The Auk 112(1):156-170, 1995

MOLECULAR SYSTEMATICS AND ZOOGEOGRAPHY OF FLOWERPIERCERS IN THE *DIGLOSSA BARITULA* COMPLEX

SHANNON J. HACKETT[1]
Museum of Natural Science and Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803, USA

ABSTRACT.—Relationships among eight species of flowerpiercers in the genus *Diglossa* (Thraupidae) are addressed using data from allozymes, mtDNA sequences, and male plumages. Molecular evolution of the mitochondrial cytochrome-*b* gene in this group parallels what has been reported for other birds. Molecular data reveal high levels of genetic differentiation among the taxa studied. There is concordance of evolution among mtDNA sequences, allozymes, and plumages for the three taxa in the *Diglossa baritula* superspecies complex. The pattern of phylogeny in the complex suggests that *plumbea* (highlands of southern Central America) is most closely related to *baritula* (highlands of northern Central America). *Diglossa sittoides* (highlands of South America) is the sister taxon to the *baritula*/*plumbea* clade. The pattern of phylogeny and genetic distances suggest that divergence of taxa in the *baritula* superspecies complex occurred as the result of both dispersal and vicariance during the Pleistocene. *Received 8 July 1993, accepted 25 May 1994.*

DIGLOSSA FLOWERPIERCERS are high-elevation species restricted to montane regions in the Neotropics (Vuilleumier 1969, A.O.U. 1983, Isler and Isler 1987). In addition, *Diglossa* are morphologically and behaviorally one of the more divergent lineages of tanagers (Bock 1985, Graves 1982, Isler and Isler 1987, Vuilleumier 1969). Indeed, their taxonomic position within the Thraupidae has been questioned; in addition to tanagers, they have been placed with emberizine finches and honeycreepers (for review of taxonomy, see Bock 1985; see also Sibley and Ahlquist 1990). The specialized bills and tongues of flowerpiercers have been the subject of several morphological studies (Vuilleumier 1969, Bock 1985). In addition, the wide range of geographic variation in several polytypic species has been used as a test case for active speciation in Neotropical highlands (Vuilleumier 1969, Graves 1982).

In this paper, I address relationships among taxa in the *Diglossa albilatera* species group (as defined by Vuilleumier 1969), in particular the three members of the *Diglossa baritula* superspecies complex, using both allozyme and DNA characters. I discuss relationships between the two independent molecular data sets and a morphological data set (based on plumage characters). Using phylogenies generated from these data, I discuss plumage and gene evolution in the group and propose a biogeographic scenario leading to the present-day distributions of the *D. baritula* superspecies complex.

METHODS

Samples and sample sizes.—Tissue samples (Table 1) for this analysis were obtained from the Louisiana State University Museum of Natural Science (LSUMNS) Frozen Tissue Collection with the exception of the *Diglossa baritula* sample, which was donated by the Field Museum of Natural History.

In this study, a number of methods of data gathering and analysis were used to try to overcome possible problems with small sample size. Sequential allozyme electrophoresis (Aquadro and Avise 1982, Hackett 1989) was performed to identify any "hidden" allelic variation that may be present in these taxa. Second, a number of different allozyme loci were resolved. Gorman and Renzi (1979) demonstrated that one or few individuals per taxon provide robust estimates of genetic distance as long as the number of loci examined is reasonably high and heterozygosity is low (but see Archie et al. 1989). In addition, Highton (1994) suggested that increasing the number of loci was important for obtaining reliable estimates of genetic distances; as the number of loci increased, statistical support increased for nodes that reflect phylogenetic relationships. Third, genetic distance data were not considered as the sole representation of the allozyme data; cladistic analysis and bootstrap analysis of allelic data also were performed. Fourth, other data sets (mitochondrial DNA sequences and plumage characters) were gathered and their results compared to those of the allozyme analysis. If concordant

[1] Present address: Field Museum of Natural History, Roosevelt Road at Lake Shore Drive, Chicago, Illinois 60605, USA.

TABLE 1. LSUMNS tissue numbers (beginning with B) and collecting localities for *Diglossa* specimens analyzed. Taxonomy following Vuilleumier (1969). *Diglossa baritula* sample donated by the Field Museum of Natural History.

Taxon	Tissue no.	Locality
1. *baritula* species group		
A. *baritula* superspecies complex		
sittoides	B1301	Bolivia: Dpto. La Paz; ca. 1 km S Chuspipata
	B5558[a]	Peru: Dpto. San Martin; 28 km by road NE Tarapoto on road to Yurimaguas
	B194	Peru: Dpto. Piura; km 34 on Olmos-Bagua Chica Hwy
baritula	MEX 350[a]	Mexico: Jalisco; Sierra de Manantlán, Las Joyas
plumbea	B9949	Costa Rica: Prov. San José; La Georgina, km 95 Pan American Hwy
	B9948	Costa Rica: Prov. San José; La Georgina, km 95 Pan American Hwy
	B16239[a]	Costa Rica: Prov. San José; La Georgina, km 95 Pan American Hwy
	B16067	Costa Rica: Prov. Heredia; Finca La Fortuna, ca. 4 km SE Virgen del Socorro
	B16068[a]	Costa Rica: Prov. Heredia; Finca La Fortuna, ca. 4 km SE Virgen del Socorro
B. *albilatera* superspecies complex		
albilatera	B231	Peru: Dpto. Cajamarca; "Batan" on Sapalache-Carmen Trail
	B256	Peru: Dpto. Cajamarca; "Lucuma" on Sapalache-Carmen Trail
	B262[a]	Peru: Dpto. Cajamarca; "Batan" on Sapalache-Carmen Trail
	B6215	Ecuador: Prov. Morona-Santiago; W slope Cordillera del Cutucú, S trail from Logroño-Yaupi
2. *lafresnayii* species group		
A. *duidae*	B7446	Venezuela: T.F. Amazonas; Cerro de la Neblina, Camp VII
	B7411	Venezuela: T.F. Amazonas; Cerro de la Neblina, Camp VII
	B7461	Venezuela: T.F. Amazonas; Cerro de la Neblina, Camp VII
	B7396	Venezuela: T.F. Amazonas; Cerro de la Neblina, Camp VII
B. *carbonaria* superspecies complex		
humeralis aterrima	B433[a]	Peru: Dpto. Piura; "Cruz Blanca," ca. 33 road km SW Huancabamba
carbonaria carbonaria	B1300	Bolivia: Dpto. La Paz; ca. 1 km S Chuspipata
carbonaria brunneiventris	B7716	Peru: Huanuco; Unchog Pass between Churrubamba and Hacienda Paty, NNW Acomayo
carbonaria carbonaria	B1294	Bolivia: Dpto. La Paz; ca. 1 km S Chuspipata
C. *lafresnayii* superspecies complex		
lafresnayii	B333	Peru: Dpto. Piura-Cajamarca; Cerro Chinguela, ca. 5 km NE Sapalache
mystacalis pectoralis	B1958	Peru: Dpto. Pasco; Cumbre de Ollón, ca. 12 km E Oxapampa
mystacalis unicincta	B7659	Peru: Huanuco; Unchog Pass between Churrubamba and Hacienda Paty, NNW Acomayo
mystacalis albilinea	B551	Peru: Dpto. Puno; Valcón, 5 km NNW Quiaca
mystacalis mystacalis	B1272	Bolivia: Dpto. La Paz; ca. 1 km S Chuspipata
3. *caerulescens* species group		
glauca	B1605	Peru: Dpto. Pasco; Santa Cruz, ca. 9 km SSE Oxapampa

[a] Specimen sequenced.

phylogenetic hypotheses result from analyses of the different character sets and methods of analysis, I conclude that small sample sizes have not had a significant effect on recovering the pattern of phylogenetic relationships among taxa.

Protein electrophoresis.—Standard horizontal starch-gel electrophoresis of proteins was performed as outlined in Hackett (1989), Hackett and Rosenberg (1990), and Murphy et al. (1990). Each locus was scored on at least two buffer systems to reduce influences of hidden variation (Aquadro and Avise 1982, Hackett 1989). Locus names follow Murphy et al. (1990). Alleles were coded by their relative mobility from the origin; the most anodally migrating allele was coded "a." Isozymes were coded in a similar manner, with a "1" indicating the most anodally migrating isozyme.

I used the computer program BIOSYS-1 (Swofford and Selander 1981) to compute genetic distances (Nei 1978, Rogers 1972), a UPGMA phenogram, and distance-Wagner (Farris 1972) trees using the multiple addition criterion of Swofford (1981). In all allozyme analyses, trees were rooted at *D. glauca*. This species is an appropriate outgroup for the following reasons: Vuilleumier (1969) placed it as part of a different species group (*caerulescens*) from the *D. albilatera* species group, and Bock (1985) considered the *caerulescens* group only distantly related to the rest of *Diglossa*.

Cladistic assessment of allelic variation was performed by coding each locus as a multistate unordered character (and alleles at each locus as character states) using the computer program PAUP 3.1.1 (Swofford 1993; Branch and Bound option). Also, in another cladistic analysis, phylogenetically informative alleles were considered as characters and coded as present or absent (see Rogers and Cashner [1987] for defense of this method of coding; see also Buth [1984], Mickevich and Mitter [1981], Murphy [1993], and Swofford and Berlocher [1987] for problems with this method of coding). For the taxa analyzed in this study, presence/absence coding of alleles, despite possible problems, resulted in trees that are more resolved, agreed with other methods of data analysis (for example, those based on genetic distances), and were concordant with phylogenetic analyses of other character systems (plumages and DNA sequences). I performed 100 bootstrap replicates on each cladistic analysis to assess confidence in the branching pattern (Felsenstein 1985, Sanderson 1989).

DNA sequences.—Following Hillis et al. (1990), a total nucleic acid preparation was made from liver tissue frozen at −80°C (see Table 1 for specimens sequenced). Amplifications of a specific region of the mitochondrial (mt) cytochrome-*b* gene were performed via the polymerase chain reaction (PCR). A 307 base-pair fragment (not including primers) was amplified using primers L14841 (5'-CCATCCAACATCTCAGCATGATGAAA-3') and H15149 (5'-CCTCAGAATGATATTTGTCCTCA-3') of Kocher et al. (1989). These primers amplify a piece of DNA from base 14991 to base 15297 (not including primers) relative to the chicken mtDNA sequence (Desjardins and Morais 1990). Double-stranded PCR amplifications were performed in 50 µl total reaction volumes [10 µl of a 10^{-2} dilution of the total DNA preparation, 2.5 µl of a 10 mM solution of each primer, 5 µl of 10× buffer (including $MgCl_2$), 2 µl of a 1.0 mM solution of dNTP's, 0.20 µl *Taq* DNA polymerase (Promega), up to 50 µl with H_2O]. Thirty to 35 cycles were performed using the following cycling parameters: (first cycle) denaturation at 94°C for 3 min, annealing at 56°C for 1 min, extension at 72°C for 30 s; (remaining cycles) denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 30 s.

Single-stranded DNA was generated following the procedure of Allard et al. (1991) in which only one primer is used (no limiting primer). I used 5 µl of the double-stranded product to generate single-stranded DNA in 100 µl reactions (5 µl double-stranded DNA, 2 µl of a 10 mM solution of one primer, 10 µl of 10× buffer [including $MgCl_2$], 4 µl dNTP's, 0.40 µl *Taq* DNA polymerase (Promega), up to 100 µl with H_2O). Twenty cycles were performed using the following cycling parameters: (first cycle) denaturation at 94°C for 3 min, annealing at 56°C for 1 min, extension at 72°C for 45 s; (remaining cycles) denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 45 s. Single-stranded DNA was generated for both the heavy and light mtDNA strands, and the products were cleaned by five washings with H_2O through Ultrafree®-MC 30,000 NNMWL filters (Millipore Corp., Bedford, Massachusetts), and concentrated to a final volume of approximately 30 µl. I used 7 µl of cleaned single-stranded DNA for DNA sequencing using T7 DNA polymerase (Sequenase® version 2.0, United States Biochemical, Cleveland, Ohio).

The DNA sequence data were analyzed cladistically with PAUP 3.1.1 (Swofford 1993; Exhaustive search option). I sequenced another tanager, *Hemispingus superciliaris*, and this sequence was used to root the DNA sequence trees. All base positions were used in the analysis; there were few transversions, non-third-position changes, or nonsynonymous changes. As with the allozyme data, 100 bootstrap replicates were performed to assess confidence in the branching pattern. Percent sequence divergence was calculated as

$$p = n_d / n, \tag{1}$$

where p is the percent sequence divergence, n_d is the number of nucleotides different between two sequences, and n is the total number of nucleotides compared (Nei 1987).

Plumages.—In addition to allozyme and sequence data, variation in adult male plumage was also assessed for *Diglossa* species (Appendix) and analyzed using the computer program PAUP 3.1.1 (Swofford 1993; heuristic search, 30 random addition-sequence replicates, TBR swapping, MULPARS). Specimens for

this analysis are housed at the American Museum of Natural History, New York. The goal of this analysis was to use plumage patterns to assess monophyly of the *baritula* superspecies complex and to test whether or not *albilatera* was the sister taxon of the *baritula* superspecies complex, as had been suggested by Vuilleumier (1969). In addition, the pattern of relationships among species within the *baritula* superspecies complex based on plumage data could be compared to the pattern based on the molecular data.

Body regions (belly, throat, breast, back, cap, rump, vent, malar region, underwing) were treated as characters, with the color or pattern in that body region as character states. In addition, the presence of sexual dimorphism in plumages was considered a character as were the presence of a blue-gray humeral patch and the presence of a breast band. The plumage matrix was composed of 12 characters (5 binary and 7 multistate). The multistate characters were divided into five unordered characters (no hypothesis of direction of evolution of character states was formed) and two ordered characters (characters 6 and 8). The ordering of characters was accomplished through the use of step-matrices in PAUP.

Justifications for the ordering of characters 6 and 8 are based on the criterion of similarity of states (Lipscomb 1992, Patterson 1982). Character 6, the back body region, had four different states (dark blue, black, gray, and gray-blue). The outgroup *D. glauca* has a solid dark-blue back; with the exception of the *baritula* superspecies complex, all other taxa have black backs. Within the *baritula* complex, *sittoides* has a solid gray-blue back and *baritula* and *plumbea* have solid gray backs. I ordered only the transformation between the states gray and gray-blue (all other transformations were left unordered) because the states differed only slightly in shade. Coding them as the same state would have obscured potentially phylogenetically informative variation within the *baritula* superspecies complex, the taxa of interest in this study; the states in these taxa are much more similar to each other than they are to any other states of this character. Character 8, the distribution of color on the rump body region, had seven different character states (solid gray-blue, dark gray, black, gray-tinged, extensive gray-tinged, faint blue-gray-tinged, and blue-gray-tinged). I ordered only the transformation of gray-tinged to extensive gray-tinged rumps and blue-gray-tinged to faint blue-gray-tinged rumps. These particular transformations influenced only those taxa in the *carbonaria* complex and the *lafresnayii* complex.

Due to the small number of plumage characters and the occurrence of all or mostly black birds in different species groups, monophyly of the *lafresnayii* and *carbonaria* superspecies complexes were not recoverable with plumage data alone. The monophyly of these two superspecies complexes has been established by a number of studies (Hellmayr 1935, Vuilleumier 1969, Graves 1980, 1982, 1990, 1991; also confirmed by allozyme results of my study). Thus, to assess monophyly of the *baritula* superspecies complex, relationships within the *baritula* complex, and possible sister taxa to the *baritula* complex, the analyses performed in PAUP constrained taxa in both the *lafresnayii* and *carbonaria* groups to be monophyletic. In addition, constraining these superspecies complexes to be monophyletic allows evaluation of relationships of taxa within each of these complexes based on plumage data.

RESULTS

Protein electrophoresis.—Levels and patterns of genetic variation at 33 presumptive gene loci were resolved (Tables 2 and 3; the three UDHs are unidentified dehydrogenases). Twenty-four (73%) loci were variable within or among species. Average genetic distance (Nei 1978) within the *baritula* superspecies complex (among *sittoides, baritula,* and *plumbea*) was 0.088 ± SD of 0.049; between the *baritula* complex and *albilatera,* genetic distances averaged 0.289 ± 0.055. The genetic distance was 0.003 between the two population samples of *D. plumbea* from the Cordillera Central and Cordillera Talamanca in Costa Rica (Table 1). Among both *lafresnayii* taxa and *carbonaria* taxa, genetic distances averaged 0.04.

In the UPGMA phenogram (Fig. 1) members of the *baritula* superspecies complex clustered together, with *baritula* and *plumbea* forming a group and *sittoides* clustering outside *baritula* and *plumbea. Diglossa albilatera* and *D. duidae* formed a group that was most genetically similar to the *baritula* complex, followed by the *carbonaria* complex and finally by the *lafresnayii* complex. Relationships among taxa within the *carbonaria* and *lafresnayii* complexes based on the allozyme distances shown in Figure 1 are uncertain. Genetic distances among taxa within each of these complexes were low, and only single individuals were analyzed. To assess relationships among taxa in the *carbonaria* and *lafresnayii* complexes based on molecular data would require collection of additional individuals from throughout the ranges of these taxa and was outside the scope of this project.

Cladistic analysis of loci with the alleles as unordered character states resulted in 1,672 equally-most-parsimonious trees, with a consistency index (C.I.) excluding uninformative characters of 0.66. The strict consensus (not shown) and majority-rule consensus (Fig. 2A)

TABLE 2. Allozyme genetic distances for *Diglossa* species analyzed. Nei (1978) genetic distances below diagonal; Rogers' (1972) genetic distance above diagonal. CT and CC refer to populations of *D. plumbea* from Cordillera Talamanca and Cordillera Central in Costa Rica.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 *sittoides*	—	0.181	0.137	0.145	0.334	0.397	0.382	0.334	0.380	0.347	0.358	0.345	0.331	0.402	0.388
2 *baritula*	0.157	—	0.097	0.129	0.279	0.339	0.329	0.280	0.325	0.333	0.314	0.333	0.318	0.409	0.333
3 *plumbea* (CT)	0.079	0.061	—	0.061	0.262	0.314	0.301	0.253	0.297	0.306	0.309	0.306	0.291	0.363	0.306
4 *plumbea* (CC)	0.089	0.089	0.003	—	0.261	0.322	0.311	0.271	0.307	0.315	0.330	0.315	0.300	0.372	0.315
5 *albilatera*	0.351	0.278	0.244	0.251	—	0.202	0.204	0.176	0.210	0.226	0.246	0.231	0.216	0.302	0.233
6 *duidae*	0.451	0.391	0.332	0.344	0.201	—	0.239	0.216	0.246	0.284	0.292	0.284	0.269	0.360	0.284
7 *humeralis aterrima*	0.439	0.382	0.325	0.341	0.200	0.255	—	0.068	0.076	0.178	0.193	0.178	0.163	0.223	0.147
8 *carbonaria carbonaria*	0.349	0.295	0.242	0.261	0.145	0.209	0.037	—	0.083	0.168	0.168	0.168	0.153	0.244	0.168
9 *carbonaria brunneiventris*	0.436	0.375	0.317	0.334	0.207	0.267	0.056	0.046	—	0.193	0.208	0.193	0.178	0.238	0.163
10 *lafresnayii*	0.379	0.375	0.317	0.334	0.224	0.308	0.178	0.142	0.189	—	0.076	0.030	0.015	0.091	0.132
11 *mystacalis pectoralis*	0.391	0.356	0.324	0.348	0.238	0.317	0.188	0.143	0.200	0.040	—	0.076	0.061	0.152	0.167
12 *mystacalis unicincta*	0.386	0.390	0.333	0.349	0.237	0.324	0.193	0.157	0.204	0.015	0.056	—	0.015	0.076	0.152
13 *mystacalis albilinea*	0.363	0.360	0.303	0.319	0.214	0.295	0.167	0.132	0.178	0.008	0.032	0.008	—	0.091	0.136
14 *mystacalis mystacalis*	0.462	0.487	0.403	0.419	0.314	0.412	0.227	0.230	0.239	0.065	0.118	0.056	0.064	—	0.178
15 *glauca*	0.451	0.390	0.333	0.349	0.242	0.324	0.157	0.157	0.167	0.131	0.159	0.164	0.139	0.177	—

of these trees resulted in little resolution. There was support for monophyly of the *baritula*, *carbonaria*, and *lafresnayii* complexes, and for a clade that included the *baritula* complex, *albilatera*, *duidae*, and the *carbonaria* complex.

With alleles coded as present or absent, parsimony analyses resulted in two most-parsimonious trees (C.I. = 0.63, excluding uninformative characters). The trees differed only in relationships among taxa in the *carbonaria* complex. The bootstrap tree from this data set (Fig. 2B) was one of the most parsimonious and also supported monophyly of the *D. baritula* complex, but leaves unresolved the relationships within the *baritula* complex. The presence/absence coding of alleles suggests that *albilatera* is the sister taxon of the *baritula* complex. This tree differs from that shown in Figure 2A by the greater degree of resolution among species. These trees (Fig. 2A and B) differ from the distance analyses mainly in the unresolved relationships among species in the *baritula* complex, which seem to differ in frequencies of alleles (Table 3).

DNA sequences.—Sequences were identical between two individuals sequenced from the same population for two of the species (*D. plumbea* and *D. sittoides*). Percent sequence divergence (Table 4) among the species ranges from 0.3% between the two populations of *D. plumbea* from Costa Rica to 9.8% between *D. sittoides* and *D. h. aterrima* (of the *carbonaria* complex).

Fifty-one (16.6%) of the 307 positions were variable among the taxa in this study (Fig. 3). Of these positions, three (5.9%) occurred at the first position of a codon, none occurred at the second position of a codon, and the remaining 48 (94.1%) occurred at the third position of a codon. The transition:transversion ratio among *Diglossa* taxa averaged approximately 9:1. Between *Diglossa* taxa and the outgroup, *Hemispingus*, the transition:transversion ratio averaged 3.4:1. Only one mutation changed the amino-acid composition of this region of the cytochrome-*b* gene.

Parsimony analysis of the sequence data (Fig. 3) resulted in one most-parsimonious tree (C.I. = 0.74, excluding uninformative characters). The bootstrap analysis (Fig. 4) resulted in a tree with the same topology as the most-parsimonious tree. The topology suggests that *D. plumbea* and *D. baritula* are sister taxa, and that *D. sittoides* is the sister taxon to the *plumbea*/*baritula* clade. In the DNA sequence analysis, *D. albilatera* is more

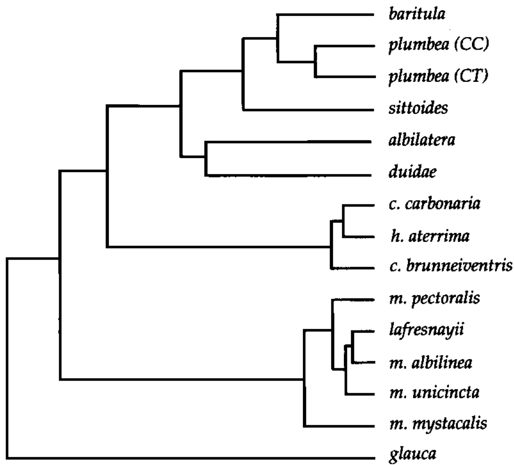
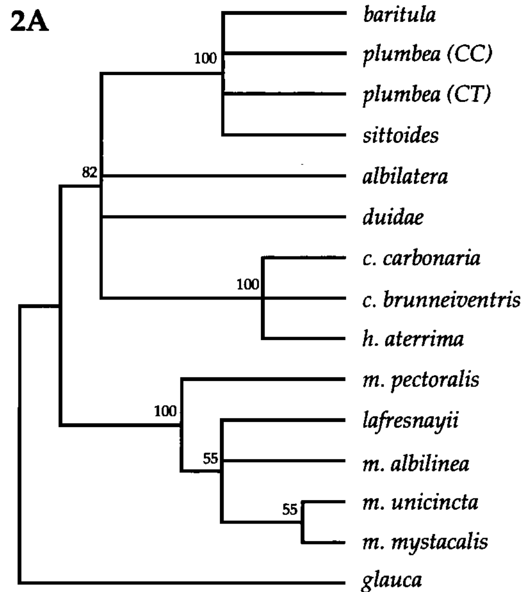


Fig. 1. UPGMA phenogram summarizing Rogers' (1972) genetic distance (Table 2) among *Diglossa* species analyzed.



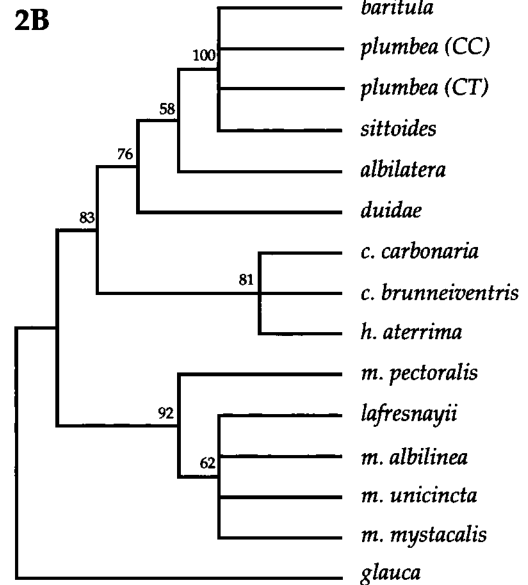


Fig. 2. Parsimony analyses of allelic data (Table 3) of *Diglossa* species. (A) Results of coding loci as characters and alleles as unordered character states. Numbers at nodes indicate percent of the most-parsimonious trees with that particular node. (B) Results of coding alleles as presence/absence. Numbers at nodes indicate percent of times that node is held in a bootstrap analysis.

closely related to *D. h. aterrima,* of the *carbonaria* complex, than to the *baritula* complex. Combining data from allozymes and mtDNA sequences into a single large data set resulted in one tree (not shown; C.I. = 0.88) that was identical to the mtDNA sequence tree (Fig. 4).

Plumages.—Analysis of the plumage characters (Appendix) resulted in 36 most-parsimonious trees (C.I. = 0.76, excluding the ordered characters). The majority-rule consensus tree (Fig. 5) indicated completely resolved relationships among the three species in the *D. baritula* complex. These relationships are identical to those of the allozyme distance tree and the mtDNA sequence tree; *baritula* and *plumbea* are sister taxa relative to *sittoides.* Based on plumage data alone, the position of *albilatera* could not be ascertained. In addition, relationships of other clades both to the *baritula* complex and to each other were unresolved.

Despite the lack of resolution at higher taxonomic levels with this plumage analysis, there is a high degree of resolution among taxa in superspecies complexes. The sister-taxon relationship of *albilatera* and *venezuelensis* supports monophyly of the *albilatera* superspecies complex of Vuilleumier (1969). Within the *lafresnayii* superspecies complex, subspecies of *mystacalis* form a clade, as do the two subspecies of *gloriosissima.* Within the *carbonaria* superspecies complex, *brunneiventris* and *carbonaria* are sister taxa; note also that the mostly black members of this complex (*humeralis humeralis, h. nocticolor,*

TABLE 3. Allozyme frequencies for *Diglossa* species analyzed. Nine loci were monomorphic and fixed for same allele across all species: ESTD, SOD1, ME2, G6PDH, ICD2, MDH1, MDH2, LDHB, EAP. CT and CC refer to populations of *D. plumbea* from the Cordillera Talamanca and Cordillera Central in Costa Rica.

Taxon	PGM1	CK1	ADA	GOT1	ICD1	MPI	GPI	LA1	LA2	LGG
sittoides	B	B	D	B	B (0.17) C (0.33) E (0.50)	D (0.83) F (0.17)	A (0.17) C (0.83)	B (0.33) C (0.67)	B (0.67) C (0.17) D (0.16)	A (0.50) D (0.50)
baritula	C	D	D	C	C (0.50) D (0.50)	D	C	C	B	B (0.50) D (0.50)
plumbea (CT)	B	D	D	B (0.83) C (0.17)	A (0.33) C (0.67)	A (0.13) D (0.87)	C	C	B	B (0.17) D (0.67) E (0.16)
plumbea (CC)	B	D	D	B	C	D	B (0.50) C (0.50)	C (0.75) D (0.25)	B	D
albilatera	A (0.13) B (0.87)	A	C (0.13) D (0.87)	C	C	D	A (0.13) C (0.87)	C	A (0.13) B (0.87)	D
duidae	A (0.13) B (0.87)	A	D	C	C	F	C	C	B	D
h. aterrima	B	A	D	C	C	C (0.50) F (0.50)	C	C	B	D
c. carbonaria	B	A	D	C	C	C (0.75) F (0.25)	C	C	B	C (0.50) D (0.75)
c. brunneiv.	B	A	D	C	C	C	C	C	B	D
lafresnayii	B	C	A (0.50) D (0.50)	C	C	E	C	C	A (0.50) B (0.50)	D
m. pectoralis	A (0.50) B (0.50)	C	D	C	C	E	C	C	B	C (0.50) D (0.50)
m. unicincta	B	C	D	C	C	E	C	C	A	D
m. albilinea	B	C	D	C	C	E	C	C	A (0.50) B (0.50)	D
m. mystacalis	B	C	B (0.50) D (0.50)	A (0.50) C (0.50)	C	E	C	C	A	D
glauca	B	C	E	C	C	B	C	C	B	D

and *h. aterrima*; see Appendix) do not form a clade. Phylogenetic relationships among taxa within the *carbonaria* and *lafresnayii* complexes should be tested with other data sets.

DISCUSSION

Molecules.—Both allozyme and mtDNA sequence data revealed a high degree of differentiation among the taxa analyzed in this study relative to that found in many comparable avian studies. This result provides additional data on the high levels of differentiation among sedentary (nonmigratory) Neotropical birds as reported by Bates (1993), Bates and Zink (1994), Capparella (1987, 1988), Hackett (1992, 1993), Hackett and Rosenberg (1990), Peterson (1992), and Peterson et al. (1992), and Seutin et al. (1993).

Results of allozyme and mtDNA sequence analyses can be compared not only at the level of evolutionary pattern (phylogenies), but also at the level of genetic differentiation. Although allozyme and mtDNA sequence divergence (Fig. 6) are highly correlated ($r = 0.90$), sequence divergence appears to level off after around 0.20 units of Nei's (1978) allozyme genetic distance. Although DNA sequence changes are limited to four character states (excluding gaps), more than four are possible at allozyme loci. Therefore, in *Diglossa*, sequence change (especially transitions) at third positions may be reaching saturation (multiple mutations at the same DNA position), whereas saturation at allozyme loci seems to have not yet been reached. Edwards et al. (1991) found 92% of changes at the cytochrome-*b* gene in a range of passerine birds occurred at third positions of codons, an expected result for a protein-coding gene evolving under functional constraints. They suggested that third positions were saturated within a genus of babblers at approximately 10% sequence divergence among species, a result consistent with the findings of my study.

Although transition:transversion ratios of 20:1

TABLE 3. Extended.

NP	FUM	SOD2	UDH1	UDH2	UDH3	SDH	ME1	LDHA	CK2	PGM2	6PGD	αGPD	GP
B	A	B	A	A	C	D	A	B	B	B	B	A	A
B	A	B	A	A	C	D	B	B	B	B	B	A	A
A (0.17) B (0.83)	A	B	A (0.83) B (0.17)	A	C	D	B	B	B	B	B	A (0.83) B (0.17)	A
B	A	B	A (0.75) B (0.25)	A	C	D	B	B	B	B	B	A (0.75) B (0.25)	A
B (0.25) D (0.75)	A	B	C	C	D	D	B	B	A (0.13) B (0.87)	B	B	B	B
F	A	A	B	B	B	D	B	A (0.25) B (0.75)	B	B	B	B	B
D	B	B	E	A	E	A	B	B	B	B	B	B	B
D	A	B	E	A	C (0.50) E (0.50)	A (0.75) B (0.25)	B	B	B	B	B	B	B
C (0.50) D (0.50)	E	B	E	A	E	A (0.50) B (0.50)	B	B	B	B	B	B	B
D	A	B	E	A	E	D	C	B	B	B	B	B	B
D	A	B	E	A	E	D	C	B	B	A (0.50) B (0.50)	B	B	B
D	A	B	E	A	E	D	C	B	B	B	B	B	B
D	A	B	E	A	E	D	C	B	B	B	B	B	B
D	C	B	E	A	E	D	C	B	B	B	A (0.50) B (0.50)	B	B
D	D	B	E	A	E	D	B	B	B	B	B	B	B

have been proposed for birds (Edwards et al. 1991), in *Diglossa* the transition:transversion ratio averaged approximately 9:1. I also find a bias of increased A's and C's at the third positions of codons on the L-strand, as has been reported by Desjardins and Morais (1990) and Edwards et al. (1991).

Phylogeny.—Mitochondrial DNA is inherited as a single linkage group, and resulting phylogenies should be interpreted as gene trees and not species trees (Neigel and Avise 1986, Pamilo and Nei 1988). Therefore, the comparisons of mtDNA sequences with allozyme loci, which are encoded by many genes in the nucleus, address how much confidence to have in a phylogeny derived from mtDNA data. Because both molecular data sets yielded similar phylogenies, I feel that the mtDNA sequences reveal species trees in this case. In addition, bootstrap values indicated relatively greater support for relationships derived from the mtDNA sequence data, perhaps as a result of more characters in the sequence data set, the presence of frequency differences separating taxa in the allozyme data, or because of polymorphisms in allozyme data sets that are difficult to analyze cladistically (Maddison 1994).

Allozyme and mtDNA data differed regarding the placement of *D. albilatera.* In the allozyme data analyses, *albilatera* was the sister taxon of the *baritula* complex, genetically similar to *duidae,* or its position was unresolved (Figs. 1 and 2). The sequence data set and combined

TABLE 4. Percent sequence divergence among species in genus *Diglossa.* CT and CC refer to populations of *D. plumbea* from Cordillera Talamanca and Cordillera Central in Costa Rica.

Taxon	1	2	3	4	5
1 *sittoides*					
2 *baritula*	7.2				
3 *plumbea* (CT)	6.2	2.6			
4 *plumbea* (CC)	6.5	2.3	0.3		
5 *albilatera*	9.8	9.4	8.8	8.5	
6 *h. aterrima*	9.8	8.8	8.8	8.5	6.5

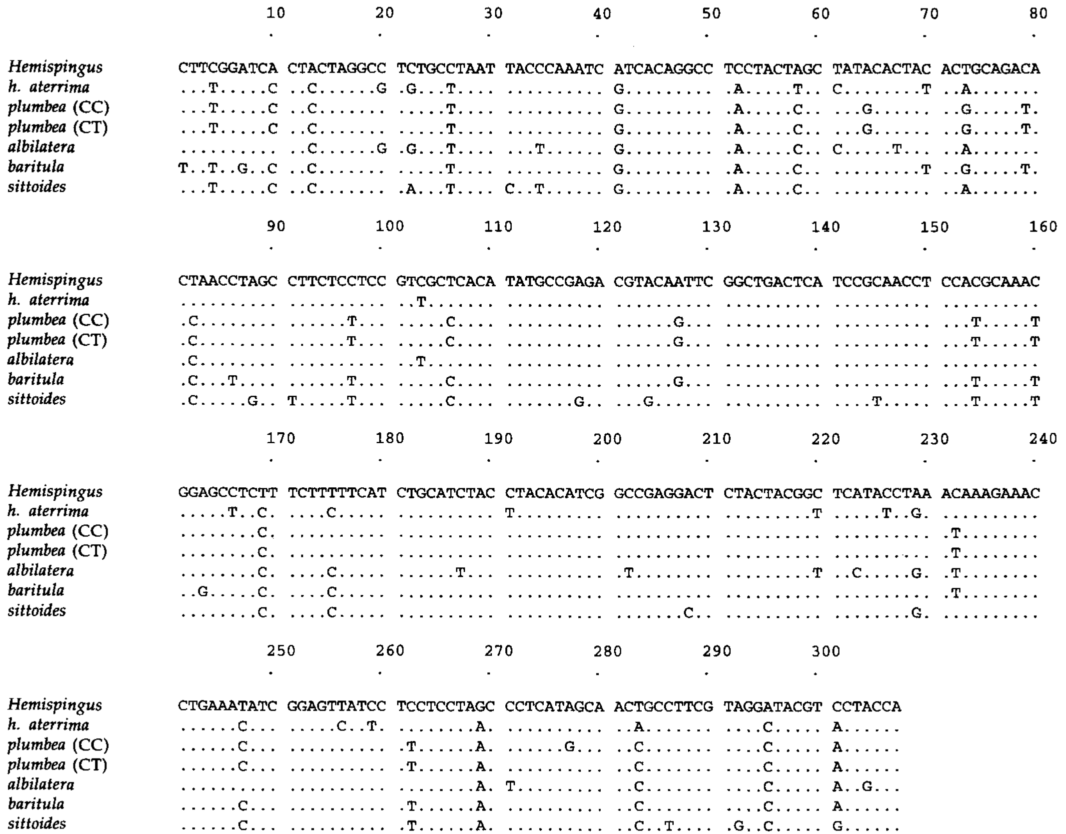


Fig. 3. Mitochondrial cytochrome-*b* sequences for *Diglossa* species. Dots indicate identity to the sequence from the outgroup, *Hemispingus*. CT and CC refer to populations of *D. plumbea* from Cordillera Talamanca and Cordillera Central in Costa Rica. First base listed corresponds to base 14991 in chicken mtDNA sequence (Desjardins and Morais 1990); last base corresponds to base 15297.

allozyme and sequence data sets suggested that *albilatera* was most closely related to *h. aterrima*, a member of the *carbonaria* complex. The placement of *albilatera* with *duidae* would make Vuilleumier's (1969) *carbonaria* and *albilatera* species groups not monophyletic. Both the *albilatera* and *baritula* superspecies complexes exhibit marked sexual dimorphism that is absent in other *Diglossa*. Although this plumage dimorphism suggests monophyly of Vuilleumier's (1969) *albilatera* species group, molecular analyses provide little support for this hypothesis. Also, the plumage analysis that included the presence of sexual dimporphism as a character (Fig. 5) could not recover a sister-taxon relationship between the *albilatera* and *baritula* complexes. It could be that historical speciation events leading to the *albilatera* species group were too closely spaced for there to be molecular characters that document monophyly of the group (Helm-Bychowski and Cracraft 1993, Lanyon 1988). In addition, *albilatera* is very different in most plumage characteristics from the *baritula* superspecies complex; it is almost completely black (a recurrent color in the *Diglossa* species groups I analyzed), whereas taxa in the *baritula* superspecies are shades of gray above and shades of rufous or gray below (Appendix). The placement of *D. albilatera* and, thus, the monophyly of the *albilatera* species group should be clarified by adding more molecular characters, especially DNA sequences, and by adding *D. venezuelensis* (the other member of Vuilleumier's *albilatera* superspecies complex), and perhaps by including more taxa in the *carbonaria* and *lafresnayii* complexes. In addition, it is possible that *Hemispingus* is too distant an outgroup for the *Diglossa* taxa sequenced in this study. Having too distant

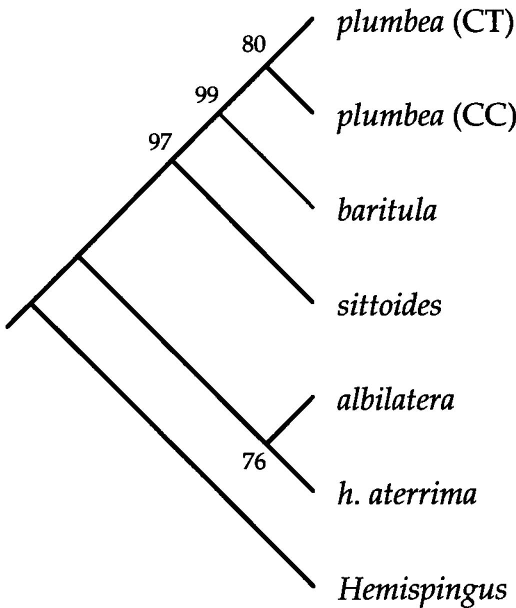


Fig. 4. Parsimony analysis of *Diglossa* mitochondrial cytochrome-*b* sequences (Fig. 3). Numbers at nodes indicate percent of times that node is held in a bootstrap analysis.

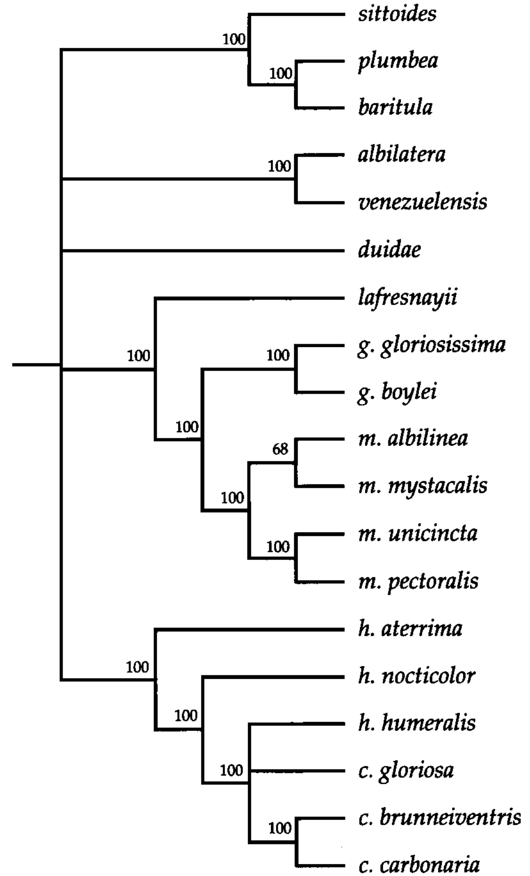


Fig. 5. Parsimony analysis of male plumage characteristics of *Diglossa* species. Numbers at nodes indicate percent of most-parsimonious trees with that particular node.

an outgroup has been suggested to cause problems for recovering avian phylogenies with DNA sequence data (Helm-Bychowski and Cracraft 1993). In my study, this may explain the *carbonaria*/*albilatera* sister-taxon relationship favored by the sequence data.

Despite uncertainty about the sister taxon of the *baritula* superspecies complex, there is strong molecular and morphological support for the monophyly of this complex and for relationships among species within it (Figs. 1, 2, 4, and 5). *Diglossa plumbea* and *D. baritula* are sister taxa, with *D. sittoides* as the sister taxon to the *plumbea*/*baritula* clade. These species are distinct from each other based on morphological (plumage) characteristics, allozyme characteristics, mtDNA sequences, and have disjunct ranges. Thus, I see no reason that these taxa should be considered subspecies of a single species (*Diglossa baritula*), and I concur with Vuilleumier (1969), the A.O.U. (1983) *Check-list*, and Sibley and Monroe (1990) in recognizing species status for these three taxa.

The phylogeny contributes insight into plumage evolution in the *D. baritula* superspecies. Male *sittoides* and *baritula* resemble each other in having rufous underparts (although the shading of rufous is different; *D. baritula* is darker), relative to the gray underparts of *plumbea.* This rufous color is either an ancestral trait retained by *sittoides* and *baritula* and lost in *plumbea,* or arose independently in both *sittoides* and *baritula.* The two taxa that share rufous underparts are not each others' closest relatives (Figs. 1, 2, 4, and 5). Other plumage characteristics support a sister-taxon relationship between *plumbea* and *baritula;* both species have gray throats, gray backs, gray rumps, and have a cap that contrasts in color with the back.

Biogeography.—The phylogeny can also provide a framework for understanding historical biogeography, and genetic distance data can permit rough estimations of the timing of divergence events. *Diglossa* flowerpiercers are high-elevation species restricted to montane

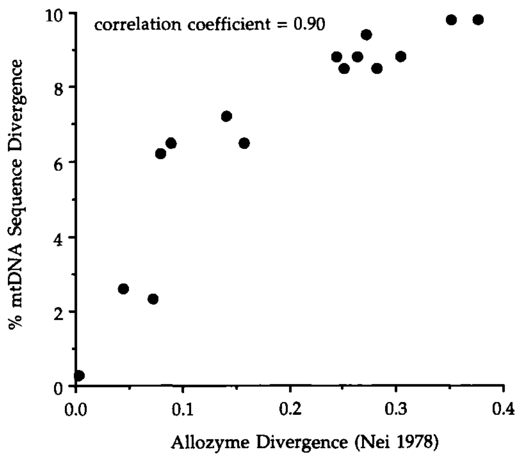


Fig. 6. Percent sequence divergence (Table 4) versus allozyme genetic distance (Nei 1978; Table 2) among *Diglossa* species.

regions in the Neotropics (Vuilleumier 1969, Graves 1980, 1982, 1990, 1991, A.O.U. 1983, Isler and Isler 1987). *Diglossa baritula* is endemic to the mountains of Mexico, Guatemala, and Honduras, with two disjunct populations on either side of the Isthmus of Tehuantepec. *Diglossa plumbea* has two disjunct populations on mountains in Costa Rica and western Panama, and *D. sittoides* is widespread throughout the Andes, from extreme northwestern Venezuela to Argentina. There are large gaps of nonmontane (unsuitable) habitat separating the ranges of these three species.

The landbridge connection between southern Central America and South America was completed three to five million years ago (Malfait and Dinkleman 1972, Pindell and Dewey 1982). One hypothesis explaining the distribution of some Central American highland birds is that South American taxa dispersed into Central America after the landbridge was completed (Chapman 1917, Vuilleumier 1969). During glacial periods in the mountains of Central and South America, it is generally accepted that montane forests were depressed in elevation and, thus, were more continuous in distribution than they are today (Vuilleumier 1969, Van der Hammen 1974, Graves 1982, Liu and Colinvaux 1985, Haffer 1987). This connection of high-elevation habitats could have provided a dispersal route for montane species from South America through southern Central America and into Mexico. During interglacial times, the high-elevation forests retreated, and forest connections were severed. This vicariant event effected the separation of ranges of taxa that had dispersed during the glacial period.

Given the pattern of phylogeny of the *D. baritula* superspecies complex, I hypothesize the following biogeographic scenario. The sister taxa of the *baritula* complex (Figs. 1, 2, 4, and 5) are found in highland regions of South America, particularly in the Andes; thus, I hypothesize that the group originated in the Andes. This also is supported by *sittoides* (the Andean member of the *baritula* complex) being basal to the two Central American members of the complex. I hypothesize that the ancestor to the *baritula* superspecies complex was in the Andes before the landbridge connection was formed. Climatic changes during the last two million years caused temporary connections between high-elevation forests in South and Central America, and the ancestor of the *baritula* complex spread northward through Central America into Mexico. During interglacial times, forests retreated, ranges of the *baritula* complex were severed, and speciation via vicariance resulted. A northward movement of taxa is supported by the phylogeny I have presented—the most basal member of the group is found in South America (*sittoides*) and the more northern forms (*plumbea* and *baritula*) are sister taxa. The alternative of a southward movement would require that the northern species, *baritula*, be basal to the other two species. This hypothesis would add eight steps to the mtDNA phylogeny (Fig. 4), an increase of 11%, and is not suggested by analyses of any of the data sets I gathered. The hypothesis of a northward movement of montane taxa and subsequent vicariance presents a testable hypothesis for other taxa.

The potential to date approximate splitting events using molecular clocks has not been widely explored (e.g. Murphy 1983, Cadle 1985, Zink 1988, Zink and Avise 1990, Hackett 1993), but two calibrations for allozyme data estimate that one unit of Nei's (1978) genetic distance corresponds to roughly 19 to 26 million years of independent evolution (Gutiérrez et al. 1983, Marten and Johnson 1986). For the biogeographic scenario outlined above, allozyme distance data suggest that the *D. baritula* complex has been evolving independently for approximately five to seven million years. Divergence among *D. sittoides, D. plumbea,* and *D. baritula* occurred during the last two million years dur-

ing the Pleistocene. Thus, the northward movement of the ancestor to the *D. baritula* complex, and subsequent speciation within the complex, occurred after the landbridge was completed between southern Central and South America.

ACKNOWLEDGMENTS

This research was supported by grants from the Fugler Fellowship of the Louisiana State University Museum of Natural Science, the Chapman Fund of the American Museum of Natural History, the American Ornithologists' Union, National Sigma Xi, and the National Science Foundation (NSF Doctoral Dissertation Improvement Grant, BSR-9101289). I am grateful to J. M. Bates, G. R. Graves, C. S. Griffiths, M. S. Hafner, D. P. Pashley, R. L. Chapman, J. V. Remsen, K. V. Rosenberg, and R. M. Zink for helpful comments on this manuscript. Also, I wish to thank the many institutions and governmental agencies in Mexico, Costa Rica, Ecuador, Venezuela, Peru, and Bolivia for their assistance in obtaining specimens used in this study.

LITERATURE CITED

ALLARD, M. W., D. L. ELLSWORTH, AND R. L. HONEYCUTT. 1991. The production of single-stranded DNA suitable for sequencing using the polymerase chain reaction. BioTechniques 10:24-26.

AMERICAN ORNITHOLOGISTS' UNION. 1983. Check-list of North American birds, 6th ed. American Ornithologists' Union, Washington, D.C.

AQUADRO, C. F., AND J. C. AVISE. 1982. Evolutionary genetics of birds. VI. A reexamination of protein divergence using varied electrophoretic conditions. Evolution 30:1003-1019.

ARCHIE, J. W., C. SIMON, AND A. MARTIN. 1989. Small sample size does decrease the stability of dendrograms calculated from allozyme-frequency data. Evolution 43:678-683.

BATES, J. M. 1993. The genetic effects of forest fragmentation on Amazonian forest birds. Ph.D. dissertation, Louisiana State Univ., Baton Rouge.

BATES, J. M., AND R. M. ZINK. 1994. Evolution into the Andes: Evidence for species relationships in the genus *Leptopogon*. Auk 111:507-515.

BOCK, W. A. 1985. Is *Diglossa* (?Thraupinae) monophyletic? Pages 319-332 *in* Neotropical ornithology (P. A. Buckley, M. S. Foster, E. S. Morton, R. S. Ridgely, and F. G. Buckley, Eds.). Ornithol. Monogr. 36.

BUTH, D. G. 1984. The application of electrophoretic data in systematic studies. Annu. Rev. Ecol. Syst. 15:501-522.

CADLE, J. E. 1985. The Neotropical colubrid snake fauna (Serpentes: Colubridae): Lineage components and biogeography. Syst. Zool. 34:1-20.

CAPPARELLA, A. P. 1987. Effects of riverine barriers on genetic differentiation of Amazonian forest undergrowth birds. Ph.D. dissertation, Louisiana State Univ., Baton Rouge.

CAPPARELLA, A. P. 1988. Genetic variation in Neotropical birds: Implications for the speciation process. Pages 1658-1664 *in* Acta XIX Congressus Internationalis Ornithologici (H. Ouellet, Ed.). Ottawa, Ontario, 1986. National Museum of Natural Science, Ottawa.

CHAPMAN, F. M. 1917. The distribution of the birdlife in Colombia: A contribution to a biological survey of South America. Bull. Am. Mus. Nat. Hist. 36.

DESJARDINS, P., AND R. MORAIS. 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., P. ARCTANDER, AND A. C. WILSON. 1991. Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. Proc. R. Soc. Lond. B 243:99-107.

FARRIS, J. S. 1972. Estimating phylogenetic trees from distance matrices. Am. Nat. 106:645-668.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 79:783-791.

GORMAN, G. C., AND J. RENZI, JR. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. Copeia 1979:242-249.

GRAVES, G. R. 1980. A new subspecies of *Diglossa (carbonaria) brunneiventris*. Bull. Br. Ornithol. Club. 100:230-232.

GRAVES, G. R. 1982. Speciation in the Carbonated Flower-Piercer (*Diglossa carbonaria*) complex of the Andes. Condor 84:1-14.

GRAVES, G. R. 1990. A new subspecies of *Diglossa gloriosissima* (Aves:Thraupinae) from the western Andes of Colombia. Proc. Biol. Soc. Wash. 103: 962-965.

GRAVES, G. R. 1991. Bergmann's rule near the equator: Latitudinal clines in body size of an Andean passerine bird. Proc. Natl. Acad. Sci. USA 88:2322-2325.

GUTIÉRREZ, R. J., R. M. ZINK, AND S. Y. YANG. 1983. Genic variation, systematic, and biogeographic relationships of some galliform birds. Auk 100: 33-47.

HACKETT, S. J. 1989. Effects of varied electrophoretic conditions on detection of evolutionary patterns in the Laridae. Condor 91:73-90.

HACKETT, S. J. 1992. Molecular phylogenies and biogeography of Central American birds. Ph.D. dissertation, Louisiana State Univ., Baton Rouge.

HACKETT, S. J. 1993. Phylogenetic and biogeographic relationships in the Neotropical genus *Gymnopithys* (Formicariidae). Wilson Bull. 105:301-315.

HACKETT, S. J., AND K. V. ROSENBERG. 1990. Evolution

of South American antwrens (Formicariidae): Comparison of phenotypic and genetic differentiation. Auk 107:473–489.

HAFFER, J. 1987. Quaternary history of tropical America. Pages 1–18 *in* Biogeography and Quaternary history in tropical America (T. C. Whitmore and G. T. Prance, Eds.). Clarendon Press, Oxford.

HELLMAYR, C. E. 1935. Catalogue of birds of the Americas. Part 8, Alaudidae-Compsothlypidae. Publ. Field Mus. Nat. Hist., Zool. Ser., vol. 13.

HELM-BYCHOWSKI, K., AND J. CRACRAFT. 1993. Recovering phylogenetic signal from DNA sequences: Relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-*b* gene. Mol. Biol. Evol. 10:1196–1214.

HIGHTON, R. 1994. Relationship between the number of loci and the statistical support for the topology of UPGMA trees obtained from genetic distance data. Mol. Phylogen. Evol. 2:337–343.

HILLIS, D. M., A. LARSON, S. K. DAVIS, AND E. A. ZIMMER. 1990. Nucleic acids III: Sequencing. Pages 318–370 *in* Molecular systematics (D. M. Hillis and C. Moritz, Eds.). Sinauer Associates, Sunderland, Massachusetts.

ISLER, M. L. AND P. R. ISLER. 1987. The tanagers: Natural history, distribution, and identification. Smithsonian Institution Press, Washington, D. C.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196–6200.

LANYON, S. M. 1988. The stochastic mode of molecular evolution: What consequences for systematic investigations? Auk 105:565–573.

LIPSCOMB, D. 1992. Parsimony, homology and the analysis of multistate characters. Cladistics 8:45–65.

LIU, K.-B., AND P. COLINVAUX. 1985. Forest changes in the Amazon Basin during the last glacial maximum. Nature 318:556–557.

MADDISON, D. R. 1994. Phylogenetic methods for inferring the evolutionary history and process of change in discretely valued characters. Annu. Rev. Entomol. 39:267–292.

MALFAIT, B. J., AND M. G. DINKLEMAN. 1972. Circum-Caribbean tectonic and igneous activity and the evolution of the Caribbean plate. Geol. Soc. Am. Bull. 83:251–272.

MARTEN, J. A., AND N. K. JOHNSON. 1986. Genetic relationships of North American cardueline finches. Condor 88:409–420.

MICKEVICH, M. M., AND C. M. MITTER. 1981. Treating polymorphic characters in systematics: A phylogenetic treatment of electrophoretic data. Pages 45–58 *in* Advances in cladistics, vol. 1 (V. A. Funk and D. R. Brooks, Eds.). New York Botanical Gardens, New York.

MURPHY, R. W. 1983. Paleobiogeography and genetic differentiation of the Baja California herpetofauna. Occas. Pap. Calif. Acad. Sci. 137:1–48.

MURPHY, R. W. 1993. The phylogenetic analysis of allozyme data: Invalidity of coding alleles by presence/absence and recommended procedures. Biochem. Syst. Ecol. 21:25–38.

MURPHY, R. W., J. W. SITES, JR., D. G. BUTH, AND C. H. HAUFLER. 1990. Proteins 1: Isozyme electrophoresis. Pages 45–126 *in* Molecular systematics (D. M. Hillis and C. Moritz, Eds.). Sinauer Associates, Sunderland, Massachusetts.

NEI, M. 1978. Estimation of average heterozygosities and genetic distance from a small number of individuals. Genetics 89:583–590.

NEI, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

NEIGEL, J., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pages 515–534 *in* Evolutionary processes and theory (E. Nevo and S. Karlin, Eds.). Academic Press, New York.

PAMILO, P., AND M. NEI. 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 3:254–259.

PATTERSON, C. 1982. Morphological characters and homology. Pages 21–74 *in* Problems of phylogenetic reconstruction (K. A. Joysey, Ed.). Academic Press, London.

PETERSON, A. T. 1992. Phylogeny and rates of molecular evolution in the *Aphelocoma* jays (Corvidae). Auk 109:133–147.

PETERSON, A. T., P. ESCALANTE P., AND A. NAVARRO S. 1992. Genetic differentiation in Mexican populations of Common Bush-Tanagers and Chestnut-capped Brush-Finches. Condor 94:244–253.

PINDELL, J., AND J. F. DEWEY. 1982. Permo-Triassic reconstruction of Western Pangea and the evolution of the Gulf of Mexico/Caribbean region. Tectonics 1:179–211.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. Univ. Texas Publ. No. 7213:145–153.

ROGERS, J. S., AND R. C. CASHNER. 1987. Genetic variation, divergence, and relationships in the subgenus *Xenisma* of the genus *Fundulus*. Pages 251–264 *in* Community and evolutionary ecology of North American stream fishes (W. J. Matthews and D. C. Heins, Eds.). Univ. Oklahoma Press, Norman.

SANDERSON, M. J. 1989. Confidence limits on phylogenies: The bootstrap revisited. Cladistics 5:113–130.

SEUTIN, G., J. BRAWN, R. E. RICKLEFS, AND E. BERMINGHAM. 1993. Genetic divergence among popu-

lations of a tropical passerine, the Streaked Saltator (*Saltator albicollis*). Auk 110:117-126.

SIBLEY, C. G., AND J. E. AHLQUIST. 1990. Phylogeny and classification of birds: A study in molecular evolution. Yale Univ. Press, New Haven, Connecticut.

SIBLEY, C. G., AND B. L. MONROE, JR. 1990. Distribution and taxonomy of birds of the world. Yale Univ. Press, New Haven, Connecticut.

SWOFFORD, D. L. 1981. On the utility of the distance Wagner procedure. Pages 25-43 *in* Advances in cladistics, vol. 1 (V. A. Funk and D. R. Brooks, Eds.). New York Botanical Gardens, New York.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony, ver. 3.1.1. Illinois Natural History Survey, Champaign.

SWOFFORD, D. L., AND S. H. BERLOCHER. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36:293-325.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-283.

VAN DER HAMMEN, T. 1974. The Pleistocene changes of vegetation and climate in tropical South America. J. Biogeogr. 1:3-26.

VUILLEUMIER, F. 1969. Systematics and evolution in *Diglossa* (Aves, Coerebidae). Am. Mus. Novit. 2381.

ZINK, R. M. 1988. Evolution of brown towhees: Allozymes, morphometrics and species limits. Condor 90:72-82.

ZINK, R. M., AND J. C. AVISE. 1990. Patterns of mitochondrial DNA and allozyme evolution in the avian genus *Ammodramus*. Syst. Zool. 39:148-161.

APPENDIX. Character-state descriptions for male plumages of *Diglossa* species.

Taxon	Sexual dimorphism	Belly color	Throat color	Breast color	Breast band	Back color	Contrasting back and cap	Rump color	Vent color	Malar stripe	Presence of blue/gray humeral patch	Presence of white underwing patch
sittoides	Yes	Light rufous	Light rufous, buffy	Light rufous	No	Solid gray-blue	No	Solid gray-blue	Light rufous, buffy	No	No	No
baritula	Yes	Rufous	Gray	Rufous	No	Gray	Yes	Dark gray	Rufous	No	No	No
plumbea	Yes	Gray	Gray	Gray	No	Gray	Yes	Dark gray	Gray	No	No	No
albilatera	Yes	Black	Black	Black	No	Black	No	Black	Black	No	No	Yes
venezuelensis	Yes	Black	Black	Black	No	Black	No	Black	Black	No	No	Yes
duidae	No	Mottled black and gray	Black	Black	No	Black	No	Black	Mottled black and gray	No	No	No
carbonaria gloriosa	No	Chestnut	Black	Chestnut	No	Black	No	Gray tinged	Chestnut	No	Yes	No
humeralis nocticolor	No	Black	Black	Black	No	Black	No	Gray tinged	Black	No	No	No
humeralis humeralis	No	Black	Black	Black	No	Black	No	Gray tinged	Black	No	Yes	No
humeralis aterrima	No	Black	Black	Black	No	Black	No	Black	Black	No	No	No
carbonaria brunneiventris	No	Rufous	Black	Rufous	No	Black	No	Extensive gray tinged	Rufous	Rufous	Yes	No
carbonaria carbonaria	No	Gray mottled with black	Black	Mainly black, gray mottled with black	No	Black	No	Extensive gray tinged	Rufous	No	Yes	No
lafresnayii	No	Black	Black	Black	No	Black	No	Faint blue-gray tinged (in some specimens, absent in others)	Black	No	Yes	No
mystacalis unicincta	No	Rufous in center, black tinged with gray towards sides	Black	Black	Yes	Black	No	Blue-gray tinged	Rufous	Whitish, blending to buffy at breast band	No	No
mystacalis pectoralis	No	Rufous in center, black tinged with gray towards sides	Black	Black	Yes	Black	No	Blue-gray tinged	Rufous	Whitish, blending to buffy at breast band	No	No
mystacalis albilinea	No	Black	Black	Black	No	Black	No	Blue-gray tinged	Rufous	Whitish, blending to buffy at breast band	Yes	No
mystacalis mystacalis	No	Black	Black	Black	No	Black	No	Blue-gray tinged	Rufous	Whitish, blending to buffy at breast band	Yes	No
gloriosissima gloriosissima	No	Rufous with lack mottling	Black	Black	No	Black	No	Faint blue-gray tinged	Rufous with black mottling	No	Yes	No
gloriosissima boyleii	No	Rufous	Black	Black	No	Black	No	Faint blue-gray tinged	Rufous	No	Yes	No