son's Phalarope are excessively afflicted with diseased ovaries. Thus, hundreds, perhaps thousands, of non-breeding females are found during the summer in the vicinity of Salt Lake, and examination shows that invariably these non-breeders are possessed of diseased and non-functioning organs. As a result of this condition, which affects perhaps two-thirds of the entire number of females, the males, if they would breed at all, must accept at least one rival, or male partner, in their family relation.

"But one who knows Phalaropine character soon suspects that this ovarian disease, which is forcing polygamy upon the race, is in itself an effect rather than a cause. The cause is the excessive development of the sex instinct in female Phalaropes. The female of *Steganopus* [*Phalaropus*] *tricolor* is a wanton who no reasonable indulgence will satisfy .... It is, without doubt, this strange excess of *libido* which has brought the females of the species first to their musky perfection of size and power, and then, lacking outlet, has deranged the sex organs themselves."

Half a century hence, our successors will no doubt find similar amusement in ideas devolving from our present ignorance.

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DNA Hybridization and Avian Systematics

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The "revolution in molecular approaches to taxonomic problems" noted by Houde (1987) is in its infancy, and neither its methods nor its concepts are yet perfected. Those who have used the methods and contributed to the data are keenly aware of their limitations, and their strengths. Since 1957 the authors of this commentary have participated in the preparation of some 70 publications pertaining to avian systematics based on the properties of proteins or DNA. We have tried various methods with varying degrees of success in a continuing effort to reconstruct the phylogeny of birds. We appreciate Houde's positive comments about DNA hybridization and our results to date. His critique contains valid points, but it does not acknowledge our current position on molecular evolutionary rates, and it is flawed by confusion about exactly what DNA hybridization measures and, thus, about the properties of the data. Our current understanding of these subjects differs from our earlier views, such as those Houde may have discussed with Sibley in 1982 (as noted by Houde 1987: 29). In this commentary we will try to clarify the issues, correct misconceptions, and state our present position on several questions.

Rates of molecular evolution.—Since 1984 we have been aware that rates of single-copy nuclear DNA (scnDNA) evolution differ among avian lineages and between birds and mammals, and we have engaged in experiments designed to determine the occurrence and extent of such differences (Bledsoe 1987; Catzeflis et al. 1987; Sheldon 1987a, b; Sibley and Ahlquist 1987; Sibley et al. 1987). The laboratory work for these papers was carried out between 1984 and 1987. While these publications were being processed, we presented the evidence for different average genomic rates in seminars and lectures, including the International Ornithological Congress in June 1986 and recent A.O.U. annual meetings.

Although the existence of different average genomic rates (AGRs) is clear, it is also clear that such variation alone does not introduce ambiguity into phylogenetic reconstructions, provided appropriate clustering algorithms are used. Thus, Houde is incorrect in claiming that the reconstruction of phylogenies from DNA hybridization data depends on the existence of the same average rate along all branches. This misunderstanding is so basic to Houde's arguments about the shortcomings of DNA hybridization studies that many of his other points are rendered irrelevant.

The relationship between polarity and divergence.—Houde (pp. 17–18) stated that the dissimilarity measures produced by DNA hybridization comparisons are "inherently phenetic," and he referred to a footnote (p. 18) concerning apomorphy and plesiomorphy in relation to distance values. He implied that, in the absence of knowing the actual nucleotide substitutions and their relative apomorphy, there will always be ambiguity in phylogenetic reconstructions from distance data. We agree with Houde that "In-

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dividual distance values are not themselves primitive or derived," but we do not agree that the criteria developed for character-state data are suitable or necessary for the interpretation of distance data, for the following reasons.

DNA hybridization distance statistics do not *describe* "overall similarity"; they *measure the median sequence divergence* between two genomes (Hall et al. 1980). Although they are phenetic, they are *genomic*, not phenotypic, and they have always been found to be metric. By virtue of these properties, DNA hybridization data differ radically from the traditional concept of overall similarity, which is based largely on subjective, qualitative evaluation of morphological characters subject to convergence and of unknown genetic content (Sibley et al. 1987: 112–113).

DNA hybridization distinguishes homology from analogy because it compares enormously complex sets of hundreds of millions of unit characters and *complexity* offers the best guide to homology (Hecht and Edwards 1977, Ghiselin 1984, Gould 1985). It measures the net divergence of the entire single-copy genome, which is ca. 10^9 nucleotide pairs in birds. At the incubation temperature of 60°C, more than 75% of the bases must be paired correctly to form a stable duplex. *Only* homologous sequences are likely to have this degree of complementarity. Although a rigorous analysis of the probability of convergence under different models of DNA evolution has not been developed, a first approximation might apply a random model in which the probability of identical substitutions at each base position in two species is ¼. For a 500 base-pair sequence, the probability of convergence at 75% of the base positions is ¼ raised to the power of 375, a vanishingly small product. This model is not entirely realistic, but it illustrates the enormous complexity contained in a single, 500 base-pair DNA strand, and it suggests that genomewide homoplasy is extremely improbable.

The ability of DNA hybridization to distinguish homology from analogy releases it from concern about symplesiomorphy, synapomorphy, and the constancy of rates. Symplesiomorphy and synapomorphy are required in character data to establish branching patterns, but DNA hybridization data provide the branching pattern without them, simply as a product of the fact that the net effect of DNA evolution is certain to be divergence (Sheldon 1987a, b; Sibley et al. 1987: 114). That this is so can be seen with the relative rate test of Sarich and Wilson (1967) described in cladistic terms (Fig. 1).

The common ancestor of A, B, and C is defined by the base sequences at Y. Similarly, the common ancestor of A and B is defined by the base sequences at X. After C branches at Y, the stem YX is the common ancestral lineage of A and B, and the changes that accumulate along YX are synapomorphous for A and B. The YC lineage accumulates autapomorphs for C. After A and B diverge at X, each of these lineages

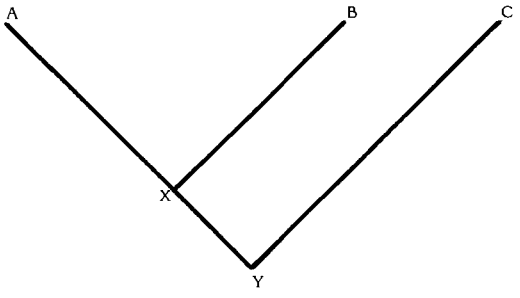

Fig. 1

accumulates autapomorphs, and the distance AB is the sum of the autapomorphous changes along XA and XB. If there is a rate difference along either XA or XB, it will be detected as a difference in distance between AC and BC because the distance CX is constant for those two measurements. What the relative rate test measures, therefore, are differences in autapomorphous changes in the lineages of two ingroups. The patterns of distance measurements directly reflect the underlying patterns of autapomorphous and synapomorphous base substitutions. This holds at more inclusive levels of phylogeny. It also holds for differences in average genomic rates of any magnitude, provided an unambiguous outgroup is employed.

Thus, Houde's claim that the reconstruction of the correct phylogeny from DNA hybridization data depends on the existence of constant rates along all branches is not true. Clustering algorithms that do not constrain sister branches to equal lengths (i.e. do not assume equal rates) will derive the correct tree. Several such algorithms have been developed to compute the correct branching pattern when average genomic rates are unequal, including Fitch-Margoliash, distance Wagner, unweighted least squares, and Felsenstein's (1987) mixed model analysis of variance method.

We have demonstrated different average genomic rates (AGRs) in scnDNA with, and without, the aid of fossils. Catzeflis et al. (1987) used the recently improved rodent fossil record to show that muroid rodents are evolving at an AGR of 2.5% nucleotide substitutions per million years. This is ca. 10 times the rate in the hominoids (=0.24%; Sibley and Ahlquist 1987), and it is twice as fast as the rodent rate proposed by Britten (1986). Li and Tanimura (1987) found similar values based on the DNA sequences of several genes. We also discovered rate differences, as did Houde, by outgroup comparisons (e.g. Sheldon 1987a, b).

The differences in average genomic rates between the rodents and hominoids suggest that the rate of generation turnover may be a factor in determining the average rate of genomic evolution. This is an old

idea (e.g. Laird et al. 1969) that has been updated by taking into account the effect of selection on the rate of coding region evolution, and of neutrality (Kimura 1983) on noncoding region evolution (e.g. Wu and Li 1985, Li and Tanimura 1987). Preliminary evidence suggests that there may be a correlation between the age at first breeding and the rate of scnDNA evolution in birds as well. We have been investigating this possibility for more than a year. Sibley and Ahlquist (1987) discussed this subject in hominoids and birds.

Congruence.—To demonstrate that the branching patterns produced by DNA hybridization are poor estimates of phylogeny, critics might argue that such estimates show little or no *congruence* with the branching patterns derived from other sources of evidence. Yet, in many instances, congruence with other data suggests the DNA estimates are accurate. Consider the New World suboscines (Sibley and Ahlquist 1985a: 401). The grouping of tyrant flycatchers, tityras, cotingas, and manakins produced by DNA hybridization departs only in details from traditional arrangements, which have long associated these groups and set them apart from the ovenbirds (Furnariidae) and the antbirds (Formicariidae and relatives). The main differences between the DNA-based and the traditional arrangements are among the Formicariidae, Rhinocryptidae, Conopophagidae, and Thamnophilidae, and for these groups morphological characters (Heimerdinger and Ames 1967, Ames et al. 1968, Ames 1971) support the DNA phylogeny. If the DNA hybridization data are giving poor estimates of phylogeny, why is such congruence evident? Houde's criticisms of the reliability, stability, or statistical significance of some of the close branches, or of the assignment of some of the tyrants to the "Mionectidae" (=Corythopinae), are valid points, but these minor problems do not perturb the overall congruence between the DNA hybridization evidence and that of other methods.

A second example concerns the Old World suboscines: New Zealand wrens (Acanthisittidae), pittas (Pittidae), philepittas (Philepittidae), and broadbills (Eurylaimidae). For these groups, Sibley et al. (1982) and Sibley and Ahlquist (1985a) found a pattern of branching that differs from that in traditional classifications (e.g. Wetmore 1960). Raikow (1986), from a study of the hindlimb myology, concluded that his results "are closely similar to those obtained by Sibley and Ahlquist using DNA-DNA hybridization; this agreement suggests corroboration of the hypothesis. Both results differ sharply from traditional views of passerine relationships." Raikow (1987) presented the evidence for his conclusion.

These are but two examples. The degree of congruence is excellent for closely related and noncontroversial taxa, for example, species within genera, or genera within families. Virtually all of the traditional clusters of species and genera have been reconstructed by the DNA comparisons. There are no examples in which, for example, a duck clusters with the pheasants, a pigeon with the sandpipers, or a passerine with any nonpasserine group. Instead, ducks cluster with the other waterfowl, pheasants with the other galliforms, etc. The DNA "sees" the same clusters of related birds that we see. The few departures from tradition (e.g. the "Pelecaniformes") are supported by independent evidence. It is difficult to see how DNA hybridization can give "correct" answers to those areas where consensus exists, and yet be "incorrect" in the areas that are controversial.

Disagreement becomes more frequent with increasing divergence, so that most of our departures from tradition are in the categorical levels at and above families. This is to be expected, given the limited resolution of traditional methods. In addition, our ability to discern relationships from morphology should be expected to decrease as the effects of divergence and convergence accumulate over time.

DNA sequence data.—The most compelling evidence for the ability of DNA hybridization to reconstruct the correct phylogeny may be congruence with DNA sequence data. It is generally believed that sequence data are probably the most precise evidence of relationships we can expect to obtain, and in addition they are character data, thus amenable to cladistic analysis. However, sequences also have their limitations. Felsenstein (1987) calculated that the sequences of at least 4,472 bases would be needed to equal the statistical power of DNA hybridization for the Sibley and Ahlquist (1987) hominoid primate data, but Roy J. Britten (pers. comm.) suggested that sequences of ca. 50,000 bases per species may be required to provide answers with the same level of confidence as those from DNA hybridization. Segments of DNA that are sequenced must be long enough to reflect the average rate and the net sequence divergence of the entire single-copy genome. Short sequences are subject to the effects of multiple hits, back mutations, and other phenomena, and very short sequences may be affected by convergent or coincidental similarities. Finally, a phylogeny reconstructed from sequence data will reflect only the branching order and evolutionary rate of the subset of the genomes upon which it is based. Such a phylogeny may or may not correspond to the actual phylogeny of the organisms in question.

Roy J. Britten (pers. comm.) noted some of the problems associated with the interpretation of DNA sequences for systematics and concluded that "Sequences of many different genes or regions must be compared to obtain definitive systematic relationships. (It has not been done). Single copy DNA hybridization is quicker, and the results are more conclusive since the whole genome is averaged."

Although there are no comparable sets of DNA sequence and DNA hybridization data for birds, there are for the hominoid primates. Sibley and Ahlquist (1984, 1987) have made two studies of the hominoids

(=gibbons, orangutan, gorilla, chimpanzees, humans), using the Old World monkeys (cercopithecoids) as the outgroup. Both studies found the branching sequence to correspond to the order given above, from oldest to most recent. The discovery that chimpanzees and humans are more closely related than either is to the gorilla was controversial; most morphological studies have concluded that the chimpanzees are closer to the gorilla than to man. There are now several sets of DNA sequences of various lengths for the hominoids, and they support the DNA hybridization results: for example, Scott et al. (1984), Nei (1985), Koop et al. (1986), Lanave et al. (1986), Saitou and Nei (1986), Britten (1986), Sakoyama et al. (1987), Li and Tanimura (1987), and Miyamoto et al. (1987). Other examples, including those that do not support their phylogeny, are noted by Sibley and Ahlquist (1987).

The hominoid data of Sibley and Ahlquist (1984, 1987) have been subjected to several statistical analyses, including those by Lausen and Degens (1986), Degens and Lausen (1986), and Felsenstein (1987); all have supported the tree derived by Sibley and Ahlquist.

The issue of the hominoid phylogeny cannot be divorced from that of the avian phylogeny because the methods of phylogeny reconstruction are the same. Houde (p. 21) broached the subject of the hominoid data of Sibley and Ahlquist when he cited the statistical study by Templeton (1985), but Houde omitted mention of the debate about the validity of Templeton's test by Nei and Tajima (1985), Saitou (1986), Ruvolo and Smith (1986), and Fitch (1986), and Templeton's (1986) response. All but Templeton supported the tree proposed by Sibley and Ahlquist (1984).

DNA melting curves.—Houde (p. 19, fig. 1) commented on some of the curves we published (Sibley and Ahlquist 1980, 1981) and drew the conclusion that there should be better ways to compare DNA hybridization melting profiles. In fact, the curves Houde criticized were some of the first we produced, and since then we have improved our methods for computing and comparing curves. The programs used to derive the curves Houde depicted have long since been replaced. In general, sigmoid, cumulative curves best represent the data because they are least subject to the effects of experimental errors. Most of the DNA hybridization studies performed in other laboratories (e.g. Benveniste 1985) have also used T_m and $T_{50}H$ values, derived from sigmoid curves. Our results have agreed with theirs in which the same taxa have been examined.

Some of the "bumps" in the curves noted by Houde, particularly in the bell-shaped frequency distributions, were caused by low temperature repeated "families" of sequences that contaminated some of our first single-copy DNA tracers. Our early DNA hybrids, notably the first set of ratite comparisons (Sibley and Ahlquist 1981), were prone to such contamination because, in 1979–1980, we were isolating single-copy DNA at Cot 500 and 60°C, conditions that are not adequate to remove some repeated DNAs. Henry Burr (pers. comm.) called Sibley's attention to this problem before the publication by Burr and Schimke (1980), and we corrected it thereafter by preparing single-copy DNA at Cot 1,000 and 50°C (e.g. Sibley and Ahlquist 1985b: 116). A "Note" about this problem was added by Sibley and Ahlquist (1981: 307) when the paper was in press.

In discussing DNA dissociation curves, it is important to emphasize that *all* homoduplex curves of organisms with the same A+T:G+C ratio will be virtually identical. Heteroduplex curves differ because they reflect the reduced percentage of hybridization relative to homoduplexes. We know that the AT:GC ratio is consistent among birds because it can be calculated from the T_m of native DNA using the following equation: $\%GC = (T_m - 69.3) \times 2.44$. There may be small variation in the AT:GC ratio in birds, but homoduplex melting curves prepared from high-quality DNA samples produce T_ms close to 86–87°C, indicating 42–43% GC content. This means that delta values reflect base-pair mismatches and are not affected to a large degree by AT:GC ratio differences.

The inclusion of taxa and the relationships of the flamingos.—Houde stated (p. 26) that "Meaningful interpretation of DNA hybridization data requires that all taxa relevant to a particular taxonomic problem be compared." He cited as one example the study by Sibley and Ahlquist (1985b) on African birds in which the relationships of the flamingos were discussed, but which did not include comparisons with the Australian Banded Stilt (*Cladorhynchus leucocephalus*), a species proposed by Olson and Feduccia (1980) as a close relative of the flamingos. Houde stated that Sibley and Ahlquist "assumed that *Cladorhynchus* will yield genetic distance values similar to those of other recurvirostrids, but the experiment has not been done." In fact, the experiment *had* been done but was omitted because *Cladorhynchus* is not closely related to the flamingos and because the paper pertained to African birds. For the record: *Cladorhynchus* is delta $T_{50}H$ 2.2 from *Recurvirostra* and *Himantopus*, delta 4.4 from four species of *Haematopus*, up to 12.8 from other groups of the parvorder Charadriida, 15.6 from the parvorder Scolopacida, but delta 18.7 from the clade that includes the flamingos and their closest relatives, the ibises and storks. The flamingos differ from the ibises and storks by delta $T_{50}H$ 11.5. These data were presented in our poster at the I.O.C. in Ottawa, in June 1986, and at the A.O.U. meeting in August 1986.

Houde (p. 26) also criticized Sibley and Ahlquist (1985b) for advocating "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." This is true, but there is other evi-

dence to support the suspicion that the American Sungrebe (*Heliornis fulica*) is not closely related to *Podica* or *Heliopais*, the African and Asian sungrebes. Alvarez del Toro (1971) called attention to the unique pockets of skin on the flanks of the male *Heliornis*, in which a hatchling can be carried, and Brooke (1984) noted other characters and proposed that *Heliornis* should be separated in a subfamily, Heliornithinae, from the Old World Podicinae. Sibley has been trying for years to obtain DNAs from *Podica* and *Heliopais*. When such material becomes available we will publish the results, but whatever the outcome, the close relationship between *Heliornis* and *Aramus* will not be altered.

No systematist using rigorous methods to obtain a large body of data can possibly include all pertinent taxa in a study of the phylogeny of a large number of species. Houde (1986) omitted pertinent taxa, such as *Rhea* (rheas), *Dromaius* (Emu), *Casuarius* (cassowaries), and the Dinornithidae (moas) in his study of some of the ratites and their presumed fossil relatives.

"Is it DNA or organisms that are to be classified?" (Houde 1987: 26).—This is an inappropriate and rhetorical ploy, because, as far as we know, no one, including us, has suggested that anything other than organisms be classified. We believe that organisms should be classified according to their phylogeny, which can be reconstructed by comparing their DNAs. In no sense does our approach constitute a classification of DNA per se. As noted above, however, if DNA sequences are used to reconstruct phylogenies, there is the risk that *gene* phylogenies, not organism phylogenies, will be produced.

Temporal calibration.—The branching pattern of the phylogeny is the primary goal, but it is challenging to try to determine the dates of divergences. Molecular distance measures are obviously *relative* to time because divergence takes time, but the conversion of relative time into *absolute* time requires an external reference date for at least one divergence node to calibrate the distance values. In addition, a given calibration factor is valid only for lineages evolving at, or near, the same average genomic rate as the lineage(s) used to calibrate the molecular "clock."

Houde (p. 27) stated that "There is no rationale for using the divergence of the orangutan (*Pongo*) for the calibration of the avian molecular clock." True, but that is *not* what Sibley and Ahlquist did. Houde (1986, 1987) misread the published record. Sibley and Ahlquist (1984: 13) calibrated the hominoid rate from the orangutan divergence date, and three avian divergences from independent geological events. By *coincidence*, the four proportionality constants came out between 4.3 and 4.6 million years = delta $T_{50}H$ 1.0. Sibley and Ahlquist have used a proportionality constant of delta $T_{50}H$ 1.0 = 4.5 MY, but they did not use the orangutan divergence to calibrate the avian clock.

Sibley and Ahlquist (1984: 13) noted that "The range of dates . . . from 16 to 80 MYA suggests that the regression is linear, and since the birds and primates lie on the same regression . . . it appears that the same average rate of DNA evolution occurs in both groups." This was one of the observations that supported the idea of a "uniform average rate" of DNA evolution, but we also noted that although "we may be close to the correct calibration . . . there are uncertainties in all of the fossil and geological datings, and additional calibration points should be obtained before concluding that the dating problem is solved."

In several papers, Sibley and Ahlquist (e.g. 1985a: 399, 1985b: 118) have stated that the proportionality constant of delta 1.0 = 4.5 MY is "tentative and subject to correction," and this remains our position. Until the corrections are made we believe that all assignments of dates to avian divergence nodes based on DNA comparisons must be viewed as tentative. The hominoid datings (Sibley and Ahlquist 1987) and the ratite datings (Sibley and Ahlquist 1981) may be close to the true divergence times, but it is not tenable to use these calibrations for other groups.

Houde (1987: 27–28) questioned whether there was evidence to support the calibration of DNA distances by various geological events, and he used his speculative account of the "volant paleognathous birds" (Houde 1986) to cast doubt on the vicariance theory of ratite distribution. It is far more likely that the Ostrich-Rhea divergence was caused by the opening of the Atlantic ca. 80 MYA than that these two extant taxa arose independently from a northern progenitor. We find Houde's arguments to be unconvincing and will maintain the conclusions in Sibley and Ahlquist (1981, 1985b: 119). Houde has no proof that his fossils had descendants; we *know* that our DNA molecules had ancestors.

Houde (1987: 27) also expressed doubt about the conclusions of Sibley et al. (1982) regarding the New Zealand wrens (Acanthisittidae), and of Sibley and Ahlquist (1981) concerning the way the ancestor of the kiwis arrived in New Zealand. The DNA hybridization evidence that the kiwis are most closely related to the Australo-Papuan Emu and cassowaries cannot be ignored, regardless of the dates assigned to the divergences. Similarly, if there were close living relatives of the New Zealand wrens outside of New Zealand, it would be reasonable to conclude that their ancestor flew across the Tasman Sea long after the Tasman Sea began to open (ca. 80 MYA). But the Acanthisittidae are the only survivors of a lineage that is delta $T_{50}H$ 17.9 from their closest living relatives. Even if their lineage has been evolving at twice the rate of the ratites, their divergence could have been ca. 40 MYA, at which time the Tasman Sea was already wide. This date would fit well, however, with the date Sibley and Ahlquist (1981) proposed for the crossing of the Tasman Sea by the ancestor of the kiwis via volcanic islands and island arcs produced by the collision of the Australian plate with the Pacific plate. The ancestor of the Acanthisittidae would not

have had to fly far between islands, judging from the numerous remnants of islands visible today on the floor of the northern Tasman Sea between northeastern Australia, New Zealand, and New Caledonia. It is difficult to account for the distinctive molecular and morphological characters of the Acanthisittidae except as the results of an ancient origin.

Houde (p. 28) cited various fossil dates to challenge the accuracy of the Sibley and Ahlquist calibration factor of delta $T_{50}H$ 1.0 = 4.5 MY. Although, as noted above, we consider it to be "tentative and subject to correction," this calibration factor does produce reasonable dates for some divergences. For example, the oldest penguin fossils are dated at ca. 50 MYA (Lower Eocene; Marples 1952), and the DNA-based date for the divergence between penguins and the procellariids is 47 MYA. The neotropical cuckoos we place in the Opisthocomidae (hoatzin), Crotophagidae (anis, guira cuckoo), and Neomorphidae (roadrunners), diverged from the Old World cuckoos at delta $T_{50}H$ 17.6, thus close to the delta 17.4 of the ostrich-rhea split, which Sibley and Ahlquist (1985b: 119) assumed to be the result of the opening of the Atlantic Ocean ca. 80 MYA. R. F. Baird and P. V. Rich (pers. comm.) are studying a fossil "cuculid from the Paleocene of Brazil" that places birds recognizable as cuckoos in South America ca. 65 MYA. If the evidence for New World vultures at 40–45 MYA (Rich 1983) is correct, the DNA divergence between storks and cathartids at ca. 36 MYA (delta 8.1) may be too recent. None of these dates, however, are discrepant by as much as "25–50%" as suggested by Houde (p. 28). It seems possible that the calibration factor of delta 1.0 = 4.5 MY will prove to be reasonably accurate for birds with delayed maturity of ca. 2–4 years of age, but not for those that breed at earlier and later ages. Let us agree that this problem, as well as the calibration factors, is "tentative and subject to correction."

Houde suggested that "it would be desirable to know the DNA hybridization values between South American and African" grebes, ducks, sungrebes, jacanas, thick-knees, pigeons, parrots, and trogons "to test the hypothesis that Atlantic seafloor spreading is appropriate for the calibration of DNA hybridization data." Some of these groups are highly vagile birds and therefore useless for this purpose. The waterbirds, especially, are irrelevant; some cross the Atlantic to this day, and most could have crossed long after the opening began. This is why we have considered only groups that reasonably might be expected to be stopped by a few miles of open water. Also, some groups may have moved between the Old and New worlds via the northern land bridges between Eurasia and North America. The divergences between the American, African, and Asian trogons must have been quite recent; the delta $T_{50}H$ values among them are 7.4, or less. African and South American parrots diverged at delta 6.8, the oldest branch among the pigeons of the southern continents is delta 8.2, and the oldest branch among the species of living flamingos is only delta 1.2. The well-known fact that the Cattle Egret (*Bubulcus ibis*) crossed the Atlantic in the 1870's (A.O.U. 1983: 51) makes it obvious that waterbirds are of little use as indicators of divergence times correlated with geological events.

Epilog.—Perfection is an elusive goal, and we must be willing to settle for progress. We believe that our application of DNA-DNA hybridization to avian systematics has made substantial progress toward the reconstruction of the phylogeny of living birds, and it is clear that many other biologists agree, including Houde and Cracraft (1987). The criticisms, in part, are that we have not yet taken full advantage of the method. We agree, but the difficulties in doing so are even more apparent to those who have participated in the work than to those who only examine the results. It may not so appear, but we are our own severest critics.

The critiques have been helpful by defining the problems perceived by other avian systematists, and we appreciate the interest and time indicated by the reviews. The technique, instrumentation, experimental design, and methods of data analysis are being improved, and we believe that the best hope for reconstructing the one correct phylogeny of birds lies with consensus among molecular methods. We urge other systematists to employ these rapidly evolving techniques to improve our understanding of the phylogenies of all groups of organisms.

Literature Cited

Alvarez del Toro, M. 1971. On the biology of the American Finfoot in southern Mexico. Living Bird 10: 79–88.

American Ornithologists' Union. 1983. Check-list of North American birds, 6th ed. Washington, D.C., Amer. Ornithol. Union.

Ames, P. L. 1971. The morphology of the syrinx in passerine birds. Bull. Peabody Mus. Nat. Hist. 37: 1–194.

———, M. A. Heimerdinger, & S. L. Warter. 1968. The anatomy and systematic position of the antpipits *Conopophaga* and *Corythopis*. Postilla 114: 1–32.

Benveniste, R. E. 1985. The contribution of retroviruses to the study of mammalian evolution. Pp. 359–417 *in* Molecular evolutionary genetics (R. J. MacIntyre, Ed.). New York, Plenum Press.

Bledsoe, A. H. 1987. Estimation of phylogeny from molecular distance data: the issue of variable rates. Auk 104: 563–565.

Britten, R. J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. Science 231: 1393–1398.

Brooke, R. K. 1984. Taxonomic subdivisions within the Heliornithidae. Ostrich 55: 171–173.

Burr, H. E., & R. T. Schimke. 1980. Intragenomic DNA sequence homologies in the chicken and

other members of the class Aves: DNA re-association under reduced stringency conditions. J. Mol. Evol. 15: 291–304.

CATZEFLIS, F. M., F. H. SHELDON, J. E. AHLQUIST, & C. G. SIBLEY. 1987. DNA-DNA hybridization evidence of the rapid rate of muroid rodent DNA evolution. Mol. Biol. Evol. 4(3) in press.

CRACRAFT, J. 1987. DNA hybridization and avian phylogenetics. Pp. 47–96 *in* Evolutionary biology, vol. 21 (M. K. Hecht, B. Wallace, and G. T. Prance, Eds.). New York, Plenum Press.

DEGENS, P. O., & B. LAUSEN. 1986. Statistical analysis of the reconstruction of phylogenies with DNA-DNA hybridization data. Res. Rept. No. 86/6. Dept. Statistics, Univ. Dortmund, W. Germany.

FELSENSTEIN, J. 1987. Estimation of hominoid phylogeny from a DNA hybridization data set. J. Mol. Evol. in press.

FITCH, W. M. 1986. Commentary. Mol. Biol. Evol. 3: 296–298.

GHISELIN, M. T. 1984. Narrow approaches to phylogeny: a review of nine books of cladism. Oxford Surv. Evol. Biol. 1: 209–222.

GOULD, S. J. 1985. A clock of evolution. Nat. Hist. 94(4): 12–25.

HALL, T. J., J. W. GRULA, E. H. DAVIDSON, & R. J. BRITTEN. 1980. Evolution of sea urchin non-repetitive DNA. J. Mol. Evol. 16: 95–110.

HECHT, M. K., & J. L. EDWARDS. 1977. The methodology of phylogenetic inference above the species level. Pp. 3–51 *in* Major patterns in vertebrate evolution (M. K. Hecht, P. C. Goody, and B. M. Hecht, Eds.). New York, Plenum Press.

HEIMERDINGER, M. A., & P. L. AMES. 1967. Variation in the sternal notches of suboscine passerine birds. Postilla 105: 1–44.

HOUDE, P. 1986. Ostrich ancestors found in the Northern Hemisphere suggest new hypothesis of ratite origins. Nature 324: 563–565.

———. 1987. Critical evaluation of DNA hybridization studies in avian systematics. Auk 104: 17–32.

KIMURA, M. 1983. The neutral theory of molecular evolution. Cambridge, England, Cambridge Univ. Press.

KOOP, B. F., M. M. MIYAMOTO, J. E. EMBURY, M. GOODMAN, J. CZELUSNIAK, & J. L. SLIGHTOM. 1986. Nucleotide sequences and evolution of the orangutan epsilon globin gene region and surrounding Alu repeats. J. Mol. Evol. 24: 94–102.

LAIRD, C. D., B. L. MCCONAUGHY, & B. J. MCCARTHY. 1969. Rate of fixation of nucleotide substitutions in evolution. Nature 224: 149–154.

LANAVE, C., S. TOMMASI, G. PREPARATA, & C. SACCONE. 1986. Transition and transversion rate in the evolution of animal mitochondrial DNA. BioSystems 19: 273–283.

LAUSEN, B., & P. O. DEGENS. 1986. Variance estimation and the reconstruction of phylogenies. *In* Proc. Gesellsch. f. Klassifikation (O. Opitz, H. J. Hermes, and P. O. Degens, Eds.). Die Klassifikation und ihr Umfeld. Frankfurt am Main, Indeks Verlag.

LI, W.-H., & M. TANIMURA. 1987. The molecular clock runs more slowly in man than in apes and monkeys. Nature 326: 93–96.

MARPLES, B. J. 1952. Early Tertiary penguins from New Zealand. New Zealand Geol. Surv., Palaeontol. Bull. 20: 1–66.

MIYAMOTO, M. M., J. L. SLIGHTOM, & M. GOODMAN. 1987. Phylogenetic relationships of humans and African apes as ascertained from DNA sequences (7.1 kilobase pairs) of the pseudo-*eta* globin region. Science in press.

NEI, M. 1985. Human evolution at the molecular level. Pp. 41–64 *in* Population genetics and molecular evolution (T. Ohta and K. Aoki, Eds.). Tokyo, Japan Sci. Soc. Press.

———, & F. TAJIMA. 1985. Evolutionary change of restriction cleavage sites and phylogenetic inference for man and apes. Mol. Biol. Evol. 2: 189–205.

OLSON, S. L., & A. FEDUCCIA. 1980. Relationships and evolution of the flamingos (Aves: Phoenicopteridae). Smithsonian Contrib. Zool. 316: 1–73.

RAIKOW, R. J. 1986. Phylogenetic relationships of the Old World suboscines. Abstr. No. 447, 19th Intern. Ornithol. Congr.

———. 1987. Hindlimb myology and evolution of the Old World suboscine birds (Acanthisittidae, Pittidae, Philepittidae, Eurylaimidae). Ornithol. Monogr. No. 41.

RICH, P. V. 1983. The fossil history of vultures: a world perspective. Pp. 3–25 *in* Vulture biology and management (S. R. Wilbur and J. A. Jackson, Eds.). Berkeley, Univ. California Press.

RUVOLO, M., & T. F. SMITH. 1986. Phylogeny and DNA-DNA hybridization. Mol. Biol. Evol. 3: 285–289.

SAITOU, N. 1986. On the delta Q-test of Templeton. Mol. Biol. Evol. 3: 282–284.

———, & M. NEI. 1986. The number of nucleotides required to determine the branching order of three species with special reference to the human-chimpanzee-gorilla divergence. J. Mol. Evol. 24: 189–204.

SAKOYAMA, Y., K.-J. HONG, S. M. BYUN, H. HISAJIMA, S. UEDA, Y. YAOITA, H. HAYASHIDA, T. MIYATA, & T. HONJO. 1987. Nucleotide sequences of immunoglobulin epsilon genes of chimpanzee and orangutan: DNA molecular clock and hominoid evolution. Proc. Natl. Acad. Sci. USA 84: 1080–1084.

SARICH, V., & A. C. WILSON. 1967. Rates of albumin evolution in primates. Proc. Natl. Acad. Sci. USA 58: 142–148.

SCOTT, A. F., P. HEATH, S. TRUSKO, S. H. BOYER, W. PRASS, M. GOODMAN, J. CZELUSNIAK, L.-YE. CHANG,

& J. L. SLIGHTOM. 1984. The sequence of the gorilla fetal globin genes: evidence for multiple gene conversions in human evolution. Mol. Biol. Evol. 1: 371-389.

SHELDON, F. H. 1987a. Phylogeny of herons estimated from DNA-DNA hybridization data. Auk 104: 97-108.

———. 1987b. Rates of single-copy DNA evolution in herons. Mol. Biol. Evol. 4: 56-69.

SIBLEY, C. G., & J. E. AHLQUIST. 1980. The relationships of the "primitive insect eaters" (Aves: Passeriformes) as indicated by DNA-DNA hybridization. Proc. 17th Intern. Ornithol. Congr.: 1215-1220.

———, & ———. 1981. The phylogeny and relationships of the ratite birds as indicated by DNA-DNA hybridization. Pp. 303-335 *in* Evolution today. Proc. 2nd Intern. Congr. Syst. Evol. Biol. (G. G. E. Scudder and J. L. Reveal, Eds.). Pittsburgh, Pennsylvania, Hunt Inst. Botan. Document., Carnegie-Mellon Univ.

———, & ———. 1984. The phylogeny of the hominoid primates, as indicated by DNA-DNA hybridization. J. Mol. Evol. 20: 2-15.

———, & ———. 1985a. Phylogeny and classification of New World suboscine passerine birds (Passeriformes: Oligomyodi: Tyrannides). Pp. 396-428 *in* Neotropical ornithology (P. A. Buckley, M. S. Foster, R. S. Ridgely, and F. G. Buckley, Eds.). Ornithol. Monogr. No. 36.

———, & ———. 1985b. The relationships of some groups of African birds, based on comparisons of the genetic material, DNA. Pp. 115-161 *in* Proc. Intern. Symp. on African Vertebrates (K.-L. Schuchmann, Ed.). Bonn, Zool. Forschungsinst. and Mus. A. Koenig.

———, & ———. 1987. DNA hybridization evidence of hominoid phylogeny: results from an expanded data set. J. Mol. Evol. in press.

———, ———, & F. H. SHELDON. 1987. DNA hybridization and avian phylogenetics: reply to Cracraft. Pp. 97-125 *in* Vol. 21, Evol. Biol. (M. Hecht, B. Wallace, and G. Prance, Eds.). New York, Plenum Press.

———, G. R. WILLIAMS, & J. E. AHLQUIST. 1982. The relationships of the New Zealand wrens (Acanthisittidae) as indicated by DNA-DNA hybridization. Notornis 29: 113-130.

TEMPLETON, A. R. 1985. The phylogeny of the hominoid primates: a statistical analysis of the DNA-DNA hybridization data. Mol. Biol. Evol. 2: 420-433.

———. 1986. Further comments on statistical analysis of DNA-DNA hybridization data. Mol. Biol. Evol. 3: 290-295.

WETMORE, A. 1960. A classification for the birds of the world. Smithsonian Misc. Coll. 139(11): 1-37.

WU, C.-I., & W.-H. LI. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proc. Natl. Acad. Sci. USA 82: 1741-1745.

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Estimation of Phylogeny from Molecular Distance Data: The Issue of Variable Rates

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Must rates of macromolecular evolution be uniform for measurements of amino acid or DNA sequence differences among taxa to be used in phylogenetic reconstruction? The evidence that rates of DNA evolution vary significantly among lineages of many organisms (Britten 1986), including birds (Sheldon 1987), makes this question especially pertinent to avian systematics. Houde (1987) contended that uniformity of rate is necessary for the use of distance data in phylogenetic reconstruction. However, his statement (p. 25) that "Satisfaction of the relative-rate test [of rate uniformity] is a prerequisite for the use of DNA data for phylogenetic reconstructions" reveals a misunderstanding of the nature of distance data and of the methods used to analyze their phylogenetic implications. This misunderstanding contributes to Houde's mistaken idea that variation in rates alone will introduce ambiguity into the reconstruction of phylogenetic branching patterns.

To see that varying rates do not inherently preclude accurate estimates of phylogeny, imagine a monophyletic set of species whose DNA sequences are evolving at the same positive rate, except for two species, which are not sister groups. These have a slow rate of DNA evolution, and thus show a smaller distance between one another than either does to any other species, including their sister groups. It is obvious that the incorrect joining of these two as sister species will produce discrepancies between the original data and any possible set of positive distances among taxa in the reconstructed (and incorrect) topology. If one would measure the level of discrepancy

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