A Comparative Summary of Genetic Distances in the Vertebrates from the Mitochondrial Cytochrome *b* Gene

Glenn C. Johns[1] *and John C. Avise*

Department of Genetics, University of Georgia

Mitochondrial cytochrome *b* (*cytb*) is among the most extensively sequenced genes to date across the vertebrates. Here, we employ nearly 2,000 *cytb* gene sequences from GenBank to calculate and compare levels of genetic distance between sister species, congeneric species, and confamilial genera within and across the major vertebrate taxonomic classes. The results of these analyses parallel and reinforce some of the principal trends in genetic distance estimates previously reported in a summary of the multilocus allozyme literature. In particular, surveyed avian taxa on average show significantly less genetic divergence than do same-rank taxa surveyed in other vertebrate groups, notably amphibians and reptiles. Various biological possibilities and taxonomic ''artifacts'' are considered that might account for this pattern. Regardless of the explanation, by the yardstick of genetic divergence in this mtDNA gene, as well as genetic distances in allozymes, there is rather poor equivalency of taxonomic rank across some of the vertebrates.

Introduction

In 1982, Avise and Aquadro summarized the multilocus allozyme literature on mean genetic distances (*D*'s) between congeneric species and confamilial genera across the major vertebrate classes. Some salient trends emerged. Notably, mean *D* values among avian congeners were typically lower than those for other vertebrate groups. Congeneric species of amphibians and reptiles often tended toward the high end of the mean genetic distance scale, whereas congeneric fishes and mammals generally were intermediate in magnitude of interspecific *D*'s. Similar trends toward smaller genetic distances for birds than for other vertebrate groups also pertained to comparisons of confamilial genera.

Here, we revisit the comparative approaches of Avise and Aquadro (1982) with another potential common yardstick: genetic differences in nucleotide sequence (*p*) of the mitochondrial cytochrome *b* (*cytb*) gene. Several rationales exist for summarizing the *cytb* literature. First, *cytb* is the gene that is perhaps most extensively sequenced to date for the vertebrates (Irwin, Kocher, and Wilson 1991; Lydeard and Roe 1997; Moore and DeFilippis 1997). Second, the evolutionary dynamics of the *cytb* gene and the biochemistry of the protein product are better characterized than most other molecular systems (Esposti et al. 1993). Third, levels of genetic divergence typically associated with sister species, congeners, and confamilial genera (the comparisons analyzed here) usually are in a range in which the *cytb* gene is phylogenetically informative and unlikely to be severely compromised by saturation effects involving superimposed nucleotide substitutions (Moritz, Dowlilng, and Brown 1987; Meyer 1994). Finally, it is of interest to compare distance trends from homologous DNA sequences of a single cytoplasmic gene vis-à-vis those from protein-level assays of multiple nuclear loci.

The comparative perspectives adopted in this paper differ somewhat from the usual immediate aim of most systematic studies, which is to reconstruct phylogenetic relationships within a taxonomic group. Rather than focusing here on the cladistic relationships of particular taxa, we address questions of the following relational sort: Are the various taxonomic ranks in existing vertebrate classifications equivalent with respect to genetic distance? In other words, by this standard, is a genus or family of birds, for example, equivalent to its hierarchical counterpart in mammals or in amphibians?

If the answer to the second question is ''no,'' possible explanations might include any one or a combination of the following: (1) identical taxonomic ranks in different vertebrate groups differ by age; (2) they are of same age but differ in rate of molecular evolution; (3) taxonomists working on some vertebrates tend to be splitters, whereas others are lumpers; or (4) relevant biological differences exist among vertebrate classes, such as in rates of morphological evolution. Such possibilities will be considered to explain the relatively small genetic distances that we summarize here for avian taxa.

Materials and Methods

Sequences Employed

Cytb sequences were retrieved from GenBank release 103.0, which includes all sequences entered prior to October 9, 1997. A total of 2,821 *cytb* sequences of length $\geq$ 200 bp were found for species classified in the subphylum Vertebrata. When multiple haplotypes for a species were recorded, only the longest of the coding sequences was analyzed (in the event of a tie, one sequence was chosen at random to represent that species). Two unpublished sequences were found to have multiple insertions and deletions when compared with congeneric sequences. They also had multiple stop codons in all three reading frames and thus were excluded from further analysis as probable pseudogenes. The culled data set represented 1,832 *cytb* sequences (1 per species). A list of the species and GenBank accession numbers for

[1] Present address: Hopkins Marine Station, Stanford University.

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Address for correspondence and reprints: John C. Avise, Department of Genetics,University of Georgia, Athens, Georgia 30602. E-mail: avise@arches.uga.edu.

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these sequences is available from the authors upon request.

Sequence comparisons involving congeners were made for any genus represented by two or more species in the data bank. Similarly, analyses of confamilial genera involved the use of a single sequence per genus for any taxonomic family represented in the data by two or more genera.

Genetic Distance Calculations

Cytb gene sequences were aligned using pileup in the GCG package (Genetics Computer Group Inc.). Genetic distances (*p*) were estimated for all pairwise comparisons using Kimura's (1980) two-parameter model as implemented in PAUP* (Swofford 1998). Altogether, 7,699 pairwise sequence comparisons were conducted.

Statistical Analyses

Separate analyses were performed on sister species, species within a genus, and genera within a taxonomic family. Various statistical procedures were employed to determine if the means in the distributions of sequence divergence estimates at a given taxonomic rank differed significantly across major vertebrate groups. To avoid the complications of nonindependence associated with multiple pairwise estimates from a single phylogeny, only the mean *p* value for a genus (in analyses of congeneric species) or for a family (in analyses of confamilial genera) was included in the statistical tests. Relatively few *cytb* sequences have been reported for amphibians and reptiles, so in some of the statistical analyses (where mentioned), these organisms were artificially pooled as herpetofauna.

The distributions of mean sequence divergence estimates within groups sometimes were significantly nonnormal, so nonparametric rank sums tests (Kruskal and Wallis 1952) using the χ^2 approximation were performed to assess differences in the means of the histograms of mean *p* among the vertebrate groups. Where a significant result warranted further inspection, Kruskal-Wallis multiple-comparisons tests were used to determine which means in the distributions were significantly different from the others (Dunn 1964). This is a conservative statistical test when α is set to 0.05 (Daniel 1990). The same procedures were also used to examine possible differences in mean *p* values associated with different taxonomic ranks within each vertebrate group.

Analyses of Sister Species

Sister species were provisionally identified as monophyletic pairs in phylogenetic analyses conducted for each genus. Phylogenies for each of 166 genera (those with three or more species represented in GenBank) were generated in PAUP* (Swofford 1998) under a Kimura (1980) two-parameter distance-based model using a neighbor-joining algorithm (Saitou and Nei 1987) with midpoint rooting. These methods were chosen because they entail simple assumptions and could be implemented on the large number of data sets examined. (No outgroup analyses were attempted, as they would have required us to make uneducated phylogenetic guesses on scores of groups [and in any event, in many cases, no obvious outgroup sequences were available].) Of course, these analyses may falsely earmark some taxa as sister species merely because intermediate sequences or species were missing from the database. Nonetheless, the approach is useful for comparative purposes when viewed conservatively as providing maximum estimates of sister species genetic distances.

These genetic distances (which are independent of one another in value) were accumulated across provisional sister species pairs for each vertebrate group. The resulting means in the frequency distributions were compared statistically using Kruskal-Wallis tests.

Results

Congeneric Species and Confamilial Genera

Figures 1–4 plot on a common scale the compilations of *cytb* genetic distances for congeneric species (in 288 genera) within each of the five major vertebrate classes. Bird species typically show relatively small values, whereas the genetic distance distributions of congeneric fishes, reptiles, and amphibians show larger variances and include some estimates that far surpass any of those recorded for birds (fig. 5). Assayed mammalian congeners generally appear intermediate in these regards.

At the level of congeneric species, the frequency distributions of mean sequence divergence estimates (fig. 5) are highly significantly different among the major vertebrate classes examined ($P < 0.0001$; table 1). A multiple-comparisons test indicates that this outcome results primarily from significant differences ($P < 0.05$) between the birds and the other major vertebrate groups, and that these other groups show no significant differences from one another at this taxonomic level (table 1).

Comparable summaries for confamilial genera are presented in figure 5. These genetic distance distributions were also significantly different among the major vertebrate classes ($P < 0.0001$; table 1). This outcome is primarily due to larger genetic distances in the assayed reptiles (and, marginally, amphibians) as compared with observed distances in birds of the same taxonomic rank (table 1). When amphibians and reptiles are pooled in the statistical analyses (because relatively few sequences within either group were available), the ''herpetofauna'' also show significantly larger genetic distances than do mammals (table 1).

Other Taxonomic Comparisons

Statistical analyses of the frequency distributions of sister species genetic distances (fig. 6) are presented in table 2. The only significant differences among the vertebrate classes involve a tendency for larger genetic distances between sister species of mammals than those for birds or fish.

Finally, comparisons were made among taxonomic ranks within each major vertebrate group. Expected trends are evident for larger genetic distances for confamilial genera than for congeneric species (fig. 5), and

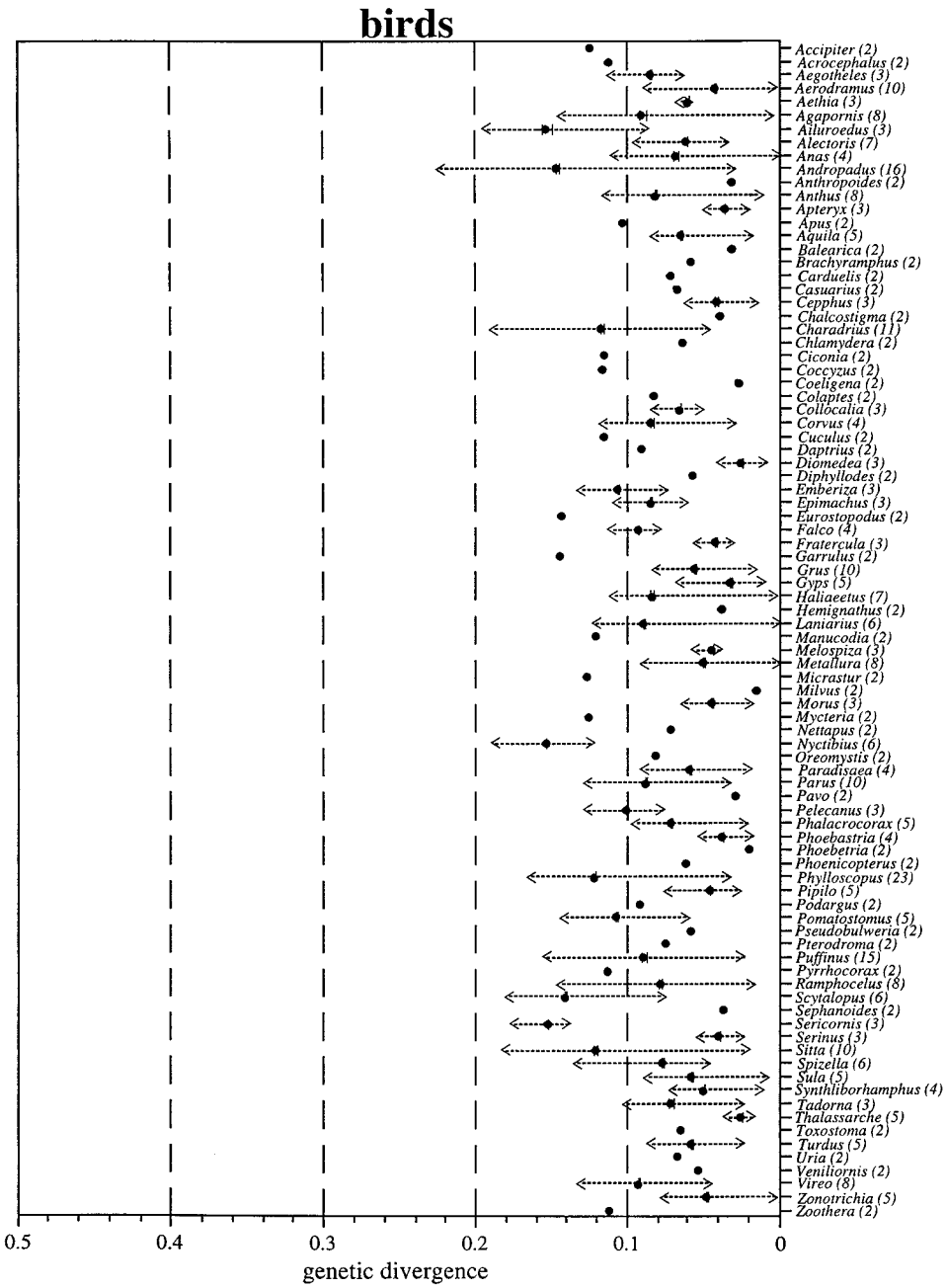

FIG. 1.—Genetic distances in *cytb* gene sequences between avian species within 88 genera. Numbers of species assayed are given in parentheses. Mean genetic divergence estimates (●), standard errors (⊢ ⊣), and ranges (<– – –>) are shown where appropriate.

for congeneric species (fig. 5) than for presumptive sister species (fig. 6). These increases in genetic distance with taxonomic rank are statistically significant in all cases, as might be expected if *cytb* genetic distances at these levels are not overtly truncated by saturation effects.

Base Composition

Table 3 reports base compositions in *cytb* for the five major vertebrate classes. Although the frequencies of the four bases are roughly similar, the large number of nucleotides examined (nearly 800,000 total) enabled detection of significant differences among the groups. For example, the birds show a somewhat higher percentage of C than do other groups in comparisons involving all sites, and in third-codon positions, the assayed reptiles show higher frequencies of G.

From these data, indices ($\hat{\imath}$) of sequence identity at equilibrium (Li 1997) were calculated (table 3). These are the genetic similarities expected in randomized sequences of given base composition. All $\hat{\imath}$ values were similar, suggesting that the asymptotes of *cytb* saturation dynamics are not greatly different across the vertebrate groups. Whether differing trajectories to these asymptotic values might conceivably act, for example, to bias avian genetic distances downward is unclear. In theory,

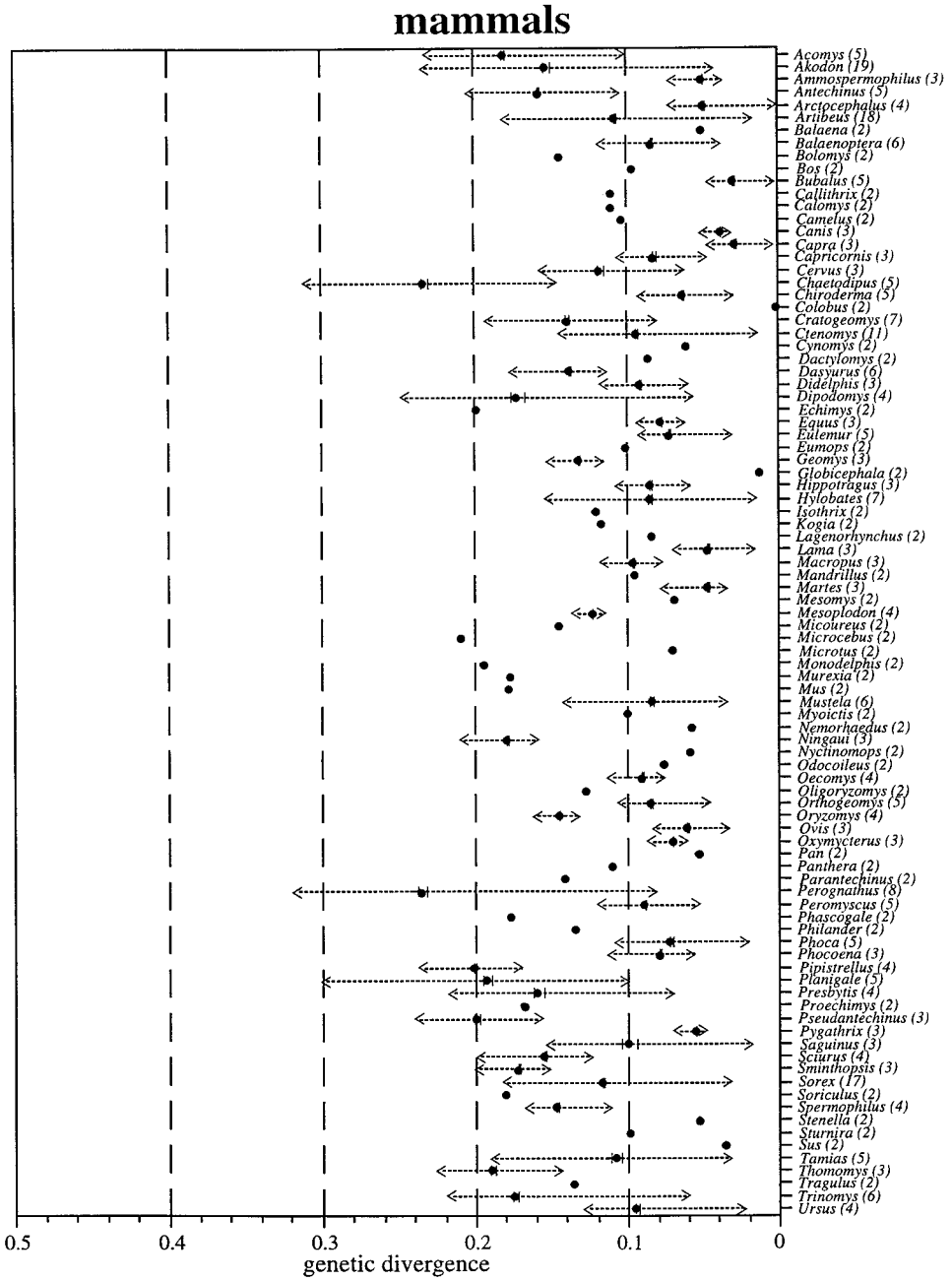

FIG. 2.—Genetic distances in *cytb* gene sequences between mammalian species within 92 genera (format as in fig. 1).

strong biases in base composition could lead to faster saturation, which would give the impression of lower percentages of sequence divergence in a given period of time. It seems doubtful, however, that the modest base compositional differences observed could alone account for the trends in *cytb* genetic distances across the vertebrates.

Discussion

The results of the *cytb* mtDNA sequence analyses parallel and reinforce the principal patterns among vertebrate groups that were previously summarized from the allozyme literature (Avise and Aquadro 1982). In both analyses, there is a significant trend toward smaller mean genetic distances among avian congeners relative to congeners in other vertebrate classes. (With respect to confamilial genera, birds and mammals also tend to show smaller mtDNA genetic distances than do comparable-rank herpetofauna.) The general agreement in genetic distance patterns in electrophoretic assays of the protein products from multiple nuclear genes versus nucleotide sequences in the mitochondrial *cytb* gene need not reflect any direct causal associations between these two qualitatively different classes of data, but instead probably signals correlations of both with additional variables (including evolutionary time; see below).

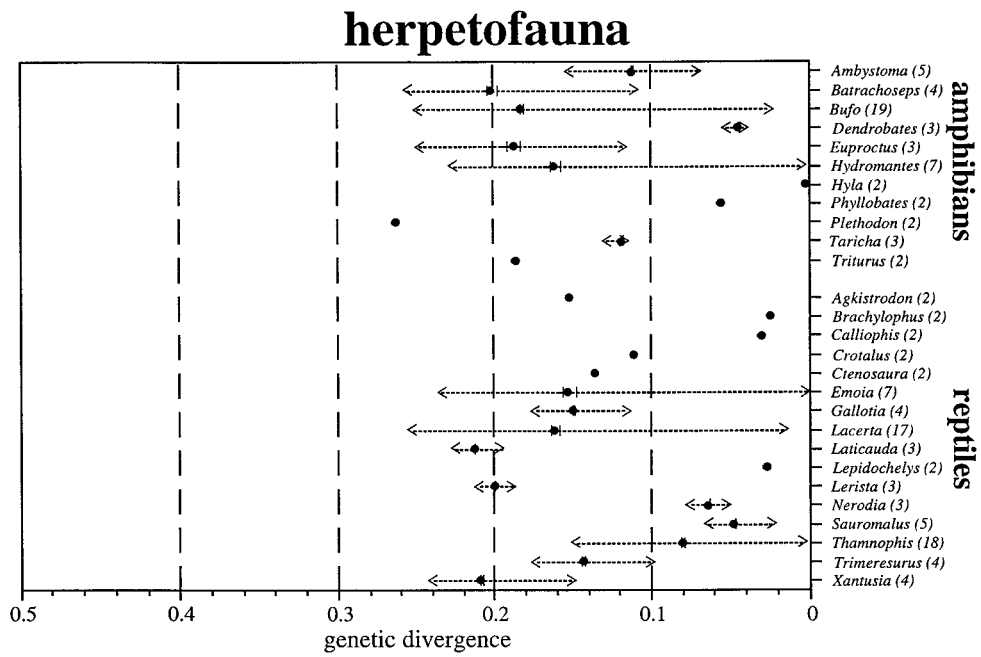

FIG. 3.—Genetic distances in *cytb* gene sequences between species within 11 genera of amphibians and 16 genera of reptiles (format as in fig. 1).

The Genetic Distance Yardstick.

By the measure of mean genetic distances in *cytb* (and allozymes), assayed avian genera often are not equivalent taxonomically to other vertebrate genera. The pattern of lower genetic distances in birds appears to result from a truncation effect wherein (for whatever reason) avian species have been placed into separate genera at a lower level of genetic divergence than is often true for other vertebrates (fig. 5). For example, the largest mean genetic distance in *cytb* for assayed avian congeners was $p \cong 0.16$, whereas mean genetic distances for a number of mammalian, amphibian, reptilian, and piscine genera exceeded this value considerably (figs. 2–4).

Statistical support for this truncation effect can be demonstrated in two ways. First, when the genetic distance distributions for the other vertebrate groups (herpetofauna pooled) are artificially truncated at the maximum *cytb* distance value observed among avian congeners ($p = 0.16$), the modified histograms are no longer significantly different across the vertebrates (Kruskal-Wallis test; $\chi^2 = 4.3$, df = 3, $P = 0.23$). Second, when the genetic distance distribution for confamilial avian species (fig. 7) is compared with those for congeneric species in all four of the other vertebrate groups (fig. 5), no statistical difference is evident (Kruskal-Wallis test; $\chi^2 = 3.1$, df = 4, $P = 0.54$).

Thus, from the perspective of sequence differences in the *cytb* molecule (and even more so in genetic distances from allozymes; Avise and Aquadro 1982), assayed avian genera tend to be oversplit relative to genera in other vertebrate classes (or, equivalently, genera in these other groups are overly inclusive relative to those in birds). This sentiment also applies to avian (and mammalian) families compared with those of the assayed herpetofauna. In other words, assayed avian genera and families tend to be taxonomically ''out of step'' at these ranks with other vertebrates with respect to *cytb* genetic distances.

Whether this conclusion also extends to higher taxonomic levels remains to be seen from examinations of appropriate molecular data (*cytb* is probably not an ideal measure because saturation effects likely would be encountered). However, considerable evidence indicates that birds are a phylogenetic subset of reptiles, such that the current taxonomic class Aves justifiably might be considered a taxonomic order within Reptilia (see Witmer 1991; Ruben et al. 1997; Forster et al. 1998). From this perspective, birds at these higher ranks also might be viewed as about one taxonomic rank out of step with the reptiles.

Such conclusions do not, however, necessarily extend in the other direction along the taxonomic hierarchy. Genetic distances in mtDNA sequences for currently reviewed avian sister species are not significantly smaller than those for herpetofauna, nor are the reported mtDNA genetic distances between primary intraspecific clades (matrilineal phylogroups) within avian as opposed to reptilian species (table 2). Thus, by these criteria, birds generally are not ''oversplit'' at the species level. Also, they are probably not oversplit at the species level with respect to the criterion of reproductive isolation. This is likely a consequence of the fundamental biological reality of the category ''species'' as a taxonomic rank (Dobzhansky 1937). Indeed, reproductive relationships in birds are often used as a partial empirical basis for making formal species-level taxonomic decisions (American Ornithologists' Union 1995).

We do not mean to imply that differences in mean genetic distance occur only between the major vertebrate

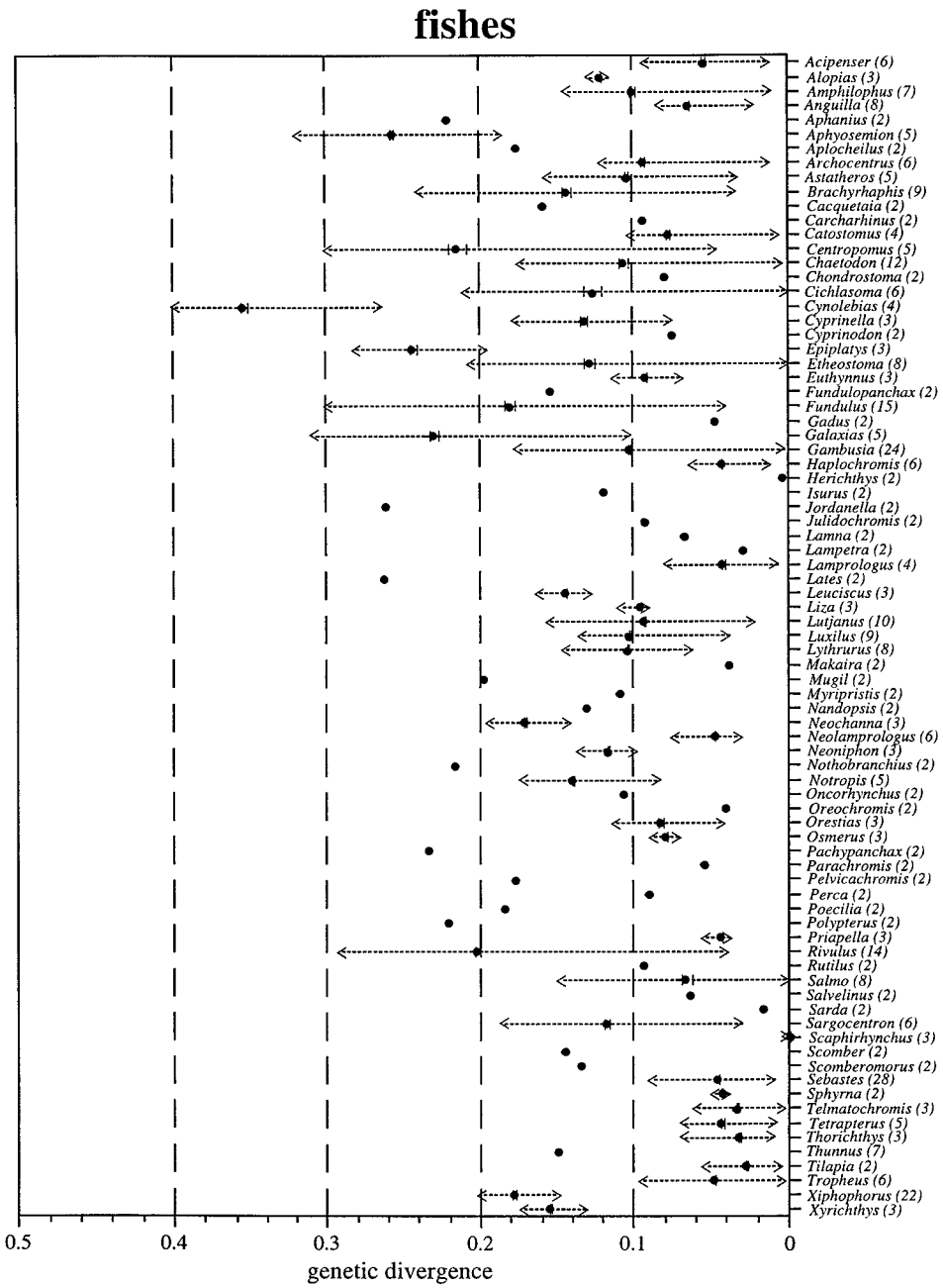

FIG. 4.—Genetic distances in *cytb* gene sequences between species within 81 genera of fishes (format as in fig. 1).

taxonomic groups. Extensive variation exists within these groups as well. One noticeable example involves rodents as compared to other mammals. In the sequences analyzed, rodents comprise about one third of the genera examined (30 of 92), and on average they are significantly more divergent genetically than are other mammals at all taxonomic levels considered here. This finding might be interpreted as consistent with suggestions that rodents have a higher rate of DNA sequence evolution than many other mammals (Wu and Li 1985; Li and Tanimura 1987). If true, this in turn would suggest that morphological characteristics used to define rodent taxa have not experienced a commensurate increase in evolutionary rate.

The Yardstick of Time

If the ''conservative'' pattern of genetic divergence between avian genera and families is reflective of more recent evolutionary separations on average than for non-avian vertebrates of the same taxonomic rank, then birds at these taxonomic levels would also appear to be oversplit by the yardstick of time. Under a ''standard'' mtDNA clock calibration (of about 2% sequence divergence per Myr between a pair of lineages; Brown, George, and Wilson 1979; Klicka and Zink 1997), the mtDNA lineages in extant avian congeners separated, on average, 3.9 MYA, whereas those in extant mammalian, reptilian, amphibian, and piscine genera appear

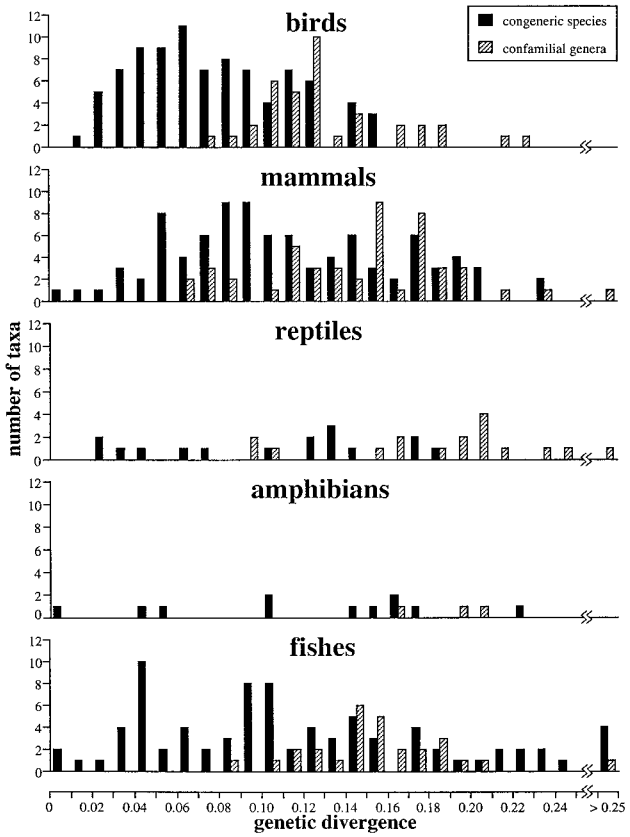

FIG. 5.—Frequency histograms of mean *cytb* genetic distances for congeneric species and confamilial genera across vertebrate groups.

significantly older (mean separation times 5.7, 6.0, 7.0, and 5.9 MYA, respectively).

Calibration of evolutionary rates in *cytb* (or other mtDNA genes) is highly problematic, particularly across lineages (Avise 1994; Li 1997). However, most suggested departures from the standard mammalian and avian mtDNA clock calibration have involved several-fold lower rates for some of the poikilothermic vertebrates (Avise et al. 1992; Martin, Naylor, and Palumbi 1992; Martin and Palumbi 1993; Canatore et al. 1994; Rand 1994; Mindell and Thacker 1996). If such slowdowns are valid and apply widely to the poikilotherms, then existing classifications by the meter of time would evidence an even greater degree of oversplitting for avian taxa (or of lumping in other groups) than is the case under a uniform mtDNA clock.

In previous discussions of the conservative pattern of allozyme divergence in avian as opposed to many nonavian groups, the possibility was raised of a slower rate of protein evolution for birds, perhaps related to physiological and metabolic constraints associated with birds' high body temperatures (Prager et al. 1974; Avise and Aquadro 1982; Avise 1983). However, recent mtDNA data do not support the notion of a general slowdown in molecular evolution in birds; if anything, they suggest just the reverse (Kocher et al. 1989; Martin, Naylor, and Palumbi 1992; Martin and Palumbi 1993; Canatore et al. 1994; Rand 1994; Mindell and Thacker 1996). In the future, comparisons of divergence patterns

Table 1
Comparisons of Genetic Distances in *cytb* Gene Sequences Between Congeneric Species and Confamilial Genera Across Major Vertebrate Groups

	Congeneric Species	Confamilial Genera
Kruskal-Wallis test		
χ^2 (approximate)	26.1	21.6
df	3	3
P	<0.0001	<0.0001
Kruskal-Wallis multiple-comparisons test (significance at $\alpha = 0.05$)		
Birds vs. mammals	Significant	NS
Birds vs. herpetofauna	Significant	Significant
Birds vs. fishes	Significant	NS
Mammals vs. herpetofauna	NS	Significant
Mammals vs. fishes	NS	NS
Herpetofauna vs. fishes	NS	NS
Multiple comparisons with amphibians and reptiles[a]		
Amphibians vs. birds	Significant	NS[b]
Amphibians vs mammals	NS	NS
Amphibians vs. fishes	NS	NS
Reptiles vs. birds	NS[b]	Significant
Reptiles vs. mammals	NS	NS
Reptiles vs. fishes	NS	NS

[a] All comparisons not involving amphibians or reptiles yielded results identical to those presented above involving the pooled herpetofauna.

[b] These results are currently statistically marginal ($P \cong 0.10$) given the conservative nature of the Kruskal-Wallis multiple-comparisons test.

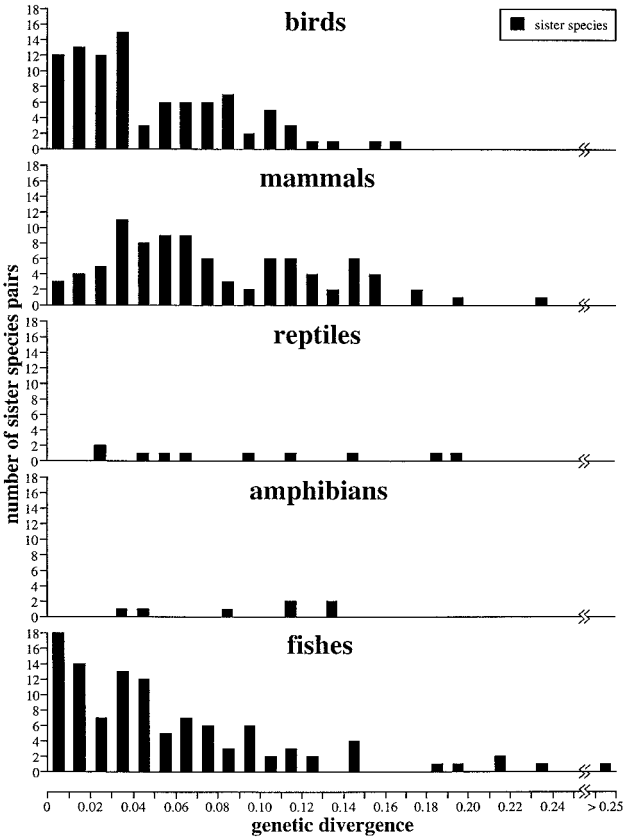

FIG. 6.—Frequency histograms of mean *cytb* genetic distances for pairs of sister species across vertebrate groups.

Table 2
Comparisons of mtDNA Genetic Distances Between Principal Intraspecific Phylogroups[a] and Between Sister Species Across Major Vertebrate Groups

	Intraspecific Phylogroups	Sister Species
Kruskal-Wallis test		
χ^2 (approximate)	7.2	22.9
df	3	3
P	0.07	<0.0001
Kruskal-Wallis multiple-comparisons test (significance at $\alpha = 0.05$)		
Birds vs. mammals	—	Significant[c]
Birds vs. herpetofauna	—	NS
Birds vs. fishes	—	NS
Mammals vs. herpetofauna	—	NS
Mammals vs. fishes	—	Significant[c]
Herpetofauna vs. fishes	—	NS
Multiple comparisons with amphibians and reptiles[b]		
Amphibians vs. birds	—	NS
Amphibians vs. mammals	—	NS
Amphibians vs. fishes	—	NS
Reptiles vs. birds	—	NS
Reptiles vs. mammals	—	NS
Reptiles vs. fishes	—	NS

NOTE.—The distances for intraspecific phylogroups were not significantly different among the vertebrates, so results from a multiple-comparisons test are provided for the sister species comparisons only.

[a] From the summary in Avise, Walker, and Johns (1998), in which a discussion of the relevance of these data to ''speciation durations'' can also be found.

[b] All comparisons not involving amphibians or reptiles yielded results identical to those presented above involving the pooled herpetofauna.

[c] Larger mean distances for mammals than for birds and fishes.

Table 3
Base Compositions in the *cytb* Sequences for Taxa Summarized in this Report

	Percentage Base Composition				Total No. of Bases	$\hat{i}$[a]
	A	C	G	T		
All sites[b]						
Birds	27.6	33.9	13.5	25.0	267,521	0.27
Mammals	29.6	28.4	13.3	28.8	283,222	0.27
Reptiles	27.7	27.6	16.1	28.6	38,703	0.26
Amphibians	27.0	24.3	16.0	32.7	24,024	0.27
Fishes	25.3	29.9	15.3	29.5	197,908	0.26
Total					811,378	
Third-position sites[b]						
Birds	37.7	46.7	3.5	12.1	89,245	0.38
Mammals	39.8	35.9	3.4	20.9	94,398	0.33
Reptiles	29.7	28.1	18.0	24.2	12,907	0.26
Amphibians	35.5	30.2	5.5	28.8	8,009	0.30
Fishes	30.2	41.3	5.5	23.0	65,959	0.32
Total					270,518	

[a] Equilibrium identity (see text).

[b] Differences between vertebrate classes were highly significant in a *G*-test of independence (Sokal and Rohlf 1995).

in synonymous and nonsynonymous substitutions in the genes of many vertebrate species might be desirable to further address these rate issues.

The Yardstick of Morphological Differences

Current vertebrate classifications have resulted primarily from morphological and other organismal-level appraisals. Thus, existing taxonomies might be interpreted as summaries of perceived morphological distances (although almost no attention has been devoted to the development of standards of morphological comparison that could be applied across major groups; Cherry, Case, and Wilson 1978).

If one accepts existing taxonomy as a general guide to morphological divergence, then avian morphological differences at the generic and familial levels appear to be somewhat out of step with those of many other vertebrates by the criteria of genetic distances (and, presumably, evolutionary time). The disparity is in the direction of an acceleration of avian morphological evolution relative to the other vertebrate groups. In other words, birds can be posited to have experienced a faster rate of morphological evolution that produced taxonomically equivalent levels of perceived morphological divergence in significantly less time on average. Any use of suspected slower mtDNA clocks to date evolutionary separations for some poikilotherms would only further

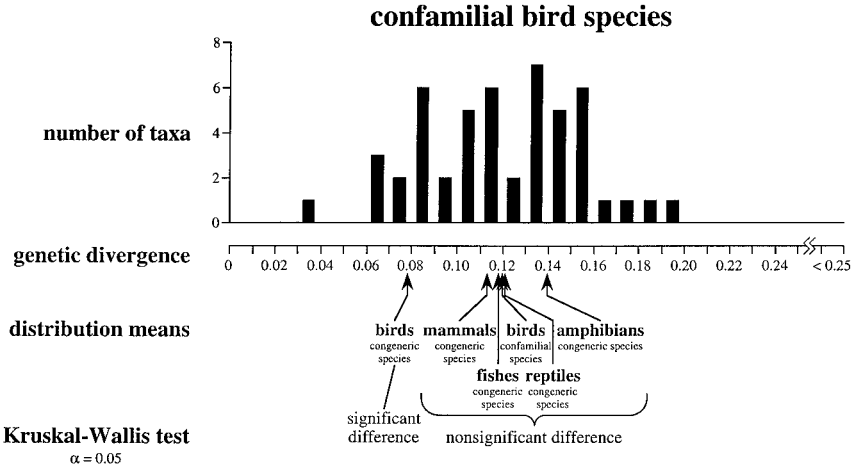

FIG. 7.—Frequency histogram of mean *cytb* genetic distances for confamilial bird species. Also shown for comparative purposes are the mean *cytb* genetic distances for congeneric species in other vertebrate groups.

increase the apparent accelerated rate of morphological evolution for birds relative to those for the other groups (notably amphibians and reptiles).

Birds often display conspicuous differences in features such as plumage coloration, song, bill shape, and foot structure that sometimes distinguish even closely related species (Gill 1995). Some other groups, such as amphibians and small mammals, may tend to be more conservative in these regards (Cherry, Case, and Wilson 1978). As emphasized by Allan Wilson and colleagues (Wilson, Maxson, and Sarich 1974; Wilson, Sarich, and Maxson 1974; King and Wilson 1975) more than 20 years ago, a primary rationale for establishing some sort of universal guidelines that would equilibrate taxonomic assignments across vertebrate (and other) groups is that such standardized classifications would greatly facilitate quantitative attempts to compare tempos and modes of evolution across different kinds of organisms and across different kinds of characters.

Acknowledgments

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