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EVOLUTIONARY DIFFERENTIATION IN THREE ENDEMIC WEST INDIAN WARBLERS

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ABSTRACT.—We explored the evolution of geographic distributions in archipelagos by comparing mitochondrial DNA (mtDNA) sequences and morphometric characters within and among conspecific populations of Adelaide's Warbler (*Dendroica adelaidae*), Plumbeous Warbler (*D. plumbea*), and Olive-capped Warbler (*D. pityophila*). Phylogenetic reconstructions were based upon 1,455 nucleotides of protein-coding mtDNA sequence from 53 individual warblers; morphological analyses employed three external measurements from a larger number of museum specimens. Of the three taxa studied, Adelaide's Warbler occupied the broadest and most fragmented geographical distribution and exhibited the greatest interpopulation differentiation in both mtDNA and morphology. Phylogenetic analyses demonstrated that the three Adelaide's Warbler populations are each reciprocally monophyletic with the Puerto Rican lineage basal to sister clades on Barbuda and St. Lucia. Genetic distances among these populations were comparable with those between some continental species. In contrast to the mtDNA pattern, the Puerto Rican and Barbudan Adelaide's Warbler populations were most similar in morphometry. We observed considerably less mtDNA and morphometric differentiation among populations of the two species with more restricted and less fragmented distributions, the Plumbeous Warbler of Dominica and Guadeloupe and the Olive-capped Warbler of the Bahamas and Cuba. High levels of molecular and morphological differentiation among the geographically disjunct Adelaide's Warbler populations and low differentiation in the two species with less fragmented ranges suggest that range disjunctions indicate the long-term evolutionary independence of geographically isolated island populations. *Received 21 August 1997, accepted 18 February 1998.*

INTERPRETATIONS OF BIOGEOGRAPHIC PATTERNS in avian taxa traditionally have relied on estimates of relationship based upon morphological characters such as size and plumage coloration. Morphological divergence among lineages is summarized in the hierarchical taxonomic classification of a group, and such classifications have often been accepted as indices of phylogenetic relationships (e.g. Ricklefs and Cox 1972). Molecular phylogenies now provide an alternative source of information on biogeographic patterns and the evolutionary processes that have molded them (Avise 1994). Species that occupy archipelagos are particularly amenable to biogeographic and phylogenetic investigations. The general distribution of a species and the boundaries of particular populations are readily defined by a taxon's presence or absence on a given island. Water barriers or unoccupied islands situated between occupied islands might retard gene flow between populations and thereby enhance their genetic subdivision. Finally, archipelagos offer the opportunity to make comparisons among taxa with different evolutionary histories superimposed upon a relatively simple common geography.

To explore evolutionary and geographic patterns in one archipelago, we investigated intraspecific variation in mitochondrial DNA (mtDNA) sequences and external morphometric characters in three West Indian *Dendroica* species, Adelaide's Warbler (*D. adelaidae*), Plumbeous Warbler (*D. plumbea*), and Olive-capped Warbler (*D. pityophila*). Adelaide's Warbler has both the broadest and most fragmented distribution, with populations on Puerto Rico, Bar-

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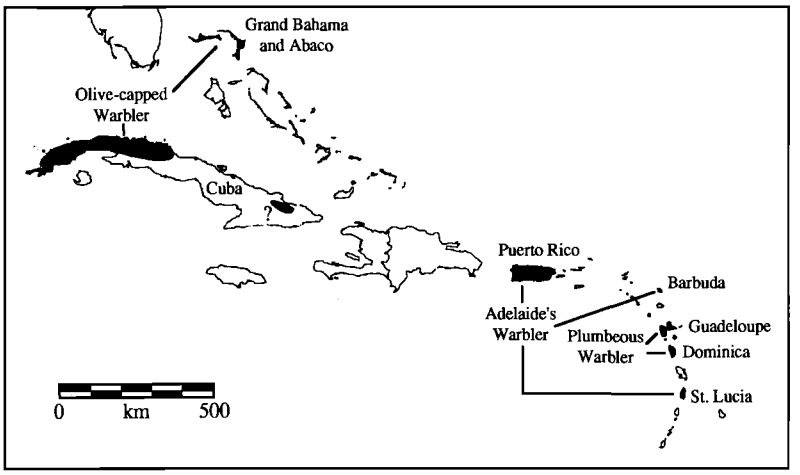

FIG. 1. Distribution of Adelaide's Warbler, Plumbeous Warbler, and Olive-capped Warbler in the eastern Caribbean. Note large disjunctions between islands occupied by Adelaide's Warbler relative to the continuous (Plumbeous Warbler) or nearly continuous (Olive-capped Warbler) distributions of the other two species.

buda, and St. Lucia (Fig. 1). The Plumbeous Warbler occupies a restricted and continuous distribution on the adjacent islands of Guadeloupe and Dominica in the Lesser Antilles (Fig. 1). The Olive-capped Warbler, found on the two northernmost islands in the Bahamas and on Cuba, is intermediate in both geographic breadth and range fragmentation (Fig. 1).

Here, we describe the geographic structure of variation in morphometry and mtDNA within each species and determine whether morphometric differentiation is proportional to genetic differentiation. We then test whether the phylogeographic data are consistent with the hypothesis that gaps in the distributions of Lesser Antillean bird species result from the extinction of geographically intermediate populations (Ricklefs and Cox 1972). Based on the assumption that both population extinctions and genetic divergence accumulate over evolutionary time, we predicted that the ancestral nodes of intraspecific phylogenetic trees should be oldest in the highly disjunct Adelaide's Warbler and youngest in the continuously distributed Plumbeous Warbler.

STUDY SPECIES AND METHODS

Adelaide's Warbler.—Adelaide's Warbler occurs on Puerto Rico (and nearby Vieques Island), Barbuda, and St. Lucia (Fig. 1). Each island population has been accorded specific status in the past (e.g. Ridgway 1902), but the three populations currently are considered to be subspecies (AOU 1983). Birds from Puerto Rico (*D. a. adelaidae*) and Barbuda (*D. a. subita*) are similar in plumage and size (Riley 1904, Hellmayr 1935), whereas those from St. Lucia (*D. a. delicata*) are about 10% larger than individuals from the two northern populations and have a diagnostically yellow supercilium and extensively yellow underparts (Ridgway 1882, 1902; Riley 1904, Curson et al. 1994). Bond (1930, 1956, 1965) also noted differences in habitat selection, foraging height, and song types among the three islands. Adelaide's Warbler is most abundant in lowland dry forest and scrub except on St. Lucia, where it ranges into humid montane forest (Bond 1956, Cruz and Delaney 1984, Raffaele 1989). Because there are no records of the species elsewhere in the Caribbean or on the mainland (Bond 1956), Adelaide's Warbler apparently is nonmigratory.

Plumbeous Warbler.—The Plumbeous Warbler is endemic to the Lesser Antilles, where it is known only from the adjacent islands of Dominica and Guadeloupe, and from Guadeloupe's satellite islands, Marie-Galante and Terre-de-Haut (Fig. 1). The species usually has been considered monomorphic (Ridgway 1902, Bond 1956, Peters 1968), but Hellmayr (1935) and Kepler and Parkes (1972) suggested that the Dominican and Guadeloupean populations each be accorded subspecific status (*D. p. plumbea* and *D. p. guadeloupensis*, respectively) based on slight differences in the coloration of the underparts. The Plumbeous Warbler is a common permanent resident of both dry and wet forests throughout its limited range.

Olive-capped Warbler.—The Olive-capped Warbler is restricted to the pine forests of the northernmost islands in the Bahamas (Abaco and Grand Bahama

Islands) and to similar habitat in eastern and possibly western Cuba (Fig. 1; Bond 1956, 1958). Ridgway (1902) noted slight plumage differences between the Bahamian and Cuban forms, which he separated into subspecies (*D. p. bahamensis* and *D. p. pityophila*, respectively), but later workers have not considered these differences substantial enough to warrant even subspecific distinction (Hellmayr 1935, Bond 1956, Peters 1968). The lack of extralimital records of Olive-capped Warblers (e.g. none from Florida, which lies 100 km west of Grand Bahama and 140 km north of Cuba) indicates that this species is sedentary.

mtDNA sample collection.—We obtained samples for genetic analyses from three Adelaide's Warblers on Puerto Rico (20 to 30 October 1993), five on Barbuda (6 to 9 May 1993), and eight on St. Lucia (18 to 26 July 1991); from 14 Plumbeous Warblers on Dominica (27 July to 3 August 1991) and 13 on Guadeloupe (21 to 27 April 1993); from two Olive-capped Warblers on Abaco, Bahamas (19 October 1993); and from one Arrow-headed Warbler (*D. pharetra*; used for outgroup rooting of Plumbeous Warbler haplotypes) on Jamaica (11 December 1995). Muscle biopsies and/or blood samples were collected nondestructively (Baker 1981) from mist-netted individuals. Muscle tissue was preserved in a DMSO/EDTA/NaCl solution, and blood was preserved in Queen's lysis buffer (Seutin et al. 1991, 1993). DNA extracts of these samples are available upon request from the authors. We obtained frozen muscle samples from four additional Puerto Rican Adelaide's Warblers and two North American Yellow-throated Warblers (*D. dominica*; used for outgroup rooting of Adelaide's Warbler haplotypes) from the Museum of Natural Science at Louisiana State University, and one muscle sample from a Cuban Olive-capped Warbler (collected near Pinas Del Rio in western Cuba) from the Academy of Natural Sciences of Philadelphia. All specimens were collected and transported under appropriate permits.

mtDNA laboratory procedures.—Total cellular DNA was extracted from each sample following the protocols of Seutin et al. (1991, 1993). We used the polymerase chain reaction (PCR) to amplify two regions of the mitochondrial genome from all individuals. The primer pair CO2GQL and CO3HMH (Seutin and Bermingham unpubl. primer sequences; all primer sequences available upon request from E. Bermingham) was used to amplify a 1,074 base-pair region that spanned the full $tRNA^{Lys}$, ATPase 8, and ATPase 6 genes. A 681 base-pair portion of the cytochrome oxidase I (COI) gene was similarly amplified using primers COIa and COIf (Kessing et al. 1989). PCRs for both ATPase and COI amplifications were conducted for 25 cycles at an annealing temperature of 54°C.

We cleaned all amplification products electrophoretically on low-melting-point agarose gels and purified them using the Geneclean procedure. We then conducted Dyedeoxy terminator cycle sequencing reactions (Applied Biosystems Division of Perkin Elmer, Inc.) following the manufacturer's protocol. We sequenced the light strand of the ATPase region with the primers CO2GQL, A6PWL, and A6TPL (Seutin and Bermingham unpubl. primer sequences). The COI region was sequenced using the two amplification primers. The cycle sequencing reactions were then electrophoresed in Applied Biosystems model 373A or model 377 automated DNA sequencers. On average, 42% of the ATPase coding region from each individual was confirmed by overlapping sequences generated from two light strand primers. In the COI region, overlap between the light-strand sequences from primer COIf and the heavy-strand sequences from primer COIa ranged between 80 and 100%, and we found no nucleotide differences between overlapping complementary sequences.

Genetic data analysis.—Analyses were conducted upon the concatenated ATPase and COI sequences because mitochondrial genes are fully linked and thus constitute a single phylogenetic marker, and because the combinability test of Farris et al. (1995), as implemented in test version 4.0d56 of David L. Swofford's Paup* package, identified no significant differences between the trees generated using the ATPase 8, ATPase 6, and COI partitions of the combined data ($P > 0.65$ for all conspecific data sets). Each concatenated sequence included the entire 842 nucleotide coding region of the overlapping ATPase 6 and ATPase 8 genes and 613 nucleotides of the COI gene corresponding to nucleotides 7342 to 7954 in the chicken mitochondrial genome (GenBank accession number X52392; Desjardins and Morais 1990). We refer to each unique concatenated sequence as a mtDNA "haplotype."

Sequences were imported into the program Sequencer 3.2 (B. Kessing pers. comm.) to generate descriptive statistics about nucleotide variation. We then used PAUP* 4.0d56 to estimate genetic distances between individuals using the LogDeterminant (LogDet; Steel 1994) distance metric. Swofford et al. (1996) discuss the advantages of this metric; in the present study, all intraspecific LogDet distances were almost identical to the corresponding distances based on uncorrected percent nucleotide difference or upon the Kimura two-parameter substitution model (Kimura 1980). In order to compare intrapopulation mitochondrial diversity across populations, we calculated the haplotype-diversity index h (Nei 1987: equation 8.5) and nucleotide-diversity index π (Nei 1987: equation 10.5) for all populations where $n > 4$. The h metric estimates the probability that two individuals sampled at random from a population will have different haplotypes based on the observed haplotype frequencies, whereas π incorporates the distribution of pairwise sequence differences into an estimate of whether randomly selected individuals from a population will differ at a nucleotide site.

We used three phylogenetic methods—maximum-likelihood (ML), neighbor-joining (NJ), and maximum-parsimony (MP)—to reconstruct the relationships between the three Adelaide's Warbler populations. ML analyses were conducted using the program PUZZLE 3.1 (Strimmer and von Haeseler 1997), which employs the quartet puzzling search algorithm of Strimmer and von Haeseler (1996) to reconstruct phylogenetic trees. These ML searches were conducted for 10,000 puzzling steps using the Hasagawa-Kishino-Yano substitution model (Hasagawa et al. 1985), and with transition:transversion ratios, nucleotide frequencies, and gamma rate heterogeneity parameters determined from the sequence data using PUZZLE's "exact" function. NJ and MP analyses were conducted using PAUP* 4.0d56. NJ analyses were based on the LogDet distance matrix, and 1,000 bootstrap replicates were performed on the NJ tree. MP analyses were conducted using the branch-and-bound search option with all characters weighted equally, and with transitions assigned a weight of 1 and transversions a weight of 15 to reflect the empirically determined bias (see Results); 1,000 bootstrap replications were performed under both weighting schemes. Alternative topologies among Adelaide's Warbler populations were compared using the Kishino-Hasegawa test (Kishino and Hasegawa 1989) as implemented in PAUP* 4.0d56.

Because the three warbler species considered here are endemic to the West Indies, we assume that populations of each species are more closely related to each other than to any non-conspecific population; molecular systematic analyses that include additional *Dendroica* support this assumption (Lovette and Bermingham unpubl. data). In all phylogenetic analyses of Adelaide's Warbler, two Yellow-throated Warbler sequences were specified as the outgroup taxon; this species was chosen because plumage and morphology place it in the same "superspecies" complex as Adelaide's Warbler (Bond 1956, Mengel 1964, Mayr and Short 1970). The use of the other members of this complex (*D. pityophila* and *D. graciae*) or other *Dendroica* warblers as outgroups did not change the phylogenetic topology among Adelaide's Warbler populations.

We investigated the relationships of Plumbeous Warbler haplotypes through ML, NJ, and MP analyses as described above, except that trees were rooted to sequences from the Arrow-headed Warbler owing to the proposed superspecies affinity between these taxa (Bond 1956, Kepler and Parkes 1972). We did not conduct phylogenetic reconstructions for the Olive-capped Warbler because it was represented by only three individuals with very similar mtDNA haplotypes.

Morphometric measurements and analyses.—All morphological measurements were made by Lovette on museum specimens from the National Museum of Natural History, Washington, D.C.; American Museum of Natural History, New York; Museum of Natural Science at Louisiana State University, Baton Rouge; and the Academy of Natural Sciences of Philadelphia. The lengths of the wing (chord of unflattened wing), bill (distal tip of the maxilla to the proximal edge of the exposed culmen), and tarsus were measured (±0.1 mm) using dial calipers. The island of origin and, when indicated, the sex of each specimen were recorded from the museum label. In some cases, Adelaide's Warbler skins with no indicated gender were sexed using plumage criteria (Ridgway 1902, Curson et al. 1994). Because sample sizes of female warbler specimens from some populations were small, we were unable to test whether these populations exhibit sexual dimorphism in wing, bill, or tarsus length. Therefore, analyses were restricted to male specimens.

All measurements were log-transformed prior to analysis. The general structure of mensural variation among populations of each species was examined via ANOVA where island of origin was specified as the independent variable. The relative magnitude of morphometric divergence among populations of the three species was assessed by comparing: (1) the Euclidian distance between island centroids in the log-transformed measurement space; and (2) the Mahalanobis distance between populations, a measure that represents the squared distance in the canonical discriminant space normalized by the pooled within-island variance along the discriminant axis. The SAS statistical analysis package (SAS Institute 1987) was used to conduct both the ANOVA (PROC GLM) and the multivariate (PROC DISCRIM and CANDISC) analyses.

Results

We obtained the entire 842-bp coding sequence of the overlapping ATPase 6 and ATPase 8 genes and 611 bp of COI coding sequence from 20 Adelaide's Warblers, 27 Plumbeous Warblers, 3 Olive-capped Warblers, 2 Yellow-throated Warblers, and 1 Arrow-headed Warbler (GenBank accession numbers U91961, AF018094 to AF018145 inclusive, and AF018200 to AF018252 inclusive; see Appendix for sequence alignments showing all nucleotide sites that varied among intraspecific haplotypes). We found no insertions or deletions in either gene region; hence, sequence alignments were unambiguous. Base frequencies were biased in both regions, especially at third-codon positions, as is typical of the avian mitochondrial genome (e.g. Desjardins and Morais 1990, Zink and Blackwell 1996). Of the 118 nucleotide sites that varied among con-

specific haplotypes, 104 involved synonymous substitutions; we found five nonsynonymous changes among ATPase 8 sequences, six among ATPase 6 sequences, and three among COI sequences. Empirical transition:transversion ratios among all conspecific Adelaide's Warbler and Plumbeous Warbler haplotypes were 15:1 and 17:1, respectively; too few nucleotide differences were found among Olive-capped Warbler haplotypes (see below) to allow a meaningful transition bias calculation for this species.

Recent discoveries of nuclear copies of avian mitochondrial genes ("pseudogenes;" e.g. Arctander 1995) have highlighted the importance of assessing the homology of the mtDNA PCR products used for phylogenetic analyses. In the present study, the presence of a single amplification product in our PCR reactions, the absence of indels and stop codons within the ATPase and COI coding regions, the high proportion of silent substitutions, the similarity to *Dendroica* sequences obtained from highly purified mtDNA samples (Lovette unpubl. data), and the complete congruency of the phylogenetic reconstructions from the two mtDNA gene regions separated by 981 nucleotides provided evidence that the sequences we obtained were mitochondrial in origin.

mtDNA diversity within West Indian warbler populations.—We found 11 haplotypes among the 20 Adelaide's Warblers from Barbuda, St. Lucia, and Puerto Rico. Two haplotypes differing by a single synonymous transition were present among the five Barbudan individuals; thus, the maximum pairwise genetic difference within this population was only 0.1%. We found slightly more variation within the St. Lucian population, where four haplotypes were distributed among the eight individuals examined. Four nucleotide sites varied among these St. Lucian haplotypes, yielding a maximum within-population divergence of 0.3%. The seven birds from Puerto Rico had five haplotypes that differed at a total of 11 nucleotide sites and had a maximum pairwise divergence of 0.6%. Although the number of samples available to us was small, our survey suggests that mtDNA diversity is proportional to island area, with the Puerto Rican (9,104 km^2) population showing the highest level of diversity (h = 0.86, π = 0.0015) relative to those from St. Lucia (h = 0.75, π = 0.0005; 620 km^2) and Barbuda (h = 0.40, π = 0.0001; 160 km^2).

Plumbeous Warbler populations on Dominica and Guadeloupe also had high levels of haplotype diversity but exhibited only moderate levels of nucleotide differentiation. We distinguished nine mtDNA haplotypes (h = 0.88) in the sample of 14 birds from Dominica and 10 haplotypes (h = 0.96) in the 13 birds from Guadeloupe. Because all haplotypes within each population were closely related, these high haplotype diversities were not paralleled by high levels of nucleotide diversity: the nine Dominican haplotypes differed at a maximum of seven nucleotide sites (π = 0.0012; maximum divergence 0.7%) and the 10 Guadeloupean haplotypes differed at a maximum of nine nucleotide sites (π = 0.0018; maximum divergence 0.8%).

We can say little about mtDNA diversity in Olive-capped Warblers based on our sample of only three individuals. The two Olive-capped Warblers from Abaco had identical haplotypes.

Haplotype diversities in the Puerto Rican and St. Lucian Adelaide's Warbler populations and in the two Plumbeous Warbler populations are among the highest reported for birds, exceeding diversities reported among continental taxa that must support much larger effective population sizes (e.g. Seutin et al. 1993, 1995; Bermingham et al. 1996; Zink 1996). We caution, however, that direct comparisons of h between studies employing different molecular techniques are problematic because h is dependent upon the sensitivity of the methodology used to assay haplotype diversity (Nei 1987). Nucleotide diversities are less influenced by this potential source of bias. Values of π observed on the larger islands (0.0012 to 0.0018) were somewhat lower than those observed in some continental populations (0.0017 to 0.0033; Ball et al. 1988, Zink 1991, Bermingham et al. 1992, Seutin et al. 1995) and in Bananaquits (*Coereba flaveola*) on larger West Indian islands (0.0019 to 0.0037; Seutin et al. 1994).

Genetic divergence and phylogenetic relationships among conspecific warbler populations.—Genetic distances among islands in Adelaide's Warbler were high relative to the modest variation within islands in this species. Pairwise divergences between Barbudan and St. Lucian haplotypes varied between 2.2 and 2.5%, and distances between Puerto Rican and Lesser Antillean haplotypes varied between 3.7 and 4.7%. ML, NJ, and MP reconstructions of the relationships be-

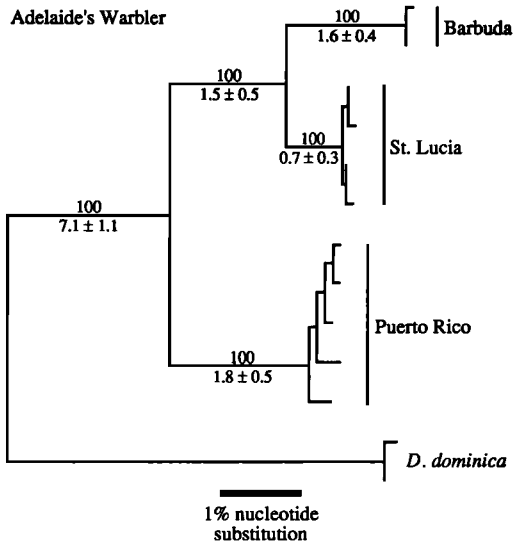

FIG. 2. Maximum-likelihood tree depicting intraspecific phylogenetic relationships in Adelaide's Warbler, based on a comparison of 1,455 nucleotides of mtDNA sequence. Numbers above branches indicate reliability values as calculated by the maximum-likelihood analysis program PUZZLE. Values below branches give branch lengths and their associated standard errors. Neighbor-joining and maximum-parsimony analyses identified an identical topology among Adelaide's Warbler populations, with 100% bootstrap support for all internal branches connecting the three haplotype groups. Scale bar shows 1% nucleotide substitution (2% sequence divergence).

tween the three Adelaide's Warbler populations identified almost identical trees that invariably supported the monophylly of each island population. All reconstructions placed the Puerto Rican population basal to a clade comprised of the Barbudan and St. Lucian populations (Fig. 2). Kishino-Hasegawa tests indicated that this topology was significantly better than alternative topologies in which the St. Lucian population (tree 14 steps longer; $t = 3.31$, $P < 0.001$) or the Barbudan population (tree 13 steps longer; $t = 2.99$, $P < 0.003$) was constrained to be basal. The same highly supported topology was obtained when the ATPase and COI regions were analyzed separately and under the two MP character-weighting schemes; topological differences among these reconstructions involved only the relationships of very similar haplotypes within single island populations. In the analyses of the combined ATPase and COI sequences, the internal branches connecting the three Adelaide's Warbler populations were invariably supported by reliability (ML) and bootstrap (NJ and MP) values of 100% (Fig. 2).

No haplotypes were shared by the Dominican and Guadeloupean Plumbeous Warbler populations, but interisland genetic divergence was modest: pairs of haplotypes from Dominica and Guadeloupe differed at 5 to 11 nucleotide sites (0.3 to 1.0% LogDet divergence). Although the overall magnitude of within- and among-population haplotype divergences was similar in this species, phylogenetic analyses suggested that the Dominican and Guadeloupean populations are monophyletic with respect to one another. When an Arrow-headed Warbler sequence was employed as an outgroup, ML, NJ, and MP analyses all placed the basal root in the internal branch separating the Dominican and Guadeloupean haplotypes (Fig. 3). Unrooted reconstructions (not shown) similarly split the Dominican and Guadeloupean haplotypes into monophyletic clades, and reliability scores (ML) and bootstrap analyses (NJ and MP) indicated high support for the internal branch separating the two island-specific clades (ML reliability = 98%; NJ bootstrap = 86%; unweighted MP bootstrap = 90%).

Divergence between the Abacan and Cuban Olive-capped Warblers was small: the Cuban haplotype differed from the Abacan haplotype by five nucleotide substitutions (0.4% LogDet difference). Without additional samples, we cannot determine whether this difference represents divergence between genetically isolated populations or diversity within a single panmictic population.

Morphometric variation.—Table 1 summarizes numbers of individuals measured and means and variances in wing length, exposed culmen length, and tarsus length. ANOVA revealed significant morphometric differences among males from different populations of each of the three warbler species: (1) the three Adelaide's Warbler populations differed in all three mensural characters, (2) the two Plumbeous Warbler populations differed only in tarsus length, and (3) the two Olive-capped Warbler populations differed in both wing length and tarsus length (Table 2).

Multivariate analyses similarly identified morphometric differences within each of the three warbler species (Table 3). In Adelaide's

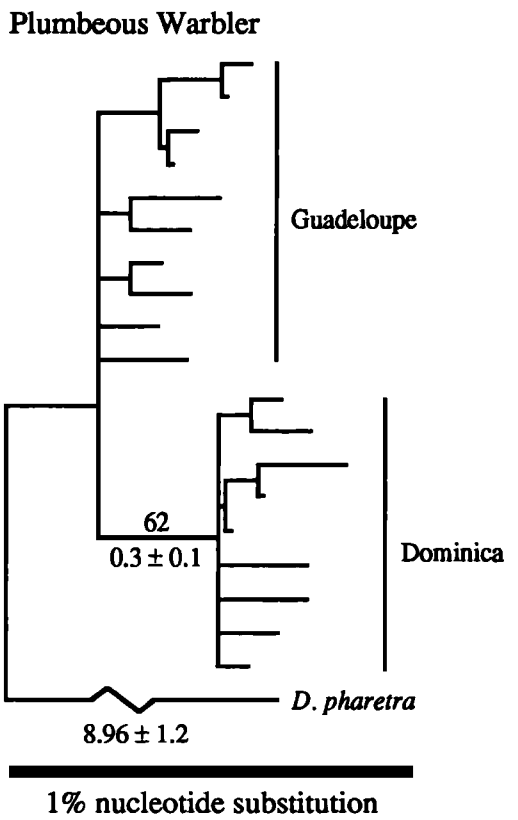

FIG. 3. Maximum-likelihood tree depicting phylogenetic relationships among Plumbeous Warbler haplotypes. Number above branch indicates reliability value; number below branch gives branch length and the associated standard error. Only one branch had a length >0.25% nucleotide substitution; values for branches <0.25% are not shown. Branch leading to outgroup taxon not drawn to scale. Unrooted maximum-likelihood, neighbor-joining, and maximum-parsimony analyses similarly separated all haplotypes into two island-specific clades. Scale bar shows 1% nucleotide substitution (2% sequence divergence).

Warbler, the Puerto Rican and Barbudan populations had overlapping distributions in the multivariate measurement space and were not significantly different; the St. Lucian population, however, had the greatest degree of morphological distinction among the intraspecific comparisons (Table 3). The overall magnitude of population differentiation was more modest in the Plumbeous Warbler and the Olive-capped Warbler, but nonetheless each island population was significantly distinct (Table 3). Considered across species, the general pattern of multivariate variation did not differ between comparisons normalized by the within-population variances and unstandardized comparisons (Table 3).

TABLE 1. Wing length, culmen length, and tarsus length of males in three species of West Indian warblers. Values are $\bar{x} \pm$ SD.

Island	*n*	Wing	Culmen	Tarsus
Adelaide's Warbler				
Puerto Rico	41	51.2 ± 1.4	9.6 ± 0.4	16.7 ± 0.6
Barbuda	19	51.6 ± 2.1	9.9 ± 0.6	17.2 ± 0.6
St. Lucia	27	57.2 ± 1.1	10.0 ± 0.4	17.9 ± 0.4
Plumbeous Warbler				
Dominica	10	65.1 ± 1.5	10.4 ± 0.6	20.3 ± 0.3
Guadeloupe	20	62.2 ± 2.4	10.5 ± 0.3	19.5 ± 0.7
Olive-capped Warbler				
Bahamas	12	58.7 ± 1.9	10.0 ± 0.4	16.8 ± 0.5
Cuba	30	59.4 ± 1.4	9.5 ± 0.7	16.0 ± 0.5

Comparison of molecular and morphometric differentation.—The pattern of interisland genetic differentiation across the three species was paralleled in part by the corresponding pattern of morphological variation. Overall, morphological diversity was highest in Adelaide's Warbler (but see below), the species with the highest interisland genetic divergence. Less interisland morphological variation was present in Plumbeous Warblers and Olive-capped Warblers, species whose populations were less genetically distinct. Nonetheless, we found significant differences among islands in morphology in each of the three species, suggesting either that

TABLE 2. Results of ANOVA of interisland variation (islands noted in Table 1) in wing length, culmen length, and tarsus length of males in three species of West Indian warblers.

Species	R^2	*F*	*P*
Adelaide's Warbler (df = 2 and 70)			
Wing length	0.81	146.9	***
Culmen length	0.17	7.1	*
Tarsus length	0.50	34.2	***
Plumbeous Warbler (df = 1 and 37)			
Wing length	0.03	1.0	ns
Culmen length	0.10	4.1	ns
Tarsus length	0.33	17.9	***
Olive-capped Warbler (df = 1 and 25)			
Wing length	0.35	13.5	**
Culmen length	0.05	1.4	ns
Tarsus length	0.43	19.0	**

ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 3. Comparisons of multivariate morphometric differences among populations of three species of West Indian warblers. Mahalanobis distance (D^2) is the squared distance in the canonical discriminant space normalized by the within-population variance. E^2 represents the squared Euclidean distance between population centroids in the log-transformed measurement space.

Species	Islands compared	df	D^2	F	P	E^2 (× 100)
Adelaide's Warbler	Puerto Rico/Barbuda	1, 59	0.73	2.4	0.078	0.016
Adelaide's Warbler	Puerto Rico/St. Lucia	1, 67	25.49	115.9	<0.0001	0.385
Adelaide's Warbler	Barbuda/St. Lucia	1, 45	18.86	53.2	<0.0001	0.268
Plumbeous Warbler	Dominica/Guadeloupe	1, 29	5.66	10.9	<0.0001	0.094
Olive-capped Warbler	Bahamas/Cuba	1, 41	2.55	7.0	<0.001	0.075

gene flow between the various pairs of conspecific populations has not been frequent enough to override morphological differentiation between them, or that differences between islands reflect individual phenotypic responses to different ecological conditions on each island.

When all pairwise comparisons among conspecific island populations are treated separately, four of the five possible comparisons between conspecific populations show a general correlation of genetic and morphometric divergence (Fig. 4). The comparison between the Puerto Rican and Barbudan Adelaide's Warbler populations is anomalous in that these populations are similar morphologically in mensural traits but highly divergent genetically.

DISCUSSION

The comparison of molecular and morphological differentiation within Adelaide's, Plumbeous, and Olive-capped warblers demonstrates that the disjunct populations of Adelaide's Warbler have been evolutionarily independent far longer than have populations of the two more continuously distributed species. Indeed, our most striking result was the high level of mtDNA divergence and phylogenetic structure in Adelaide's Warbler, a species with three island-specific and highly distinct monophyletic groups of closely related haplotypes (Fig. 2). In contrast, the low mtDNA divergence between the two Plumbeous Warbler populations (Fig. 3) indicates a relatively recent common ancestry, but the presence of reciprocally monophyletic haplotype groups on Dominica and Guadeloupe suggests that even these geographically adjacent populations have attained evolutionary independence. Our reconstruction of phylogeographic variation among populations of Olive-capped Warbler was less detailed owing to small sample sizes. Nevertheless, the very low level of mtDNA divergence between the Cuban and Bahamian samples (Fig. 3) demonstrates a recent or continuing evolutionary connection between these populations.

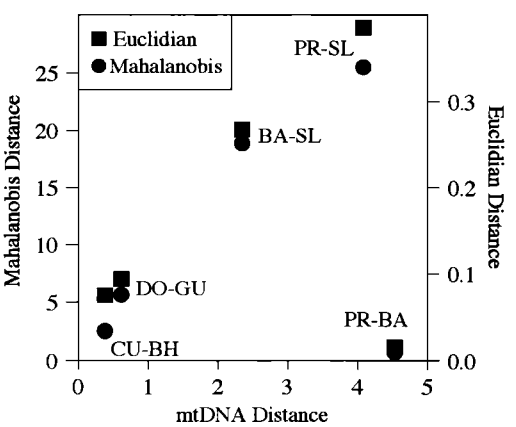

FIG. 4. Comparison of mean interisland mtDNA divergence with corresponding mean Mahalanobis and Euclidean distances in multivariate morphometric measurement space.

The low intraspecific genetic divergences in Plumbeous Warblers and Olive-capped Warblers were paralleled by correspondingly little morphometric variation in these taxa. Nonetheless, we found significant differences in morphology among islands within both species, indicating either that gene flow has been insufficient to override morphological differentiation, or that individuals respond developmentally to different ecological conditions on each island. The relationship between genetic and morphometric differentiation in Adelaide's Warbler was more complex, because the Puerto Rican and Barbudan populations exhibited highly divergent mtDNA haplotypes but similar external measurements. The morphological

similarity between northern Adelaide's Warbler populations is probably not restricted to size traits, because previous workers (Riley 1904, Curson et al. 1994) have noted plumage similarities between Barbudan and Puerto Rican Adelaide's Warblers. Several processes could account for the lack of congruency between mitochondrial divergence and morphological divergence in this species. First, if the mtDNA tree accurately reflects the evolutionary relationships of the three Adelaide's Warbler populations, then either morphological evolution in the St. Lucian lineage has been accelerated relative to that in the two northern lineages, or the Puerto Rican and Barbudan populations have evolved similar morphologies independently. Alternatively, morphological similarity between the Puerto Rican and Barbudan populations may result from male-mediated gene flow, which would not be reflected in the maternally inherited mitochondrial genome. Although a lack of records of Adelaide's Warblers from other islands and the great distances between islands argue for the genetic isolation of the three extant populations, confirmation of an absence of male-mediated gene flow would require additional study based on paternally or biparentally inherited genetic markers.

The 2.2 to 4.7% mitochondrial divergences among Adelaide's Warbler populations equals or exceeds those between many pairs of closely related bird species (e.g. Bermingham et al. 1992, Helbig et al. 1995, Klicka and Zink 1997). Our ongoing studies of parulid warbler relationships suggest that the large divergences among Adelaide's Warbler populations are not a result of high rates of nucleotide substitution in the genes we sequenced. For example, the interpopulation divergences in Adelaide's Warbler are higher than corresponding divergences among species in the "Black-throated" group (i.e. *D. nigrescens, D. occidentalis, D. townsendi,* and *D. virens*; see Bermingham et al. 1992) in which combined ATPase and COI sequence divergence ranges between 0.9 and 4.2% (Lovette, Bermingham, and S. Rohwer unpubl. data). The much lower magnitudes of divergence among island populations in Plumbeous Warblers and Olive-capped Warblers are more typical of genetic divergence within continental species (Bermingham et al. 1992, Helbig et al. 1995, Zink 1997).

Several independent calibrations have found that avian mtDNA sequences diverge at a rate of approximately 2% per million years (Shields and Wilson 1987, Tarr and Fleischer 1993, Nunn et al. 1996, Randi 1996, Klicka and Zink 1997). This rate calibration must be applied cautiously because it was derived primarily from RFLP-based estimates of genetic divergence and because the process of nucleotide substitution is not perfectly clock-like (e.g. Ayala 1986, Gillespie 1986). Nonetheless, the high degree of differentiation among Adelaide's Warbler populations provides strong evidence that they have been isolated for a long time; the split that isolated the Puerto Rican Adelaide's Warbler lineage probably occurred in the late Pliocene (ca. 1.8 to 2.4 million years ago), whereas the more recent split between the Barbudan and St. Lucian lineages occurred in the mid-Pleistocene (ca. 1.1 to 1.3 million years ago). In contrast, the small mtDNA divergences within Plumbeous Warblers and Olive-capped Warblers suggest that populations of these species have been connected by gene flow within the past 150,000 to 200,000 years.

The range of intraspecific divergence among the three warblers is interesting given the possibility that Antillean bird distributions have been influenced by recent changes in habitat distribution and quality. Fossil remains from the Bahamas suggest a pattern of late Pleistocene extinctions in West Indian vertebrates typical of xeric habitats (Pregill and Olson 1981). Pregill and Olson attributed these extinctions to a regional increase in rainfall over the past 10,000 to 20,000 years stemming from climate changes associated with the end of the last glacial period. Because Adelaide's Warblers favor dry lowland habitats (Bond 1956, Cruz and Delaney 1984, Raffaele 1989), this species may have been susceptible to the loss of xeric forests in the Lesser Antilles. Biogeographic considerations support the recent extinction of at least one population of Adelaide's Warbler. Barbuda and Antigua formed parts of a single large island during the last glacial maximum (ca. 20,000 years ago), and presumably a population of Adelaide's Warbler on what is now Antigua went extinct after the present-day islands were separated by rising sea levels. This extinction may have resulted from anthropogenic causes; Steadman et al. (1984) and Pregill et al. (1988) characterized a 4,300 to 2,500 year-old faunal assemblage from Antigua and found the

remains of a number of vertebrates, including seven bird species, that are no longer found on the island, possibly due to habitat degradation (Steadman et al. 1984).

Evidence against a more general recent extinction scenario is twofold. First, the presence of extensive dry forest on several islands between St. Lucia and Puerto Rico argues against the hypothesis that recent loss of suitable habitat caused the extinction of Adelaide's Warbler populations on these islands. Second, our genetic data strongly demonstrate that the evolutionary separation of the three extant Adelaide's Warbler lineages greatly predates the end of the most recent glaciation. The high levels of molecular divergence between the three extant Adelaide's Warbler populations show that they have persisted as evolutionarily isolated units through several Pleistocene glaciation cycles.

What biogeographic and evolutionary processes might account for distributional gaps such as those seen in Adelaide's Warbler? In general, range disjunctions might be created either by haphazard dispersal events that pass over intervening suitable islands, or by the extinction of populations on intermediate islands. Cases in which disjunct populations lack genetic divergence would support the long-distance dispersal scenario, because the absence of divergence between disjunct populations would allow little time for the extinction of intervening populations. Genetic similarity among disjunct populations is unlikely to be maintained by gene flow, because continued movement between disjunct populations should facilitate the colonization of intervening islands and hence close distributional gaps. Support for the alternative extinction-based scenario could come from paleontological evidence of a species' presence on an island where it does not presently occur, but avian fossils are not known from most West Indian islands. The proposition that gaps result from extinctions over evolutionary periods of time could also be supported statistically; under the extinction scenario, we would expect a positive relationship between range disjunction and interisland genetic divergence. In any particular case, however, large genetic divergences among disjunct populations could also result from old haphazard colonization events with subsequent differentiation in isolation.

Because we found a greater degree of genetic divergence among the highly disjunct populations of Adelaide's Warbler than among the less-disjunct populations of Plumbeous Warblers and Olive-capped Warblers, our data support the hypothesis that distributional gaps arise in long-established taxa by the extinction of geographically intermediate populations (Ricklefs and Cox 1972). Although other mtDNA-based studies of West Indian birds have documented a variety of phylogeographic histories superimposed on a common geography (Seutin et al. 1993, 1994; Klein and Brown 1994; Bermingham et al. 1996; Ricklefs and Bermingham 1997), Adelaide's Warbler is the only taxon with a large West Indian range disjunction for which genetic data have been published. A number of other Lesser Antillean species, including *Mimocichla plumbea, Cichlherminia lherminieri, Myadestes genibarbis, Troglodytes aedon,* and the *Icterus "dominicensis"* orioles also have conspicuous gaps within their distributions, and studies of these taxa are currently in progress.

Taxonomic Implications

The large mitochondrial differentiation between the three Adelaide's Warbler populations raises the issue of whether each population warrants species status. As noted above, mtDNA differences between the three populations exceed those between some North American *Dendroica* species, and each Adelaide's Warbler population appears to be a monophyletic entity. Although the pattern of morphological differentiation is not completely congruent with the mtDNA pattern, each population is also characterized by unique morphological differences. Thus, the criteria of the phylogenetic species concept (Cracraft 1983, Zink and McKittrick 1995) argue for elevating the three Adelaide's Warbler populations to species-level status. The genetic distinctiveness of the three populations also suggests that each should be considered an evolutionarily significant unit (Ryder 1986, Moritz 1994) for purposes of conservation.

As is common in situations where differentiated populations exist in allopatry, their potential classification under the biological species concept (Mayr 1963, 1969) or the recognition species concept (Patterson 1985) is problematic due to a lack of evidence on how

individuals from different islands would behave if they came into contact. Song recognition may play a key role in reproductive isolation or the lack thereof in birds, and Bond (1930, 1965) noted qualitative differences in song structure among Adelaide's Warbler populations. Even within an island, however, Adelaide's Warbler song varies on a microgeographic scale (Stacier 1996). Nonetheless, we found that Barbudan Adelaide's Warblers responded to recordings of St. Lucian individuals (Seutin pers. obs.); unfortunately, logistical constraints and a lack of recordings from the Barbudan population precluded controlled tests. Formal song-recognition experiments could provide important information on the degree of behavioral differentiation among populations of Adelaide's Warbler. If the three Adelaide's Warbler populations are accorded species status, the Puerto Rican population should remain *Dendroica adelaidae* Baird 1865, the Barbudan population should be referred to *Dendroica subita* Riley 1905, and the St. Lucian population to *Dendroica delicata* Sclater 1871.

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APPENDIX. Variable nucleotide sites among conspecific mtDNA haplotypes in three West Indian *Dendroica* warblers. Dots indicate a match to the uppermost conspecific sequence. Sample size (*n*) indicates the number of individuals with identical haplotypes. PR = Puerto Rico; BA = Barbuda; SL = St. Lucia; DO = Dominica; GU = Guadeloupe; BH = Bahamas; CU = Cuba.

Island and haplotype	*n*	ATPase 8	ATPase 6	Cytochrome oxidase I
			Adelaide's Warbler	
PR A	3	CTTAGATGT	TTCCTTGCGCGGAGTGATTCCATTCATTATCACACTAGTG	CTTGCCACTTGTTTTGGCCTTATCTTGGAC
PR B	1	...G.....	.....	.....C.....A..T
PR C	1	...G.....	.....G.....	.....C.....T
PR D	1	...G.....	.....T.....	.....C.....
PR E	1	...G..C..	...T.....C.....G...	.....T...C.....
BA A	4	TCC.AG.A.	.CT.CCAAATAA.T.A.CC.AGC.TGCC.CTG.G.C.A.A	TCCTAT..TCACCCCAATTCCG.TCC.A..
BA B	1	TCC.AG.A.	.CT.CCAAATAA.T.A.CC.AGC.TGCC.CTG.G.C.A.A	TCCTAT...CACCCCAATTCCG.TCC.A..
SL A	2	.C..AGCA.	.CT..CA.A.AA.TC.GCC...C.T.CCGC..TGT.GACA	TCC.A.G...ACCCCA.T.CCGC.CC.AG.
SL B	1	.C..AGCA.	CCT..CA.A.AA.TC.GCC...CCT.CCGC..TGT.GACA	TCC.A.G...ACCCCA.T.CCGC.CC.AG.
SL C	4	.C..AGCA.	.CTT.CA.A.AA.TC.GCC...C.T.CCGC..TGT.GACA	TCC.A.G...ACCCCA.T.CCGC.CC.AG.
SL D	1	.C..AGCA.	CCT..CA.A.AA.TC.GCC...CCT.CCGC..T.T.GACA	TCC.A.G...ACCCCA.T.CCGC.CC.AG.
			Plumbeous Warbler	
DO A	1	AGATG	GCCAGTCCGGTGGCCTAT	CAGATCATTACTT
DO B	1	C...A	A.....	.G.G.....G..C
DO C	1	C....	.....G.	.....G.CC
DO D	1	C....	.....	...G.....G..C
DO E	1	C....	.....A.....G.	..A.....G..C
DO F	2	C....	.T.....	.....G.....C
DO G	1	C....	.....G.	C
DO H	1	C....	.T.....	.....G..C
DO I	5	C....	.....	.....G..C
GU A	1	C.G..	...G.C....C.AT...C	.....G..C
GU B	1	C....	...G.C...AC.A....C	.....T...G..C
GU C	2	C....	...G.C....C.A....C	.....T...G..C
GU D	1	C....	..A..C....C....C.C	.....T...GT.C
GU E	1	C....	.T..AC....C.....C	.....T...G..C
GU F	2	C....	.T...C.T..C...T..C	.....T...G..C
GU G	1	CA.C.	.....C....CA.....C	.....T..CG..C
GU H	2	C.G..	...G.C....C.AT...C	.....T...G..C
GU I	1	C....	.....CT...C.....C	T....T..CG..C
GU J	1	C....	.....C....C.....C	....CT.C.G..C
			Olive-capped Warbler	
BH A	2		ACTA	G
CU A	1		TTCG	A