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CONFIRMATION OF A PORTION OF THE SIBLEY-AHLQUIST "TAPESTRY"

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ABSTRACT.—DNA-DNA hybridization was used to compare seven taxa from five avian orders, with an alligator as outgroup. Complete matrices of $\Delta T_{50}H$ and ΔNPH (both symmetrized and unsymmetrized) gave the same FITCH topology, which was supported in 100% of bootstrapped and jackknifed trees. The outgroup alligator rooted the tree between anseriform-galliform and coliiform-strigiform-columbiform clades, and resolution within the latter favored a strigiform-columbiform association. In contrast, ΔT_m gave differing and more poorly supported FITCH resolutions for deeper nodes because the distances were compressed due to greatly reduced *NPH*s. An *F*-ratio test between FITCH and KITSCH trees based on symmetrized Jukes-Cantor-corrected $\Delta T_{50}H$s indicated significant rate variation among the lineages. Despite this result, the UPGMA algorithm applied to symmetrized data gave a topology identical to the $\Delta T_{50}H$ and ΔNPH FITCH trees, whether or not the outgroup alligator was included. However, phenograms calculated from unsymmetrized Δs of all three indices associated *Bubo* and *Colius*, as did the FITCH tree based on a completed matrix reconstructed from Sibley and Ahlquist's original data. Thus, our results support Sibley and Ahlquist's use of $\Delta T_{50}H$ to assess ordinal patterns in avian phylogeny, replicate a portion of their "tapestry" based on the same DNA-DNA hybridization technique, and show that for these taxa least-squares and phenetic algorithms generate much the same topology. *Received 31 August 1993, accepted 21 November 1993.*

SIBLEY AND AHLQUIST'S (1990) summary publication of their long series of avian DNA-DNA hybridization experiments provoked a number of critical reviews, many challenging the authors' assertion that numerous aspects of higher-category phylogeny had been resolved. In particular, critics questioned whether the technique has sufficient range or resolution, whether the correct measure of thermal stability had been used, and whether the experimental design (utilizing relatively few labeled taxa) was adequate to support Sibley and Ahlquist's claims for the structure of avian phylogeny and classification (e.g. Krajewski 1991, O'Hara 1991, Raikow 1991, Lanyon 1992). Thus, the implications of Sibley and Ahlquist's work for systematic ornithology remain unclear pending resolution of these issues.

We believe that much of the debate surrounding Sibley and Ahlquist's work can be settled empirically. The traditional view of corroboration of phylogenetic hypotheses requires comparison of results from independent characters. However, a central question for the debate about Sibley and Ahlquist's results remains whether their findings can be replicated with the same technique. While strict replication would entail data production and analysis following protocols used by Sibley and Ahlquist, the criticism about the validity of these methods mandates a second level of replication in which Sibley and Ahlquist's supposed errors in data collection and analysis are avoided. If both levels of replication should yield the same results, one could at least conclude that Sibley and Ahlquist's trees are the ones given by DNA-DNA hybridization and are robust to departures from ideal design and analysis. Differences, however, would call Sibley and Ahlquist's results into question with the very same technique. We view reconciliation of any discrepancies between trees produced by DNA-DNA hybridization and those produced by other methods (e.g. DNA sequencing) as a separate issue.

Unfortunately, few investigations have repeated any part of the Sibley-Ahlquist system using identical or near-identical techniques, although studies addressing particular points of intrafamilial relationships have obtained similar outcomes (e.g. Sheldon 1987, Krajewski 1989, Sheldon et al. 1992, Bleiweiss et al. 1994b). As a further step toward such replication, we here chose to examine seven avian taxa from major

branches of the Sibley-Ahlquist tree. Unlike those authors, we constructed a complete, balanced matrix of comparisons among these birds and an unequivocal outgroup, the crocodilian *Alligator mississippiensis*. Otherwise, our hybridization protocols differed only trivially from those employed by Sibley and Ahlquist, mostly with respect to the amounts of material used to fabricate hybrids, narrower temperature intervals in the elution regime, and modifications that enhance the precision of percent hybridization. Our results using least-squares analysis are entirely congruent with the equivalent threads of the so-called "tapestry" of avian relationships in Sibley and Ahlquist (1990). These results also indicate that distances among major avian clades are easily within the technique's range and, furthermore, validate Sibley and Ahlquist's choice of the $T_{50}H$ statistic to determine higher-level relationships among birds. Moreover, UPGMA and FITCH algorithms gave the same topology (except on one point of resolution using data uncorrected for label compression and involving a very short internode), suggesting that despite modest rate inequalities, our and Sibley and Ahlquist's data are robust to diverse analytical assumptions.

METHODS

To provide a methodological test of Sibley and Ahlquist's results, we chose to analyze relatively few taxa, and ones whose associations are relatively uncontroversial. Any discrepancies between our results and those of Sibley and Ahlquist would raise serious questions indeed about the validity of the technique. In particular, we used two obvious sister pairings as internal controls: two members of the Columbiformes (the Rock Dove [*Columba livia*] and Mourning Dove [*Zenaida macroura*]) and two galliforms (the domestic fowl [*Gallus gallus*] and the Blue-breasted Quail [*Coturnix chinensis*]). Additional avian taxa were chosen to represent other presumed major clades (the Mallard [*Anas p. platyrhynchos*], an anseriform; the Speckled Mousebird [*Colius striatus*], a coliiform; and the Great Horned Owl [*Bubo virginianus*], exemplifying Strigiformes). All but one of the taxa were species labeled by Sibley and Ahlquist (they labeled *Francolinus natalensis*, a relative of our *Coturnix chinensis*). As our aims were principally directed to analytical rather than taxonomic issues, no attempt was made at more extensive sampling within major clades.

One extract from each of the avian taxa was prepared from ethanol-preserved tissues, while an alligator extract was made from whole blood drawn from a living animal. Procedures for extraction, sonication, separation of single- from multiple-copy sequences, iodination, fabrication of hybrids, and thermal elution followed those outlined in Kirsch et al. (1990) and in Bleiweiss and Kirsch (1993a), except that the amount of driver in each hybrid was 50 μg and the tracer : driver ratio was 1:500. In addition, the elution regime included two room-temperature washes that presumably remove free iodine and small unhybridizable fragments, thus increasing the measured normalized percent hybridization (*NPH*) and improving its precision.

The experimental design was straightforward. Each of the eight taxa was labeled, then hybridized three times with itself and the seven others; all hybrids with a particular label were eluted in a single run. For each hybrid, we calculated not only the $T_{50}H$ index used by Sibley and Ahlquist (1990), but also its two constituents, T_m and *NPH*, all based on radioactive counts above 56°C. Modes were unrecoverable for the most distant hybrids. Both unsymmetrized and symmetrized (Sarich and Cronin 1976) matrices of "delta" values for the three measures were analyzed by the FITCH program in Felsenstein's PHYLIP (ver. 3.3; Felsenstein 1990), with the global branch-swapping, subreplicate, and Cavalli-Sforza and Edwards options enabled. The rationale for symmetrization is that it alleviates systematic experimental error due to "compression" of some labels (Springer and Kirsch 1991), and may help to identify rate variation.

The effect of measurement imprecision on the topology was assessed for each of the six matrices (i.e. based on three indices, each unsymmetrized and symmetrized) by Krajewski and Dickerman's (1990) adaptation of bootstrapping for distances (with the order of taxa randomized for each of 1,000 runs), and the consistencies of the matrices were tested by jackknifing on taxa (Lanyon 1985). We used Felsenstein's *F*-ratio test (Sheldon 1987, Springer and Kirsch 1989) to detect rate variation in DNA change using symmetrized $\Delta T_{50}H$ values adjusted with the Jukes-Cantor one-parameter correction for multiple hits (Jukes and Cantor 1969), comparing the sums-of-squares of corresponding FITCH and KITSCH trees generated from these data. The Jukes-Cantor correction provides a closer approximation to true evolutionary distances (Springer and Krajewski 1989), and so may increase the sensitivity of the *F*-ratio test.

Because Sibley and Ahlquist used phenetic methods on matrices without specific outgroups to derive the tapestry, we also analyzed the data with UPGMA as implemented in NTSYS-pc (ver. 1.70; Rohlf 1992), both with and without inclusion of the outgroup alligator, to allow more direct comparisons between their results and our own. Those comparisons included computing correlations between Sibley and Ahlquist's tapestry tree matrix and our UPGMA tree matrix from symmetrized $\Delta T_{50}H$s (alligator omitted), between the branch lengths of the two trees, and

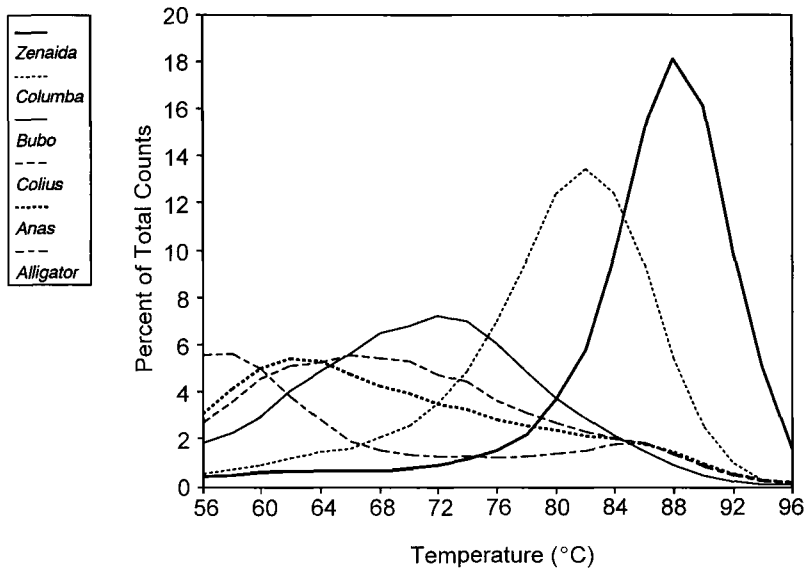

Fig. 1. Representative stepwise elution curves for hybrids with labeled *Zenaida macroura*. Individual points normalized for percentage of hybridization.

between Sibley and Ahlquist's tree matrix and our symmetrized $\Delta T_{50}H$ data.

In addition, we obtained most of Sibley and Ahlquist's raw data for taxa common to their and our studies, and calculated a correlation among measurements common to the two data sets. We also used a reconstruction method to complete their matrix (Lapointe and Kirsch 1995), and generated a FITCH tree from it, comparing the topology with one generated from our own $\Delta T_{50}H$ matrix with the same lacunae introduced. The reconstruction procedure used to fill missing cells in an incomplete matrix has three steps: (1) symmetrization (after Sarich and Cronin 1976), based on the available information; (2) reflection of missing cells where one of a pair of reciprocals is absent; and (3) estimation of both reciprocals (after DeSoete 1984) when neither is available.

In simulations with real and invented data, we have found that this procedure is capable of good metric and topological recovery provided that about 60% of the cells of a matrix are filled and that at least one reciprocal measurement between each pair of terminal sister taxa is available (Lapointe and Kirsch 1995).

Results

Data.—Figure 1 depicts representative stepwise elution curves for hybrids with labeled *Zenaida macroura*. Tables 1 to 3 indicate the Δ-values for T_{50}Hs, T_ms, and *NPH*s, respectively, except that absolute mean melting temperatures are given in Tables 1 and 2 for the homologues to permit comparison of the quality of labels (i.e. mean temperatures of homoduplex melts). Additional one-way comparisons with mammal and lizard extracts used as drivers verify that the avian-crocodilian hybridizations were within the range of our protocols, because distances from birds to mammals and lizards were greater than the avian-crocodilian Δs listed in Tables 1 to 3 (e.g. mean Δ*NPH*s for *Bubo* hybridized with an agamid lizard or two mammals ranged from 69.85 to 77.84%).

Table 4 lists the $\Delta T_{50}H$ distances assembled from 16 runs performed by Sibley and Ahlquist, with the numbers of replicates indicated in parentheses. In some cases the species were not identical to ours, and tracers and drivers for *Colius* and *Urocolius* were combined. Only 19 of the 49 cells are filled, or 39% of the possible comparisons. As only homologous hybrids (which are by definition zero) are available for the *Bubo* and *Columba* labels, these taxa are effectively unlabeled with respect to this matrix. Moreover, the four comparisons involving *Bubo* and its hypothesized sister taxon *Columba*-plus-*Zenaida* are lacking. Table 5 shows the same matrix as completed by the reconstruction procedure of Lapointe and Kirsch (1995).

TABLE 1. Matrix of uncorrected $\Delta T_{50}H$ distances among seven avian species and an outgroup crocodilian; $n = 192$ with each measurement replicated three times. Columns are tracers. Each cell lists average distance and standard deviation (SD) for that comparison, except that absolute mean melting temperatures (rather than zero distances) are given for homologous comparisons. At bottom are corrections for asymmetry representing the row/column ratio in each case. These were used as basis for up to 10 cycles of multiplication, after each of which row/column ratios were recalculated, until all ratios reached unity.[a]

	Alligator	*Anas*	*Gallus*	*Coturnix*	*Colius*	*Bubo*	*Columba*	*Zenaida*
Alligator	84.10 ± 0.62	31.28 ± 1.22	37.87 ± 0.23	35.11 ± 5.00	29.96 ± 0.57	29.36 ± 1.01	33.59 ± 0.96	33.76 ± 2.05
Anas	32.39 ± 1.16	82.32 ± 0.22	24.85 ± 0.47	22.61 ± 0.10	24.66 ± 0.36	20.90 ± 1.73	27.27 ± 0.85	26.41 ± 0.10
Gallus	31.21 ± 1.82	24.23 ± 0.40	85.64 ± 0.06	9.74 ± 0.25	24.07 ± 0.77	23.79 ± 1.24	28.56 ± 0.67	29.77 ± 0.17
Coturnix	32.61 ± 0.78	25.44 ± 0.12	11.30 ± 0.17	84.24 ± 0.47	25.51 ± 1.62	25.20 ± 2.01	29.22 ± 0.25	30.57 ± 0.59
Colius	31.40 ± 1.85	27.05 ± 0.27	31.67 ± 0.33	21.41 ± 2.17	83.87 ± 0.48	18.41 ± 0.56	26.75 ± 0.10	23.56 ± 0.21
Bubo	35.94 ± 0.92	23.96 ± 0.37	30.17 ± 0.17	25.70 ± 1.22	17.18 ± 0.16	81.29 ± 0.23	21.02 ± 1.48	19.66 ± 0.41
Columba	31.20 ± 1.07	24.07 ± 0.73	28.33 ± 0.94	20.72 ± 2.34	17.40 ± 0.26	13.60 ± 0.21	83.89 ± 0.05	6.79 ± 0.24
Zenaida	29.50 ± 0.96	25.39 ± 0.15	28.10 ± 0.49	22.02 ± 0.89	17.73 ± 0.59	14.19 ± 0.51	7.60 ± 0.22	86.28 ± 0.02
Correction	1.018	0.980	0.886	1.123	1.116	1.164	0.832	0.863

[a] Asymmetry before correction = 8.07%; asymmetry after correction = 3.37%; unweighted average SD for all cells = 0.76; correlation of SD with distance = 0.37; FITCH sum of squares, unsymmetrized data = 1,070.44; FITCH sum of squares, symmetrized data = 367.26.

TABLE 2. Matrix of uncorrected ΔT_m distances among seven avian species and an outgroup crocodilian; $n = 192$ with each measurement replicated three times. Conventions as for Table 1.[a]

	Alligator	*Anas*	*Gallus*	*Coturnix*	*Colius*	*Bubo*	*Columba*	*Zenaida*
Alligator	84.17 ± 0.21	18.68 ± 0.29	21.96 ± 0.15	20.70 ± 0.27	16.84 ± 0.93	18.38 ± 0.77	16.53 ± 2.69	23.12 ± 1.24
Anas	20.16 ± 0.07	82.32 ± 0.19	18.53 ± 0.47	16.22 ± 0.52	15.78 ± 0.57	14.06 ± 0.60	19.83 ± 1.58	19.62 ± 0.31
Gallus	19.65 ± 0.52	17.97 ± 0.55	85.64 ± 0.06	8.97 ± 0.30	13.09 ± 1.16	15.20 ± 0.33	20.98 ± 1.81	20.20 ± 0.26
Coturnix	19.42 ± 0.76	18.72 ± 0.18	9.87 ± 0.10	84.27 ± 0.29	14.49 ± 0.68	15.41 ± 0.51	21.46 ± 0.37	20.00 ± 0.21
Colius	19.30 ± 0.65	19.95 ± 0.14	21.72 ± 0.38	12.33 ± 2.97	83.88 ± 0.45	13.02 ± 0.32	19.32 ± 0.32	18.16 ± 0.11
Bubo	20.13 ± 0.35	18.34 ± 0.15	20.32 ± 0.10	14.55 ± 1.12	13.25 ± 0.10	81.29 ± 0.20	16.30 ± 0.90	16.01 ± 0.21
Columba	20.12 ± 0.24	17.67 ± 0.87	20.36 ± 0.51	12.56 ± 2.22	13.87 ± 0.36	12.06 ± 0.33	83.89 ± 0.08	6.54 ± 0.24
Zenaida	19.58 ± 0.14	19.26 ± 0.21	20.81 ± 0.34	14.71 ± 0.61	13.89 ± 0.08	12.01 ± 0.24	6.49 ± 0.30	86.29 ± 0.04
Correction	0.979	0.942	0.860	1.155	1.165	1.145	0.860	0.871

[a] Asymmetry before correction = 8.63%; asymmetry after correction = 3.94%; unweighted average SD for all cells = 0.53; correlation of SD with distance = 0.15; FITCH sum of squares, unsymmetrized data = 677.45; FITCH sum of squares, symmetrized data = 261.56.

FITCH analyses, jackknifing, bootstrapping, and rate test.—FITCH trees based on symmetrized or unsymmetrized $\Delta T_{50}H$ or ΔNPH data, and on the Jukes-Cantor-corrected $\Delta T_{50}Hs$, all have the same topology (Fig. 2). Moreover, all nodes are consistent with every jackknife pseudoreplicate tree and are supported in 100% of the trees generated from 1,000 pseudoreplicate bootstrap matrices. The Jukes-Cantor-corrected data were not bootstrapped, because these were based on mean symmetrized $\Delta T_{50}Hs$, and Krajewski and Dickerman's (1990) program requires individual measurements.

In contrast, FITCH trees based on unsymmetrized or symmetrized ΔT_ms differ from the $T_{50}H$ and *NPH* trees, and some subterminal nodes bootstrapped poorly (Figs. 3A and 3C, which give bootstrap numbers for nodes in trees). In addition, little of the structure survived jackknifing. Only three pairings in the tree based on unsymmetrized data and two in that calculated from symmetrized ΔT_ms are consistent with all jackknife pseudoreplicate trees (Figs. 3B and 3D).

A KITSCH tree calculated from the symmetrized Jukes-Cantor-corrected $\Delta T_{50}H$ values differs from the corresponding FITCH tree in placing *Anas* with the *Colius-Bubo*-Columbiformes branch. Because the KITSCH and FITCH topologies differed, the user-tree option was employed for KITSCH to permit the *F*-ratio test. The results indicate significant rate variation among the lineages: SS (KITSCH) = 1,864.73, SS (FITCH) = 1,062.93, df (KITSCH) = 185, df (FITCH) = 179; $P < 0.001$ for $F = 10.43$ in a test with 6 and 179 df (degrees of freedom based on number of replicate measurements).

UPGMA analyses.—Phenograms calculated from symmetrized data (for all three indices) using UPGMA have topologies identical to those derived using FITCH on symmetrized $\Delta T_{50}Hs$ and $\Delta NPHs$; those calculated from unsymmetrized data differ in linking *Bubo* and *Colius* as a cluster closest to the two columbiforms, a result probably due to the combined effect of rate variation and compression of the *Bubo* and some other labels. In addition, phenograms from unsymmetrized ΔT_ms (with or without *Alligator*) differed in resolution among *Anas* and other clades, echoing FITCH trees obtained from the same data. Other algorithms (complete linkage, neighbor joining, and often even single linkage) gave parallel results.

TABLE 3. Matrix of uncorrected ΔNPH distances among seven avian species and an outgroup crocodilian; $n = 192$ with each measurement replicated three times. Conventions as for Table 1, except that zero distances are listed for homologous comparisons.[a]

	Alligator	*Anas*	*Gallus*	*Coturnix*	*Colius*	*Bubo*	*Columba*	*Zenaida*
Alligator	0 ± 4.69	59.50 ± 3.22	64.83 ± 0.90	61.40 ± 9.99	50.23 ± 1.94	56.17 ± 2.71	59.07 ± 2.57	53.63 ± 5.69
Anas	55.17 ± 2.52	0 ± 0.36	33.07 ± 1.33	30.83 ± 0.55	37.13 ± 1.85	36.07 ± 4.92	40.63 ± 2.11	36.73 ± 1.01
Gallus	52.73 ± 4.21	34.43 ± 1.31	0 ± 0.17	6.50 ± 0.66	37.83 ± 2.74	42.67 ± 3.36	43.70 ± 3.54	45.50 ± 0.36
Coturnix	55.67 ± 1.55	39.13 ± 0.57	11.37 ± 0.91	0 ± 2.09	40.17 ± 3.26	45.50 ± 4.53	47.30 ± 1.11	46.87 ± 1.16
Colius	52.97 ± 4.21	44.73 ± 1.25	50.10 ± 0.87	30.30 ± 1.95	0 ± 0.53	31.10 ± 0.79	39.20 ± 0.40	31.13 ± 0.98
Bubo	63.00 ± 1.65	34.50 ± 1.39	47.03 ± 0.47	41.53 ± 2.63	22.57 ± 0.47	0 ± 0.44	26.43 ± 2.70	25.43 ± 0.93
Columba	52.53 ± 2.48	33.50 ± 4.00	41.50 ± 2.74	30.47 ± 4.45	20.20 ± 0.70	12.57 ± 1.85	0 ± 1.21	3.20 ± 0.10
Zenaida	48.37 ± 2.65	37.80 ± 0.40	39.47 ± 1.81	29.87 ± 2.08	20.23 ± 2.02	15.93 ± 2.66	10.07 ± 0.35	0 ± 0.46
Correction	1.049	0.950	0.899	1.204	1.117	1.081	0.755	0.853

[a] Asymmetry before correction = 13.30%; asymmetry after correction = 7.75%; unweighted average SD for all cells = 2.02; correlation of SD with distance = 0.39; FITCH sum of squares, unsymmetrized data = 4,119.05; FITCH sum of squares, symmetrized data = 2,112.64.

TABLE 4. Sibley and Ahlquist's $\Delta T_{50}H$ data for genera the same as or closely related to those examined in our study. Columns are labels. Numbers of replicates for each comparison given in parentheses following mean distances. One homologue included in each of 16 runs. Dashes indicate comparisons not performed.

	Anas	*Gallus*	*Francolinus*	*Colius*[a]	*Bubo*	*Columba*	*Zenaida*
Anas	0(3)	22.89(1)	16.99(1)	—	—	—	—
Gallus	16.68(2)	0(1)	8.66(1)	—	—	—	—
Francolinus	—	9.28(1)	0(2)	—	—	—	—
Colius[a]	—	—	—	0(4)	—	—	16.29(2)
Bubo	—	—	—	14.78(2)	0(1)	—	—
Columba	17.72(1)	23.08(1)	—	16.07(1)	—	0(1)	5.32(9)
Zenaida	—	—	—	16.34(1)	—	—	0(4)

[a] Labels and drivers of *Colius* and *Urocolius* were combined.

Figure 4 compares the tree inferred from Sibley and Ahlquist's tapestry (A) with that derived by UPGMA from our symmetrized $\Delta T_{50}H$ data for the seven avian taxa (B). The correlation between matrices representing these trees is 0.95; that between the two trees' branch lengths is 0.93. Moreover, the correlation between the tapestry tree matrix and our matrix of symmetrized $\Delta T_{50}H$s (with the alligator omitted) is 0.92. Because the compared matrices represent different data sets, it is appropriate to use a Mantel test, which returned a probability of 1.00 that the matrices show identical structure.

Comparison with Sibley and Ahlquist's original data.—The correlation of the 12 heterologous distances common to our and Sibley and Ahlquist's studies is 0.94, although Sibley and Ahlquist's numbers average about 81% of ours, probably because of differences in the ways in which they and we calculated *NPH* (compare Tables 1 and 4). The FITCH tree calculated from Table 5 is depicted in Figure 5. This topology is fully supported by a jackknife strict consensus. Our estimation procedure predicts that when all comparisons of a terminal taxon with its putative sister group (which may be a singleton or cluster) are missing, the relationship of that taxon will collapse to a trichotomy or near trichotomy with the next-nearest clade or taxon (Lapointe and Kirsch 1995). This is exactly the result shown in Figure 5, which associates *Bubo* with *Colius* by a short internode (0.72°C) and coincidentally matches (topologically) phenograms based on our own, complete unsymmetrized $\Delta T_{50}H$ and ΔNPH matrices. The FITCH tree obtained from a reconstructed matrix based only on comparisons in our own data corresponding to Sibley and Ahlquist's was identical in topology to Figure 5, linked *Bubo* and *Colius* for the same reason, and also was supported by a jackknife strict consensus.

DISCUSSION

Technical and analytical issues.—Sibley and Ahlquist's (1990) advocacy of the $T_{50}H$ index is based on the fact that T_m measures only the stability of those sequences similar enough to form stable hybrids at criterion temperature. For distant comparisons, where many sequences do not hybridize, ΔT_ms may seriously underestimate true divergence and could distort the topology, particularly among taxa separated by short internodes or where ΔNPH is greater than 50% (e.g. most comparisons of birds with

TABLE 5. Matrix of Table 4 as completed by the estimation procedure of Lapointe and Kirsch (1995). Replicate numbers of reflected and estimated values set equal to one for FITCH analysis.

	Anas	*Gallus*	*Francolinus*	*Coliusa*[a]	*Bubo*	*Columba*	*Zenaida*
Anas	0(3)	18.03(1)	18.20(1)	24.31(1)	24.31(1)	24.31(1)	24.31(1)
Gallus	22.89(2)	0(1)	9.28(1)	18.17(1)	18.17(1)	18.17(1)	18.17(1)
Francolinus	18.20(1)	7.31(1)	0(2)	18.17(1)	18.17(1)	18.17(1)	18.17(1)
Colius[a]	24.31(1)	18.17(1)	18.17(1)	0(4)	14.73(1)	16.02(1)	16.34(2)
Bubo	24.31(1)	18.17(1)	18.17(1)	14.73(2)	0(1)	16.02(1)	16.31(1)
Columba	24.31(1)	18.17(1)	18.17(1)	16.02(1)	16.02(1)	0(1)	5.34(9)
Zenaida	24.31(1)	18.17(1)	18.17(1)	16.29(1)	16.31(1)	5.34(1)	0(4)

[a] Labels and drivers of *Colius* and *Urocolius* were combined.

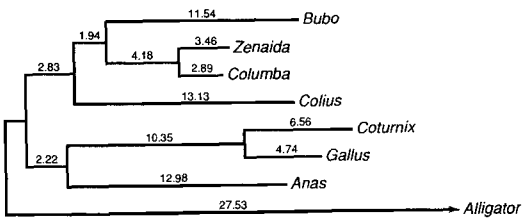

Fig. 2. Best-fit FITCH topology with estimated branch lengths based on symmetrized Jukes-Cantor-corrected $\Delta T_{50}Hs$.

the alligator; Table 3). By incorporating *NPH*, $\Delta T_{50}H$ extends the range of comparisons and provides a more realistic measure of genetic distance. However, $\Delta T_{50}H$ has been criticized in principle because it is a composite of two other measures (Sarich et al. 1989), and because the variance of *NPH* is regarded as too high to give (either alone or in combination with T_m) resolution among short internodes (Marks et al. 1988). If *NPH* is inaccurate, as well as imprecise, $\Delta T_{50}Hs$ may prove positively misleading.

We do not agree that $T_{50}H$ should be discarded just because of its composite nature. Corrections routinely are applied to molecular data to compensate for the limitations in measures that can be made experimentally (e.g. for multiple hits in DNA sequences). While we concur that *NPH* is much less precise than other indices (Bleiweiss and Kirsch 1993b; compare standard deviations in Tables 1 to 3), ΔNPH may be the only index with sufficient range to allow for extremely distant comparisons (e.g. Kirsch et al. 1991). Appropriate amounts of replication also may confer sufficient accuracy. The degree of resolution and specific topology obtained with ΔNPH (or $\Delta T_{50}H$) will then obviously depend upon the length of internodes joining taxa in any particular investigation. These are essentially empirical issues, not ones of principle.

However, we believe that it is important to decompose a composite measure in order to determine if the constituent indices give similar or conflicting results: analogously with the results of consensus or "total-evidence" proce-

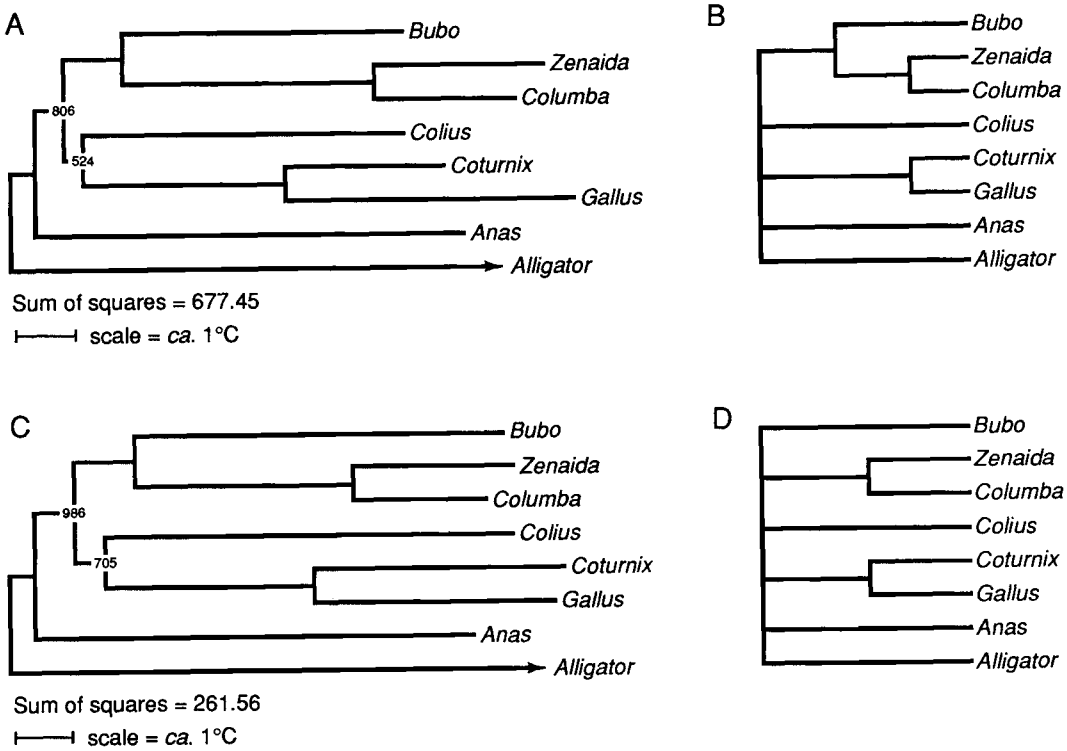

Fig. 3. Best-fit FITCH topologies for (A) unsymmetrized and (C) symmetrized $\Delta T_m s$. Bootstrap numbers, based on 1,000 pseudoreplicate trees, indicated only for those nodes where support was less than 100%. Jackknife strict-consensus topologies indicated alongside each FITCH tree (B and D).

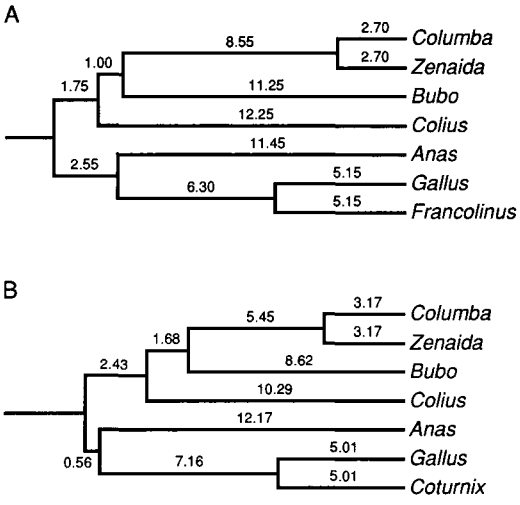

Fig. 4. (A) UPGMA phenogram for seven avian taxa inferred from Sibley and Ahlquist's (1990:842–847) tapestry, with phenon levels apportioned as branch lengths. (B) UPGMA phenogram for seven avian taxa as calculated from symmetrized data of Table 1, with phenon levels apportioned as branch lengths.

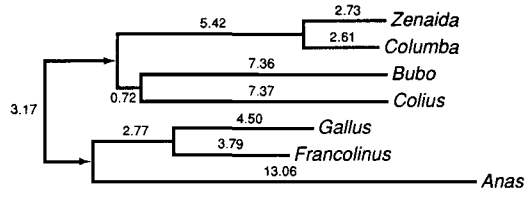

Fig. 5. Best-fit FITCH topology with estimated branch lengths calculated from completed matrix of Sibley and Ahlquist's $\Delta T_{50}H$ data on depicted taxa (Table 5).

dures, a composite index could give a topology different from or incompatible with either or both of those based on its components (Bleiweiss and Kirsch 1993a; Table 5). Thus, we have presented results based on ΔT_m and ΔNPH, as well as on $\Delta T_{50}H$. Given the great degree of sequence divergence among the birds chosen, or among all of them and the crocodilian, it is no surprise that jackknifing of the FITCH analyses of ΔT_ms failed to support any nodes except those for the less-divergent terminal or near-terminal pairs. Nevertheless, the supported nodes are congruent with those of the ΔNPH and $\Delta T_{50}H$ trees, while the lack of resolution among earlier divergences does not falsify the indications of $\Delta T_{50}H$ and ΔNPH. We therefore conclude that the deeper structure in our $\Delta T_{50}H$-based trees is provided by *NPH*, and that the topology is not distorted by inclusion of either *NPH* or T_m in $T_{50}H$ calculations.

At the same time, nonadditive ΔNPHs would violate one assumption of least-squares analysis and, thus, a $\Delta T_{50}H$ topology would be of dubious value. Nonadditivity is expected due to measurement error alone and, in fact, none of the three indices are strictly additive: all 70 quartets of distances for each of the three symmetrized matrices fail this severe test (however, all but 1 of 168 triplets [56 for each index] satisfy the triangle inequality; i.e. they are metric). The central questions are how much each index departs from additivity, and how they compare to each other in this respect. The relative performance of the three indices cannot be judged by comparing the sums-of-squares of FITCH trees calculated from each, because the sum-of-squares is an absolute, not relative measure: ΔNPH trees should have larger values and ΔT_m trees smaller values because of the relative magnitudes of the distances obtained with each index. However, a direct indication of relative additivity is provided by the correlations of measured distances with the fitted pathlengths of corresponding trees. By this criterion, ΔNPH compares favorably with either ΔT_m or $\Delta T_{50}H$; symmetrized ΔNPHs and $\Delta T_{50}H$s each have a correlation of 0.99, while that of symmetrized ΔT_ms is 0.98. This result indicates the suitability of ΔNPH for least-squares analysis in our study, and suggests that, despite its high variance, it also is an accurate measure.

It may be objected that trees obtained by a least-squares method, rather than the phenetic algorithm utilized by Sibley and Ahlquist, do not truly replicate their results. While all algorithms will give the same tree if the data are close to ultrametric, and equivalent results using different tree-building methods certainly provide an important kind of confirmation (i.e. robustness to different analytical assumptions), we did subject our data to UPGMA. The symmetrized data (with or without the alligator) gave topologies among the birds identical to those depicted in Figure 2. Trees based on unsymmetrized data joined *Bubo* with *Colius*, a result we believe is most likely due to compression of some labels enhanced by modest rate variation. Again, symmetrization removes the systematic error associated with compression,

highlighting apparent rate differences; yet, it is significant that FITCH trees based on either unsymmetrized or symmetrized matrices recovered the same branching sequence even with respect to *Bubo* and *Colius*.

We also compared the tree matrix for the seven avian taxa inferred from Sibley and Ahlquist's tapestry (Fig. 4A) with the cophenetic matrix obtained from our own UPGMA tree based on symmetrized $\Delta T_{50}H$s (Fig. 4B), and with the matrix of symmetrized $\Delta T_{50}H$s (after Table 1). In both cases, the correlation was high (0.95 and 0.92, respectively), indicating that the structures of the three matrices were very similar. Comparison of branch lengths derived from the two UPGMA trees gave a similarly high correlation of 0.93.

Finally, one might ask what sort of tree Sibley and Ahlquist's original data could produce. Only 39% of the relevant comparisons were common to their and our studies, and no heterologous hybrids with two of the taxa were made (thus, *Bubo* and *Columba* were, for the purposes of this study, unlabeled). Although it may seem unlikely that any least-squares tree could be constructed from such sparse data, the estimation procedure we have developed allows completion of the table (Lapointe and Kirsch 1995). The resulting FITCH topology matches Figure 2 except in the pairing of *Bubo* with *Colius*, which is evidently based on the single (relatively short) measured distance between them. This result, while similar to phenograms based on our unsymmetrized data, may be coincidental. Incorrect resolution is expected when no measurements among terminal sister clades are available (Lapointe and Kirsch 1995), and our own data gave the same association of *Bubo* and *Colius* when reconstructed from a reduced matrix. Sibley and Ahlquist's decision that *Bubo* and *Colius* are successive outgroups to the columbiforms must have been based on evidence from other labels (Sibley and Ahlquist 1990:362).

Phylogenetic relationships.—If least-squares trees are taken as the better representations of phylogeny, then in every respect relationships among these birds shown by our study are as concluded by Sibley and Ahlquist (1990). Both pairs of terminal sister taxa used as controls always associate with one another, consistent with monophyly of the Columbiformes (pigeons and doves) and Galliformes (fowl and quail). Although we did not carry out these comparisons to test specific higher-level relationships, our results also are consistent with a sister-group relationship between Galliformes and Anseriformes, and their separation from other nonpasserine birds. Placement of *Bubo* closest to the two members of the Columbiformes, and of *Colius* outside all three (in FITCH trees based on $\Delta T_{50}H$s and *NPH*s, and in phenograms based on symmetrized data for all three indices), further emphasizes the relatively isolated position of Coliiformes.

As to the rate question, while our results detected significant rate variation and agree with Sibley and Ahlquist's conclusion that strigiforms evolved slowly, the distribution of apparently slow or fast rates among other lineages (Fig. 2) militates against an easy explanation based on generation times. Furthermore, the close topological similarity of most phenograms and FITCH trees suggests that rate variation does not affect the recovered phylogeny, except in the case of the association of *Colius* with *Bubo* in phenograms based on unsymmetrized data, where rate variation acts synergistically with systematic experimental error (i.e. compressed labels).

Conclusions.—Our results replicate Sibley and Ahlquist's quite closely, whether the data are analyzed by a least-squares or ultrametric method, and whether the tapestry tree or raw data are compared with our findings. This close correspondence obtains not only because our experimental protocols were similar to Sibley and Ahlquist's, but also because six of our seven avian species were ones labeled by these authors; even the absolute distances obtained by Sibley and Ahlquist and ourselves were very highly correlated and similar in magnitude. However, Sibley and Ahlquist clearly did not run their labels as part of a single matrix, and their experiments included many intermediate taxa tested as drivers only. Thus, the respective positions of the corresponding labeled species in the tapestry apparently were inferred by aggregating values for these and for taxa which were never labeled. This procedure has been criticized because topological inferences about unlabeled drivers may be compromised by nonuniform rates (Lanyon 1992). For example, in Table 1 the relatively short distance from (labeled) *Anas* to *Bubo* implies a special relationship between them. Only the information provided by other tracers reveals that these taxa belong to separate clades, their apparent special similarity probably being due to a slower rate

of change in the *Bubo* lineage. Still, rate variation did not compromise recovery of relationships among the suite of taxa common to Sibley and Ahlquist's and our studies, except regarding the association of *Colius* with *Bubo* in some phenograms. Whatever the reasons for Sibley and Ahlquist's success in the face of their admittedly incomplete matrices, the causes are not likely to include inappropriate choice of index or misconstrual of the results.

While our success in replicating part of the tapestry supports some of Sibley and Ahlquist's contentions, due caution must be taken in generalizing from this fragment to the body of their work. One reason that our experiments gave such consistent results may be that we selected taxa covering much of the range of distances among major branches of the tree. Too often in higher-level comparisons, the suite of taxa chosen comprises a set of singletons with no intermediate relatives. The problem of long, undivided branches, and the spurious relationships that they may suggest in trees based on either distances or character data is well recognized (Swofford and Olsen 1990). We think that we have circumvented this analytical artifact through our choice of taxa. On the other hand, many nodes in the tapestry are more closely spaced than the ones recovered here, creating a severe problem of resolution. In these cases, it may be expected that confirmation of the corresponding parts of Sibley and Ahlquist's work will be difficult (e.g. Bleiweiss et al. 1994a). Thus, different parts of the tapestry may prove less robust to replication, although we stress that further repetition can only help to isolate and understand problematic areas of the tapestry more fully.

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