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Molecular Phylogenetics and Evolution 32 (2004) 512–523
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogeny and plumage signal evolution in a trans Andean and circum Amazonian avian species complex

Irby J. Lovette*

Evolutionary Biology Program, Cornell Laboratory of Ornithology, Cornell University, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA

Received 18 July 2003; revised 15 December 2003
Available online 3 March 2004

Abstract

Species with fragmented distributions are particularly useful models for investigating processes underlying biological diversification in the Neotropics. The *Phaeothlypis* wood-warbler complex (Aves: Parulidae) is comprised of six disjunct or parapatric populations. The geographic distribution of these six populations mirrors the classic map of Neotropical areas of endemism that were originally proposed as putative Pleistocene forest refugia, but the magnitude of mitochondrial DNA divergence between these populations suggests that they are each substantially older, with origins in the late Pliocene. Phylogenetic reconstructions based on long mtDNA coding sequences show that the Guiana Shield and Atlantic Forest populations are sister lineages, and group this combined lineage and the remaining four population-specific lineages in a five-way hard polytomy. MtDNA-based phylogenetic reconstructions provide no evidence that the three populations with conspicuous yellow rump and tail feathers currently grouped as the Buff-rumped Warbler (*P. fulvicauda*) form a monophyletic group. Furthermore, there is a broad discordance between mtDNA and plumage along a transect just east of the Andes, where the contact zone between highly divergent mtDNA clades is more than 1000 km north of the phenotypic hybrid zone between the bright and dark plumage forms. This discordance between mtDNA genotype and plumage phenotype is similar to patterns seen on a finer geographic scale in other avian hybrid zones and may result from asymmetric introgression of the bright plumage trait.

1. Introduction

Despite the extremely high phylogenetic diversity of most groups of Neotropical organisms, detailed reconstructions of geographic and temporal patterns of diversification have been obtained for only a small proportion of Neotropical clades. This paucity of information extends to relatively well-studied groups such as birds, and the lack of comparable data limits our ability to test for patterns and mechanisms of Neotropical diversification (Bates et al., 1998; García-Moreno and Fjeldsa, 2000). Phylogenetic information is similarly critical for reconstructing patterns of morphological evolution, for examining the historical roles of geographic or habitat barriers in fostering differentiation, and for assaying biodiversity for applied conservation purposes.

Here, I reconstruct the evolutionary relationships among geographic populations in the *Phaeothlypis* complex of Neotropical wood-warblers (Aves: Parulidae). These small passerine birds are riparian habitat specialists, occurring along low and mid-elevation streams in southern Central America and widely through tropical and subtropical South America (Ridgely and Tudor, 1989; Robbins and Parker, 1997). Two features of this group make it particularly interesting from an evolutionary perspective. First, the complex is comprised of six geographic populations with disjunct or parapatric distributions. Most of these populations are separated by habitat or topographic barriers that have been implicated in the differentiation of many other Neotropical groups, including the Andes highlands, the central Panama gap, the central Amazonian lowlands, and past marine incursions (e.g., Brumfield and Capparella, 1996; Cracraft and Prum, 1988; Hackett, 1993; Patton and da Silva, 1997). On a continent-wide scale, the geographic

* Fax: 1-607-255-8088.
E-mail address: ijl2@cornell.edu.

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doi:10.1016/j.ympev.2004.01.007

pattern of these populations contains some of the classic areas of avian endemism proposed by Haffer (1969) and elaborated upon—and often contested—by many subsequent workers (e.g., Bush, 1994; Clapperton, 1993; Colinvaux and de Oliveira, 2001; Colinvaux et al., 2001; Fjeldsa, 1994; Haffer, 1974; Haffer and Prance, 2001; Liu and Colinvaux, 1985; Prance, 1982), suggesting that the processes causing the fragmented geographic distribution of the *Phaeothlypis* group may have been similar to those acting on some other Neotropical taxa.

Second, a conspicuous and highly distinctive morphological character—a bright yellow versus dark olive rump patch—separates the two putative species taxa in the *Phaeothlypis* complex, the brightly colored Buff-rumped Warbler (*P. fulvicauda*) and the dark-colored Neotropical River Warbler (*P. rivularis*). These taxa are primarily terrestrial and are most common at the edges of streams or rivers, and the bright plumage trait is likely to have a signaling function in a noisy environment where acoustical communication modalities may be unreliable (Klump, 1996). These species both are well known for a conspicuous tail-swinging behavior that is also seen in several other allied species in the more inclusive *Basileuterus* wood-warbler assemblage (Lovette and Bermingham, 2002; Ridgely and Tudor, 1989). In *fulvicauda*, this tail-bobbing motion highlights the brightly contrasting rump patch, and several workers have suggested that this combination of plumage and behavioral signals is an important isolating mechanism in western Amazonia where the dark-rumped and bright-rumped forms come into contact (Miller, 1952; Ridgely and Tudor, 1989). Morphological intermediates between these forms have been described from southeastern Peru (Zimmer, 1949), however, raising the possibility of hybridization and potential introgression between these differentiated forms. An additional apparent hybrid zone (Wetmore et al., 1984) in central Panama links the Central American and northwestern South American populations, both of which exhibit the bright-rump trait typical of *fulvicauda*.

Phylogenetic approaches offer the prospect of both reconstructing the historical pattern of geographic population structure in the *Phaeothlypis* complex and of exploring the evolution of this bright plumage character. The goals of the present study are to use mtDNA-based phylogenetic reconstructions to: (1) explore the historical pattern and approximate timing of divergence among the various disjunct populations in the *Phaeothlypis* complex; (2) determine whether the disjunct populations that share the bright plumage trait form a monophyletic group; and (3) explore potential mtDNA and plumage introgression in the zone of potential hybridization in Peru and Bolivia.

2. Materials and methods

2.1. Study taxa and sampling

Taxonomically the *Phaeothlypis* complex is comprised of two species, the Neotropical River Warbler (*Phaeothlypis rivularis*) and the Buff-rumped Warbler (*P. fulvicauda*). These species are closely allied sister taxa and together comprise the genus *Phaeothlypis* in the recent systematic treatments of Lowery and Monroe (1968) and American Ornithologists' Union (1998), but many other workers (e.g., Hellmayr, 1935; Ridgely and Tudor, 1989; Ridgway, 1902; Sibley and Monroe, 1990; Zimmer, 1949) have placed both species in the much more diverse wood-warbler genus *Basileuterus*. MtDNA-based phylogenetic reconstructions that include almost all *Basileuterus* species support this latter classification by showing that the *rivularis* and *fulvicauda* lineages described in this paper together form a monophyletic group nested within the more ancient *Basileuterus* radiation (Lovette and Bermingham, 2002; Lovette, unpublished data). However, for convenience and congruence with current taxonomic practice, I use the term "*Phaeothlypis*" here when referring to the *fulvicauda/rivularis* clade, even though the formal recognition of the genus *Phaeothlypis* renders *Basileuterus* paraphyletic. Each of the two putative *Phaeothlypis* species is comprised of three geographically disjunct populations (Fig. 1A).

I obtained mtDNA sequences from 32 individuals representing all geographically disjunct populations within the *Phaeothlypis* complex (Fig. 1A). Genetic samples were derived from frozen muscle tissue specimens archived in the collections of a number of institutions (Table 1). Almost all specimens were originally obtained as part of general collecting programs.

I conducted two rounds of phylogenetic analysis based on mtDNA sequences. Initially I surveyed general patterns of relationship among individuals and among geographic sites using the 842-bp ATPase coding region obtained from all 32 *Phaeothlypis* samples. Analyses of these ATPase sequences revealed multiple geographically structured mtDNA clades that were highly divergent from one another, but support for a bifurcating topology among these clades was absent. To explore whether a more nucleotide-intensive sample of mtDNA sequences would help resolve relationships among these divergent geographic populations, I sequenced a total of 5840 mtDNA nucleotides from 11 individuals representing each highly differentiated geographic *Phaeothlypis* lineage and four outgroup taxa (Table 1). The long mtDNA sequences obtained from this subset of samples included the 4.6 kb region that spans the complete NADH dehydrogenase subunit II (ND2; 1041 bp), cytochrome oxidase subunit I (COI; 1551 bp), cytochrome oxidase subunit II (CO2; 684 bp), ATP-synthase

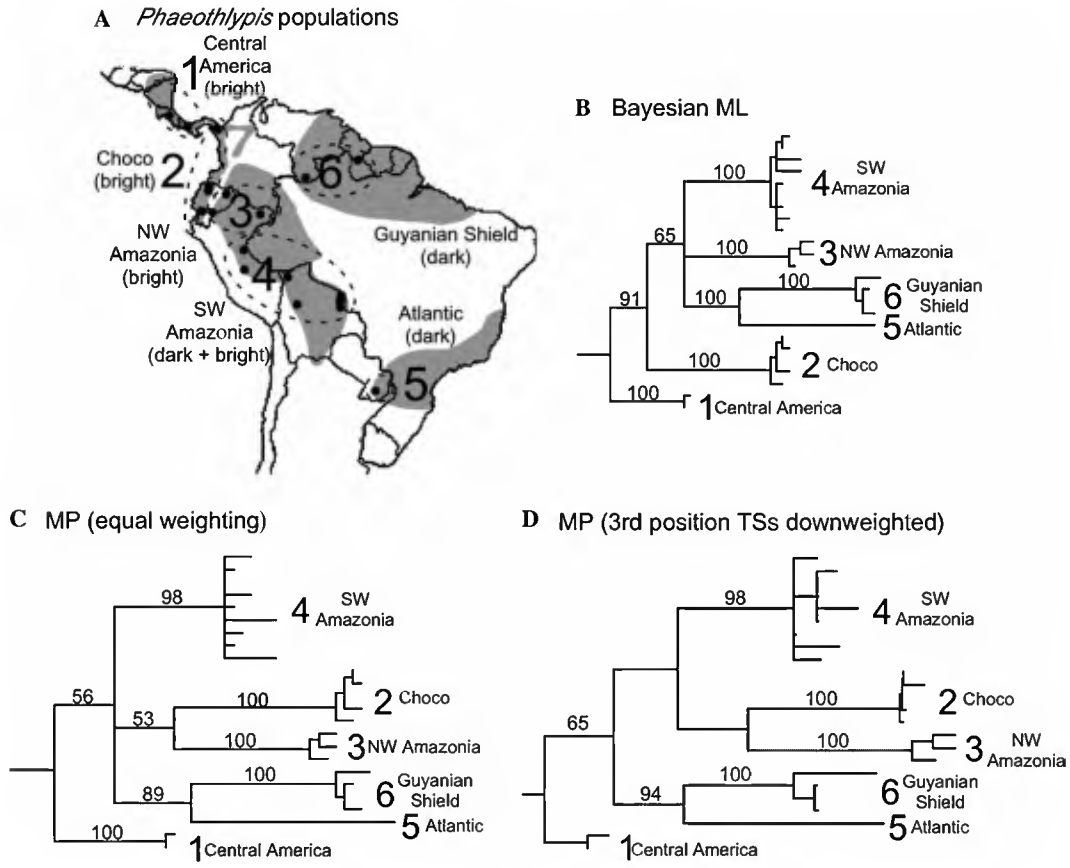

Fig. 1. Phylogeographic reconstructions of the relationships among *Phaeothlypis* populations in Central and South America. (A) Map showing the geographic haplotype affinities of the 32 sampled individuals. Dots indicate sampling locations. Named and numbered clusters of sampling sites encircled by dashed lines correspond to the haplotype groups in the tree-based figures. "Dark" and "bright" refer to plumage phenotypes. (B) Bayesian maximum likelihood phylogram. Numbers above branches indicate posterior probability scores. (C) Maximum parsimony phylogram with all nucleotide changes equally weighted. (D) Maximum parsimony phylogram with third codon-position transitions downweighted relative to other nucleotide substitutions. In both parsimony reconstructions, numbers above branches indicate bootstrap scores. All reconstructions were rooted to *Basileuterus flaveolus* and *B. leucoblepharus* (not shown).

8 (ATPase8; 168 bp), and ATP-synthase 6 (ATPase6; 684 bp) genes, and the complete coding sequence of the cytochrome *b* gene (cyt *b*; 1143 bp). These combined protein-coding regions totaled 5251 nucleotide sites; the remaining 579 nucleotides included the eight intervening tRNA and spacer sequences in the ND2 through ATPase6 region.

2.2. Laboratory protocols

DNA extractions were conducted using standard phenol–chloroform-based methods (e.g., Sambrook and Russell, 2001). General laboratory protocols for PCR amplification and DNA sequencing on an ABI 377 or ABI 3100 automated sequencer have been described elsewhere (Lovette and Bermingham, 1999, 2002; Lovette et al., 1999), as have many of the primers used for amplification and sequencing (see Hunt et al., 2001). Primers not reported elsewhere are described in Table 2. All PCR amplifications employed the reaction mixture and thermal cycling protocols detailed in Hunt et al. (2001), with only the primers and annealing temperature (range 50–56 °C) differing among reactions. Overlapping sequences were aligned and checked by eye using Sequencher 3.1 (Gene Codes).

2.3. Phylogenetic analyses

I assessed patterns of molecular variation and generated phylogenetic reconstructions using PAUP*4.0 (Swofford, 2002) and MrBayes 1.11 (Huelsenbeck and Ronquist, 2001). ATPase-based reconstructions were rooted using *Basileuterus flaveola* and *B. leucoblepharus*, which are closely allied to *Phaeothlypis* based on both behavioral evidence (Lowery and Monroe, 1968; Meyer de Schauensee, 1966; Ridgely and Tudor, 1989; but see also Olson, 1975) and mtDNA- and nuclear DNA-based phylogenetic reconstructions (Lovette and Bermingham, 2002; Lovette, unpublished data). Reconstructions based on long mtDNA sequences included *flaveola* and *leucoblepharus* as well as a more distantly allied

Table 1
Species, sample sources, mtDNA and plumage affinities, and sequence accession numbers

Taxon	MtDNA plumage					Long		
	Clade[a]	Score[b]	Source[c]	Sample[d]	Locality	mtDNA[e]	ATPases[e]	Cytochrome *b*[e]
Phaeothlypis sp.	1		LSUMNS	B-16093	Costa Rica: Puntarenas Prov.	AY327392		AF382997
Phaeothlypis sp.	1		NMNH	B5341	Panama: Chiriqui Prov.	AY327397		AY340214
Phaeothlypis sp.	2	B	ANSP	3614	Ecuador: Azuay Prov.		AY340196	
Phaeothlypis sp.	2	B	ANSP	2145	Ecuador: Esmeraldas Prov.		AY340195	
Phaeothlypis sp.	2	B	LSUMNS	B-11873	Ecuador: Esmeraldas Prov.	AY327393		AY340210
Phaeothlypis sp.	2	B	LSUMNS	B-11834	Ecuador: Esmeraldas Prov.		AY340194	
Phaeothlypis sp.	2	B	LSUMNS	B-2233	Panama: Darien Prov.		AY340200	
Phaeothlypis sp.	2	B	LSUMNS	B-2240	Panama: Darien Prov.	AY327396		AY340213
Phaeothlypis sp.	3	B	ANSP	1527	Ecuador: Morona-Santiago Prov.	AY327394		AY340211
Phaeothlypis sp.	3	B	ANSP	5820	Ecuador: Sucumbios Prov.		AY340197	
Phaeothlypis sp.	3	B	LSUMNS	B-7061	Peru: Loreto Dept.	AY327400		AY340216
Phaeothlypis sp.	4	B	LSUMNS	B-14518	Bolivia: Santa Cruz Dept.		AY340183	
Phaeothlypis sp.	4	D	LSUMNS	B-1142	Bolivia: La Paz Dept.		AY340189	
Phaeothlypis sp.	4	D	LSUMNS	B-1146	Bolivia: La Paz Dept.	AY327391		AY340209
Phaeothlypis sp.	4	B	LSUMNS	B-9307	Bolivia: Pando Dept.		AY340192	
Phaeothlypis sp.	4	NA	LSUMNS	B-9760	Bolivia: Pando Dept.		AY340193	
Phaeothlypis sp.	4	B	LSUMNS	B-14656	Bolivia: Santa Cruz Dept.		AY340184	
Phaeothlypis sp.	4	B	LSUMNS	B-14472	Bolivia: Santa Cruz Dept.		AY340182	
Phaeothlypis sp.	4	B	LSUMNS	B-12500	Bolivia: Santa Cruz Dept.		AY340191	
Phaeothlypis sp.	4	B	LSUMNS	B-12450	Bolivia: Santa Cruz Dept.		AY340190	
Phaeothlypis sp.	4	B	LSUMNS	B-18572	Bolivia: Santa Cruz Dept.		AY340188	
Phaeothlypis sp.	4	B	LSUMNS	B-18264	Bolivia: Santa Cruz Dept.		AY340187	
Phaeothlypis sp.	4	B	LSUMNS	B-18189	Bolivia: Santa Cruz Dept.		AY340185	
Phaeothlypis sp.	4	B	LSUMNS	B-18240	Bolivia: Santa Cruz Dept.		AY340186	
Phaeothlypis sp.	4	B	LSUMNS	B-2000	Peru: Pasco Dept.		AY340204	
Phaeothlypis sp.	4	B	LSUMNS	B-2050	Peru: Pasco Dept.	AY327398		AY340215
Phaeothlypis sp.	4	NA	LSUMNS	B-11223	Peru: Ucayali Dept.		AY340203	
Phaeothlypis sp.	5		UKNHM	88464	Paraguay: Caazapa Prov.	AY327399		
Phaeothlypis sp.	6		ANSP	6191	Guyana: Waruma		AY340199	
Phaeothlypis sp.	6		NMNH	B-05027	Guyana: Waruma	AY327395		AY340212
Phaeothlypis sp.	6		NMNH	B-05039	Guyana: Waruma		AY340198	
Phaeothlypis sp.	6		LSUMNS	B-7499	Venezuela: Amazonas Terr.	AY327401		AY340217
Basileuterus flaveolus			LSUMNS	B-6545	Bolivia: Santa Cruz Dept.		AY340207	
B. flaveolus			LSUMNS	B-14692	Bolivia: Santa Cruz Dept.	AY498538		AF382994
B. leucoblepharus			UWBM	GAV777	Argentina: Corrientes Prov	AY498539		
B. leucoblepharus			UWBM	DAB848	Argentina: Corrientes Prov	AY498537		
B. rufifrons			STRI	PABRU28	Panama: Panama Prov.	AY327390		AF383012
Dendroica fusca			ANSP	2011	Ecuador:	AY327389		AY340208

[a] Numbers correspond to clades indicated in Figs. 1, 3, and 4.

[b] Plumage scores for specimens from eastern and western Andean foothills sites (B, buffy yellow rump plumage; D, dark olive rump plumage; and NA, voucher not available or voucher prepared as a skeleton).

[c] Museum tissue sources. Abbreviations: LSUMNS, Louisiana State University Museum of Natural Science, Baton Rouge, LA, USA; NMNH, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ANSP, Academy of Natural Sciences, Philadelphia, PA, USA; UKMNH, University of Kansas Museum of Natural History, Lawrence, KS, USA; UWBM, Burke Museum of Natural History and Culture, University of Washington, Seattle, WA, USA; and STRI, Smithsonian Tropical Research Institute, Balboa, Panama.

[d] Museum source sample or catalog number.

[e] Long mtDNA sequences include the ND1l, COI, COII, ATPase8, and ATPase6 coding sequencing and the intervening tRNA and spacer regions, as described in the text. ATPase sequences comprise the complete ATPase8 and ATPase6 coding region (842 bp). Cytochrome *b* sequences comprise the complete coding sequence (1143 bp).

Basileuterus species (*B. rufifrons*), and were rooted to sequences of *Dendroica fusca*.

In analyses of the longer nucleotide dataset, a partition homogeneity test (Farris et al., 1995) conducted in PAUP* (1000 heuristic replicates with 10 stepwise addition replicates/heuristic replicate) identified no significant differences among the six protein-coding gene partitions when all taxa were included ($P = 0.18$) or when analysis was restricted to the ingroup samples ($P = 0.20$), and I therefore combined the sequences obtained from each individual for subsequent analyses of phylogenetic relationships. The phylogenetic analyses reported here were conducted using only the 5251 bp protein-coding sequences, owing to uncertainties about how to parameterize search models or weight characters appropriately in the tRNA and spacer regions.

Table 2
Novel primers[a] used for amplification and sequencing of a 4.6 kb region of avian mtDNA

Primer	Strand	Primer sequence 5′–3′	Location[b]
IL5524	Light	TCACCCACCCAACATCCTG	5524 (ND2)
IL6052	Light	CTCATCATCCAAGAACTRAC	6052 (ND2)
IL6052.Bfla	Light	CTTATTATCCAAGAATTGAC	6052 (ND2)
IL6591	Light	GCCGATAAGAAGAGGAATTG	6591 (tRNA Tyr)
IL6673	Heavy	GCCAATGTCTTTGTGGTTGGTTG	6673 (CO1)
IL6673.Bfla	Heavy	CCCGATRTCTTTGTGGTTRGTTG	6673 (CO1)
IL7389	Heavy	GAGATGATTCCAAATCCTGG	7389 (CO1)
IL7925	Light	KGTAAACYTAACCTTCTTCCCC	7925 (CO1)
IL8587	Light	CCCATCCTTACGAATCCTCTACATAAT	8587 (CO2)
IL8760	Heavy	GACTCGGATAGTRGAGTTTATGGG	8760 (CO2)
IL9240	Heavy	GTCRAAGAARCTTAGGTTCAT	9240 (ATPase8)
IL9288	Heavy	GTTATTGAGATAAGGATGAGTGGGAT	9288 (ATPase6)
IL9518	Heavy	ATKGATAGTTGGGTRGTTGGGGTG	9518 (ATPase6)
IL9538	Light	CACCCCAACYACCCAACTATC	9538 (ATPase6)

[a] Additional primers have been reported previously (see text).
[b] Numerical location gives the position of the 3′ nucleotide in the chicken mitochondrial genome (GenBank X52392; Desjardins and Morais, 1990); text indicates the mitochondrial gene that includes the primer binding region.

The invariable, frame-shifted 10-bp overlap region between the ATPase6 and ATPase8 genes was similarly excluded from all analyses because those nucleotide positions could be not be assigned a single codon position.

I used both maximum parsimony (MP) and maximum likelihood (ML) techniques to explore the phylogenetic relationship among mtDNA haplotypes in both the ATPase and multigene mtDNA datasets. MP reconstructions were generated in PAUP* via heuristic searches with 1000 stepwise addition replicates. MP searches were conducted using three character weighting schemes: (1) equal weighting of all substitutions, (2) downweighting of all transitions by 0.2 relative to transversions, and (3) downweighting of third-position changes by 0.2 relative to all other substitutions. The support for individual nodes was assessed via MP heuristic bootstrap with 1000 bootstrap replicates, each with 10 addition sequence replicates.

Bayesian ML analyses were conducted using MrBayes 1.11 (Huelsenbeck and Ronquist, 2001), in which a Markov chain Monte Carlo search was parameterized using the general time reversible model (nst = 6), with site-specific rate variation partitioned by codon. Bayesian analyses were run for 1,000,000 generations and sampled every 1000 generations; the topologies sampled from the first 250,000 generations were discarded as burn-in. Consensus topologies with the mean branch lengths from the remaining 751 sampled trees were generated in MrBayes.

An additional ML analysis of the 5251-bp dataset was conducted in PAUP*. This heuristic ML search (100 stepwise addition replicates) was parameterized with empirical base frequencies, and with the transition:transversion ratio (6.25), proportion of invariable sites (0.694), gamma rate variation value (3.60, with four rate categories) estimated in PAUP* during the search. A ML bootstrap was performed using the same model for 100 bootstrap replicates, each with a single stepwise starting tree.

2.4. Molecular clock comparisons

The general timing of the *Phaeothlypis* radiation is relevant to the many competing hypotheses about the causes of avian speciation in South America and to the growing body of evidence suggesting that clades of South American birds exhibit a variety of temporal patterns of diversification (e.g., Bates et al., 1999; Chesser, 2000; García-Moreno and Fjeldsa, 2000; García-Moreno et al., 2001; Hackett, 1993; Lovette and Bermingham, 2001; Roy et al., 1999). However, few molecular clock calibrations are available for birds, including only a single robust calibration for passerines (Fleischer et al., 1998), which was based on cytochrome *b* divergence in Hawaiian honeycreepers and on the emergence dates of the larger islands in the Hawaiian archipelago. Although the applicability of this rate calibration to other passerine taxa is untested, the Hawaiian honeycreepers (Drepaniidae) and wood-warblers (Parulidae) are closely allied groups that contain species of similar body size, and the range of cytochrome *b* divergences used in the Fleischer et al. calibration overlaps the range of divergence among the *Phaeothlypis* populations considered here. Fleischer et al. (1998) found a consistent divergence rate of 1.6% per million years in their comparisons, which were based on gamma-corrected Kimura 2-parameter (Kimura, 1980) cytochrome *b* distances. I used that distance metric (with a rate variation parameter of 0.25) and substitution rate to estimate divergence dates among *Phaeothlypis* lineages. I found that pairwise divergences based on cytochrome *b* alone were nearly identical to pairwise divergences based on the entire 5251 bp coding-region dataset (Fig. 2). I therefore used distances from these longer sequences in

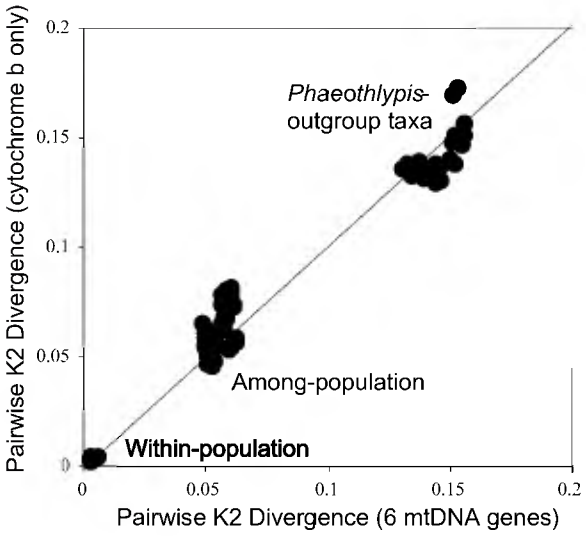

Fig. 2. Concordance of substitution rates in cytochrome *b* versus the combined six mtDNA coding genes used for molecular clock comparisons. Points indicate pairwise distances among haplotypes. The line indicates parity between the two axes.

molecular clock calculations because this increased nucleotide sampling will tend to reduce the stochastic variation in estimates of pairwise differentiation. I did not correct these date estimates for the possible effects of ancestral polymorphism (as reviewed in Edwards and Beerli, 2000). Such corrections require assumptions about ancestral population size, which are usually based on observations of present-day intrapopulation variation. Furthermore, under this line of reasoning the low magnitude of intrapopulation haplotype differentiation in present-day *Phaeothlypis* populations (see Section 3) suggests that applying such a correction would only very slightly reduce estimates of interpopulation divergence.

3. Results

3.1. ATPase-based phylogenetic analyses

I obtained the complete 842-nucleotide coding sequence of the ATPase6 and ATPase8 genes from 32 *Phaeothlypis* specimens and several outgroup taxa (Table 1). These ATPase sequences were identical in length and sequence alignments were unambiguous. I identified 24 unique ATPase haplotypes with 128 variable nucleotide sites, of which 93 sites were potentially parsimony informative. The two outgroup taxa differed from one another by 9.4% K2-gamma sequence divergence, and from the *Phaeothlypis* haplotypes by a mean of 13.1%. ATPase haplotypes within the *Phaeothlypis* ingroup differed by a maximum of 9.4%.

All ATPase-based phylogenetic reconstructions identified six well-differentiated haplotype groups (Fig. 1). Nucleotide variation within each of these groups was very low (maximum pairwise difference among any within-group haplotypes = 7 nucleotide substitutions, 0.8% uncorrected divergence). Pairwise divergence among haplotypes from different groups was considerably larger (range: 30–56 substitutions; 3.6–6.8%).

These results show that the *Phaeothlypis* complex is comprised of six geographically structured mtDNA clades (Fig. 1), and the six haplotype groups identified in the ATPase reconstructions have distributions that are broadly concordant with the patterns of geographic disjunction and morphological variation. The one partial exception is an apparent zone of secondary contact in the upper Amazon basin of central Peru and northwestern Bolivia. On morphological criteria, these sites were expected to harbor individuals with close affinities to the *P. fulvicauda* populations sampled from the more northerly upper Amazon sites in northeastern Peru and eastern Ecuador (group 6 in Fig. 1). Instead, haplotypes from these geographically intermediate sites were similar or identical to the remaining group 5 haplotypes (geographically *P. rivularis*) sampled from sites in central and eastern Bolivia.

Considered in concert, the ATPase-based likelihood and parsimony reconstructions (Figs. 1B–D) are broadly congruent in that they all: (1) identify the same six clusters of haplotypes; (2) group three (ML) or four (MP) of the remaining populations in a polytomy; and (4) give strong support to the placement of the Guiana and Atlantic groups as the most recently derived sister lineages.

3.2. Long mtDNA-based phylogenetic analyses

To further explore the phylogenetic relationships of the six haplotype groups, I analyzed a total of 5251 nucleotides from 11 *Phaeothlypis* samples and four outgroup taxa. The 4.6 kb region spanning the ND2 through ATPase6 genes had the typical avian gene order (ND2–tRNA Trp–tRNA Ala–tRNA Asn–tRNA Cys–tRNA Tyr–CO1–tRNA Ser–tRNA Asp–CO2–tRNA Lys–ATPase8–ATPase6). Complete cytochrome *b* sequences were also obtained from 10 of the 11 *Phaeothlypis* samples and all outgroup taxa (Table 1). Alignments were straightforward, as all protein-coding genes had identical lengths, with indels occurring only in the short intergenic spacer regions and within several tRNAs. In all taxa the COI gene had a valine (GTG) start codon, as has been found in a number of other vertebrate mtDNAs, including those of several birds (e.g., Härlid et al., 1997, 1998). In comparisons that included only the 5251 bp of protein-coding sequence from the 11 *Phaeothlypis* samples, a total of 604 nucleotide sites varied, of which 483 were potentially parsimony informative. In comparisons that included all outgroup taxa, 1111 sites varied, of which 773 were potentially parsimony informative. The maximum

K2-gamma divergence among any two *Phaeothlypis* samples was 6.2%, whereas the *Phaeothlypis* representatives differed from the outgroup taxa *flaveola* and *leucoblepharus* by 10.0–12.7%, from *B. rufifrons* by 12.1–13.7%, and from *D. fusca* by 13.2–15.0%.

Rooted phylograms based on the ML, Bayesian, and MP analyses of the 5251-bp dataset (Fig. 3) shared all of the features seen in the ATPase-only reconstructions. Support for the sister relationship of the Guiana Shield and Atlantic Forest populations was very high in all reconstructions (populations 5 and 6; all bootstrap and probability scores = 97–100%). The remaining relationships among population-level lineages were poorly resolved regardless of the tree reconstruction method. In the Bayesian reconstruction, the Choco lineage was basal to the remaining *Phaeothlypis* lineages, but the posterior probability for the internode defining this relationship was only 58%. In the ML reconstruction, the southwestern Amazonia population was basal, and again the support for this topology was negligible. Topologies varied among MP analyses with the three character weighting schemes, but the short internodes at the base of the Phaeothlypis group either collapsed in strict consensuses of equally shortest trees or received bootstrap support below 50%; the single exception was an internode with a 56% bootstrap that placed the southwestern Amazonian population basal in the MP reconstruction with 0.2 downweighting of transitions. Considered together, these reconstructions suggest that five of these six lineages originated rapidly and demonstrate that the basal lineage cannot be identified with confidence even in a dataset with a very high phylogenetic information content at the relevant level of molecular divergence.

4. Discussion

4.1. Historical biogeography

The mtDNA evidence suggests that the *Phaeothlypis* complex is comprised of six highly differentiated

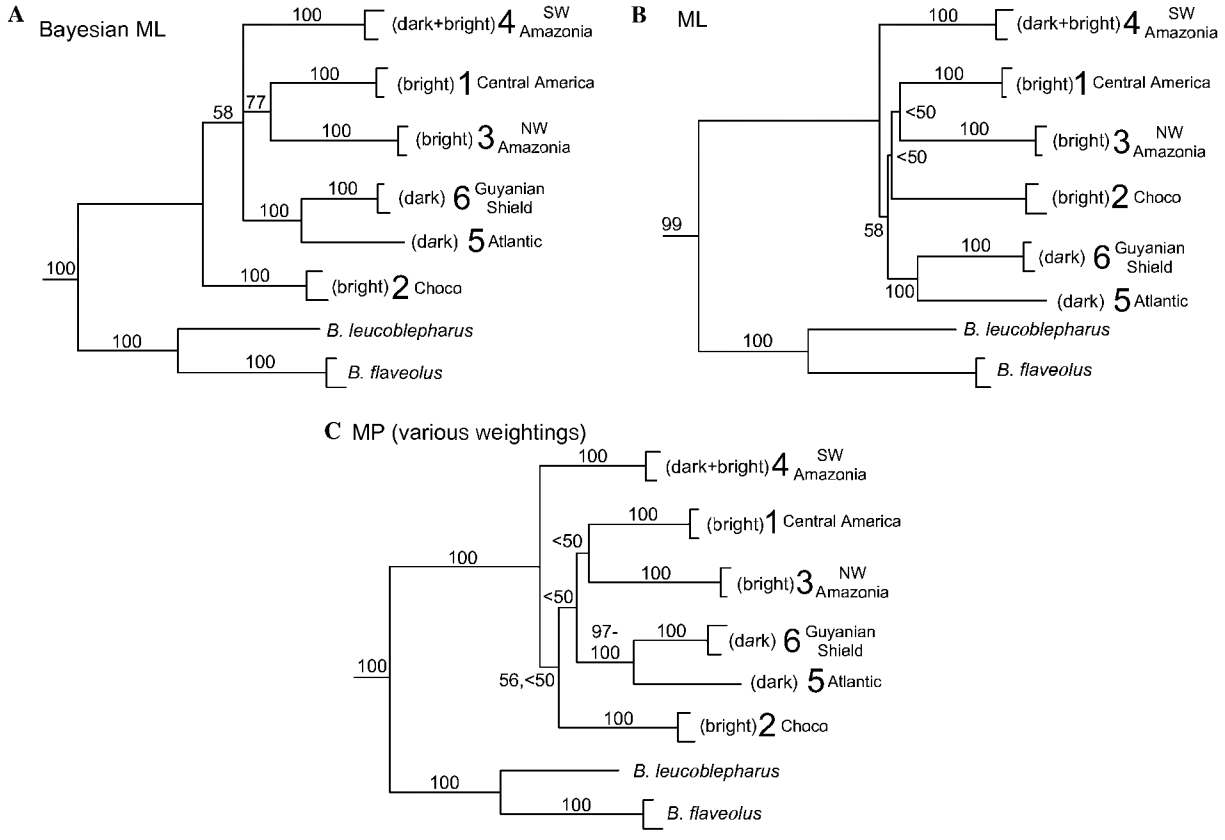

Fig. 3. Relationships among *Phaeothlypis* haplotype groups based on analyses of long (5251 bp) protein-coding mtDNA sequences. Named and numbered lineages correspond to the populations mapped in Fig. 1A. Considered together, these analyses support the presence of a "hard polytomy" involving the five most basal *Phaeothlypis* lineages. (A) Bayesian likelihood phylogram with posterior probability values. (B) Maximum likelihood phylogram with bootstrap scores. (C) Topology of single shortest parsimony tree with transition downweighting. Values show range of bootstrap values across analyses employing three character weighting schemes. The only topological variation across these reconstructions involved the three basal *Phaeothlypis*-clade internodes with <50% bootstrap support, all of which collapsed in consensuses among the four equally shortest trees found under equal character weighting and/or among the two shortest trees found with third-position downweighting.

geographic populations (Fig. 1), including: (1) a southern Central American population represented in this study by samples from Costa Rica and western Panama; (2) a Choco region population represented by samples from eastern Panama and the Pacific slope of Ecuador; (3) a northern upper Amazon basin population represented by samples from eastern Ecuador and northeastern Peru; (4) a southeastern upper Amazon basin represented by samples from central Peru and northern Bolivia, (5) a Guiana Shield population represented by samples from Guyana and Venezuela, and (6) an Atlantic population represented by a sample from Paraguay. The distribution of the two highly divergent mtDNA lineages in the western upper Amazon basin (populations 3 and 4) is inconsistent with the pattern of plumage variation in this region, but otherwise the pattern of mtDNA lineage diversity is congruent with the geographic separation among disjunct or parapatric populations (Fig. 1).

Only two of these geographic clades show a clear sister relationship: the monophyly of the two easternmost populations (lineages 5 and 6 in Fig. 1) was highly supported in all reconstructions (Figs. 1 and 3). The common ancestry of these Guiana and Atlantic populations is somewhat surprising, as they are separated by a wide geographic disjunction that corresponds to the present-day belt of savanna and dry woodland that extends from northwestern Argentina to northeastern Brazil. These sister populations each occupy areas of endemism identified from distributions of birds and other Neotropical organisms (e.g., Haffer, 1969), but broad-scale comparisons of patterns of taxon sharing (Bates et al., 1998; Santiago, 2000) suggest that the Guiana and Atlantic regions do not cluster on the basis of shared vertebrate species and subspecies. The sister relationship of the Guiana and Atlantic *Phaeothlypis* populations appears to be unusual (e.g., Cracraft and Prum, 1988; Da Silva and Patton, 1998; Hall and Harvey, 2002) but has been seen in a few other South American vertebrate groups (e.g., bushmaster snakes: Zamudio and Greene, 1997).

Also unusual is the lack of a clear sister relationship between the Central American and Choco *Phaeothlypis* populations (populations 1 and 2 in Fig. 1). Phylogeny-based comparisons of a range of avian taxa with broad Neotropical distributions have shown that the Central American and Choco regions tend to contain lineages that are recently derived from one another relative to lineages found east of the Andes (Brumfield and Capparella, 1996; Cracraft and Prum, 1988; Hackett, 1993; Santiago, 2000). The *Phaeothlypis* pattern is anomalous in that the two populations west of the Andes appear no more closely allied to one another than they each are to the various populations east of the Andes, including the most geographically distant populations (Table 3; Fig. 3). The deep mtDNA divergence separating the Central American and Choco *Phaeothlypis* populations is particularly interesting given the apparent hybridization of these forms in a narrow zone of contact centered on the Panama Canal (Wetmore et al., 1984). Molecular investigations of potential gene flow between these populations are warranted but will be difficult to implement because these taxa are presently rare in central Panama, as recent habitat alteration (Robinson et al., 2000) may be causing the secondary separation of these previously parapatric forms.

Table 3
Mean pairwise mtDNA differentation[a] among *Phaeothlypis* populations

Population[b]	1	2	3	4	5	6
1	—	3.2	3.0	3.0	3.2	3.2
2	0.051	—	3.6	3.4	3.8	3.6
3	0.048	0.058	—	3.4	3.4	3.6
4	0.048	0.055	0.055	—	3.6	3.4
5	0.050	0.061	0.055	0.058	—	2.9
6	0.051	0.058	0.058	0.055	0.046	—

[a] Below diagonal: mean gamma-corrected K2 distances based on 5251-bp mtDNA coding sequence (see text for details). Above diagonal: estimated divergence date (MY) using the Fleischer et al. (1998) calibration for passerine mtDNA.

[b] Population numbers correspond to the mtDNA lineages in numbered Fig. 1.

A similar lack of phylogenetic resolution is apparent among most pairs of *Phaeothlypis* populations: exclusive of the Guiana/Atlantic sister lineages, the relationships among the other population-level lineages were universally poorly resolved (Figs. 1, 3, and 4). The hard mtDNA polytomy at the base of the *Phaeothlypis* tree suggests that these five lineages became reciprocally isolated at approximately the same period. An absence of spatio-temporal patterning is also apparent in simple comparisons of pairwise genetic distances among populations (Table 3), in which geographically adjacent populations do not show lower pairwise differentiation than non-adjacent populations.

The various *Phaeothlypis* populations have a mosaic distribution that closely mirrors the centerpiece of the seminal Pleistocene refugia hypothesis of Neotropical speciation, the map of centers of endemism associated with hypothesized pockets of forested habitat that might have persisted through the arid periods of Pleistocene glacial maxima (Haffer, 1969). Although the refugia hypothesis has come under increasing criticism (e.g., Colinvaux et al., 2001), the magnitude of mtDNA differentiation separating the *Phaeothlypis* populations is inconsistent with a Pleistocene origin. Instead, the application of the Fleischer et al. (1998) molecular clock calibration suggests that the separation of the five basal *Phaeothlypis* lineages occurred between 3.0 and 3.8 MY before present, a date range considerably earlier than the Pleistocene/Pliocene boundary at 1.8 MY before present. The *Phaeothlypis* warblers therefore join a

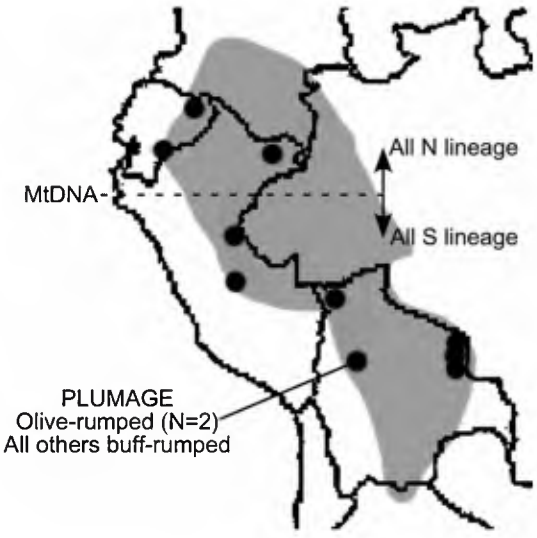

Fig. 4. Plumage and mtDNA discordance in the upper Amazon basin region. Shading indicates the approximate distribution of *Phaeothlypis* populations. MtDNA haplotypes from collecting sites (black dots) north of the dashed line fall within clade 3 (see Fig. 1), whereas haplotypes from sites south of line fall within clade 4. Specimens from all sites have the typical "*fulvicauda*"-type plumage consisting of a bright yellow rump patch, except for the two individuals from a single location in central Bolivia, as indicated, which have the dark plumage typical of "*rivularis*."

growing list of avian taxa that appear to have diversified intraspecifically long before the late Pleistocene. The earlier origin of these *Phaeothlypis* lineages occurred instead during a geologically active period that saw the uplift of the northeastern Andes and the closure of the Panama seaway. As the *Phaeothlypis* complex is derived within a clade of *Basileuterus* warbler species that otherwise includes taxa restricted to South America (Lovette, unpublished data), this ancestral *Phaeothlypis* lineage probably occurred in South America and later spread northwards into Central America across the Central Panama gap at approximately the same time as the various South American *Phaeothlypis* populations became isolated from one another.

4.2. Plumage evolution and potential hybrid introgression

At the broadest geographic level, I found no support for clades corresponding to the bright- or dark-rumped taxa traditionally recognized as separate *Phaeothlypis* species (Figs. 3 and 4). Instead, the mtDNA data suggest that five regional lineages diverged from one another nearly simultaneously. Given the absence of the bright-rump character in all other *Basileuterus* species, this plumage pattern represents a derived state in *Phaeothlypis*. Although the three populations showing this derived state are geographically adjacent to one another (Fig. 1A), there is no genetic evidence that these populations are particularly closely allied. It remains possible that the three bright-rumped populations form a monophyletic group, but in that case the common ancestral lineage in which the bright-rump character emerged must have originated and persisted for only a short period before the three bright-rumped mtDNA lineages separated from one another.

An alternative scenario of asymmetric introgression of the bright-rump character into formerly dark-rumped populations could also account for the pattern of geographically concordant plumage variation superimposed on populations with no clear phylogenetic clustering. This introgression hypothesis is consistent with the contrasting patterns of mtDNA and plumage variation in the upper Amazon basin, where I found a large discordance between the pattern of mtDNA variation and rump plumage color in samples from a transect spanning northeastern Ecuador to central Bolivia (Fig. 4). Populations from almost all sampling sites along this transect exhibit the bright-rump trait, with the olive-rumped form occurring only at a southwestern sampling site near La Paz, Bolivia. This geographic pattern of plumage variation conflicts with previous descriptions of these forms, which have suggested that all birds from central Peru northwards have the buff-rumped characters and all birds from Bolivia have the olive-rumped characters (e.g., Lowery and Monroe, 1968; Ridgely and Tudor, 1989), with a potential zone of hybrid introgression in southeastern Peru (Zimmer, 1949). These intermediate birds, described by Zimmer (1949) as *B. r. significans*, have the bright buff tail coverts seen in more northerly individuals but have largely olive retrices in which the buffy band is confined to the base, where it is obscured in live birds by the uppertail coverts. There were no such intermediate plumages in the vouchers for the samples I sequenced (S. Cardiff, personal communication), which are recently collected specimens that were unavailable to Zimmer (1949).

The buff-rumped plumage trait is hence considerably more widespread in the southern upper Amazon basin than has been appreciated previously, and the olive-rumped trait is apparently confined to the most southwesterly populations at the southern extension of the upper Amazon basin transect (Fig. 4). I found no evidence of mtDNA differentiation between the dark-rumped and bright-rumped forms in Bolivia: ATPase haplotypes from the two dark-rumped birds differed by as little as a single nucleotide substitution (0.1%) from those of bright-rumped individuals from elsewhere in Bolivia and Peru. Instead, the geographic breakpoint between the two highly differentiated mtDNA lineages in the transect was in northeastern Peru, more than 1000 km north of the zone of plumage intergradation in an area where only bright-rumped individuals are found.

This mtDNA/plumage discordance suggests that either the southern mtDNA lineage has introgressed northwards, or that the bright-rump plumage trait has introgressed southwards. Although it is impossible to

distinguish between these two possibilities given existing information, asymmetric introgression of plumage traits has been found in other avian hybrid zones (Brumfield et al., 2001; Parsons et al., 1993), and it could occur in the *Phaeothlypis* system, particularly if the derived buff-rumped character is associated with behavioral dominance or increased reproductive success. Furthermore, Haldane's rule (Haldane, 1922) suggests that introgression of female-linked characters such as mtDNA across avian hybrid zones is somewhat less likely than the introgression of non-sexed linked or male-linked traits (Moore, 1995; Sperling, 1993; Tegelström and Gelter, 1990), as the heterogametic female hybrids are more likely to be sterile or inviable and are hence less likely to serve as conduits for organellar genome introgression. The most likely scenario is therefore that the plumage trait has introgressed southwards into the southern Amazon basin population.

Given the potential for asymmetric plumage introgression between these Andean populations, it becomes interesting to speculate that the derived, bright-rump plumage originated within a single geographic population and subsequently spread through the Central American, Choco, and northern Amazon basin populations via previous periods of asymmetric introgression. Unfortunately, rigorous tests of this scenario would require multilocus genetic analysis of large numbers of individuals and measures of reproductive success, a challenging task for a taxon that occurs at low densities in remote areas.

4.3. Taxonomic implications

The *Phaeothlypis* complex provides an interesting example of a group in which the application of different criteria leads to opposing systematic decisions. The current two-species taxonomy (sensu American Ornithologists' Union, 1998; Lowery and Monroe, 1968; Ridgely and Tudor, 1989; Ridgely and Greenfield, 2001; Robbins and Parker, 1997; Sibley and Monroe, 1990) is based primarily on a single morphological character, the bright-rump character shared by the populations grouped as *fulvicauda*. In contrast, the mtDNA reconstructions presented here suggest that the *Phaeothlypis* complex is comprised of six well-differentiated, geographically structured mtDNA lineages and that five of these lineages originated nearly simultaneously in the mid-Pliocene. The magnitude of mtDNA differentiation separating the six *Phaeothlypis* populations exceeds that between a number of pairs of other parulid warbler taxa that are widely considered valid species (e.g., Lovette and Bermingham, 1999; Lovette et al., 1999; Zink et al., 2000). From a phylogenetic species perspective, the *Phaeothlypis* complex would likely be subdivided into six species-level taxa. From a biological species concept perspective, the morphological hybrids from the zone of contact between the Central American and Choco populations (Wetmore et al., 1984) and the apparent introgression between the northern and southern Amazon basin populations (this study) show that these parapatric populations are not completely reproductively isolated. By extension, the allopatric populations with equivalent mtDNA differentiation also seem unlikely to be fully isolated by factors other than geography. Therefore, application of the biological species concept would support recognition of only a single species taxon, as advocated by Meyer de Schauensee (1966). Although these disparate systematic perspectives cannot be reconciled with existing information, I advocate the recognition of six species-level taxa, a taxonomic approach that has the advantage of acknowledging the deep phylogenetic divisions between most of these populations, and which would be problematic only in the area of potential introgression between the northern and southern Amazon basin populations where plumage phenotype may not be a reliable guide to genome-wide ancestry.

Acknowledgments

I thank the Louisiana State University Museum of Natural Science; National Museum of Natural History, Smithsonian Institution; Academy of Natural Sciences, Philadelphia; University of Kansas Museum of Natural History; Burke Museum of Natural History and Culture, University of Washington; and Smithsonian Tropical Research Institute for loaning material from their frozen tissue collections, and D. Agro, J. Bates, M. Braun, S. Cardiff, D. Dittmann, R. Ridgely, M. Robbins, S. Rohwer, F. Sheldon, and C. Wood for facilitating those loans. E. Bermingham provided extensive advice and support at the Smithsonian Tropical Research Institute. S. Cardiff generously scored plumage characters of the preponderance of voucher specimens in the collections of the Louisiana State University Museum of Natural Science, and I. Fiorentino and L. Stenzler provided laboratory assistance. I thank M. Kimura, J.V. Remsen, K. Zamudio, and two anonymous reviewers for their helpful comments on the manuscript.

References

American Ornithologists' Union, 1998. Check-list of North American Birds, seventh ed. American Ornithologists' Union, Washington, DC.

Bates, J.M., Hackett, S.J., Cracraft, J., 1998. Area-relationships in the neotropical lowlands: an hypothesis based on raw distributions of passerine birds. J. Biogeogr. 25, 783–793.

Bates, J.M., Hackett, S.J., Goerck, J.M., 1999. High levels of mitochondrial DNA differentiation in two lineages of antbirds (*Drymophila* and *Hypocnemis*). Auk 11, 1093–1106.

Brumfield, R.T., Capparella, A.P., 1996. Historical diversification of birds in northwestern South America: a molecular perspective on the role of vicariant events. Evolution 50, 1607–1624.

Brumfield, R.T., Jernigan, R.W., McDonald, D.B., Braun, M.J., 2001. Evolutionary implications of divergent clines in an avian (Manacus:Aves) hybrid zone. Evolution 55, 2070–2087.

Bush, M.B., 1994. Amazonian speciation: a necessarily complex model. J. Biogeogr. 21, 5–17.

Chesser, R.T., 2000. Evolution in the high Andes: the phylogenetics of *Muscisaxicola* ground-tyrants. Mol. Phylogenet. Evol. 15, 369–380.

Clapperton, C.M., 1993. Quaternary Geology and Geomorphology of South America. Elsevier Press, Amsterdam.

Colinvaux, P.A., de Oliveira, P.E., 2001. Amazon plant diversity and climate through the Cenozoic. Palaeogeogr. Palaeoclimatol. Palaeoecol. 166, 51–63.

Colinvaux, P.A., Irion, G., Rasanen, M.E., Bush, M.B., deMello, J.A., Nunes, S., 2001. A paradigm to be discarded: geological and paleoecological data falsify the HAFFER & PRANCE refuge hypothesis of Amazonian speciation. Amazoniana 16, 609–646.

Cracraft, J., Prum, R.O., 1988. Patterns and processes of diversification: speciation and historical congruence in some Neotropical birds. Evolution 42, 603–620.

Da Silva, M.N.F., Patton, J.L., 1998. Molecular phylogeography and the evolution and conservation of Amazonian mammals. Mol. Ecol. 7, 475–486.

Desjardins, P., Morais, R., 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. J. Mol. Biol. 212, 599–634.

Edwards, S.V., Beerli, P., 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogenetic studies. Evolution 54, 1839–1854.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

Fjeldsa, J., 1994. Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. Biodivers. Conserv. 3, 207–226.

Fleischer, R.C., McIntosh, C.E., Tarr, C.L., 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Mol. Ecol. 7, 533–545.

García-Moreno, J., Fjeldsa, J., 2000. Chronology and mode of speciation in the Andean avifauna. Bonner Zoologische Monographien 46, 25–46.

García-Moreno, J., Ohlson, J., Fjeldsa, J., 2001. MtDNA sequences support monophyly of Hemispingus tanagers. Mol. Phylogenet. Evol. 21, 424–435.

Hackett, S.J., 1993. Phylogenetic and biogeographic relationships in the Neotropical genus *Gymnopithys*. Wilson Bull. 105, 301–315.

Haffer, J., 1969. Speciation in Amazonian forest birds. Science 165, 131–137.

Haffer, J., 1974. Avian Speciation in Tropical South America. Publications of the Nuttall Ornithological Club No. 14, Cambridge, MA.

Haffer, J., Prance, G.T., 2001. Climatic forcing of evolution in Amazonia during the Cenozoic: on the refuge theory of biotic differentiation. Amazoniana 16, 579–607.

Haldane, J.B.S., 1922. Sex ratio and the unisexual sterility in hybrid animals. J. Genet. 12, 101–109.

Hall, J.P.W., Harvey, D.J., 2002. The phylogeography of Amazonia revisited: new evidence from riodinid butterflies. Evolution 56, 1489–1497.

Härlid, A., Janke, A., Arnason, U., 1997. The mtDNA sequence of the ostrich and the divergence between paleognathous and neognathous birds. Mol. Biol. Evol. 14, 754–761.

Härlid, A., Janke, A., Arnason, U., 1998. The complete mitochondrial genome of *Rhea americana* and early avian divergences. J. Mol. Evol. 46, 669–679.

Hellmayr, C.E., 1935. Catalogue of birds of the Americas and the adjacent islands, Part 8, Field Museum of Natural History Publications in Zoology, Series 13.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hunt, J.S., Bermingham, E., Ricklefs, R.E., 2001. Molecular systematics and biogeography of Antillean thrashers, tremblers, and mockingbirds (Aves:Mimidae). Auk 118, 35–55.

Kimura, M., 1980. A simple method for estimating the evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

Klump, G.M., 1996. Bird communication in a noisy world. In: Kroodsma, D.E., Miller, E.H. (Eds.), Ecology and Evolution of Acoustic Communication in Birds. Cornell University Press, Ithaca, pp. 321–338.

Liu, K.B., Colinvaux, P.A., 1985. Forest changes in the Amazon Basin during the last glacial maximum. Nature 318, 556–557.

Lovette, I.J., Bermingham, E., 1999. Explosive speciation in the New World *Dendroica* warblers. Proc. R. Soc. Lond. B 266, 1629–1636.

Lovette, I.J., Bermingham, E., 2001. A mitochondrial sequence-based phylogenetic analysis of *Parula* wood-warblers (Aves: Parulidae). Auk 118, 211–215.

Lovette, I.J., Bermingham, E., 2002. What is a wood-warbler? The molecular characterization of a monophyletic Parulidae. Auk 119, 695–714.

Lovette, I.J., Bermingham, E., Rohwer, S., Wood, C., 1999. Mitochondrial RFLP and sequence variation among closely related avian species and the genetic characterization of hybrid *Dendroica* warblers. Mol. Ecol. 8, 1431–1441.

Lowery, G.H., Monroe Jr, B.L., 1968. Family Parulidae. In: Paynter, R.A. (Ed.), Checklist of Birds of the World, vol. 14. Mus. Comp. Zool, Cambridge, MA, pp. 5–93.

Meyer de Schauensee, R., 1966. The Species of Birds of South America. Livingston, Wynnewood, PA.

Miller, A.H., 1952. Two new races of birds from the upper Magdalena Valley of Columbia. Proc. Biol. Soc. Washington 65, 13–20.

Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49, 718–726.

Olson, S.L., 1975. Geographic variation and other notes on *Basileuterus leucoblepharus* (Parulidae). Bull. Br. Ornithol. Club 95, 101–104.

Parsons, T.J., Olson, S.L., Braun, M.J., 1993. Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. Science 260, 1643–1646.

Patton, J.L., da Silva, M.N.F., 1997. Rivers, refuges, and ridges: the geography of speciation of Amazonian mammals. In: Berlocher, S., Howard, D. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, Oxford, pp. 202–213.

Prance, G.T. (Ed.), 1982. Biological Diversification in the Tropics. Columbia University Press, New York.

Ridgely, R.S., Tudor, G., 1989. The Birds of South America, Vol. One. University of Texas Press, Austin, TX.

Ridgely, R.S., Greenfield, P.J., 2001. The Birds of Ecuador, Volume One: Status, Distribution, and Taxonomy. CU Press, Ithaca, NY.

Ridgway, R., 1902. The birds of North and Middle America, part II. Bull. US Nat. Mus. No. 50.

Robbins, M.B., Parker III, T.A., 1997. What is the closest living relative of Catharopeza (Parulinae)? Ornithol. Monogr. 48, 595–599.

Robinson, W.D., Brawn, J.D., Robinson, S.K., 2000. Forest bird community structure in central Panama: influence of spatial scale and biogeography. Ecol. Monogr. 70, 209–235.

Roy, M.S., Torres, M.J.C., Hertel, F., 1999. Molecular phylogeny and evolutionary history of the tit-tyrants (Aves: Tyrannidae). Mol. Phylogenet. Evol. 11, 67–76.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, third ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Santiago, R.R., 2000. Biogeographic area relationships of lowland Neotropical rainforest based on raw distributions of vertebrate groups. Biol. J. Linn. Soc. 71, 379–402.

Sibley, C.G., Monroe, B.L., 1990. Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven, CT.

Sperling, F.A.H., 1993. Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. Heredity 71, 227–233.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Tegelström, H., Gelter, H.P., 1990. Haldane's rule and sex biassed gene flow between two hybridizing flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). Evolution 44, 2012–2021.

Wetmore, A., Pasquier, R.F., Olson, S.L., 1984. The Birds of the Republic of Panamá, Part 1V. Smithsonian Institution Press, Washington, DC.

Zamudio, K.R., Greene, H.W., 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. Biol. J. Linn. Soc. 62, 421–442.

Zimmer, J.T., 1949. Studies of Peruvian Birds, No. 54: The Families Catamblyrhynchidae and Parulidae. Am. Mus. Novi. No. 1428.

Zink, R.M., Blackwell-Rago, R.C., Ronquist, F., 2000. The shifting roles of dispersal and vicariance in biogeography. Proc. R. Soc. Lond. B 267, 497–503.