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Mitochondrial Perspective on the Phylogenetic Relationships of the *Parula* Wood-warblers

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Studies of intraspecific genetic variation in Neotropical resident birds have frequently revealed a high degree of phylogeographic differentiation (e.g. Capparella 1988, Hackett and Rosenberg 1990, Peterson et al. 1992, Bates and Zink 1994, Hackett 1996). That trend suggests tropical bird species are particularly likely to be subdivided geographically and that their constituent lineages tend to be evolutionarily old. Only a few counterexamples have been reported in which molecular surveys of Neotropical avian species did not reveal phylogeographic structure. Brawn et al. (1996) found no differences in mtDNA RFLP haplotypes between populations of three passerine species on the Perlas Islands in the Bay of Panama and conspecific populations on the nearby mainland. However, the geographic distances (<100 km) and putative period of genetic isolation (<10,000 years) in that system were both short. Gutierrez (1994) found similarly low mtDNA divergence in Oilbirds (*Steatornis caripensis*) from colonies 400 km apart in northern Venezuela. On a broader geographic scale, Brumfield and Capparella (1996) observed considerable protein divergence between Central and South American House Wrens (*Troglodytes aedon*), but found little differentiation among locations along a 7,000 km transect from Panama to Tierra del Fuego.

Here we explore the magnitude of mtDNA differentiation among geographically distant populations of the Tropical Parula (*Parula pitiayumi*), and we use mtDNA sequences to explore the relationship of *P. pitiayumi* to the three other species currently placed in *Parula* (AOU 1998). *Parula pitiayumi* has a breeding distribution that extends from Northern Mexico south through much of South America and has been divided into 14 subspecies (Lowery and Monroe 1968). Based on its broad geographic distribution and high phenotypic diversity, our *a priori* expectation was that *P. pitiayumi* would display evidence of a high degree of phylogeographic variation in mtDNA. Instead, we found surprisingly modest mitochondrial variation both within *P. pitiayumi* and between that taxon and its congener *P. americana*. As described below, our mtDNA-based phylogenetic reconstructions suggest that *P. pitiayumi* and *P. americana* are conspecific, and reconstructions that included the other two *Parula* species, as well as representatives of several other wood-warbler genera, suggest that the four species currently placed in the genus *Parula* (AOU 1998) do not form a monophyletic group.

Methods.—Accession numbers, sources, and tissue collecting localities are given in Table 1. Our study included 15 *P. pitiayumi* individuals and two samples each of the other three currently recognized *Parula* species (*P. americana, P. gutteralis,* and *P. superciliosa*). Multiple representatives of two additional parulid genera (*Vermivora ruficapilla, V. peregrina, Dendroica adelaidae, D. tigrina*) thought to be closely allied to *Parula* (see AOU 1998:533) and a *Coereba flaveola* sample were also included in some analyses. DNA extraction, amplification, and sequencing procedures followed standard laboratory protocols described elsewhere (Lovette et al. 1998, 1999). We obtained the complete sequence (842 nucleotides) of the overlapping mitochondrial ATPase 8 and ATPase 6 genes from all individuals. From one or two individuals per species, we also obtained an additional 2,797 nucleotides of mtDNA sequence representing the complete cytochrome *b* and ND2 genes and 613 nucleotides of the cytochrome oxidase I gene. Cytochrome *b* sequences from *V. peregrina* were not included in analyses owing to the probable co-amplification of a nuclear-encoded pseudogene (Numt; I. Lovette unpubl. data) All sequences have been archived in GenBank (AFO18097, 18207, 256468–256519).

We used PAUP* 4.0b2 (Swofford 1999) to estimate genetic distances among individuals and to generate phylogenetic hypotheses. In analyses that included distantly related taxa, distances were estimated using the Hasegawa-Kishino-Yamura (HKY) metric (Hasagawa et al. 1985) with the transition : transversion ratio set to 6 and the gamma parameter set to

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TABLE 1. Collecting localities of specimens included in phylogenetic analyses. Tissue accession numbers and sources given in parentheses. Acronyms refer to the following institutions: STRI (Smithsonian Tropical Research Institute); LSUMNS (Louisiana State University Museum of Natural Science, Baton Rouge); ANSP (Academy of Natural Sciences, Philadelphia); FMNH (Field Museum of Natural History, Chicago); BMNH (Burke Museum of Natural History, University of Washington, Seattle).

Taxon	Collecting locality and voucher or tissue accession number(s)
Parula pitiayumi	Argentina: Tucumán-San Miguel de Tucumán (BMNH 54442, GAV649, JAG1752)
	Bolivia: La Paz Dept., Cerro Asunta Pata. (LSUMNS 22750)
	Bolivia: Santa Cruz Dept., Cordellera. (LSUMNS 18914, 19104)
	Bolivia: Santa Cruz Dept., Parque Nacional Noel Keonpff Mercado. (LSUMNS 18431, 18571)
	Costa Rica: Heredia Prov., Virgin del Socorro. (LSUMNS 16034)
	Panama: Darien Prov., Cana. (LSUMNS 2150)
	Panama: Veraguas Prov., Azuero Peninsula. (ANSP 5762, 7223)
	Trinidad: Chacachacare Island. (STRI CCPPI1, CCPPI2)
	USA: Louisiana, Cameron Parish. (LSUMNS 105302)
Parula americana	Jamaica: St. Elizabeth Parish. (STRI JAPAM1, JAPAM2)
Parula gutteralis	Panama: Chiriqui Prov., District Boquete. (LSUMNS 26458, 26459)
Parula superciliosa	Mexico: Guerrero St., Sierra de Atoyac. (FMNH 6142)
	Mexico: Michoacan St., Cerro de Tancitaro. (FMNH 5730)
Vermivora peregrina	Honduras: La Ceiba. (STRI HAVPE62)
Vermivora ruficapilla	USA: Washington, Yakima Co. (BMNH CSW5040)
Dendroica adelaidae	Barbuda: Martello Tower (STRI BUDAD1)
Dendroica tigrina	Jamaica: St. Elizabeth Parish (STRI JADTI1)
Coereba flaveola	Bahamas: Abaco. (STRI ABCFA2)

0.12 with eight rate categories (see Lovette and Bermingham 1999). In ATPase-based comparisons of our focal taxa *P. pitiayumi* and *P. americana*, pairwise divergences among haplotypes were very low and we estimated distances based on the uncorrected % transition + transversion divergence across all sites. We employed two phylogenetic methods—maximum-likelihood (ML) and maximum-parsimony (MP)—to reconstruct phylogenetic relationships among mtDNA haplotypes using Paup*. ML analyses were conducted using the quartet puzzling search algorithm of Strimmer and von Haeseler (1996), with parameters set as described above. Exhaustive MP searches on the subset of taxa from which we obtained long mtDNA sequences were run using only transversion substitutions owing to the rapid saturation of mitochondrial transitions at large genetic distances. We weighted all changes equally in our MP heuristic searches of trees best representing the relationships of the *P. pitiayumi* and *P. americana* ATPase haplotypes. In both MP analyses, bootstrap values were based on 1,000 heuristic replications.

Results and discussion.—Phylogenetic reconstructions based upon long mitochondrial sequences indicated that the four species currently placed in *Parula* constitute two pairs of closely related taxa, *P. gutteralis/P. superciliosa* and *P. pitiayumi/P. americana*. MtDNA sequence divergence between these two species pairs was high (9.4–10.0% HKY divergence). Rooted hypotheses of phylogenetic relationship (Fig. 1) that included representative *Vermivora* and *Dendroica* species suggested that those two *Parula* clades are not each others' closest relatives, with *gutteralis* and *superciliosa* being allied to *Vermivora* and *pitiayumi*, and *americana* allied to *Dendroica*. We tested that inference by comparing observed trees to trees identified in searches where the four *Parula* species were constrained to be monophyletic. In transversion parsimony searches, the observed tree (249 steps) was considerably shorter than the shortest constraint tree (276 steps). Similarly, a likelihood ratio test (Kishino and Hasegawa 1989) based on those alternative topologies demonstrated that the observed HKY ML tree (−Ln 10,597) was a highly significant improvement over the constraint tree (−Ln 10,705; $T = 5.49$; $P < 0.0001$). Those results indicate that the four currently recognized *Parula* species do not form a monophyletic group, at least in terms of their mitochondrial DNA gene tree. That pattern is consistent with previous suggestions that all or some members of the genus should be merged into the closely allied genera *Vermivora* or *Dendroica* (Eisenmann 1955, AOU 1998). On the basis of the present evidence, *gutteralis* and *superciliosa* appear to have affinities with *Vermivora*, and *pitiayumi* and *americana* with *Dendroica*. Evidence from additional, unlinked molecular loci are required to test that hypothesis, however, as hybrids between *P. americana* and several *Dendroica* species have been documented (Haller 1940, Cochrum 1952, Graves 1993) and the mtDNA gene tree (Fig. 1) could be incongruent with the corresponding organismal tree owing to past mtDNA introgression.

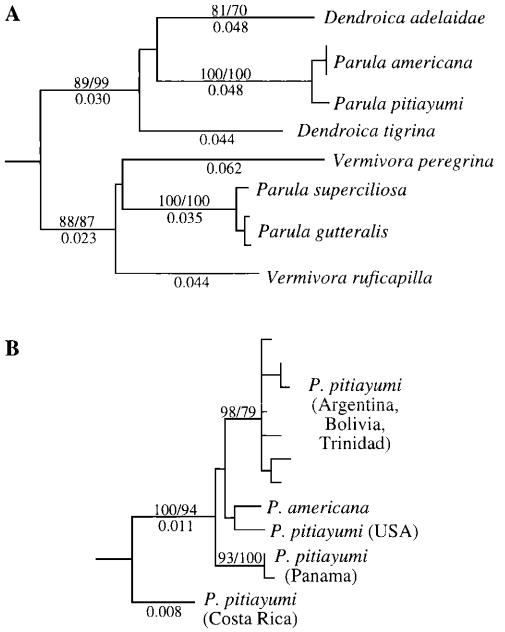

FIG. 1. (A) Maximum-likelihood tree depicting the phylogenetic relationships between the four *Parula* species and several representative *Dendroica* and *Vermivora* taxa. Reconstruction is based upon 3,639 nucleotides of protein-coding mtDNA sequence per individual and is rooted to sequences from *Coereba flaveola* (not shown). A transversion-based parsimony search identified an identical topology. (B) Maximum-likelihood tree depicting the phylogenetic relationships between *P. pitiayumi* and *P. americana* ATPase sequence haplotypes. In both trees, numbers below branches indicate HKY branch lengths >0.005; numbers above branches indicate ML reliability scores (left) and MP bootstrap proportions (right).

The *P. pitiayumi* and *P. americana* mitochondrial haplotypes were very similar and we included both taxa in our survey of geographic ATPase variation. The ATPase haplotype shared by the two *P. americana* individuals differed from the most similar *P. pitiayumi* haplotype by only six nucleotide substitutions (0.7% divergence), and that *P. americana* haplotype fell within a cluster of *P. pitiayumi* haplotypes in all reconstructions (Fig. 1). The mitochondrial evidence bolsters previous suggestions that *P. pitiayumi* and *P. americana* are conspecific (e.g. Hellmayr 1935, Paynter 1957, Lowery and Monroe 1968, Mayr and Short 1970, AOU 1983) by demonstrating that those taxa are not very different genetically and are not reciprocally monophyletic (Fig. 1B).

In situations such as this, where mitochondrial sequences show unexpectedly low divergence, it is particularly important to explore the possibility that the sequences represent slowly evolving Numts rather than the target mitochondrial sequences (e.g. Sorenson and Quinn 1998). Three lines of evidence suggest that the ATPase sequences reported here are mitochondrial. First, the pattern and magnitude of ATPase divergence was mirrored by the other three mitochondrial gene regions we sequenced from a subset of samples; those four gene regions together span more than 10 kb of the ~17 kb mitochondrial DNA genome and thus would represent an extraordinarily long nuclear translocation. Second, we found no unexpected nonsense or stop codons within coding regions, and levels of ATPase amino acid conservation between the *Parula* sequences were similar to those we have documented in other studies of closely related wood-warbler taxa (Lovette et al. 1998, 1999; Lovette and Bermingham 1999). Third, the sequences appear typically mitochondrial with a highly biased transition:transversion ratio (8.2) and a striking antiguanine bias at third positions (4.6%). We caution, however, that the second and third lines of evidence are considerably weaker than the first, because of the fact that translocated sequences maintain characteristic mitochondrial attributes for some time owing to the slower rate of molecular evolution in the nucleus (I. Lovette and E. Bermingham pers. obs.).

In our geographic survey of ATPase variation in *P. pitiayumi/americana*, we identified 12 unique haplotypes in our sample of 16 individuals. Only 36 nucleotide sites varied among those haplotypes and the 2 most divergent individuals differed by 22 nucleotide substitutions (2.6% uncorrected nucleotide divergence). We found little genetic divergence among haplotypes drawn from some geographically distant localities, particularly among South American samples (Fig. 1B). For example, haplotypes from Trinidad and Argentina differed at only two to four nucleotide sites (0.2 to 0.5% divergence) and we observed individuals with identical haplotypes in Bolivian and Argentinean collecting localities (Table 1) separated by 800 km.

In contrast to the near absence of mtDNA variation in South American *P. pitiayumi*, haplotypes from North and Central American locations showed modest differentiation. The largest differences (2.0 to 2.6%) among *P. pitiayumi/americana* haplotypes involved the single individual from Costa Rica, which was basal in the phylogenetic reconstructions (Fig. 1B). PCR or sequencing artifacts were unlikely to have produced the divergence seen in that individual, because we obtained an identical ATPase sequence from two separate rounds of extraction, amplification, and sequencing. Three individuals from eastern and western Panama had almost identical haplotypes that differed by 1.1–2.3% from those at other localities. Finally, the two samples of *P. americana* had identical haplotypes and formed a weakly differentiated clade along with an individual collected in Louisiana and identified by plumage traits

(S. Cardiff pers. comm.) as a vagrant *P. pitiayumi nigrilora*, the subspecies normally found in northeastern Mexico and southern Texas.

Although our geographic sampling of *P. pitiayumi* was coarse and a number of disjunct Central American and Mexican populations that could represent distinct evolutionary units were not sampled, the magnitude of mitochondrial variation among our widely spaced sampling locations was remarkably low. Considered together, *P. pitiayumi* and *P. americana* have a breeding range that extends from the boreal forests of northeastern Canada south through much of tropical Central and South America. The latitudinal breadth of that distribution, the phenotypic variation within *P. pitiayumi*, and the differences in migratory (AOU 1998) and song (Regelski and Moldenhauer 1996) behavior between some geographic populations suggested that those taxa would harbor considerable phylogeographic diversity, as has been found for other paruline warblers with both temperate- and tropical-zone breeding populations (Klein and Brown 1994, Milá et al. 2000). Nonetheless, the low magnitude of mitochondrial variation in *P. pitiayumi* and *P. americana* demonstrates that those taxa have experienced a recent genetic connection across vast geographic distances, either via expansion of an ancestral population or via gene flow among established populations. That pattern of low genetic variation contrasts markedly with the highly structured mitochondrial differentiation we have noted within many Neotropical *Basileuterus* warbler species (Lovette and Bermingham unpubl. data) and the large intraspecific genetic diversity documented within many other Neotropical resident birds. Although long-distance gene flow could result from migratory *P. americana* females remaining to breed with *P. pitiayumi* males south of *P. americana*'s normal breeding range, the close similarity of the Argentina, Bolivia, and Trinidad *P. pitiayumi* haplotypes and the lack of records of *P. americana* from continental South America (Ridgely and Tudor 1989: 494) argue against that scenario. An alternative and more likely possibility is that *P. pitiayumi* and *P. americana* have undergone an evolutionarily recent range expansion from a common ancestral population and that much of the phenotypic diversity that characterizes the present-day geographic populations we sampled is of relatively recent origin.

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Genetic Monogamy in Carolina Wrens (*Thryothorus ludovicianus*)

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Molecular comparisons have shown that socially monogamous passerines often have mixed reproductive strategies (Birkhead and Møller 1992, 1996). Pairs often cooperate in raising a brood, but each sex may pursue additional extrapair matings (e.g. Westneat 1987, Morton et al. 1990, Kempenaers et al. 1992). Further, females of some species lay eggs in nests of conspecifics (i.e. intraspecific brood parasitism, ISBP; reviewed in Hughes 1998).

Although considerable intra- and interspecific variation has been found in rates of extrapair paternity (EPP), causes for that variation remain unclear and additional data are warranted (Petrie and Kempenaers 1998). Further, few studies have been conducted on temperate-zone species that defend a territory and maintain a pair bond year round. In this study, we use multilocus DNA fingerprinting to examine paternity and intraspecific brood parasitism in Carolina Wrens (*Thryothorus ludovicianus*), a socially monogamous species that maintains a year-round pair bond and territory (Haggerty and Morton 1995). In addition, we report on breeding synchrony in Carolina Wrens because it is an ecological factor that may be related to paternity (Møller and Ninni 1998).

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