CRITICAL EVALUATION OF DNA HYBRIDIZATION STUDIES IN AVIAN SYSTEMATICS

PETER HOUDE

Division of Birds, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560 USA

ABSTRACT.—It is frequently claimed that equal rates of DNA evolution are observed in birds, but specific tests necessary to demonstrate this are rarely performed. I demonstrated statistically significant differences in rates of DNA evolution for a few passerine birds that vitiate the role of DNA hybridization as the direct indicator of kinship. The differences in evolutionary rates observed may be substantial enough to introduce ambiguity into the clustering of taxa. Researchers frequently fail to perform the specific experiments needed to distinguish between real differences in relative rates of DNA evolution vs. differences that can be attributed to experimental error. They also fail to draw attention to and account for either erroneous or problematical data for some birds, or fail to perform the experiments necessary to determine the cause of unexpectedly problematical results. The DNA molecular clock is shown to be calibrated using speculative and questionable data. It involves diverse organisms that cannot be shown to evolve at the same rate, and that probably did not. Yet, DNA hybridization is a valuable tool that probably cannot lead one to make major systematic errors providing the data are not incorrect because of technical or computational errors and are taken from a sufficient diversity of relevant taxa. *Received 29 November 1985, accepted 8 May 1986.*

THE past two decades have witnessed a revolution in molecular approaches to taxonomic problems (methods summarized by Scott and Smith 1982, Thorpe 1982). In particular, DNA hybridization studies seem to promise overall measures of species divergence (Schildkraut et al. 1961, McCarthy and Bolton 1963, Hoyer et al. 1964). The comparison of only "single copy" DNA was a major technical advance (Britten and Kohne 1968, Kohne et al. 1971, Hoyer et al. 1972). Sibley and Ahlquist (1981a) provided a more complete history of the development of these techniques. Schultz and Church (1972), Shields and Straus (1975), Eden et al. (1978), and Burr and Schimke (1980) hybridized avian DNA, and Sibley and Ahlquist (1980, 1981a–c, 1982a–i, 1983, 1984a, c, 1985a–c, 1986), Sibley et al. (1982, 1984a, b), and Ahlquist et al. (1984) employed this technique extensively in the study of avian relationships. Their work has received great praise from some popular reviewers (Diamond 1983, Lewin 1984, Gould 1985), although some systematists remain skeptical of such studies for a variety of reasons. These include discrepancies between conclusions drawn from DNA hybridization and more traditional (e.g. morphological) studies (e.g. Sibley and Ahlquist 1980, 1984a, 1985c, 1986) and apparent disagreement among biochemists about the uniformity of macromolecular evolutionary rates (e.g. Fitch 1976, Bonner et al. 1981, Dover et al. 1981, Avise and Aquadro 1982, Ayala 1982, Holmquist et al. 1982, Thorpe 1982, Britten 1986). I examined the few avian DNA hybridization studies in which adequate data are presented to determine whether ambiguities or discrepancies exist in the assumptions, the methodology, or the interpretations.

STATISTICS THAT DESCRIBE THERMAL STABILITY CURVES

DNA hybridization data are plotted as thermal stability (dissociation, elution) curves. They express either a fraction or a cumulative percentage of hybridization against temperature. Several statistics, or "distances," can be derived from these curves. These are single-value statistics that describe the overall similarity of the single-copy, or nonrepeated, DNA of two organisms. They are, therefore, inherently phenetic. The definitions of these statistics are adapted from Sibley and Ahlquist (1981a).

"Delta mode" is the difference in temperature between the modes of thermal stability curves of homologous (same species) and heterologous (different species) DNA hybrids.

"The normalized percent of hybridization" (NPH) is the percentage of hybridization in a heterologous hybrid divided by that of its homolog times 100 (Sibley and Ahlquist 1981a). Sibley and Ahlquist (1981a) wrote that NPH "has a range greater than that of delta mode and is probably more nearly linear with respect to time." The reciprocity of NPH, however, is the poorest of all the statistics that describe thermal stability curves. NPH may deviate from values predicted by the relative-rate test (see below) by as much as 35% (e.g. NPH = 35.7 ± 1.7 [n = 6] for tinamou × Ostrich [*Struthio camelus*] and NPH = 56.1 ± 2.0 [n = 5] for tinamou × kiwi; Sibley and Ahlquist 1981a).

"Delta $T_{50}H$" is the difference between the temperatures at which 50% of the homologous DNA is hybridized and 50% of the heterologous DNA is hybridized. This statistic is often extrapolated when the hybridized species are not closely related. Delta $T_{50}H$ is currently the statistic most widely used in DNA hybridization studies of birds.

These statistics describe parameters, but not the shapes, of different thermal stability curves. Drastically different curves hypothetically could yield identical single-point statistics. Examination of thermal stability curves of ratite DNA (Sibley and Ahlquist 1981a) reveals that comparisons within different monophyletic assemblages exhibit characteristically different-shaped curves (Fig. 1). Multivariate comparisons made over a range of temperatures would represent an improvement over delta mode or delta $T_{50}H$ values by accommodating differences in the shapes of curves. Such an approach might be more useful for distantly related taxa (e.g. interfamilial or interordinal comparisons) than delta $T_{50}H$, which must be extrapolated for taxa with less than 50% nucleotide homology.

THE "UNIFORM AVERAGE RATE" OF DNA EVOLUTION

The relative advantage of genetic distance data over morphological data for the formulation of phenetically based phylogenetic reconstructions depends on the extent to which macromolecular evolution is "clocklike" (Sarich and Wilson 1967, Farris 1981, Ayala 1982).[1] The rate of macromolecular evolution must be proportional to time and consistent across taxa to be clocklike. Full understanding of the various hypotheses of neutrality (that is, random evolution of the genome, unconstrained by selective forces) is not central to systematic problems *if* clocklike DNA evolution can be documented clearly (Thorpe 1982). It is sufficient that the extent to which DNA change is clocklike can be tested (independent of paleontological data; Fitch 1976) using the relative-rate test of Sarich and Wilson (1967). Simply stated, uniform rates of molecular evolution should be manifested as equal distances between any pair of sister taxa (A and B) relative to any given outgroup (C) (Fig. 2). This test determines the difference, if any, in the rates of evolution of the two sister taxa since they shared their ancestry as a single species. Equal distances presumably measure the same sequences, shared because of common ancestry, in the two hybrids A × C and B × C. This probably is not uniformly true because A and B may lose or retain different primitive sequences after their divergence. Such tests have already been used to show that different proteins or portions of DNA do not, in fact, evolve at equal rates, and individual proteins do not evolve uniformly through time (e.g. Fitch 1976, Ayala 1982). The average (either several proteins averaged together or individual proteins averaged across time, or both) of these different rates, however, can approximate stochastically a constant rate of molecular evolution (Ayala 1982). Sibley and Ahlquist asserted further that

[1] Sibley and Ahlquist (1980) used a cladistic algorithm, the Distance Wagner (Farris 1981), to cluster avian DNA distance data. High distance values are considered to reflect primitive similarities of heterologous DNA, whereas low distance values are considered to indicate derived similarities. Measures of genetic distance, however, are phenetic descriptions of the overall primitive and derived homology of two taxa. Individual distance values are not themselves primitive or derived, but the difference in two values (d) can indicate primitive and derived polarity. Because evolutionary rate differences can lead to ambiguity as to the magnitude and sign of d (i.e. the polarity of d), satisfaction of the relative-rate test is a prerequisite for the use of genetic distance data in cladistic, as well as phenetic, clustering algorithms. Distance values that do not measure homologous sequences in two heterologous hybrids involving one shared taxon (A × C, B × C, where C is a sister group of A and B), furthermore, will convey meaningless information of character-state polarity.

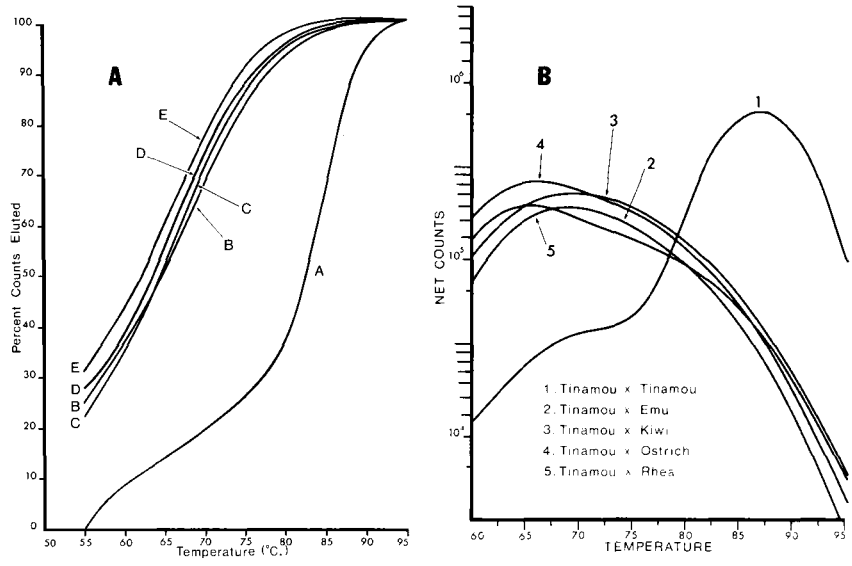

Fig. 1. Thermal stability curves of (A) *Acanthisitta* × *Acanthisitta* and *Acanthisitta* × suboscines, and (B) tinamou × tinamou and tinamou × ratites, reproduced from Sibley et al. (1982) and Sibley and Ahlquist (1981a), respectively. In both figures the curves of the homologous hybrids stand well apart from those of the heterologous hybrids, but the interpretation of these data are quite different. (See text.) The fact that the abscissa is net counts eluted in one graph and percentage counts in the other accounts for the difference in the shapes of the curves (i.e. sigmoid vs. bell shaped). Figure 1A: curve A = *Acanthisitta* × *Acanthisitta*, B = average of 9 different New World suboscine × *Acanthisitta* hybrids, C = average of 3 different broadbill × *Acanthisitta* hybrids, D = average of 4 different pitta × *Acanthisitta* hybrids, E = average of 41 different oscine × *Acanthisitta* hybrids. Figure 1B was mislabeled by Sibley and Ahlquist: one curve is labeled twice and another is not labeled. In my opinion, the tinamou × Emu curve is correctly labeled and the unlabeled curve is actually the tinamou × rhea hybrid. I base this conclusion on my observation that the shapes of thermal elution curves convey phylogenetic information. Ostrich × outgroup and rhea × outgroup elution profiles almost always resemble each other but differ from hybrids made with the DNA of Emu, cassowary, or kiwi.

although different genes exhibit different rates of mutation, the nuclear genome is sufficiently large that, when different genes that evolve at different rates are averaged, the entire genomes of different lineages always show the same average rate of evolution. Sibley and Ahlquist (1984b) also argued that the "uniform average rate" (UAR) of DNA evolution is the same for both mammals and birds. They claimed (Sibley and Ahlquist 1981a, 1982d, 1983, 1984b) that equal rates are observed, but empirical evidence is scant. Data from other laboratories contradict their conclusion. First, grossly variable rates in the evolution of single-copy nuclear DNA across taxa have already been observed in primates (Bonner et al. 1981) and rodents (Brownell 1983) using DNA hybridization techniques. Second, the rate of evolution of rodent DNA was approximately twice that of ratite birds (Brownell 1983, Wu and Li 1985). Third, apparent differences in evolutionary rates among higher taxonomic groups, identified by single-copy DNA hybridization, were correlated with known differences in rates of nucleotide substitution (Britten 1986).

I analyzed statistically the genetic distances (delta mode) of primitive insect-eating passerine birds (Sibley and Ahlquist 1980) to test relative rates of DNA evolution between genera of birds (Tables 1 and 2). The relative-rate test dictates that any two or more taxa in a monophyletic group must exhibit the same genetic distance to any given outgroup. Friedman's χ^2_R test specifically tests the null hypothesis that no differences exist in the genetic distances of sister taxa when hybridized with each of several outgroups, as predicted by the relative-rate test. This test (BMDP statistical package, Dixon and Brown 1979) shows that highly significant differences (Table 1: Friedman's $\chi^2_R = 14.85$, $P =$

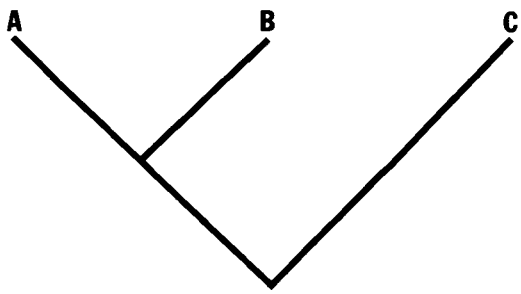

Fig. 2. Relative-rate test: AC = BC. Ultrametric inequality: AB < AC, BC.

0.0019; Table 2: Friedman's $\chi^2_R = 15.63$, $P = 0.0004$) exist and the null hypothesis is rejected. Second, the Student Newman Keuls (SNK) procedure on the rank sums (Zar 1974) identifies specifically which of the taxa in the monophyletic group differ and violate the relative-rate test. This second test shows that distance values of the sylvioids *Sylvia* and *Parus* differ significantly ($P < 0.05$) from those of the sylvioids *Trichastoma* and *Chamaea*. The values for the fringilloids *Prunella* and *Motacilla* differ significantly ($P < 0.05$) from that of the fringilloid Ploceinae. I used nonparametric statistics because it is the constancy of the rank of each variable (species belonging to the monophyletic group) across all cases (outgroup hybrids) that is important to the relative-rate test, not the actual distance values (Tables 1 and 2). In other words, *Parus* and *Sylvia* consistently exhibit greater genetic distances than *Trichastoma* and *Chamaea* relative to all outgroups examined, contrary to the prediction of UAR. Consistently higher distance values between one member of the monophyletic group and *all* outgroups than between other members of the monophyletic group and the same outgroups indicate that this lineage evolved faster than the other members of the group. Consistently lower values indicate slower evolution in that lineage (Bonner et al. 1981). Sibley and Ahlquist (1981a, 1983) argued that deviations from the expectation of no difference in relative rates of evolution between members of a monophyletic assemblage, as predicted by UAR, can be attributed to experimental error. Experimental error should be manifested as random variation. Nonrandom deviations, such as among the sylvioids, probably indicate actual differences in rates of DNA evolution across lineages. Although it would be desirable to examine larger matrices of DNA hybridization values to investigate relative rates further, such data for birds have never been published.

TABLE 1. Relative-rate test of Sylvioidea using Friedman's χ^2_R and SNK test on the rank sums. Matrix of delta mode values for the sylvioids, *Sylvia, Trichastoma, Chamaea,* and *Parus,* hybridized with 14 outgroups, is taken from Sibley and Ahlquist (1980). *Sylvia* and *Parus* differ significantly ($P < 0.05$) from *Trichastoma* and *Chamaea,* as indicated by asterisks. The variables A and B refer to taxa and assume the values of rank order. NS = not significant.

	Parus	*Cham-aea*	*Trich-astoma*	*Sylvia*
Turdinae	11.5	9.6	9.6	10.6
Erithacus	10.3	10.9	10.9	11.1
Myadestes	11.0	10.0	10.0	11.0
Muscicapa	11.4	9.2	8.8	9.2
Miminae	10.8	12.9	10.9	12.5
Sturnus	10.7	13.0	11.0	13.1
Cinclus	10.8	10.4	10.4	12.1
Phainopepla	9.8	7.7	7.7	9.8
Thryomanes	10.8	10.3	10.4	12.4
Monarcha	9.5	9.1	9.1	9.3
Corvus	10.3	9.0	9.0	9.0
Prunella	12.0	8.5	8.5	12.4
Motacilla	10.1	9.0	9.8	9.7
Ploceinae	10.6	8.4	8.4	8.9
Rank sums (R)	43.0	26.5	25.0	45.5
Rank order	2	3	4	1

Friedman's $\chi^2_R = 14.85$, $P = 0.0019$*

SNK test of rank sums

SE = 4.83

B vs. A	$R_B - R_A$	q	p	$q_{0.05,\infty,p}$
1 vs. 4	20.5	4.24	4	3.633*
1 vs. 3	19.0	3.93	3	3.314*
1 vs. 2	2.5	0.52	2	2.772 NS
2 vs. 4	18.0	3.73	3	3.314*
2 vs. 3	16.5	3.42	2	2.772*
3 vs. 4	1.5	0.31	2	2.772 NS

Thus, at least some avian lineages appear to exhibit significantly different rates of genetic evolution. Is this statistically detectable difference substantial enough to lead to erroneous conclusions about phylogenetic relationships? Obviously, it depends on the magnitude of the rate differences relative to the closeness of the taxa in question. Polar ordination (Fig. 3) of the complete matrix of delta mode values for primitive insect-eating passerines (Sibley and Ahlquist 1980) forms clusters of taxa that accord with presumed monophyletic groups. Taxa that occupy the center of the plot (*Sylvia, Cinclus,*

TABLE 2. Relative-rate test of Fringilloidea using Friedman's χ^2_R and SNK test on the rank sums. Matrix of delta mode values for the fringilloids, *Prunella*, *Motacilla*, and Ploceinae, hybridized with 15 outgroups, is taken from Sibley and Ahlquist (1980). The Ploceinae differ significantly ($P < 0.05$) from *Motacilla* and *Prunella*, as indicated by asterisks. The variables A and B refer to taxa and assume the values of rank order. NS = not significant.

	Ploceinae	*Motacilla*	*Prunella*
Turdinae	10.5	10.5	10.7
Erithacus	9.1	10.3	11.8
Myadestes	10.0	9.5	11.0
Muscicapa	8.6	9.6	12.2
Miminae	8.8	9.7	9.3
Sturnus	9.3	11.0	10.4
Cinclus	9.7	11.6	10.8
Phainopepla	7.1	7.6	9.7
Thryomanes	9.0	10.1	11.6
Monarcha	8.2	8.3	9.6
Corvus	10.0	9.8	13.2
Sylvia	8.9	9.7	12.4
Trichastoma	8.4	9.8	8.5
Chamaea	8.4	9.0	8.5
Parus	10.6	10.1	12.0
Rank sums (R)	18.5	31.5	40.0
Rank order	3	2	1

Friedman's $\chi^2_R = 15.63$, $P = 0.0004$*

SNK test of rank sums

SE = 3.87

B vs. A	$R_B - R_A$	q	p	$q_{0.05,\infty,p}$
1 vs. 3	21.5	5.56	3	3.314*
1 vs. 2	8.5	2.20	2	2.772 NS
2 vs. 3	13.0	3.36	2	2.772*

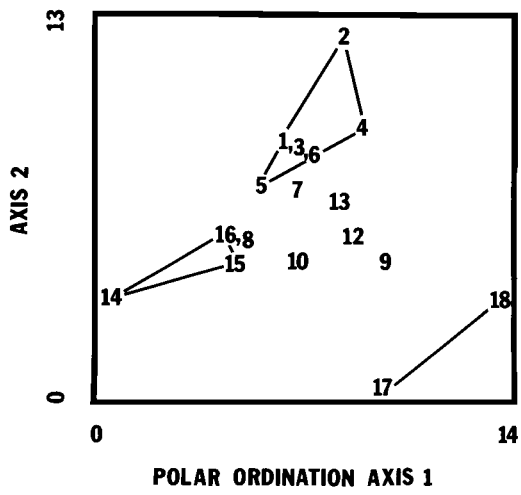

Fig. 3. Polar ordination of primitive insect-eating passerines based on delta mode values of Sibley and Ahlquist (1980). 1 = Turdinae, 2 = *Erithacus*, 3 = *Myadestes*, 4 = *Muscicapa*, 5 = Miminae, 6 = *Sturnus*, 7 = *Cinclus*, 8 = *Phainopepla*, 9 = *Sylvia*, 10 = *Trichastoma*, 11 = *Chamaea*, 12 = *Parus*, 13 = *Thryomanes*, 14 = *Prunella*, 15 = *Motacilla*, 16 = Ploceinae, 17 = *Monarcha*, 18 = *Corvus*. Coordinates of *Thryomanes* and *Prunella* are tied. Monophyletic groups, clustered unambiguously, are joined by lines.

Thryomanes, and *Phainopepla*) might be grouped in any of several ways. Different methods of clustering (UPGMA, WPGMA, single and complete linkage methods), applied to the same data set, yield trees in which some taxa are not grouped in the same way each time (Fig. 4). Differences in branching patterns are due at least in part to the fact that the distance data do not fit the trees as well as they should if DNA evolution were purely metric. Branch lengths are stretched and squeezed to fit conflicting data. Templeton (1985) showed that Sibley and Ahlquist (1984b) did not compute the statistical confidence of the different phylogenetic trees that can be generated from the same data set. When this is done, it becomes evident that other topologies are possible.

Lanyon (1985) found that the clustering of some taxa differs when a single taxon is omitted from the DNA hybridization data set. He assembled a matrix of delta $T_{50}H$ values from linear tables (see below) of tyrannoids (Sibley and Ahlquist 1985a). Ideally, omitted taxa should result only in a missing terminal branch and should not affect the clustering of other taxa. I subjected portions of Sibley and Ahlquist's (1985a) data set to the same relative-rate test as applied to the primitive insect eaters (Tables 3–5) to test whether the unstable branches identified by Lanyon were the consequence of evolutionary rate differences. (Lanyon's matrix consisted of only ingroups and thus does not provide the necessary data to test relative rates across all variables, i.e. all seven taxa from the monophyletic Tyrannidae.) Consistent rate of differences are evident between the monophyletic clade *Schiffornis* and *Pachyramphus* (Table 3) even though the branching of these two taxa was stable when jackknifed (Lanyon 1985). Rate differences were not detectable for other taxa treated by Lanyon, but the hybrid values for some taxa were consistently higher than the hybrid values for other taxa in the same monophyletic group (Tables 4

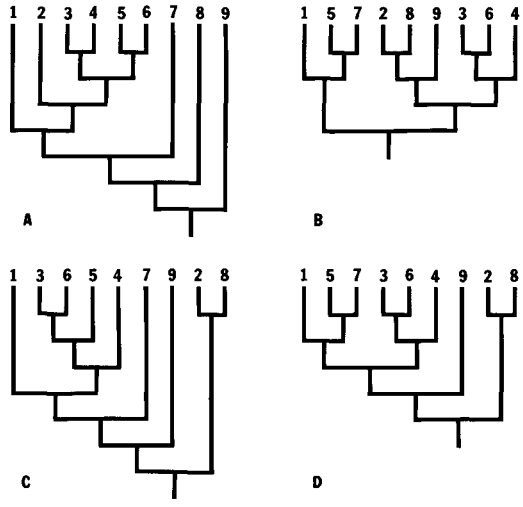

Fig. 4. Trees produced by alternate clustering techniques using delta mode values of primitive insect-eating passerines of Sibley and Ahlquist (1980). (A) Single linkage, (B) complete linkage, (C) UPGMA, (D) WPGMA. Branch lengths do not correspond to genetic distance. Multiple branches for taxa that are clustered identically in all four trees are lumped as single terminal branches, e.g. *Monarcha* and *Corvus*. 1 = *Monarcha* and *Corvus*; 2 = Turdinae, *Myadestes, Erithacus, Muscicapa,* Miminae, and *Sturnus*; 3 = *Phainopepla*; 4 = *Prunella, Motacilla,* and Ploceinae; 5 = *Sylvia*; 6 = *Trichastoma* and *Chamaea*; 7 = *Parus*; 8 = *Cinclus*; 9 = *Thryomanes*.

TABLE 3. Relative-rate test of Tityrinae using Friedman's χ^2_R. Matrix of delta $T_{50}H$ values for the tityrines, *Schiffornis turdinus* and *Pachyramphus polychopterus,* hybridized with 6 tyrannoid outgroups, *Myiarchus tyrannulus, Sayornis phoebe, Elaenia frantzii, Mionectes olivaceus, Pipra erythrocephala,* and *Pipreola arcuata,* is taken from Lanyon (1985). First values "represent the $T_{50}H$ for the indicated hybrid or the mean value when the reciprocal test was conducted" (Lanyon 1985). Second values "represent the mean $T_{50}H$ for hybrids between the indicated genera (including species other than those listed)" (Lanyon 1985). Friedman's test statistic and significance levels are identical for both data sets. *Schiffornis* differs significantly ($P < 0.05$) from *Pachyramphus,* as indicated by an asterisk.

	Schiffornis		*Pachyramphus*	
Myiarchus	8.70	9.80	7.80	8.13
Sayornis	10.60	10.60	9.35	9.37
Elaenia	9.10	9.10	8.85	8.73
Mionectes	11.10	11.10	9.85	9.93
Pipra	8.75	9.02	8.55	8.80
Pipreola	10.20	9.67	10.00	9.45

Friedman's $\chi^2_R = 6.0$, $P = 0.0143$*

and 5; note rank sums). The inability to demonstrate clear rate differences in these taxa probably occurs because the statistical significance of these tests was limited by the number of outgroups used. The number of outgroups available for relative-rate tests were low because (1) only non-Tyrannidae can be used as outgroups, and (2) the same outgroups generally were not used by Sibley and Ahlquist for hybridization with the seven Tyrannidae treated by Lanyon (1985).

Another study included the superfamilies Sylvioidea and Fringilloidea (the specific taxa were not indicated), which together form a monophyletic group, compared with two species of Sturnidae and two species of Mimidae (Sibley and Ahlquist 1984a: table 5). The relative-rate test showed equal rates of evolution in the sylvioids and fringilloids in the 1984 study even though I demonstrated statistical differences in rates between members of these superfamilies (Tables 1 and 2). This exists because genetic distance values of many species within each of these superfamilies were averaged, and slowly and rapidly evolving lineages tend to balance each other when combined. This is an important point. Even highly significant statistical differences in evolutionary rates do not necessarily preclude the formulation of valid phylogenetic reconstructions, provided a sufficient diversity of taxa is averaged and the overall distributions of evolutionary rates within the different groups are not skewed in opposite directions. Sibley and colleagues routinely employ this method of averaging. However, potentially interesting rate differences may be obscured if only these average distances, but not the raw data, are presented. Moreover, the specific hybrid comparisons that were actually made in the formation of phylogenetic trees usually are not indicated. Average nodal distances are provided instead. Higher-level divergences based primarily on some specific taxa might not agree with distances based on different taxa within the same monophyletic groups.

Certainly, distance values that do not satisfy the "ultrametric inequality" (Farris 1981) are not clocklike and thus have dubious application in phylogenetic analysis. The ultrametric inequality simply requires that the distance between any two taxa, A and B, in a monophyletic group should never exceed the distance

TABLE 4. Relative-rate test of Tyrannidae using Friedman's χ^2_R. Matrix of delta $T_{50}H$ values for the Tyrannidae, *Myiarchus tyrannulus, Elaenia frantzii, Pipra erythrocephala,* and *Pachyramphus polychopterus,* hybridized with 4 mionectid outgroups, *Leptopogon amaurocephalus, Leptopogon superciliaris, Mionectes olivaceus,* and *Mionectes oleaginea,* is taken from Sibley and Ahlquist (1985a). Parenthetic rank values follow data. Note constancy of rank values for *Pipra.* "Ideal" rank sums are the most extreme values that can be obtained if the evolution of *Pipra* has been faster than the evolutionary rates of *Myiarchus, Elaenia,* and *Pachyramphus.* The power of Friedman's test is limited by the small number of outgroups used here. NS = not significant.

	Myiarchus	*Elaenia*	*Pipra*	*Pachyramphus*
L. amaurocephalus	9.3 (2.5)	9.1 (1)	9.9 (4)	9.3 (2.5)
L. superciliaris	9.4 (2)	9.8 (3.5)	9.8 (3.5)	9.1 (1)
M. olivaceus	9.4 (1)	10.0 (3)	10.2 (4)	9.8 (2)
M. oleagineus	9.9 (2)	9.2 (1)	10.3 (3.5)	10.3 (3.5)
Rank sums	7.5	8.5	15	9
Ideal rank sums	8	8	16	8
Friedman's $\chi^2_R = 5.17$, $P = 0.1594$ NS				

between either A or B and some outgroup, C, if these distances are clocklike (Fig. 2). Nonconformity with the ultrametric inequality requires negative branch lengths, which are biologically meaningless and historically impossible. Violations of the ultrametric inequality can result from unequal rates of evolution or because the sequences measured by distance data for two heterologous hybrids, A × C and B × C, are not entirely homologous. Sibley and Ahlquist (1984b: 10) wrote, "These definitions are satisfied by our DNA hybridization data, all 18,000+ hybrids in more than 900 experimental sets, without exception," and (1985c: 118), "Our DNA hybridization data fit the definitions . . . for metric and ultrametric distance measures." Yet, another example of differences in rates of DNA evolution in birds comes directly from violation of the ultrametric inequality by genetic distances between suboscine and oscine passerines. In their study of the New Zealand wrens (Acanthisittidae), Sibley et al. (1982) used labeled *Acanthisitta* DNA in hybridization experiments with 14 suboscines ($T_{50}H$ 16.4–18.6) and 41 oscines ($T_{50}H$ 18.6–21.7). Sibley et al. considered *Acanthisitta* a suboscine, the sister group of all other suboscines. According to their phylogenetic reconstruction, the nonacanthisittid suboscines are strictly monophyletic, and the living oscines may be either monophyletic or paraphyletic. Sibley and Ahlquist (1982c) hybridized the DNA of an oscine, *Vireo olivaceus* (labeled), and a suboscine, *Elaenia flavogaster.* Both *Vireo olivaceus* and *Elaenia flavogaster* were hybridized with *Acanthisitta chloris.* This provides data for a test of the ul-

TABLE 5. Relative-rate test of Tyranninae using Friedman's χ^2_R. Matrix of delta $T_{50}H$ values for the Tyranninae, *Myiarchus tyrannulus, Sayornis phoebe,* and *Elaenia frantzii,* hybridized with 5 nontyrannine tyrannoids, *Mionectes olivaceus, Schiffornis turdinus, Pipra erythrocephala, Pachyramphus polychopterus,* and *Pipreola arcuata,* is taken from Lanyon (1985). First values "represent the $T_{50}H$ for the indicated hybrid or the mean value when the reciprocal test was conducted" (Lanyon 1985). Second values "represent the mean $T_{50}H$ for hybrids between the indicated genera (including species other than those listed)" (Lanyon 1985). "Ideal" rank sums are the most extreme values that can be obtained if the evolution of *Myiarchus* has been slower than *Sayornis* and *Elaenia.* The power of Friedman's test is limited by the small number of outgroups used here. NS = not significant.

	Myiarchus		*Sayornis*		*Elaenia*	
Mionectes	9.40	9.50	9.45	9.45	9.75	9.93
Schiffornis	8.70	9.80	10.60	10.60	9.10	9.10
Pipra	8.50	8.14	8.40	8.40	8.95	8.97
Pachyramphus	7.80	8.13	9.35	9.37	8.85	8.73
Pipreola	8.80	8.82	9.25	9.25	8.90	9.55
Rank sums	6	7	12	11	12	12
Ideal rank sums	5		12.5		12.5	
Friedman's $\chi^2_R = 4.8, 2.8$; $P = 0.0907$ NS, 0.2466 NS						

trametric inequality. The $T_{50}H$ values for these hybrids are: *Vireo* × *Acanthisitta*, 19.5; *Vireo* × *Elaenia*, 16.1; *Acanthisitta* × *Elaenia*, 17.7. The distance between two taxa (*Acanthisitta* and *Elaenia*) in a monophyletic group (suboscines) exceeds the distance between one of these (*Elaenia*) and an outgroup (*Vireo*). The ultrametric inequality is violated. This cannot be corrected by an alternative phylogenetic model without invoking additional violations of the ultrametric inequality in comparisons that involve other species. $T_{50}H$ 16.1 is smaller than even the smallest value ($T_{50}H$ 16.4) found between *Acanthisitta* and 14 suboscines. Clearly, any suboscine × oscine hybrid should always yield $T_{50}H$ values greater than 18.6–21.7 and never be less than any suboscine × suboscine hybrid if the UAR theory is to be upheld.

Assuming the above data are reproducible, it would seem that the rate of genetic evolution in *Acanthisitta* exceeds that of *Elaenia* (and possibly *Vireo*) by a substantial amount. Technical errors in the preparation of the *Elaenia* DNA for hybridization, however, could be responsible for these results (C. G. Sibley pers. comm.). For example, if the specimen was not fixed properly within a critical time period after collection, then endogenous DNase might have broken the DNA into smaller segments than normally are used (500 nucleotides on average). Smaller segments are less unique and, therefore, have greater affinity for heterologous DNA than do larger segments. Thus, a nonbiological explanation potentially could account for this inconsistency. But no other hybrids involving *Elaenia* indicate that this sample was improperly prepared. The specific experiments (e.g. simple repetition) necessary to determine the cause or validity of these results were not performed. Sibley and Ahlquist (1985c: 117) wrote, "delta [$T_{50}H$] values may be corrected for some sources of error (e.g. variation in fragment size)," implying that they have run into this problem elsewhere. Corrected values ought to be clearly identified in publications.

In all the most recent avian DNA hybridization studies, linear tables of genetic distances relative to one reference (labeled) taxon are published, rather than a matrix of values. Linear tables make impressive presentations because they appear to point to unambiguous phylogenetic conclusions. They are inappropriate tests of relative rates unless the reader is prepared to assemble matrices from them. If the rates of DNA evolution for the taxa in a linear table differ from one another, then the resultant phylogenetic reconstruction could be undetectably erroneous.

Linear tables can show some trends even though they are not particularly amenable to relative-rate tests. Sibley and Ahlquist (1985a) provided several linear tables of distance data for the Tyrannoidea. Although they consider their "Mionectidae" to be an outgroup of the Tyrannidae, delta $T_{50}H$ values of Tyranninae × Mionectidae hybrids overlap extensively with delta $T_{50}H$ values of Tyranninae × nontyrannine Tyrannidae. This overlap is manifested by further violations of the ultrametric inequality (e.g. *Sayornis phoebe* × *Schiffornis turdinus*, 10.6; *Mionectes olivaceus* × *Sayornis phoebe*, 9.2; *Mionectes olivaceus* × *Schiffornis turdinus*, 11.5; reciprocal hybrid 10.7). The data in these tables could not have been clustered to form the phylogenetic tree without extensive averaging of delta $T_{50}H$ values. The biological validity of the family "Mionectidae" is questionable.

Sibley and Ahlquist (1983: table 2) performed tests that strongly suggest a correlation of evolutionary rate differences and phylogenetic disparity, although the tests were not designed specifically for that purpose. Ten identical hybrids formed with *Acanthiza chrysorrhoa* (Passeres: Acanthizidae) and *Anthochaera carunculata* (Passeres: Meliphagidae) had a range of variation of delta $T_{50}H$ values of 0.6. Nine hybrids formed with *Acanthiza chrysorrhoa*, and 9 different confamilial genera of honeyeaters (Passeres: Meliphagidae) had a somewhat higher range of variation, 1.7. Hybrids formed with *Acanthiza chrysorrhoa* and 19 genera of oscines belonging to different families had a still higher range of variation, 2.3. Sibley and Ahlquist (1983) maintained that the observed increase of variation with taxonomic disparity is the result of the experimental design. The 10 identical hybrids were formed with the same specimen preparations. The 9 hybrids formed with honeyeaters had to be made with different specimen preparations for each of the different species. The use of different specimen preparations could increase the experimental error (Sibley and Ahlquist 1983: table 4). The high range of 2.3 could be the result of the correspondingly larger sample size. The authors failed to discern between these alternative explanations. Their table 2 shows the data

needed for relative-rate tests, but the methods are inconsistent. The table does not indicate the role of experimental error. Their table 4 was designed to show the effect of sample size and the number of radio-labeled taxa used on experimental error. It relates no information, however, about relative rates in monophyletic groups. The ranges of $T_{50}H$ values in their table 4 are generally higher than those of table 2 and others (e.g. Sibley and Ahlquist 1983: table 5), regardless of sample size and number of specimen preparations.

It is clear from relative-rate tests that either the DNA of some lineages of birds has evolved at different rates or differences in the preparation of specimens can lead to nonrandom errors in distance values. Errors or the difference in rates may rarely be large enough to preclude the use of genetic distances for the formulation of phylogenetic reconstructions, but they may lead to ambiguous phylogenetic reconstructions. Farris (1981: 14), in reference to electrophoretic clocks, wrote that "Proponents of Nei's version of the clock nonetheless persist in interpreting this distance as if it were purely clocklike—and for a familiar reason, if exceptions are admitted the method loses its claim to the role of direct indicator of phylogenetic kinship." Rate differences would have their greatest effect when the branching nodes of taxa in a dendrogram are very close. Unstable branches may in turn lead to nomenclatural problems because Sibley and Ahlquist have attempted to coin taxonomic subdivisions for many phylogenetic branches. It is important that data are scrutinized for statistical variations in rates that are subtle enough to be overlooked yet substantial enough to cause an error in data clustering. Again, the data necessary for such analysis (i.e. complete matrices) are almost never published. This is presently the single most objectionable aspect of the DNA hybridization studies of avian relationships.

Satisfaction of the relative-rate test is a prerequisite for the use of DNA data for phylogenetic reconstructions. It would be difficult to demonstrate equal rates of evolution in different orders. To test relative rates of evolution among orders, sister orders must be compared with even more distantly related outgroups. Genetic distances that appear to satisfy relative-rate tests between such distant taxa can be the result of diminished sensitivity of the technique for distantly related organisms. Reduced sensitivity is the result of the increasingly large percentage of mismatched base pairs and reduced rate of reassociation in heterologous duplexes formed at low temperatures. Reduced sensitivity begins abruptly at about $T_{50}H$ 15 (Sibley and Ahlquist 1985c: 152). Values above $T_{50}H$ 25 may be beyond the sensitivity of the technique (Brownell 1983). Because comparisons between distantly related taxa may be outside the range of sensitivity of DNA hybridization, there is now no way to test relative rates of DNA evolution in distantly related birds without relying on other, more conservative measurements for calibration.

There are three reasons relative-rate tests might suggest that evolutionary rates have differed among avian lineages. (1) Differences may be nonbiological, resulting from differences in the fragment size of the single-copy DNA, DNA concentration and driver : tracer ratio, or reduced stringency conditions. These are errors introduced by the experimenter. (2) Differences may be biological (e.g. differences in genome size) but still not the result of evolutionary rate differences. Some species may possess a greater number of similar, but nonrepeated, nucleotide sequences than other species that are not removed in the initial preparation of single-copy DNA. These sequences would tend to form duplexes with themselves rather than with the DNA of the hybridized species, and would appear to have evolved slowly. Hybrids involving such species should characteristically give poor reciprocity. Both of the single strands in a duplex would be radio-labeled (hence, each heteroduplex counted twice) when this was the tracer species. Neither strand would be labeled or counted when this was the driver species. There is no evidence for this phenomenon to date, possibly because a great excess of driver DNA is always used to minimize the formation of homoduplexes. (3) Rate differences may actually exist. The possible causes for rate differences are many and varied. Environmental mutagens are one possible cause. Differences in generation length was suggested as another (Wu and Li 1985), but Sibley and Ahlquist (1983) showed that this was not the case in Procellariiformes. Rate differences were attributed to a possible founder-effect in one case (Bonner et al. 1981). Wyles et al. (1983) suggested that accelerated rates of morphological, and possibly biochemical, evolution in birds could be adaptively linked with behavioral

plasticity. Adaptive mechanisms could also retard evolution through gene repair and nonrandom segregation during gametogenesis. Other effects (Dawkins 1976, Dover 1982) may also accelerate evolutionary rates, but they should not have a noticeable impact on the hybridization of nonrepeated DNA.

TAXONOMIC CONSIDERATIONS

Interpretation of DNA hybridization data is subject to ambiguities, as are other systems. These include definitions of adequate criteria for the identification of monophyletic groups, the reliance on other studies, and the differing goals of molecular vs. organismal taxonomists.

Consider the criteria for determining monophyly of taxa. Sibley and Ahlquist (1981a) claimed to have shown (i.e. satisfied the relative-rate test) the monophyly of the ratites. Although the published delta $T_{50}H$ values were miscalculated, supplemental data (Diamond 1983) support their claim. This was not a rigorous test of monophyly. Apparent satisfaction of the relative-rate test might be artificially simulated by reduced sensitivity or other unknown mechanisms. To show that ratites are monophyletic, hybrids formed with polyphyletic or paraphyletic outgroups should yield different genetic distances. These distances should be greater than those used to show equal rates for the monophyletic group. If the paleognathous birds and all neognathous birds are each strictly monophyletic (i.e. neither was ancestral to the other), then a rigorous test of monophyly is not possible.

A potential ambiguity in the interpretation of hybridization data is elucidated by comparing thermal stability curves from two different studies. The curves of New Zealand wrens × other passerines (Sibley et al. 1982: fig. 1; Fig. 1A in this paper) and the curves of tinamou × ratites (Sibley and Ahlquist 1981a: fig. 10; Fig. 1B in this paper) are very similar to each other, but they are interpreted differently. In both figures, the homologous hybrids stand well apart from a tight cluster of curves that represent heterologous hybrids. The ratites are interpreted as being monophyletic relative to the tinamou outgroup, but the suboscine and oscine passerines are considered to be diphyletic relative to the New Zealand wrens. This interpretation is justified because the sample means, standard deviations, and standard errors show that delta $T_{50}H$ values for suboscine and oscine passerines, relative to *Acanthisitta*, are significantly different from one another (Sibley et al. 1982: 123). They are not significantly different in the ratite × tinamou hybrids. Yet there remains the question of whether statistical significance goes hand in hand with biological significance. Clearly, *Acanthisitta* is far removed from all other passerines. Given the possibility of evolutionary rate differences in passerine birds, the slightly lesser average genetic distance of *Acanthisitta* to suboscines than to oscines does not provide convincing evidence for the suboscine relationships of *Acanthisitta*. As with the ratites, convincing evidence of such a relationship could come from unequal distances to ingroups, and equal distances to monophyletic outgroups but unequal distances to polyphyletic or paraphyletic outgroups.

Meaningful interpretation of DNA hybridization data requires that all taxa relevant to a particular taxonomic problem be compared. In practice, however, the DNA of some taxa are usually unavailable. Researchers have been inconsistent in the reliance on or disregard for traditionally accepted classifications when integrating missing taxa into phylogenetic reconstructions. For example, Sibley and Ahlquist (1985c: 124) rejected Olson and Feduccia's (1980) hypothesis of a relationship between the Australian Banded Stilt (*Cladorhynchus leucocephalus*) and the flamingos. Sibley and Ahlquist's temporal calibration indicated that the oldest divergence among the Charadriiformes or among the Ciconiiformes was older than Olson and Feduccia's purported flamingo-stilt fossil. Sibley and Ahlquist assumed that *Cladorhynchus* will yield genetic distance values similar to those of other recurvirostrids, but the experiment has not been done. Elsewhere, Sibley and Ahlquist (1985c: 121) advocated a distant relationship between South American and African sungrebes (Heliornithidae) to highlight unexpectedly low $T_{50}H$ values between *Heliornis* and the Limpkin (*Aramus guarauna*), even though intraheliornithid hybrids have not been made.

The merit of a system in which taxonomic hierarchy is determined by genetic distance is debatable. Sibley and Ahlquist advocate a classificatory system in which all divergences between $T_{50}H$ X and Y are generic, those between $T_{50}H$ Y and Z are familial, and so on. Is it DNA or organisms that are to be classified? If organisms evolved at constant rates, or even if ge-

notypic and phenotypic evolutionary rates were closely correlated, then there would be no discrepancy. A classificatory system based strictly on genetic-distance data groups diverse species that share a relatively recent common ancestry. At the same time, it separates groups that are morphologically conservative.

Temporal Calibration

The extent to which DNA hybridization data satisfy the relative-rate test will determine the accuracy of the branching pattern of phylogenetic reconstructions based on those data. Converting measures of genetic distance to absolute time presents difficulties. "The question is not what should be done, which is obvious, but to find trustworthy divergences based on fossils and/or geological records. For birds, there may be no dated divergences based on fossils that are accurate enough for this purpose, but general constraints of the avian fossil record are useful" (Sibley and Ahlquist 1984b). In practice, however, Sibley and Ahlquist do not seem to have considered the general constraints of the fossil record. The calibration of the DNA molecular clock rested on only five data points older than 10 million years. The first of these is based on the divergence of the orangutan (*Pongo*) from other hominoids at 16 MYBP, following Pilbeam (1983). There is no rationale for using the divergence of primates for the calibration of the avian DNA molecular clock. Mammalian and avian DNA clocks have already been shown to run at different rates (Brownell 1983, Britten 1986). Furthermore, Pilbeam (1984) recently suggested that the *Pongo*-hominoid divergence could be as recent as 12 MYBP and is subject to an uncertainty of 25%. Other authors cited by Sibley and Ahlquist (1984b) also advocated this later divergence time. The significance of this divergence even to mammalian molecular clocks is open to question.

The other four published data points for the calibration of the DNA molecular clock were inferred from geotectonic data and vicariance biogeographic hypotheses of the diversification of selected avian taxa. These are based on dichotomies among the ratites, the suboscines and New Zealand wrens, Sibley and Ahlquist's New World suboscine and "Old World suboscine" passerines, and the South American and African barbets. The first two of these are mutually inconsistent.

Ostriches (Struthionidae) and rheas (Rheidae) are presumed to have diverged from a common ancestor as the result of the separation of South America and Africa (Cracraft 1974, Prager et al. 1976). Sibley and Ahlquist (1981a) felt that by 80 MYBP the Atlantic was wide enough to form an effective barrier between the hypothetical South American and African ratite populations. Hence, the Ostrich-rhea divergence was used as the baseline for their temporal calibration of genetic distance. New fossil evidence undermines the significance of vicariance biogeography to the divergence of Ostriches from other ratites. Paleogene fossils of volant paleognathous birds (Houde and Olson 1981) are the sister group of Ostriches, which apparently evolved in the Northern Hemisphere long after the breakup of Gondwanaland (Houde in press a). The fossils do not establish the divergence of Ostriches and rheas, but suggest that the divergence was not necessarily correlated with the spreading of the Atlantic seafloor.

Another calibration date comes from the study of the New Zealand wrens. Sibley et al. (1982) presumed the New Zealand wrens to have diverged from an unnamed and hypothetical Australian sister group as the result of the formation of the Tasman Sea at about 80 MYBP (Sibley and Ahlquist 1981a, 1984b; Sibley et al. 1982). Sibley and Ahlquist assumed that this geotectonic event was of primary importance to the divergence of New Zealand wrens and their sister group. From their own phylogenetic reconstruction and dating based on the Ostrich-rhea divergence, however, Sibley and Ahlquist (1981a) concluded that kiwis (Apterygidae) did not diverge from the Papuan-Australian ratites until the middle or late Eocene, at about 40–50 MYBP. To account for the dispersal of the flightless kiwi from Australia, across the Tasman Sea to New Zealand, they envisioned an archipelagic sweepstakes route for which there may be some evidence (Fleming 1975). It seems inconceivable that, as flightless birds, kiwis could find a way to cross the Tasman Sea but the volant New Zealand wrens could not. "Many of the recent Australian immigrants to New Zealand have been volant species aided by the prevailing westerly wind pattern" (Sibley and Ahlquist 1981a: 323).

As their last calibration points, Sibley and Ahlquist (1983, 1985c) presumed New World suboscine passerines (Tyrannides) and South

American barbets (Capitonidae) to have diverged from Old World "suboscines" (Eurylaimides) and African barbets ("Lybiidae"), respectively, at 75–80 MYBP, again owing to the breakup of the Gondwana supercontinent. Passerine birds are not known from pre-Miocene fossils, despite a relatively good representation of small arboreal birds from older deposits (Feduccia and Martin 1976; Olson 1976, 1985; Olson and Feduccia 1979; Mourer-Chauviré 1982). Regardless, Sibley and Ahlquist (1985a) extended families of modern passerines (e.g. Furnariidae, Formicariidae, Thamnophilidae) back as far as the Paleocene, a period from which even the ancestors of few orders of modern birds are known. Barbets, on the other hand, are present in early Tertiary deposits of the Northern Hemisphere and were not endemic to Gondwanaland. Sibley and Ahlquist (1986) subsequently placed South American barbets in the Ramphastidae and African barbets in the Megalaimidae, and, without explanation, advocated their divergence at 40–50 MYBP. It would be desirable to know the DNA hybridization values between South American and African grebes (Podicipedidae), ducks (Anatidae), sungrebes (Heliornithidae), jacanas (Jacanidae), thick-knees (Burhinidae), pigeons (Columbidae), parrots (Psittacidae), and trogons (Trogonidae) to test the hypothesis that Atlantic seafloor spreading is appropriate for the calibration of DNA hybridization data. Recently publicized (1986, Intern. Ornithol. Congr. Ottawa) genetic distances for psittacids and anatids do not agree with each other or the other calibration data discussed above, suggesting seafloor spreading is inappropriate for calibration.

Any attempt to calibrate a molecular clock must keep within the constraints dictated by the paleontological record. The fact that the fossil record is less than perfect is not an adequate rationale for ignoring it altogether. A major stumbling block is distinguishing between reliable paleontological occurrences and careless assignments of isolated scraps of fossil bone to modern taxa (see Olson 1977, Steadman 1981). Several orders of modern birds are erroneously reported to occur in Mesozoic deposits (Brodkorb 1963, 1964, 1971, 1976; Harrison and Walker 1975; Rich 1983; and others), but only primitive Charadriiformes and possibly Pelecaniformes are as yet known in the Mesozoic (i.e. late Cretaceous) (Elzanowski 1983, Steadman 1983, Olson 1985). Incomplete fossils from the early Eocene cannot be assigned confidently to modern orders. For example, isolated portions of the skeleton of *Lithornis*, a paleognathous carinate, have been assigned incorrectly to no fewer than six different orders of modern neognathous birds (Owen 1840, Lydekker 1891, Lambrecht 1933, Hoch 1973, Harrison and Walker 1977), and some of these have become firmly established in the literature as the earliest representatives of modern families (Houde in press b).

Actually, the patterns of diversification of mammals and birds through time are very similar. The Mesozoic avian and mammalian faunas consist primarily of bizarre reptile-like forms with no obvious relationships to extant taxa. The earliest avian and mammalian faunas of modern aspect occur in the late Cretaceous. Those with clear relationships to modern orders do not appear until the late Paleocene and early Eocene (Novacek 1982). Thus, the early origins of modern families and orders of birds envisioned by Sibley and Ahlquist is probably overestimated (Wyles et al. 1983, Helm-Bychowski and Wilson 1986). One example is the opinion (Sibley and Ahlquist 1983) that some taxa of modern birds began to diverge as long ago as 130 MYBP or at about the time of *Archaeopteryx* (140 MYBP). Such hypotheses are unlikely because of the great amount of morphological convergence that this would require in the various lineages of birds. For example, the carpometacarpus, pygostyle, carinate sternum, triosseal canal, heterocoelous vertebrae, neognathous palate, and rhynchokinetic skull are, in most cases, absent in birds until the late Cretaceous.

Therefore, the calibration of the DNA clock in birds should be more conservative by a factor of 25–50%, at least at high delta $T_{50}H$ values, in accordance with the suggestion of Helm-Bychowski and Wilson (1986). This agrees with the 12 MYBP date for the *Pongo*-hominoid divergence. The only hope for reconciliation of rodent and ratite DNA clocks is to modify the calibration for both by 25% or more. There are fossil vultures (Cathartidae), however, at least as old as Sibley and Ahlquist's (1985c) hypothesized time of divergence of vultures from storks (Ciconiidae) in the late Eocene (Mourer-Chauviré 1982). If rates of molecular evolution in the vulture lineage have been similar to rates

in other birds, then Sibley and Ahlquist's calibration may be correct for low $T_{50}H$ values.

Sibley and Ahlquist (1981a, 1983) originally advocated a curvilinear relationship of time to genetic distance based on DNA hybridization. More recently (Sibley and Ahlquist 1984b, 1985c) they argued for a linear relationship, relying on the combined mammalian and avian calibration points discussed above. The avian calibration dates are so nearly identical (75–80 MYBP), however, that they do not form a regression. These values are not independent because they all are based on Gondwanaland vicariance and because the $T_{50}H$ values are precisely at the point of diminished sensitivity of the DNA hybridization technique. The calibration dates are speculative, and two are mutually inconsistent. The nonavian data are of no value for establishing the linearity or nonlinearity of the correlation of genetic distance to time because of the uncertainty of the nonavian dates and the evidence for different rates of evolution within and between mammals and birds. Thus, it calls the relationship of genetic distance to time into question.

Conclusions

The DNA hybridization technique has received much praise but little critical appraisal. Surely, it is a powerful new tool for systematic problems, chiefly below the ordinal level (Prager and Wilson 1980). It would be incorrect to maintain that the problems discussed here are sufficient to reject completely the DNA hybridization method and all conclusions drawn from it. The precise branching patterns of phylogenetic reconstructions based on DNA hybridization data may be debatable, but these data should not lead to gross errors when the data are free of computational and technical errors. It is equally important to sample a sufficient diversity of taxa, and to compare all the taxa that are relevant to a particular taxonomic problem.

The claims for the clocklike behavior of DNA evolution have been shown to be overoptimistic. This is demonstrated by the DNA data itself, without the need of other evidence. In spite of mild differences in evolutionary rates, DNA hybridization studies can be useful if the differences in rates are acknowledged and accounted for. It is self-defeating, however, to ignore differences in evolutionary rates in the interest of selling the method as the direct indicator of phylogenetic kinship and, consequently, to incorporate these biases in the phylogenetic analysis (see Holmquist et al. 1982). The most worthwhile research objective now might be a more detailed examination of differences in evolutionary rates between taxa. This could begin with the repetition of studies that already have provided evidence for rate differences to see if the results are reproducible.

Other improvements that should be incorporated into future studies are: the presentation of complete data matrices, with clear indications of sample sizes and ranges; relative-rate tests that employ methods to distinguish between experimental error and rate differences; labeling of ambiguous branches in phylogenetic trees; calculation of confidence limits for alternative phylogenetic trees; and clear indications of where values have been "corrected." Material that would best be omitted includes: linear tables of genetic distances using only one reference taxon; figures of thermal stability curves in which the curves of different taxa are averaged together (e.g. Sibley et al. 1982); and unsupported conclusions about the calibration of the DNA molecular clock and the relationship of genetic distance to time.

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Nominations for the **William Brewster Memorial Award** and the **Elliott Coues Award** for 1987 should be sent to the Brewster and Coues Awards Committee by **1 March 1987.** Nominations must include a curriculum vitae and bibliography of the nominee and a clear and precise letter stating the bases on which the nominator makes the nomination. The Brewster Award recognizes notable contributions to the ornithology of the Western Hemisphere in the past 10 years; the Coues Award recognizes impact on the ornithology of the Western Hemisphere, with no time limit. Send applications to **R. F. Johnston, Museum of Natural History, University of Kansas, Lawrence, KS 66045.**