 1 in the electronic supplementary material for details about locality and other voucher specimen data). Similar to the first dataset, we generated a Bayesian inference phylogeny using MRBAYES for this second dataset. 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 the electronic supplementary material.

(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 v. 1.06 (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 (non-parametric rate smoothing, NPRS; Sanderson 1997) topology for a dataset consisting of the *Mionectes RAG-1* sequences and a variety of *RAG-1* 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

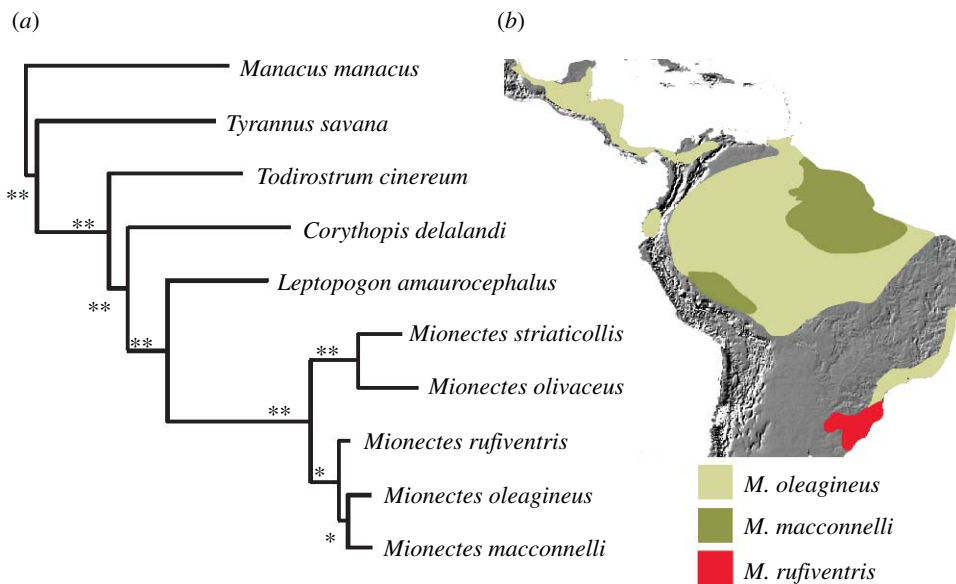


Figure 1. (a) Relationship of *Mionectes* and related genera determined by Bayesian inference using partial sequences of the mtDNA cytochrome *b* gene (999 bp) and the nuclear exons *RAG-1* (930 bp) and *c-myc* (477 bp). The monophyly of *Mionectes* and a basal split between montane and lowland clades 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. Posterior probabilities: double asterisk, 100%; single asterisk 95%.

alternative calibration was evaluated by bootstrapping the expanded *RAG-1* data matrix. The NPRS molecular dating analysis is described in further detail in the electronic supplementary material.

(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 2 and 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 (HPDs) 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 than 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 the 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 v. 1.21 (Clement *et al.* 2000). The resulting network was redrawn by hand.

3. RESULTS

Our multi-locus phylogeny recovered all five *Mionectes* species as a monophyletic clade with 100% posterior probability (figure 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 *Corythopsis* were the closest out-groups 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 1a). This split is old: average model-corrected *cyt b* distance between the montane and lowland *Mionectes* clades was 14.3%, dating to *ca* 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

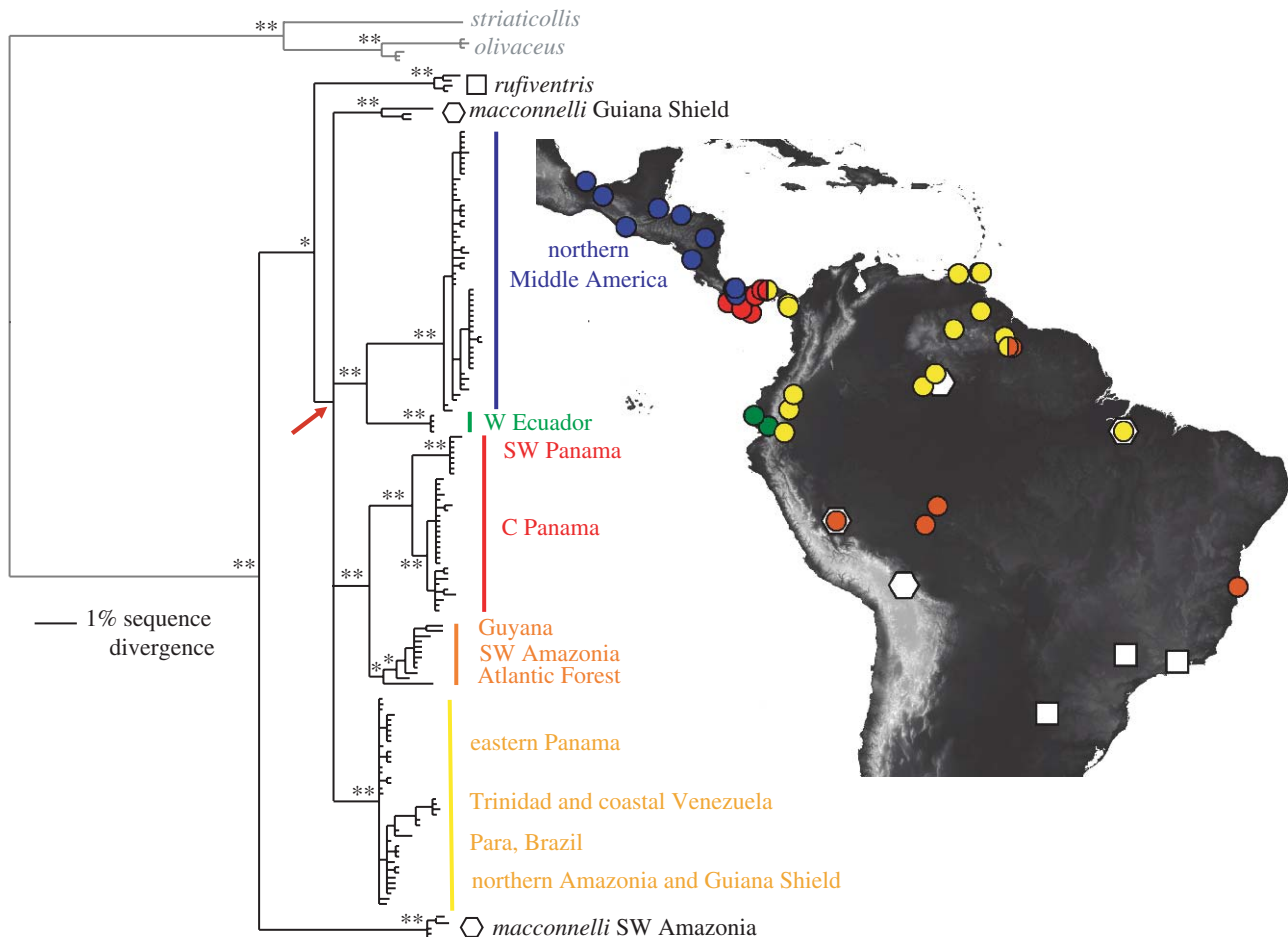


Figure 2. Majority-rule consensus Bayesian inference topology for 163 individuals of *Mionectes* flycatchers (156 lowland and 7 highland birds) based on complete *ND2* sequences. Posterior probabilities for bifurcations indicated at node: double asterisk, 100%; single asterisk, greater than 95% (omitted from the 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* colour coded to correspond to the major clades at left. Two sites (central Panama and Guyana) are bicoloured 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 (figure 4).

model-corrected distance between these two clades was 2.0%. Owing to a lack of widespread geographical sampling in *M. striaticollis*, we have no phylogeographic results for this montane species.

Our broad geographical sampling of *ND2* sequences from birds collected throughout the range of the three lowland *Mionectes* species identified a series of strongly supported clades (figure 2) with posterior probability nodal support greater than 95% (figure 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 2), one found east and west of the Andes (YELLOW; figure 2), and one exclusively east of the Andes (ORANGE; figure 2). For heuristic purposes, we refer to each clade by its colour in figure 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, and 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*, the 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*, and ORANGE: *oleagineus*. Furthermore, in two instances,

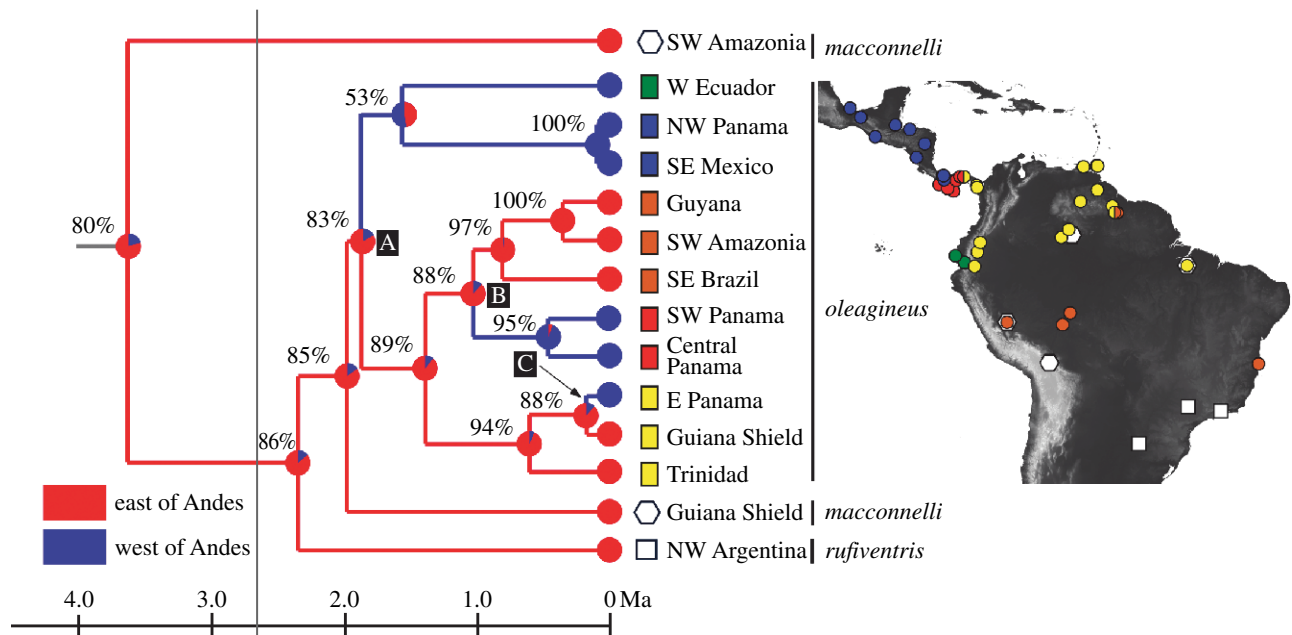


Figure 3. Ancestral area reconstruction for lowland *Mionectes* flycatchers. The phylogenetic tree represents the consensus Bayesian inference topology obtained from cytochrome *b* and *ND2* sequences (2184 bp) modified to conform to an enforced molecular clock (see text). Posterior probabilities of all nodes were 100% except node A (98%). Branch colour reflects the most parsimonious state (east or west of the Andes) for that branch, while coloured circles at nodes represent relative likelihoods of each state. For *M. oleagineus*, colour coding follows figure 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 BP assuming a rate of mtDNA diversification of $2.0\% \text{ Myr}^{-1}$ (Fleischer *et al.* 1998). The vertical grey line at 2.7 Myr BP indicates completion of uplift in the northern Andes (Gregory-Wodzicki 2000).

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 Iwokrama Reserve (Guyana), we recovered three ORANGE haplotypes and one YELLOW haplotype (figure 2). This broad sampling of *ND2* sequences from *M. oleagineus* did not resolve sister relationships among clades in every instance (figure 2).

The addition of *cyt b* sequences to a subsample of birds provided a phylogeny with greatly improved nodal support throughout the tree (figure 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 neighbour, 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 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 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 $0.38\% \text{ Myr}^{-1}$, and for node C (the third crossing) slower than $0.06\% \text{ Myr}^{-1}$. The former is slower than any reported rate for birds and less than half of the typical result for passerines such as *Mionectes* (Lovette

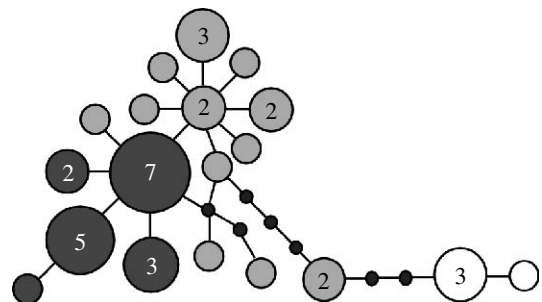


Figure 4. Statistical parsimony-based haplotype network for the YELLOW clade (see figure 2) of *M. oleagineus* showing incomplete lineage sorting between populations east and west of the Andes. Dark grey, eastern Panama; light grey, Amazon Basin and Guiana Shield; white, coastal South America. Black 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.

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 *RAG-1* calibration (see §2 and the electronic supplementary material) 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 $2\% \text{ Myr}^{-1}$ 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 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, $p = 0.003$).

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 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 out-groups are 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 *ca* 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 geographical sampling of montane *Mionectes* (table 1 found in electronic supplementary material), 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 geographical span, *Myadestes* solitaires showed identical mtDNA divergence (Miller *et al.* 2007). If we assume a constant rate of mtDNA divergence of *ca* 2.0% Myr⁻¹, then both montane *Mionectes* and *Myadestes* in southern Middle America began to differentiate across the Isthmus of Panama *ca* 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 three times that of montane *Mionectes* and *Myadestes* (approx. 5–6%; Weir *et al.* in press). 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* probably 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 3). *Mionectes macconnelli*, which has a disjunct distribution in southwestern Amazonia and the Guiana Shield (figure 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 3). Also, *M. oleagineus* was recovered as a monophyletic clade with pronounced phylogeographic structure among mtDNA haplotypes (figure 3).

The geographical 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 §1). 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 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 3).

The *Mionectes* mtDNA phylogeny (figure 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 3) might represent vicariance associated with the final uplift of the northern Andes. Assuming typical rates of passerine mtDNA evolution, these populations split *ca* 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 and C, figure 3). Forcing the date of the splits represented by nodes B and C to be coincident with the northern Andean uplift would imply unreasonably slow rates of mtDNA evolution (see §3). 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 and C (figure 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 3), ancestral area analysis suggests that birds from southwestern Amazonia or the Guiana Shield colonized lowlands west of the Andes (figure 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 2). In the most recent split (node C, figure 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 *Mionectes oleagineus parvus*, 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 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% HPD: 0.3–115 females per 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 (Cracraft & Prum 1988; Prum 1988; Brumfield & Capparella 1996; Bates *et al.* 1998; Ron 2000; Brumfield *et al.* 2001). An exception occurs in the lowland forest woodcreeper *Glyphorhynchus 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 geographical pattern of populations west of the Andes. Descendants of the first cross-Andean split (figures 2 and 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 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. Approximately 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 haplotypes, 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 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 geographical 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 suggest that these mountains can play a more persistent role in Neotropical biogeography and diversification.

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