Response to A. H. Bledsoe and J. E. Ahlquist et al.

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Bledsoe's (1987) commentary, while basically sound, does not address the methods that have been generally used in DNA hybridization studies of avian relationships. Nonuniformity of rates of phenotypic evolution never precluded the use of morphology in phylogenetic analysis, so, obviously, differences in rates of DNA evolution do not *preclude* the use of DNA distance data from phylogenetic analysis either (Houde 1987: 25; *contra* Bledsoe's account of my position). The *relative* advantage of DNA distance data over phenotypic data in phenetic analyses is, however, directly dependent on the extent to which molecular evolution is clocklike (Ahlquist et al. 1987, Houde 1987). No one can deny the central role that the "uniform average rate" (UAR) of nucleotide substitution has played in the interpretation and promulgation of avian DNA hybridization data, until Sheldon (1987a) and I (Houde 1987) demonstrated evolutionary rate differences in birds. We have yet to see in the published record how the new "average genomic rates" (AGR) differ from UAR.

Differences in rates of evolution *alone* will produce ambiguity in the clustering of taxa when phenetic algorithms are employed (Sourdis and Krimbas 1987). The algorithms used in all but 3 (Sibley and Ahlquist 1980; Sheldon 1987a, b) of the roughly 26 studies using DNA hybridization to estimate avian phylogeny were of this type. Bledsoe's ability to correctly reconstruct six "true" phylogenies with a nonphenetic algorithm inspires confidence, but it falls substantially short of demonstrating statistically the complete rate independence of the least-squares method. Nonphenetic algorithms, such as those in the PHYLIP package, do not produce unambiguously unique solutions (Lanyon 1985, Hobish 1986), and "tree topologies that differ by only a few steps are not significantly different" (Felsenstein 1985: 157). Bledsoe was able to recognize his correct reconstructions because he already knew the "true" phylogenies, but this is never the case in nature. As it is also clear that no single method of analysis is most appropriate in all instances (Hobish 1986), when is least squares appropriate and when is it not? Considering Bledsoe's results, least squares is a promising method of analysis that deserves considerably more attention and testing.

I enthusiastically endorse Ahlquist et al.'s (1987) commentary and their DNA hybridization studies in general, but I do not think that these authors have fully exploited the analytical and presentational resources currently available. My purpose here, as before (Houde 1987), is to distinguish between the part of their work that reports results, which is almost certainly correct, and the part of their work that is couched in assumptions and therefore susceptible to ambiguity in interpretation, as other methods. The following criticisms apply: (1) Ahlquist et al. discussed the congruence of DNA distances for closely related and noncontroversial taxa, i.e. "a duck always clusters with the other waterfowl . . . ." Sibley et al. (1987) admitted that they rarely have sufficient data to construct complete matrices of nominal taxa, but they claimed to have "virtually complete representation at the level of categorical hierarchy" (p. 119). Thus, different members of a taxon may be substituted to fill the empty cells of incomplete distance matrices. The dubiousness of substituting species in a matrix is best exemplifed by the procellariiform data set. In 1983 Sibley and Ahlquist supported UAR by claiming that Procellariiformes yielded similar distances to an outgroup, despite different generation lengths. In 1987 they interpreted the same data set as supporting different rates of evolution—the result of differences in generation length. Are these values the same or are they not? If not, then can one species be legitimately substituted for another in data matrices of categorical hierarchy? If an average value is used instead, then how does the proportion of species of long and short generations skew this AGR from UAR?

(2) Ahlquist et al. did not address the most objectionable dates of divergences (the origin of passerine families), which are probably 25–50% too great. The fossil "cuckoo" (Cuculidae) to which they refer is known from only a half of a bone—precisely the questionable practice I warned against (Houde 1987); yet, Ahlquist et al. are of the *opinion* that the series of complete skeletons on which I reported (Houde 1986) is "unconvincing." This "cuckoo" does not date the divergences of cuculiform families, because the morphology of basal Cuculiformes is unknown. On the other hand, the fossil limpkin, *Badistornis*, does tell about the divergence of finfoots (Heliornithidae) and limpkins (Aramidae), if Sibley and Ahlquist (1985) are correct that Old World finfoots are a sister group of the neotropical Sungrebe (*Heliornis*) and limpkin (*Aramus*). The finfoot morphology is primitive and the limpkin morphology is derived if they are correct, but their *Aramus-Heliornis* divergence (20 MYA ± 10%) is 30% younger than *Badistornis*, which has been unambiguously interpreted as a limpkin (Wetmore 1940, Brodkorb 1967, Cracraft 1973, Olson 1985).

Because different groups of birds (and mammals) have evolved at different rates, no single temporal calibration or single correction coefficient will apply to more than one group of organisms except by co-

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incidence. Thus, Ahlquist et al.'s examples of the congruence of their data to selected fossil evidence have no bearing on the larger picture of dating avian divergences.

(3) There is no evidence that trogons (Trogonidae), parrots (Psittacidae), and pigeons (Columbidae) are either more vagile than passerines (Passeriformes), barbets (Capitonidae), and cuckoos or had a past distribution that would have better facilitated their dispersal, except that their delta $T_{50}H$ values of trans-Atlantic sister taxa are lower. Evolutionary rate differences are another explanation, or possibly a contributing factor, for the low values.

(4) Sibley and Ahlquist (1981) first calibrated their molecular clock by the hypothesized (Cracraft 1974) divergence of ratites, and subsequently strengthened their calibration by the addition of other divergence dates, including apes (Sibley and Ahlquist 1984), that seemed to agree. I (Houde 1987) may have been incorrect semantically, but not in essence, to say that they calibrated the *avian* clock on a primate divergence. They advocated a *single* DNA molecular clock, partly based on primates, which they did not specify as avian but applied universally to birds (Sibley and Ahlquist 1985).

(5) Ahlquist et al. missed my point when I said, "Is it DNA or organisms that is to be classified?" The taxonomic levels indicated by genetic distance may be so different from that indicated by morphology as to obscure diversity. For example, in an unpublished poster presentation (19th Intern. Ornithol. Congr., Ottawa) Sibley and Ahlquist included 9 or 10 of the traditionally recognized avian orders (Mayr and Amadon 1951, Wetmore 1960) in a single order, Ciconiiformes.

(6) It was not my intention to expressly refute any of Ahlquist et al.'s phylogenetic conclusions, only to show what constitutes the best criteria for drawing such conclusions. Templeton's (1985) complaint that Sibley and Ahlquist (1984) did not statistically weigh alternative hypotheses does not depend on the veracity of his own phylogenetic hypothesis and statistical test. I am, furthermore, baffled by Ahlquist et al.'s rationale for excluding *Cladorhynchus* in their treatment of flamingos (Phoenicopteridae; Sibley and Ahlquist 1985) on the grounds that this is not an African bird, when in the same paper they included several neotropical and Australian taxa (e.g. *Aramus, Heliornis, Pedionomus*).

(7) Ahlquist et al. (1987) adopted an adversarial position, broadly criticizing traditional systematics as "subjective" and "mysterious; . . . it could only be done by those blessed with the power of intuition coupled with long experience" (Sibley et al. 1987: 113). Yet, Ahlquist et al. cast similar aspersions on the relative abilities of other molecular biologists (e.g. Bonner et al. 1981, criticized by Sibley 1981; Brownell 1983, criticized by Sibley et al. 1987). Ahlquist et al. further criticized the "limited resolution of traditional methods," especially in the "categorical levels at and above families." Sheldon (1987a, b), however, pointed out that only delta $T_{50}H$, not delta mode and delta T_m, can be used to measure the divergence of disparate taxa (e.g. families and orders); yet, delta $T_{50}H$ values clearly violate one of the assumptions required for the clustering of interordinal DNA distance data, viz. homologs exist between all sequences of heterologous DNA. Homologs of all sequences clearly do not exist in interordinal hybrids, because the slope of the melting curve must be extrapolated to the point at which 50% of the DNA would hypothetically be in heteroduplex form to calculate $T_{50}H$. Both molecular and traditional analyses are subject to uncertainties at higher taxonomic levels.

(8) Do DNA distance data provide any advantage over traditional phenotypic data? Yes; if DNA hybridization were not such a powerful tool, then it would not deserve the severe critical scrutiny I have given it. Reproducibility, in both data collection and analysis, is one clear advantage of DNA hybridization. Although all the coding information that makes up an organism is contained in DNA, counterintuitively, this is not necessarily another advantage of DNA hybridization. Phenotypic expression is influenced by the number of copies of a gene, its promoters, enhancers, and location in relation to these and other genes (Borst and Greaves 1987). Traditional studies incorporate this information in the form of phenotypic characters; such potentially useful information can be invisible to DNA hybridization, which uses single copies of sheared DNA.

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