NUCLEAR DNA EVOLUTION AND PHYLOGENY OF THE NEW WORLD NINE-PRIMARIED OSCINES

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ABSTRACT.—Estimates of phylogeny were derived from measures of dissimilarity of single-copy nuclear-DNA sequences for 13 species that represent the currently recognized major groups of New World nine-primaried oscines and an outgroup (*Passer*). The dissimilarity coefficients (delta mode and delta $T_{50}H$) calculated from thermal dissociation curves of reassociated DNA sequences exhibited the properties of a metric. No statistically significant increase in goodness-of-fit of the raw data to a phylogeny estimated from a least-squares analysis of the 13 × 13 matrix of distances was achieved when the lengths of sister branches were allowed to vary. "Jackknife" and negative branch-length analyses identified unstable stems that resulted from non-additivity caused in part by measurement error. Such stems were collapsed to produce a more robust topology, which served as the basis for estimating the positions of taxa not included in the 13 × 13 matrix.

The clade that subsumed several "typical" tanagers (e.g. *Tachyphonus rufus*) also included *Sicalis luteola* and *Diuca diuca* (usually allied with the North American emberizine sparrows); *Cyanerpes cyaneus*, two species of *Diglossa*, and *Coereba flaveola* (often split among several major groups); and *Tersina viridis*, *Catamblyrhynchus diadema*, and *Nephelornis oneilli* (whose affinities are often considered uncertain). This "tanager" clade and its sister group, the cardinals (represented by *Cardinalis cardinalis*), together formed one fork of a trichotomy. Several emberizine sparrows (e.g. *Pooecetes gramineus*) formed the second fork, and wood-warblers (e.g. *Dendroica striata*) and New World orioles (e.g. *Psarocolius angustifrons*) formed the third. The chaffinches (represented by *Fringilla coelebs*) and several cardueline finches (e.g. *Carduelis pinus*) together formed the sister group of the other New World nine-primaried oscines included in the study. This phylogeny implies that convergence in feeding specializations among lineages is more extensive than traditional arrangements of the assemblage would suggest. *Received 27 October 1987, accepted 18 March 1988.*

MOST workers include in the "New World nine-primaried oscines" various combinations of: vireos (Vireonidae); wood-warblers (Parulinae); bananaquits (Coerebinae); tanagers (Thraupinae); cardinals (Cardinalinae); emberizine sparrows (Emberizinae); New World orioles (Icterinae); Hawaiian honeycreepers (Drepanidinae); chaffinches (Fringillinae); and cardueline finches (Carduelinae). The Swallow-Tanager (*Tersina viridis*), Plush-capped Finch (*Catamblyrhynchus diadema*), and Pardusco (*Nephelornis oneilli*, Lowery and Tallman 1976) are included, but their relationships have been considered particularly uncertain (family and subfamily names, A.O.U. 1983; for a similar classification that includes South American and Old World species, see Paynter 1968, Paynter and Storer 1970). The monophyly of the New World nine-primaried assemblage as traditionally construed was rigorously tested when Raikow (1978) performed a cladistic analysis that delimited the assemblage and excluded the vireos. DNA (Sibley and Ahlquist 1982a) and protein studies (Johnson et al. 1988) also indicate that the vireos are not part of the monophyletic New World nine-primaried assemblage. However, these studies have not broadly resolved phylogenetic relationships within the assemblage.

I present herein the results of DNA hybridization comparisons among members of the New World nine-primaried assemblage and estimate their phylogenetic relationships from the DNA comparisons. I used the thermal stability of reassociated single-copy nuclear-DNA (scnDNA) to calculate dissimilarity values that reflect the extent of divergence of single-copy nuclear genomes since the genetic isolation of lineages from one another. I then examined the mathematical properties of the dissimilarity

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values, used a method of phylogenetic inference suitable for these properties, and investigated how potential sources of error might affect the resulting phylogenetic estimates.

I constructed a matrix of distances from all pairwise comparisons among a set of species containing an outgroup (*Passer*) and 12 New World nine-primaried oscines. These species were selected as sources of DNA for radioactive labeling based on a review of the history of classification of the assemblage (Bledsoe 1984). At least one representative of each of the taxa listed above was included except the vireos and Hawaiian honeycreepers. In addition, I compared the scnDNAs of the labeled species to DNA of 16 species not used in radiolabeling, to expand the estimate of phylogeny to include one species of Hawaiian honeycreeper and 15 other New World nine-primaried oscines.

Methods

Biochemical techniques.—I measured scnDNA sequence differences among 13 species (Table 1) by DNA-DNA hybridization, using sheared, radioiodine-labeled scnDNA fractions hybridized with excesses of unfractionated, sheared nuclear-DNA chromatographed on hydroxyapatite across a thermal gradient. Details of the methodology are given by Bledsoe (1984).

DNA samples were prepared from preserved erythrocytes (most species) or liver and kidney tissues (*Fringilla coelebs* only) pooled from 1–4 individuals. One DNA sample was prepared for each species except *Cardinalis cardinalis*, for which two samples were prepared. One sample of radioiodine-labeled scnDNA was prepared for each species (Table 1) except *Tachyphonus rufus* and *C. cardinalis*, for which 2 were prepared, and for 3 species of *Passer*, for which DNA was not radioactively labeled.

Data analysis.—The raw data consisted of the number of radioactive counts representing scnDNA sequences eluted at each of 17 temperatures from 55.0–95.0 C at 2.5 C increments. The sum of the radioactive counts eluted at the 55.0, 57.5, and 60.0 C steps, minus the radioactive coounts contributed by ^{125}I not bound to scnDNA, represented sequences that did not hybridize at the temperature of incubation (60.0 C). The radioactive counts eluted at each temperature from 62.5–95.0 C represented the dissociation of scnDNA sequences that formed stable hybrids during incubation. The data were analyzed on an IBM 3170 computer with a non-linear, least-squares regression program written by T. F. Smith. The program used the raw experimental counts to fit the portion of the thermal dissociation curve above the incubation temperature to a modified Fermi-Dirac distribution (discussed by Sibley and Ahlquist 1983), which describes

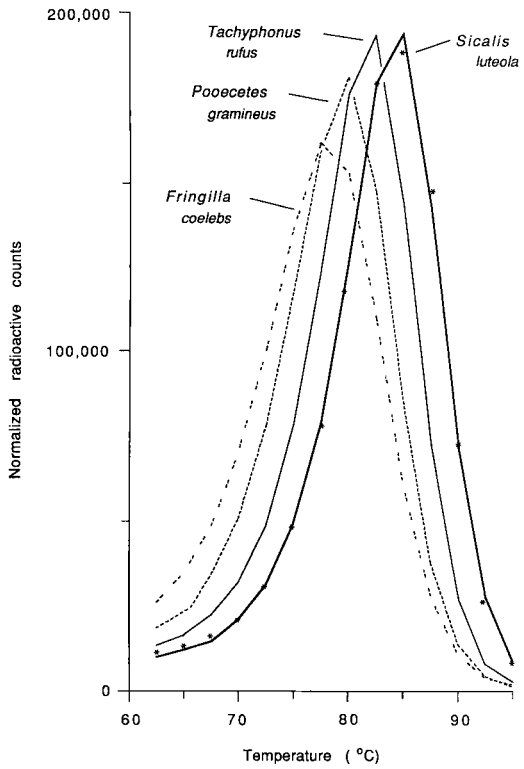

Fig. 1. Thermal dissociation curves for radioiodine-labeled scnDNA of *Sicalis luteola* hybridized to excesses of unfractionated, unlabeled nuclear DNA of *Tachyphonus rufus*, *Pooecetes gramineus*, and *Fringilla coelebs*. For each curve, the lines connect values fitted to a modified Fermi-Dirac distribution. For the conspecific (*Sicalis*) curve, the experimental values are denoted by asterisks. The Fermi-Dirac fitted modes of the curves are: 84.35 (*Sicalis*), 81.55 (*Tachyphonus*), 79.72 (*Pooecetes*), and 78.22 (*Fringilla*).

DNA-DNA dissociation curves of closely similar genomes (Fig. 1). The mode of the fitted distribution provided a measure of temperature of dissociation of reassociated sequences. For each experimental set, the interspecific mode was subtracted from the conspecific mode to yield the delta mode for each DNA-DNA hybrid, which provided a measure of dissimilarity of the reassociated sequences. In addition, the data were used to calculate another statistic, delta $T_{50}H$, by linear-interpolation method (Bledsoe and Sheldon MS). Delta $T_{50}H$ is a measure of the median dissimilarity of sequences, including those that did not hybridize. For most experiments, at least two conspecific DNA-DNA hybrids were included, and their average mode and $T_{50}H$ were used to calculate delta values. The delta values of replicated interspecific comparisons were averaged to yield mean dissimilarity values for most pairwise combinations of taxa (Table 1).

TABLE 1. Folded matrices of mean delta mode (below diagonal) and delta $T_{50}H$ values (above diagonal) among 12 species of New World nine-primaried oscines and an outgroup (*Passer*). The mean is followed by the standard deviation, below which the sample sizes of the comparisons are given.[1]

Species	NO	CD	SL	TR	TV	CC
Nephelornis oneilli	—	3.0 ± 0.35 (1, 1)	3.6 ± 0.21 (1, 1)	3.8 ± 0.36 (1, 2)	4.1 ± 0.14 (1, 1)	5.1 ± 0.66 (2, 2)
Catamblyrhynchus diadema	2.8 ± 0.07 (1, 1)	—	3.7 ± 0.85 (2, 2)	4.1 ± 0.21 (1, 1)	4.2 ± 0.07 (1, 1)	5.2 ± 0.50 (1, 1)
Sicalis luteola	3.0 ± 0.28 (1, 1)	3.2 ± 0.85 (2, 1)	—	3.9 ± 0.15 (1, 6)	4.1 ± 0.42 (2, 0)	5.5 ± 1.13 (1, 1)
Tachyphonus rufus	3.7 ± 1.04 (2, 2)	3.6 ± 0.00 (1, 1)	3.6 ± 0.21 (1, 6)	—	3.9 ± 0.17 (2, 5)	5.6 ± 0.67 (1, 3)
Tersina viridis	3.8 ± 0.07 (1, 1)	4.3 ± 0.28 (1, 1)	3.7 ± 0.28 (2, 0)	3.8 ± 0.26 (5, 2)	—	5.4 ± 0.35 (2, 1)
Cardinalis cardinalis	5.1 ± 0.70 (2, 2)	5.0 ± 0.57 (1, 1)	5.3 ± 0.67 (1, 2)	5.2 ± 0.51 (3, 1)	5.0 ± 1.02 (2, 2)	—
Junco hyemalis	5.3 ± 0.29 (2, 1)	5.4 ± 0.00 (1, 1)	5.7 (0, 1)	5.8 ± 0.42 (1, 1)	5.9 ± 1.01 (1, 2)	5.6 ± 0.21 (2, 2)
Pooecetes gramineus	5.6 ± 0.17 (2, 1)	5.6 ± 0.52 (2, 1)	5.3 ± 0.87 (2, 2)	5.7 ± 0.39 (2, 5)	5.0 (1, 0)	5.9 ± 0.55 (2, 1)
Dendroica striata	5.0 ± 0.70 (2, 0)*	5.5 ± 0.44 (5, 1)	5.5 (1, 0)	5.6 ± 0.11 (0, 5)	5.0 (1, 0)*	6.0 ± 0.07 (1, 1)
Psarocolius angustifrons	5.3 (1, 0)*	5.3 ± 0.34 (5, 1)	4.9 (1, 0)*	5.3 ± 0.35 (4, 5)	5.2 (1, 0)*	5.4 ± 0.23 (4, 1)
Fringilla coelebs	6.1 (0, 1)	6.2 ± 0.00 (1, 1)	6.3 ± 0.15 (1, 2)	6.2 ± 0.11 (2, 3)*	6.6 ± 0.14 (1, 1)	6.6 ± 0.75 (4, 1)
Carduelis pinus	5.9 (0, 1)*	6.0 (1, 0)	6.2 (1, 0)*	6.0 ± 0.15 (2, 1)*	6.8 ± 1.20 (0, 2)*	6.9 (0, 1)
Passer (species)	7.7 (0, 1)	6.9 ± 0.14 (0, 2)	7.2 ± 0.71 (0, 2)	7.2 (0, 1)	7.7 ± 0.99 (0, 2)	7.5 ± 0.00 (0, 2)

[1] The first value in parentheses refers to the sample size of the comparison using labeled DNA of the species at the side of the matrix; the second value refers to the sample size of the comparison using labeled DNA of the species at the top of the matrix. The asterisks indicate replacement values (see Methods).

The data presented here were originally reported in an unpublished thesis (Bledsoe 1984), but were reanalyzed for this study using more robust methods. The raw data and a complete description of the data analysis are available upon request.

Data correction.—In 5 of 31 experimental sets, conspecific mode or $T_{50}H$ values differed markedly between sets using the same radioactive DNA sample while interspecific values did not. In such instances, the conspecific comparison was corrected (Bledsoe 1987a) to rectify the overall scale of delta values between experiments. This method was unlikely to alter branching patterns because the ranks of interspecific modes and $T_{50}H$ values of such experiments were generally stable. The correction removed some discrepancies, but other delta values nonetheless differed by 1.5 or greater from the mean of other clumped replicate values and were excluded from the cell means (Table 1). When it was unclear which value among few replicates was aberrant, the value with the poorest Fermi-Dirac fit was excluded. Delta $T_{50}H$ values were occasionally grossly discrepant because their percent reassociations (normalized to the conspecific comparison) differed by greater than 10%. For these, an approximate correction was used, in which the normalized percent reassociation (NPR) of one comparison was set equal to that of the comparison with an NPR closest to 95%, the average value expected of delta modes in the range of 3–7 and close to most NPR values in this study (Bledsoe unpubl. data).

Delta values were not available for several comparisons because sequences failed to hybridize properly, the experimental apparatus malfunctioned, or taxa of interest were not available initially. Because the algorithm for estimating phylogeny requires a dissimilarity value for each pairwise comparison, the following replacements were used to complete the matrix: *Icterus nigrogularis* (in place of *Psarocolius angustifrons*) for 3 comparisons (to *Tersina viridis*, *Nephelornis oneilli*, and *Sicalis luteola*); *Vermivora pinus* (in place of *Dendroica striata*) for 3 comparisons (to *T. viridis*, *N. oneilli*, and *Passer domesticus*); and *Carduelis tristis* (in place of *C. pinus*), *Carpodacus mexicanus* (in place of *C. pinus*), *S. columbiana* (in place of *S. luteola*), and *Plocepasser mahali* (in place of *Passer* sp.) for 1 comparison each (to *N. oneilli*, *T. viridis*, *C. pinus*, and *P. angustifrons*, respectively). No suitable replacements were available for two cells (*T. rufus* to *F. coelebs* and *C. pinus*), for which an average of the values of the sister group of *T. rufus* (*S. luteola*, *C. diadema*, and *N. oneilli*) in a rooted network constructed by UPGMA analysis (using the algorithm described by Sneath and Sokal 1973: 230–234) was used. Replaced values (marked with an asterisk in Table 1) probably did not

TABLE 1. Continued.

JH	PG	DS	PA	FC	CP	PS
6.2 ± 0.25	6.0 ± 0.35	6.0 ± 0.42	5.4	7.3	7.2	8.6
(1, 2)	(1, 2)	(0, 2)*	(0, 1)*	(1, 0)	(1,0)*	(1, 0)
6.1 ± 0.14	6.0 ± 0.55	5.9 ± 0.37	5.9 ± 0.15	7.1 ± 0.14	6.7	7.8 ± 0.28
(1, 1)	(1, 2)	(1, 5)	(1, 5)	(1, 1)	(0, 1)	(2, 0)
5.6 ± 1.48	5.9 ± 0.85	6.0	6.2	7.4 ± 0.17	6.8	7.2 ± 0.64
(2, 0)	(2, 2)	(0, 1)	(0, 1)*	(2, 1)	(0, 1)*	(2, 0)
6.3 ± 0.42	6.3 ± 0.40	6.4 ± 0.11	5.7 ± 0.52	7.3 ± 0.18	6.9 ± 0.33	8.0
(1, 1)	(5.2)	(5, 0)	(5, 4)	(3, 2)*	(1, 2)*	(1, 0)
6.3 ± 0.76	6.3 ± 1.41	6.1	6.3	7.4 ± 0.21	7.3 ± 0.42	8.5 ± 1.06
(2, 1)	(0, 2)	(1, 0)*	(0, 1)*	(1, 1)	(2, 0)*	(2, 0)
6.2 ± 0.06	6.3 ± 0.45	6.1 ± 0.35	5.5 ± 0.10)	7.5 ± 0.44	7.5	8.8 ± 0.71
(1, 2)	(3, 3)	(1, 1)	(1, 4)	(1, 4)	(1, 0)	(2, 0)
—	3.3 ± 0.59	5.2 ± 0.61	5.3 ± 0.10)	7.8	7.8	7.6 ± 0.60
	(0, 4)	(1, 2)	(1, 1)	(1, 0)	(1, 0)	(2, 0)
2.8 ± 0.58	—	5.3 ± 0.48	6.6 ± 0.74	7.6 ± 0.35	7.7	7.3
(4, 0)		(5, 1)	(0, 9)	(1, 1)	(0, 1)	(1, 0)
4.9 ± 0.30	4.9 ± 0.15	—	4.6 ± 0.46	7.8 ± 0.50	7.4	8.0
(2, 1)	(1, 4)		(5, 1)	(1, 1)	(0, 1)	(1, 0)*
5.0 ± 0.07	5.3 ± 0.46	4.1 ± 0.24	—	6.9	6.4	8.1
(2, 0)	(8, 1)	(1, 5)		(0, 1)	(0, 1)	(1, 0)*
6.3	6.9 ± 0.00	6.4 ± 0.63	6.4	—	5.4 ± 0.98	8.0 ± 0.92
(0, 1)	(1, 1)	(1, 1)	(1, 0)		(3, 1)	(2, 0)
6.4	6.8	6.8	6.8	4.8 ± 0.71	—	7.9
(0, 1)	(1, 0)	(1, 0)	(1, 0)	(1, 3)		(1, 0)
7.0	7.0	7.0	6.6	7.3 ± 0.28	7.2	—
(0, 1)	(0, 1)	(0, 1)*	(0, 1)*	(0, 2)	(0, 1)	

greatly affect the estimation of branching pattern because only close relatives of the missing taxa were used and because most (85%) of the dissimilarity measures in Table 1 are original values. Distances between each of the labeled species and 1 of 3 species of *Passer* (*domesticus, melanurus,* or *hispaniolensis*) were included to root the least-squares networks discussed below.

Phylogenetic estimation.—The matrix of pairwise delta mode and delta $T_{50}H$ values (Table 1) was analyzed with the computer program PHYLIP (Phylogenetic Inference Package, version 2.6), written by J. Felsenstein. The KITSCH and FITCH algorithms, which respectively constrained and did not constrain sister branches to be equal in length, were employed to reconstruct phylogenies. Because delta mode variance does not increase with increasing delta mode values (Bledsoe 1987a), and because the correlation between variance and mean delta $T_{50}H$ ($r = 0.116$) was not significantly different from zero ($P > 0.05$), the appropriate measure of the fit of the original distances to the inferred topologies is the least-squares measure of Cavalli-Sforza and Edwards (1967). The SHUFFLE program of PHYLIP was used to vary the order of addition of taxa to search for the topology with the best least-squares fit.

Because the delta values conformed to the axioms of a metric (see Results), negative branch-lengths were not allowed in the initial estimations (Felsenstein 1984). However, such lengths were permitted in a second series of estimations to suggest areas where the topologies might be based on inconsistent (nonadditive) distances. "Jackknife" comparisons (Lanyon 1985), in which each of the labeled taxa was sequentially excluded from the analysis and negative lengths were not permitted, were also performed to identify unstable portions of the topologies.

Branches that were both unstable in jackknife trees and negative in reconstructions permitting such lengths were collapsed in estimating the phylogeny of the species used as sources of DNA for radioactive labeling. Such estimates were based on FITCH analysis, to avoid assumptions about rates of DNA evolution, and on delta mode. The fits of the delta mode trees in general were better than for the delta $T_{50}H$ topologies, even considering that the delta $T_{50}H$ values are roughly 10% larger than delta mode and that the measure of fit is a sum of squares. The lengths of collapsed branches were apportioned to other stems so that as few pairwise distances as possible were altered.

I estimated the phylogenetic positions of species not used as sources of radioactive DNA but for which distances to several labeled taxa were measured by assuming that their rates of DNA evolution were syn-

TABLE 2. Differences between reciprocal delta $T_{50}H$ values calculated for each labeled species.

Labeled species	Mean difference	Standard deviation	Range	*n*
Tersina viridis	0.450	0.451	0.1–1.3	6
Tachyphonus rufus	0.512	0.242	0.2–0.8	8
Catamblyrhynchus diadema	0.410	0.372	0.0–1.0	10
Nephelornis oneilli	0.400	0.245	0.0–0.7	7
Sicalis luteola	0.650	0.547	0.2–1.6	6
Cardinalis cardinalis	0.540	0.460	0.0–1.6	10
Junco hyemalis	0.450	0.476	0.0–1.3	6
Pooecetes gramineus	0.671	0.275	0.4–1.1	7
Dendroica striata	0.550	0.437	0.0–1.1	6
Psarocolius angustifrons	0.475	0.408	0.1–0.9	4
Fringilla coelebs	0.529	0.562	0.0–1.7	7
Carduelis pinus	1.700	—	—	1

chronous or nearly so with those of the labeled taxa, which themselves exhibit closely similar rates (Bledsoe 1987a; see below). Under this assumption, a complete set of distances between unlabeled and labeled species is not required, although such values would be necessary to specify any sister-group relationships between unlabeled taxa only. Unlabeled species were placed on the estimate of branching among labeled taxa (Fig. 3), using the distances of the KITSCH delta-mode tree (to reflect the assumption of synchronous rates). For an unlabeled species, the smallest distance (*d*) to a labeled species (defined as A) was found. If that distance differed from the next largest distance (to a species designated B) by 0.8 or greater (an approximate estimate of error) for both delta mode and delta $T_{50}H$, the unlabeled species was linked to A at a distance *d*. If not, an average was calculated from the distances to the labeled species in the least inclusive set (designated C) of Fig. 3 that contained both A and B. If that average differed from the next largest distance (to a species designated D) by 0.8 or greater, it was linked with inclusive set C. If not, the averaging procedure was repeated until the species was placed. *Diglossa albilatera* and *D. lafresnayii* were considered as a single unit for the analysis. The method worked for all unlabeled species but *Cyanerpes cyaneus*, for which distances were not available to enough taxa to realize a 0.8-unit gap. *C. cyaneus* was placed with the last inclusive set identified in the procedure.

RESULTS

Metric properties.—The mathematical properties of the delta mode values (Table 1) do not violate the axioms of a metric (Bledsoe 1987a). All of the delta $T_{50}H$ values (Table 1) conform to the triangle inequality and the axiom of distinctness. Table 2 lists the average differences of the delta $T_{50}H$ values of reciprocal comparisons. Given the sample sizes and small level of differences, the symmetry was generally good, which suggests the delta $T_{50}H$ values conform to the axiom of symmetry. (See Bledsoe and Sheldon [MS] for a more complete analysis of the metric properties of DNA-DNA hybridization dissimilarity values.)

FITCH and KITSCH trees.—The branching patterns of the KITSCH delta $T_{50}H$ (Fig. 2) and the FITCH and KITSCH delta mode trees (Bledsoe 1987a) were identical. The FITCH delta $T_{50}H$ topology (Fig. 2) differed in placing *Tersina* and *Tachyphonus* as sister groups.

When the branching pattern of the 3 identical topologies was used for the FITCH analysis of delta $T_{50}H$, the resulting tree had a sum of squares (ss) of 14.26. This was statistically not significantly different from that of the topologically identical KITSCH tree (F-ratio test [Felsenstein 1984], ss [FITCH] = 14.26, ss [KITSCH] = 17.61, F-ratio = 1.18, $F_{.05[11,55]} = 1.97$). The value of 14.26 was slightly higher than the ss value (14.03) for the best FITCH delta $T_{50}H$ tree (Fig. 2). The statistical significance of this difference could not be tested because branching patterns differed. The difference between the topologically identical sums of squares of the FITCH and KITSCH delta mode trees was not statistically significant (Bledsoe 1987a).

When negative branches were permitted for the delta mode trees, the FITCH tree exhibited two negative branches (ss = 7.69). The *Fringilla-Carduelis* and *Junco-Pooecetes-Psarocolius-Dendroica* groups formed a clade linked to the other taxa by a short negative branch (−0.47). *Tachyphonus* was linked to the *Tersina-Catamblyrhynchus-Nephelornis-Sicalis* clade by a short negative branch (−0.13). In the KITSCH tree (ss = 11.36), a short negative branch (−0.16) joined the *Dendroica-Psarocolius* and *Pooecetes-Junco* clades, which were successive sister groups of the *Car-*

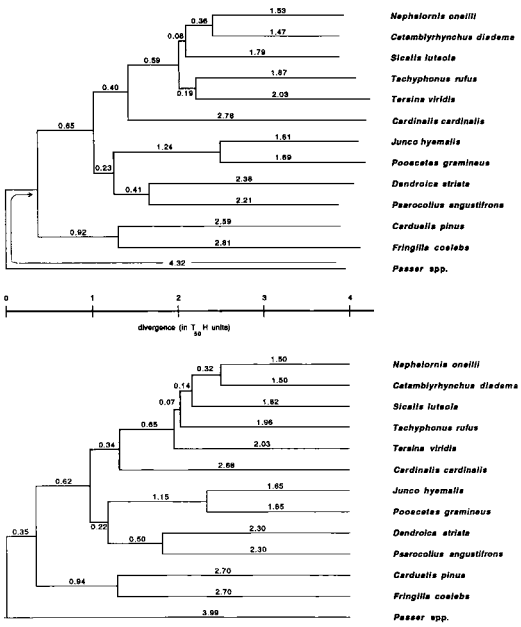

Fig. 2. Least-squares topologies based on the delta $T_{50}H$ values (Table 1) and constructed so that branches from each node are not constrained to be equal (FITCH tree, top) and so that such branches are required to be equal in length (KITSCH tree, bottom). The FITCH tree was rooted to *Passer* (see text for species) and had a ss = 14.03. The KITSCH tree had a ss = 17.61. The numbers next to the stems denote branch-lengths in units of $\Delta T_{50}H$. The axis indicates divergence in $\Delta T_{50}H$ units.

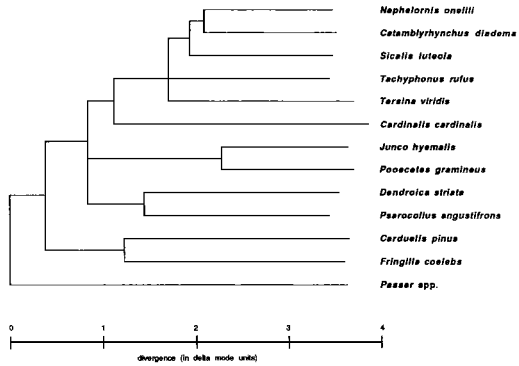

Fig. 3. Estimate of phylogeny among 12 species of New World nine-primaried oscines used as sources of labeled scnDNA. The tree was rooted to *Passer* (see text for species) and was derived from the FITCH delta-mode topology (Bledsoe 1987a, Fig. 1) by collapsing those portions of the tree characterized by instability in "jackknife" and negative-branch comparisons (see Results).

dinalis-tanager group. *Sicalis* and *Catamblyrhynchus* were linked as the sister group of *Nephelornis* by a small negative branch (−0.15).

In the delta $T_{50}H$ trees allowing negative branches, the FITCH tree (ss = 12.27) changed so that the *Pooecetes-Junco* group was the sister group of *Psarocolius*, and was linked as the sister group of *Dendroica* by a negative branch (−0.59). The KITSCH tree (ss = 17.11) also showed this same change, with a slightly smaller negative branch (−0.46). In addition, *Tachyphonus* was linked as the sister group of the *Tersina-Sicalis-Nephelornis-Catamblyrhynchus* clade with a short negative branch (−0.10).

"Jackknife" comparisons.—Jackknifing based on delta mode yielded 11 FITCH trees congruent with the FITCH delta-mode topology (Bledsoe 1987a), the same pattern as the KITSCH tree in Fig. 2. The twelfth tree (with *Pooecetes* omitted) placed *Tersina* and *Tachyphonus* as sister groups. In each analysis, *Passer* was included to root the topology. Twelve KITSCH trees were congruent with the KITSCH delta-mode branching pattern (Bledsoe 1987a), as in Fig. 2. The remaining tree (*Dendroica* omitted) differed and placed *Psarocolius* as the sister group of the clade that included *Cardinalis* and the tanagers. (Because each KITSCH tree is rooted at the topology's midpoint, an outgroup is not required and an additional jackknife tree [with *Passer* omitted] was available.)

Jackknifing based on delta $T_{50}H$ yielded 10 FITCH trees congruent with the FITCH branching pattern (Fig. 2). One tree (*Nephelornis* omitted) differed in placing *Tersina* and *Tachyphonus* as sister groups, which formed the sister group of *Sicalis*, with *Catamblyrhynchus* as the sister group of these 3 taxa. Another analysis (*Dendroica* omitted) linked the *Junco-Pooecetes* clade as the sister group of *Cardinalis* and the tanagers, with *Psarocolius* as the sister group of the *Junco-Pooecetes-Cardinalis*-tanager clade. Jackknifing based on delta $T_{50}H$ produced 10 trees congruent with the KITSCH tree (Fig. 2). One tree (*Nephelornis* omitted) placed *Tersina* and *Tachyphonus* as sister groups. Another analysis (*Dendroica* omitted) yielded the same tree as obtained in the delta mode analysis when *Dendroica* was omitted (see above). When *Junco* was omitted, *Pooecetes* was placed as the sister group of the other taxa except *Carduelis*, *Fringilla*, and *Passer*.

Estimate of phylogenetic branching among labeled taxa.—The differences in branching pattern among the trees in Fig. 2, the jackknife topologies, and the trees permitting negative lengths

TABLE 3. Distances from unlabeled species to labeled species (see Table 1 for abbreviations of the labeled species). In each cell, the delta mode value is followed by the $\Delta T_{50}H$ value, below which the sample size (if $n > 1$) and standard deviation (if $n > 2$) for the comparison are listed.

Unlabeled species	Labeled species									
	NO		CD		SL		TR		TV	
Chloronis riefferii	2.7	3.3	3.0 2	3.6 2	2.8 2	3.2 2	3.4	4.0	3.3	3.1
Urothraupis stolzmanni			4.8	4.4			4.4	4.2	4.0 2	3.7 2
Diuca diuca	3.2	3.5	4.1	4.5	3.4 2	3.6 2				
Cyanerpes cyaneus			4.2	4.0			5.2	5.2	3.4	4.5
Diglossa albilatera			3.2	3.2			3.6	4.2		
Diglossa lafresnayii	3.0	3.3	4.0	4.3						
Coereba flaveola							4.3	4.8	4.6 2	5.2 2
Cacicus cela	4.7	5.0	5.7	6.0	4.9	5.4	5.1 5 0.09	5.0 5 0.14		
Gymnomystax mexicanus	7.1	7.3					7.0	7.3		
Myioborus miniatus	6.0	6.5	5.5	6.8			5.5	6.2		
Helmitheros vermivorus	4.6	6.1	6.4	6.8			5.8 4 0.10	6.3 5 0.26		
Spizella arborea										
Carpodacus mexicanus										
Pinicola enucleator	6.7	6.9	6.6	6.7	6.4	6.7				
Leucosticte arctoa										
Himatione sanguinea										

point to 4 unstable arrangements, each in sections with short branches.

In two cases, a change in topology occurred only when negative lengths were allowed and only in a single such tree (*Sicalis* and *Catamblyrhynchus* as the sister group of *Nephelornis*; *Fringilla* and *Carduelis* as the sister group of *Junco, Pooecetes, Psarocolius* and *Dendroica*). However, these rearrangements occurred neither in Fig. 2 nor in the delta-mode (Bledsoe 1987a) or jackknife trees, and are not as consistent as these trees with the original distances (Table 1). In addition, a sizable (>0.45) branch linked *Fringilla* and *Carduelis* as the sister group of the other labeled taxa in all positive-length topologies. In the negative-length version, this branch was simply reflexed onto the stem leading to the *Junco-Psarocolius* group, while the stem linking *Carduelis* and *Fringilla* was correspondingly lengthened.

In contrast, two portions of the trees in Fig. 2 were unstable in several reconstructions. *Tersina* and *Tachyphonus*, linked as successive sister groups of the other "tanagers" in most arrangements, were placed as each other's sister group in the FITCH tree (Fig. 2), in two of the FITCH jackknife trees (based on both distance statistics), and in one of the KITSCH jackknifes. They switched positions in several negative-length reconstructions and their placement (Fig. 2) was based on very short lengths (<0.20). Thus, their relationships are probably best expressed by a trifurcation (Fig. 3).

A second area of instability involved the *Dendroica-Psarocolius* and *Junco-Pooecetes* clades. With *Dendroica* omitted, jackknife trees placed either *Psarocolius* (KITSCH delta mode) or *Junco* and *Pooecetes* (FITCH and KITSCH delta $T_{50}H$) as the sister group of the cardinals and tanagers. When negative lengths were allowed, they occurred twice (KITSCH delta mode and FITCH delta $T_{50}H$) in this portion of the topology (see above). The branches joining *Junco* and *Pooecetes* as the sister group of *Dendroica* and *Psarocolius* (Fig. 2) are short (<0.25), and the pattern of delta-mode distances for these taxa (Table 1) is character-

TABLE 3. Continued.

Labeled species													
CC		JH		PG		DS		PA		FC		CP	
				5.1 2	5.7 2								
5.2	5.9												
				5.4 2	5.6 2								
		5.6	5.6										
4.7	5.2												
						6.7	6.7						
4.8 2	5.1	5.4	6.0	4.6 3 0.50	5.1 3 0.64	4.6 5 0.29	4.6 5 0.61	1.4 5 0.50 2.9 5 0.45	1.7 5 0.24 3.7 5 0.56				
5.2 2	6.2	4.8	5.3			1.2	1.4						
						2.4 5 0.56	2.2 5 0.54						
5.7	6.3	2.8	3.6							7.0 2	7.6 2	6.3	7.3
6.2	6.6									4.8 2	5.5 2	3.7	2.0
6.6	7.9			7.0	6.7	6.8	7.0			5.1	5.6	4.3	2.7
5.9	6.9									4.7	5.2	3.4	1.7
6.7	7.9									5.2	5.6		

ized by inconsistency and uncertain inferred rates of DNA evolution (Bledsoe 1987a). Thus, a trifurcation between the *Psarocolius-Dendroica, Junco-Poolcetes,* and cardinal-tanager clades is warranted (Fig. 3).

Placement of unlabeled taxa.—Distances between the species used as sources of radioactively labeled DNA and the species that were not used in preparing tracer DNAs are listed in Table 3. An estimate of the relationships of the unlabeled taxa is depicted in Fig. 4. Of the unlabeled species (Table 3), inconsistency characterized distances of *Cacicus cela.* Although clearly the sister group of *Psarocolius* in this analysis, *C. cela* nonetheless exhibited distances to other taxa consistently lower than might be expected if it were conforming to the assumption of synchronous rates. (For a discussion of rate asynchrony in *Psarocolius,* see Bledsoe 1987a.) In addition, the delta-mode distances of *Carpodacus mexicanus, Pinicola enucleator,* and *Leucosticte arctoa* to *Carduelis pinus* are substantially higher than the delta $T_{50}H$ values, contrary to the usual pattern (see Table 1). However, even using the latter values, the taxa were placed unambiguously.

DISCUSSION

VALIDITY OF THE ESTIMATES OF PHYLOGENY

Several factors affect the estimates of phylogeny. Measurement error produced inconsistency among pairwise distances and contributed to the collapse of certain stems of the original reconstructions (Fig. 2; Bledsoe 1987a, Fig. 1). However, it is difficult to determine precisely the effect of measurement error on the pattern of inclusive sets (Figs. 2–4) because the algorithms employed do not permit the calculation of error estimates for the stems. The statistics of dispersion for the raw pairwise values cannot be used directly to assess these limits, because each branch was estimated in part by many pairwise distances. With most pairwise algorithms, no satisfactory method exists to specify such

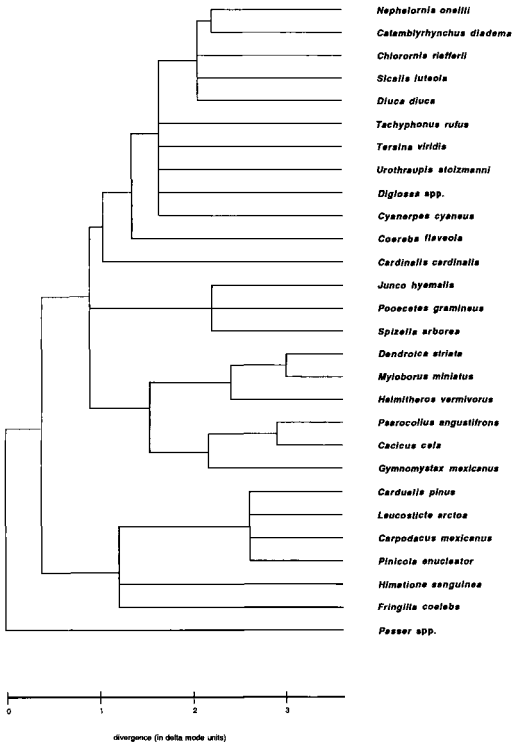

Fig. 4. Estimate of phylogeny of the New World nine-primaried oscines, including both radio-labeled species (Table 1) and other species (Table 3). The tree is based on the branching pattern in Fig. 3 and an estimate of the positions of unlabeled taxa (Table 3). See Table 3 for the two species of *Diglossa* (treated here as a unit) and the text for the species of *Passer*.

error (although P. DeBenedictis [pers. comm.] has proposed such a method for distance Wagner procedures). Furthermore, the values in Table 1 underestimate the distances between taxa because they do not reflect multiple substitutions at a single site within each phyletic line. Unless the distribution of multiple hits is markedly non-random, Poisson or other similar corrections that approximate the effect would increase branch lengths by roughly 4% for a distance of 7.5 delta units but should not change the pattern of branching. For example, a simple Poisson correction (Fitch 1976) applied to delta-mode distances in Table 1, transformed to values of percent base-pair mismatch using the calibration of Caccone et al. (in press), did not change the topology.

The limitations of the least-squares algorithm have potentially more serious implications for phylogenetic accuracy. The FITCH algorithm does not assume uniformity in rates of DNA evolution and is appropriate for inferring both rates and branching patterns (Felsenstein 1984, Bledsoe 1987b). The algorithm seeks a solution for two problems simultaneously: (1) to adjust the length of a given tree to achieve the lowest sum of squares and (2) to identify among possible trees that which yields the lowest such value. Such a solution is NP-complete (Day 1983). For limited taxa it can either be optimal or computationally efficient, but not both. Thus, all possible topologies were not assessed for the best least-squares fit. This problem should not cause a highly inaccurate result because the data (Table 1) are metric and sufficiently consistent to exclude nearly all of the possible trees.

A second problem concerns the assumption of the least-squares algorithm that distances are additive, independent, and drawn from a normal distribution. The true distribution of distances is not known, although a normal distribution appears to be a reasonable approximation for some groups (Felsenstein 1987). In addition, a correlation among distances could occur because measurement error might affect all values of a single experimental set. The phylogeny-independent method of correction (see Methods) should reduce such correlations. Departures from additivity, either through measurement error or underlying nonlinearity of distances, could compromise the validity of trees (Figs. 3, 4). Felsenstein (1987) demonstrated statistically that primate delta $T_{50}H$ values were nonlinear and reconstructed branch lengths were longer than those corrected for nonlinearity. The greater the distance, the more pronounced the truncation of nonlinear values. Farris (1981) maintained that branch-length fitting of Euclidean distances exaggerates terminal lengths relative to internal ones, a contention consistent with Felsenstein's (1987) observation.

In Figs. 2–4, possible biases would most likely arise in the short lengths. In view of these considerations, I constructed a final estimate of phylogeny (Fig. 4) so that poorly supported or unstable stems were collapsed (as in Fig. 3) and the positions of taxa not compared to all of the labeled species were estimated conservatively. The result is by no means final but should provide a robust framework for developing more accurate estimates from additional comparisons.

Other Phylogenetic Evidence

Molecular studies.—Shields and Straus (1975) used DNA-DNA hybridization to compare distances (based on two replicates) from *Junco hyemalis* (DNA tritium-labeled) to *Spizella arborea* (delta Tm = 3.1), *Cardinalis cardinalis* (6.4), *Carpodacus purpureus* (7.7) and *Passer domesticus* (8.3). Their values are close to those in Tables 1 and 3 and the branching pattern derived from them was identical to that in Figs. 3 and 4.

Avise et al. (1980) measured the level of differentiation of protein-encoding loci in 13 species of songbirds, including *J. hyemalis, C. purpureus,* and two species of *Spizella* (*pusilla* and *passerina*). They obtained a branching pattern identical to that among *Junco, Spizella,* and *Carpodacus.* None of their results, nor of Shields and Straus's (1975), is incongruent with those I obtained.

A previous analysis (Sibley and Ahlquist 1985: figs. 17 and 20) of an incomplete set (ca. 25%) of the data in Tables 1 and 3 yielded a tree that differed in retaining very short branches and in the pattern of branching among the icterines, wood-warblers and North American emberizine sparrows. That analysis assumed that rates of DNA evolution were equal in all bird lineages (Sibley and Ahlquist 1983) and thus employed methods of data correction and phylogenetic estimation substantially different from those used in the present study.

Morphological evidence.—The distributions of morphological characters among the New World nine-primaried oscines are often inconsistent with one another (Baird 1864, Coues 1872, Wallace 1874, Parker 1878, Stejneger 1885, Ridgway 1902, Sushkin 1925, Hellmayr 1935, Amadon 1950, Storer 1969, Raikow 1978). An analysis of the phenotypic evidence is thus lengthy and complex (Bledsoe unpubl. data). The phenotypic evidence is consistent with some relationships in Figs. 3 and 4, but is not extensive enough to support or refute a conclusion of broad congruence between the morphological and DNA studies.

Adaptive Radiation

The traditional concept of adaptive radiation in the New World nine-primaried assemblage assumed diversification into several trophic zones (insectivory, frugivory, arboreal granivory, terrestrial granivory, etc.), each occupied by a major group. This view, implicit in earlier classifications, was formalized by Raikow (1978: 34), who employed the idea that "the major groups are the products of adaptive radiations into discrete adaptive zones defined mainly in terms of feeding specializations." Raikow offered his treatment of such shifts as derived behavioral character-states as "an attempt to see whether this concept is a valid model for a theory of phylogenetic relationships within the group" (Raikow 1978: 34).

The present study suggests that such a model is not valid, because feeding specializations appear to have arisen in a more diverse and complex manner than previously suspected. DNA phylogenies (Figs. 3, 4) indicate that adaptive radiation produced changes in feeding specializations that repeatedly converged upon the morphological and trophic attributes of other lineages. For instance, the clade subsuming *Tersina, Catamblyrhynchus, Tachyphonus, Sicalis, Cyanerpes,* and *Conirostrum* includes, respectively, a frugivore and aerial insectivore (Schaefer 1953), a bamboo-specialist (Hilty et al. 1979), a terrestrial granivore, a nectarivore (Skutch 1962), and a foliage-gleaning insectivore. This clade occupies several of the niches typical of other members of the assemblage.

This pattern of diversification is not limited to a few species within the group. Clades such as the New World orioles and the group that consists of the carduelines and Hawaiian honeycreepers are each delimited by molecular distances (this study; see also Sibley and Ahlquist 1982b for data on the Hawaiian honeycreepers) and derived myological character-states (Raikow 1978, Bledsoe unpubl. data). Each subsumes a diversity of adaptive "types" convergent upon the morphologies of members of other clades. Thus, convergence characterizes adaptive radiation at several levels of the New World nine-primaried assemblage. Because the cranial and appendicular musculature (Beecher 1953, Raikow 1978, Bledsoe unpubl. data) and skeletal anatomy (Tordoff 1954) were not modified extensively in the process of adaptive radiation, clades within the assemblage may prove difficult to delimit phenotypically. Additional molecular comparisons might thus be required to expand the estimate of phylogeny to include other New World nine-primaried oscines.

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