The Auk 115(1):127–141, 1998

MOLECULAR AND OSTEOLOGICAL HERON PHYLOGENIES: SOURCES OF INCONGRUENCE

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ABSTRACT.—Payne and Risley's (1976) comparison of 33 osteological characters of herons was the first cladistic estimate of heron phylogeny. Among their findings were two major clades: (1) Boat-billed Heron (*Cochlearius cochlearius*), night-herons, and bitterns; and (2) tiger-herons and day-herons. In contrast, more recent DNA-DNA hybridization comparisons, cladistic analyses of vocalizations, and mtDNA sequence data portray a more asymmetric phylogeny, with day-herons and night-herons forming a clade with bitterns as their sister group, and tiger-herons and the Boat-billed Heron branching basally. To explore the source of the disagreement between these phylogenetic estimates, we reanalyzed the osteological data using modern cladistic methods and compared the results with the DNA-DNA hybridization tree using taxonomic congruence analysis. Character-by-character comparisons between trees and among lineages within trees suggest that similar cranial morphology in the relatively unrelated tiger-herons and day-herons has resulted in the misleading attraction of these two lineages in osteological estimates of phylogeny. Apparent convergence in bill morphology and modifications of orbital structures for nocturnal feeding in night-herons and Boat-billed herons have led to further disagreement between data sets. In part, problems in the osteological data stem from the relatively small character matrix of Payne and Risley (1976), but ultimately they may derive from using highly adaptive characters to reconstruct phylogeny. In this case, the cranial characters are functionally correlated as part of the piscivorous heron *Bauplan*. As such, they relate to the forces responsible for speciation and divergence in the early history of the group but may not be useful for phylogenetic inference. The discovery of bias in cranial characters underscores the value of taxonomic congruence analysis and the need to explore cases of phylogenetic incongruence. *Received 19 June 1996, accepted 24 June 1997.*

CONGRUENCE ANALYSIS of independent estimates of phylogeny is a useful method for judging phylogenetic accuracy (Mickevich and Johnson 1976). Agreement in branching patterns in trees from different data sets provides strong evidence of phylogeny (Bledsoe and Raikow 1990, Swofford 1991, Miyamoto and Fitch 1995). When phylogenies disagree, however, interesting lessons about evolution and the mechanics of phylogenetic estimation also may be learned. Incongruence can result from biased data derived from poor sampling strategies (e.g. using correlated characters) or idiosyncrasies of evolution (e.g. differences in rates of character change or character convergence) in one or both data sets (Wiley 1981, Patterson et al. 1993). Because the discovery and explication of characters that have evolved in unusual ways are main goals of evolutionary biology, the analysis of incongruence is an interesting pursuit. Nonetheless, when phylogenies are incongruent, phylogeneticists tend not to pursue the matter. When available, molecular data sets often are preferred because they are considered more objective than morphological or other data in which characters are judged prior to tree-building (Sibley and Ahlquist 1987). Not only does such an approach fail to recognize the many problems of molecular data, it ignores potentially useful information in the rejected data set. Advocates of character congruence (e.g. Kluge 1989, Kluge and Wolfe 1993) avoid this problem by combining data into a single tree-building effort according to the principle of total evidence. But, in certain instances, it is not possible or desirable to combine data sets, e.g. if one set consists of obligate distances, like those of DNA-DNA hybridization (Sheldon and Whittingham 1997), or if one is suspected of containing non-phylogenetic information (Bull et al. 1993, de Queiroz 1993, Miyamoto and Fitch 1995, Page 1996). In such cases, taxonomic congruence (Mickevich 1978),

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which maintains independent data sets, is the appropriate method of analysis.

Among bird groups, the herons (Ardeidae) offer a particularly promising opportunity to study sources of phylogenetic incongruence. There have been three rigorous studies of heron phylogeny using three different kinds of data and two types of analysis: (1) a cladistic analysis of osteological characters (Payne and Risley 1976), (2) DNA-DNA hybridization distance comparisons (Sheldon 1987a, b; Sheldon and Kinnarney 1993; Sheldon et al. 1995), and (3) a cladistic analysis of vocal characters (McCracken and Sheldon 1997). The analyses based on DNA-DNA hybridization and vocalizations are largely congruent (Figs. 1A, B), depicting: (1) day-herons and night-herons as the sister taxon of bitterns, and (2) the Boat-billed Heron (*Cochlearius cochlearius*) and tiger-herons as branching basally. This configuration is identical to that suggested by preliminary cladistic analysis of mitochondrial nucleotide sequences (1,065 bp) of the cytochrome-*b* gene for the same taxa (K. McCracken, C. Jones, and F. Sheldon unpubl. data). However, this configuration is incongruent with major portions of the osteological tree (Fig. 2), which consists of two main clades of herons: (1) Boat-billed Heron, night-herons, and bitterns; and (2) tiger-herons and day-herons. This incongruence must stem

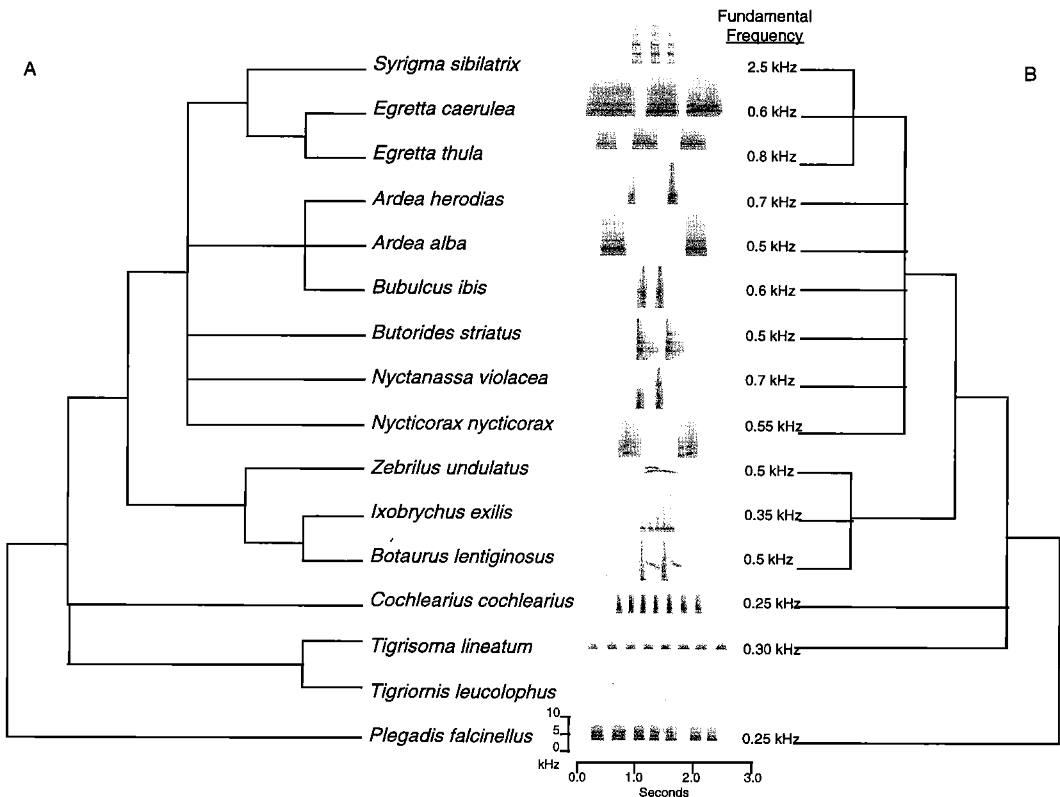

FIG. 1. DNA-DNA hybridization and vocal estimates of heron phylogeny. (A) Branch topology represents the best estimate of heron phylogeny based on DNA-DNA hybridization (Sheldon 1987 a, b; Sheldon et al. 1995). (B) Strict consensus of two trees (length = 7, CI = 1.0, RI = 1.00) estimated with PAUP using three informative vocal characters that track heron phylogeny (McCracken and Sheldon 1997). Vocal characters include, fundamental frequency (kHz), syllabic structure (tonal/harmonic), and number of syllables. Fundamental frequency was ordered because it is continuous. Syllabic structure and number of syllables are discrete and were coded as unordered. A randomization test for phylogenetic conservativeness (Maddison and Slatkin 1991) suggested that the arrangement of characters into shared ancestral and derived states corresponds strongly with the hierarchical arrangement of branches on the DNA-DNA hybridization tree ($P < 0.0001$). Vocal recordings not available for *Tigriornis leucolophus*.

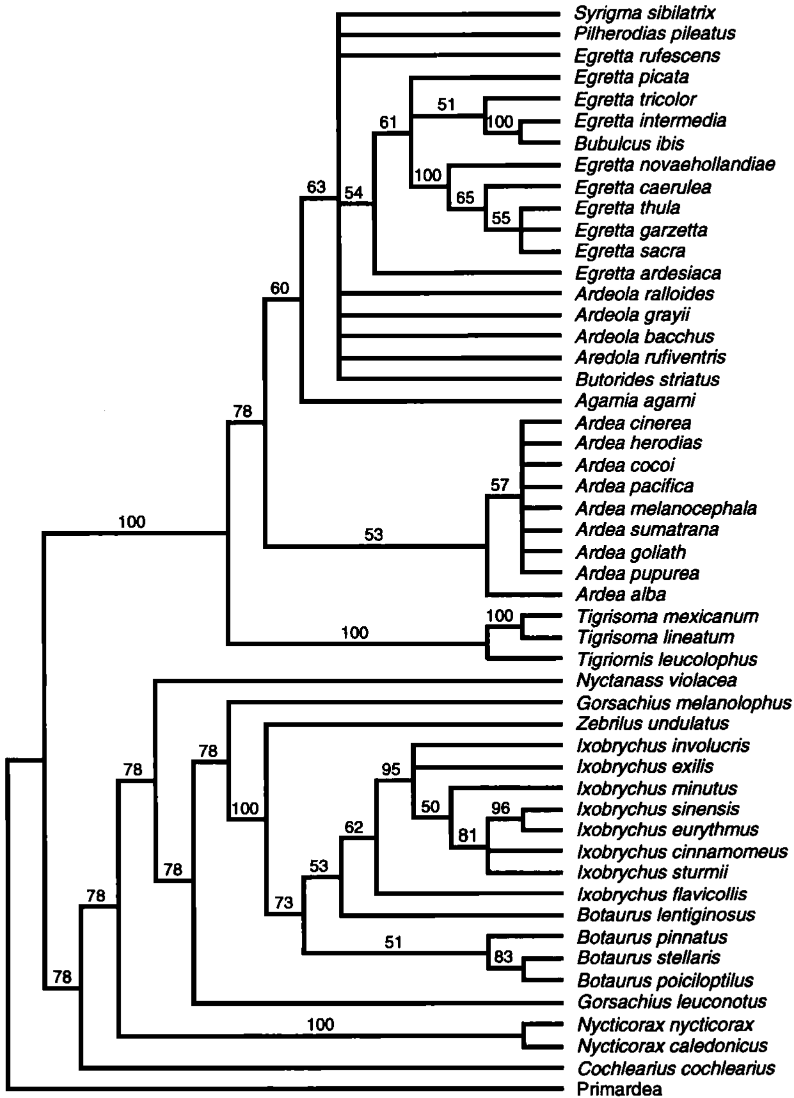

FIG. 2. Osteological estimate of heron phylogeny using 30 skeletal characters and "primardea" as the outgroup. Fifty-percent majority-rule consensus (Rohlf's CI[1] = 0.717) of 4,100 most- parsimonious trees (length = 143, CI = 0.336, RI = 0.790).

from evolutionary phenomena, a mistake in character selection, problems with analysis, or some combination of these factors.

The first task is to determine whether DNA-DNA hybridization / vocalizations / cytochrome *b* or the osteological analysis provides the most accurate estimate of phylogeny. In the absence of this information, either phylogenetic hypothesis might be taken to be correct, whereby data from the other could be fitted accordingly to observe patterns. However, the fact that three very different data sets (i.e. DNA-DNA hybridization, vocalizations, and mtDNA sequences) yield highly congruent trees suggests that these analyses yield the best estimate of phylogeny. Using this logic, we demonstrate that disagreement between DNA-DNA hybridization, vocalizations, and cytochrome *b* versus Payne and Risley's (1976) estimate of phylogeny based on osteological characters largely results from the influence of cranial characters in the osteological data. Under the rubric of cla-

TABLE 1. Corrections to Payne and Risley's (1976) matrix of skeletal characters.

Species	Character, state
Pilherodius pileatus	Character 3, state 1
Egretta novaehollandiae	Character 3, state 1
Egretta caerulea	Character 9, state 0
Nyctanassa violacea, Nycticorax nycticorax, N. caledonicus, Gorsachius melanolophus	Character 22, state 2
Gorsachius leuconotus (UMMZ 201761)	Previously undescribed states same as *G. melanolophus* except for characters 6(2), 21(1), and 30(0)
Zebrilus undulatus (LSUMNS 136695)	Previously undescribed character states for characters 3(1), 4(1), 13(1), 23(1), 24(1), 25(1), and 26(0)
Ixobrychus minutus	Character 3, state 1

distics, the problem can be attributed to correlated symplesiomorphic characters in tiger-herons and day-herons, and convergence and specialization in night-herons, Boat-billed Herons, and to a lesser extent in bitterns. We also compare rates of molecular and morphological change, but find no evidence that high rates of skeletal-character evolution are related to high rates of single-copy DNA evolution.

METHODS

Reanalyzing the osteological data.—We reanalyzed Payne and Risley's (1976) skeletal character matrix using PAUP 3.1.1 (Swofford 1993), a parsimony-based software package that was not available to Payne and Risley (1976). Corrections to the original matrix included state changes for eight taxa (R. B. Payne pers. comm.) and the inclusion of new character states for the White-backed Night-Heron (*Gorsachius leuconotus;* R. B. Payne pers. comm.) and the Zigzag Heron (*Zebrilus undulatus;* coded by us; Table 1). Unfortunately, skeletons of *Ardea imperialis, Egretta eulophotes,* and *Zonerodius heliosylus* were unavailable for analysis. Appendix 1 contains a complete list of taxa included in our analyses.

Payne and Risley (1976) used a hypothetical heron ancestor, "primardea," as an outgroup in their analyses. They derived character states for primardea from 10 non-heron ciconiiforms using phenetic methods. We repeated Payne and Risley's (1976) analysis using primardea. However, because using real taxa as outgroups requires no *ad hoc* assumptions about primitive character states (Nixon and Carpenter 1993), we also used the same non-heron ciconiiforms from which Payne and Risley (1976) derived primardea as an outgroup. Another substantial difference in our reanalysis concerned the Lesser Flamingo (*Phoeniconaias minor*). Payne and Risley (1976:37) noted a problem with this species as an outgroup, and we found that it appeared as an ingroup member in our initial analysis. The relationships of flamingos remain poorly understood, but current evidence indicates that they are not herons and may not be a close outgroup (see Sibley and Ahlquist 1990, Feduccia 1996). Thus, we excluded the Lesser Flamingo from all further comparisons.

Character states were coded and ordered according to Payne and Risley (1976). Because only 33 skeletal characters were used to compare 49 heron species, we faced two problems in our reanalysis. First, the large number of species precluded use of exhaustive and branch-and-bound search algorithms, which yield the most-parsimonious tree, and instead required the use of a heuristic-search algorithm. Heuristic-search algorithms do not necessarily find the most- parsimonious tree but, in some cases, may converge on a local parsimony minimum rather than the global minimum. Second, the probability of discovering a single most-parsimonious tree is small with less than two or three characters per taxon. Accordingly, we employed a meticulous search strategy to circumvent the problems of too many taxa and too few characters. We used the tree bisection-reconnection branch-swapping procedure to increase the probability that the heuristic search would find the most-parsimonious tree, and repeated the search 100 times. We initiated each search with a random addition sequence to ensure unbiased sampling of tree space. One hundred trees were saved at each step, potentially yielding a maximum of 10,000 trees per analysis.

Tracing character evolution and testing congruence.—We optimized Payne and Risley's (1976) skeletal characters onto the DNA-DNA hybridization phylogeny (Fig. 1A) and the skeletal tree using PAUP to study skeletal character patterns. We used both accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN). ACCTRAN and DELTRAN analyses were consistent, so we report only the ACCTRAN optimizations. We recorded each state change for each character on both the DNA tree and the skeletal tree (Appendix 2). Parallel and reverse state changes were determined for each character by the character state at the outgroup node. Multifurcations were considered uncertain and optimized most parsimoniously to avoid inflating the

number of character changes in unresolved parts of the trees.

To test null hypotheses of no difference in number of character changes between the molecular and morphological branch topologies, we performed a series of nonparametric winning-sites tests (Prager and Wilson 1988; see also Templeton 1983). Parametric Kishino-Hasegawa (1989) winning-sites tests generally are applied to sequence data. However, nonparametric tests offer a more appropriate test statistic for data sets such as Payne and Risley's (1976), which contain small numbers of characters that are not normally distributed. Moreover, parallel and reverse steps can be scored separately to partition the effects of these processes on tree topology. The testing process was as follows. We scored the difference in number of steps for each variable character between the morphological and molecular trees, identifying parallel and reverse step events in each instance. We then compared the distribution of scores for each variable character with the binomial distribution to test hypotheses of overall tree similarity. Like other statistical tests, winning-sites tests rely upon the assumption that each character is independent. To the extent that one or more skeletal characters are not independent, the effective number of degrees of freedom may be overestimated. We investigated different tree topologies and calculated step differences for alternative constructions of contentious nodes.

Next, we used *a priori* criteria to group skeletal characters. Payne and Risley (1976) included 33 skeletal characters in their analyses (one of the bill, three palatal, two orbital, three lacrimal, three ectethmoidal, one of the foramen magnum, three vertebral, three sternal, one coracoidal, two furcular, four humeral, five synsacral, and two tarsometatarsal). We applied winning-sites tests to each of these groups to determine whether parallelisms, reversals, and consistency indices differed between molecular and morphological estimates of phylogeny. Character groups that did not differ significantly between trees ($P < 0.05$) were pooled with the nearest anatomical group(s). Groups that differed significantly were retained. Using this procedure: (1) pelvic, vertebral, and pectoral characters were combined to form a single group consisting of all postcranial characters; and (2) bill, palatal, orbital, lacrimal, ectethmoid, and foramen magnum characters were combined to form a group consisting of all cranial characters.

Following these results, we tested whether cranial and postcranial frequencies of character evolution differed among heron lineages using the DNA-DNA hybridization tree (Fig. 1A). Sheldon (1987b) discovered differences in rates of single-copy DNA evolution among the three main heron clades; bittern DNA evolved ~25% faster than day-heron and night-heron DNA, which in turn evolved ~19% faster than Rufescent Tiger-Heron (*Tigrisoma lineatum*) and Boat-billed Heron DNA. We calculated the number of parallel and reverse state-change events for each character in the DNA tree in each of these heron lineages (Appendix 3). State-change polarity was determined by the character state at the outgroup node. Next, we used a contingency analysis (PROC FREQ SAS 1990) to test the null hypothesis that frequencies of cranial and postcranial parallel and reverse events do not differ among bitterns, day-herons and night-herons and between tiger-herons and the Boat-billed Heron.

RESULTS

Reanalysis of Payne and Risley's data.—Our tree including 49 heron species, 30 informative skeletal characters, and the composite outgroup primardea (Fig. 2) is nearly identical to Payne and Risley's (1976) Wagner tree. This tree distinguishes two main clades: night-herons and bitterns, and tiger-herons and day-herons.

When we included 10 non-heron ciconiiform species as the outgroup, the Lesser Flamingo emerged as sister to the tiger-herons within the day-herons. This arrangement was supported unambiguously by 26 skeletal characters and strict consensus (Rohlf's CI[1] = 0.503). Deleting the Lesser Flamingo from the analysis had no substantial effect on the overall branching pattern in Figure 3. However, Figures 2 and 3 differ substantially in the positions of the Boat-billed Heron, night-herons, day-herons, and tiger-herons, indicating the strong influence of real outgroups versus a hypothetical outgroup. In Figure 3, *Cochlearius* does not form a monophyletic group with night-herons and bitterns, but rather is the sister taxon of all other herons. Night-herons do not form a monophyletic group with the bitterns. Instead, the bitterns are the sister taxon of the day-herons and tiger-herons. The outgroup topology is concordant with Sibley and Ahlquist (1990).

Molecular and morphological congruence.—Comparisons of the DNA-DNA hybridization estimate of heron phylogeny (Fig. 1A) and the skeletal trees (Fig. 3) agree in that the Boat-billed Heron is basal to all other herons, and the bitterns are the sister taxon of the day-herons. However, the DNA-DNA hybridization and skeletal trees do not agree as to the positions of tiger-herons and night-herons.

We entered the DNA-DNA hybridization tree and a reduced skeletal tree of the same taxa and a topology consistent with Figure 3 into MacClade (Maddison and Maddison 1992) and

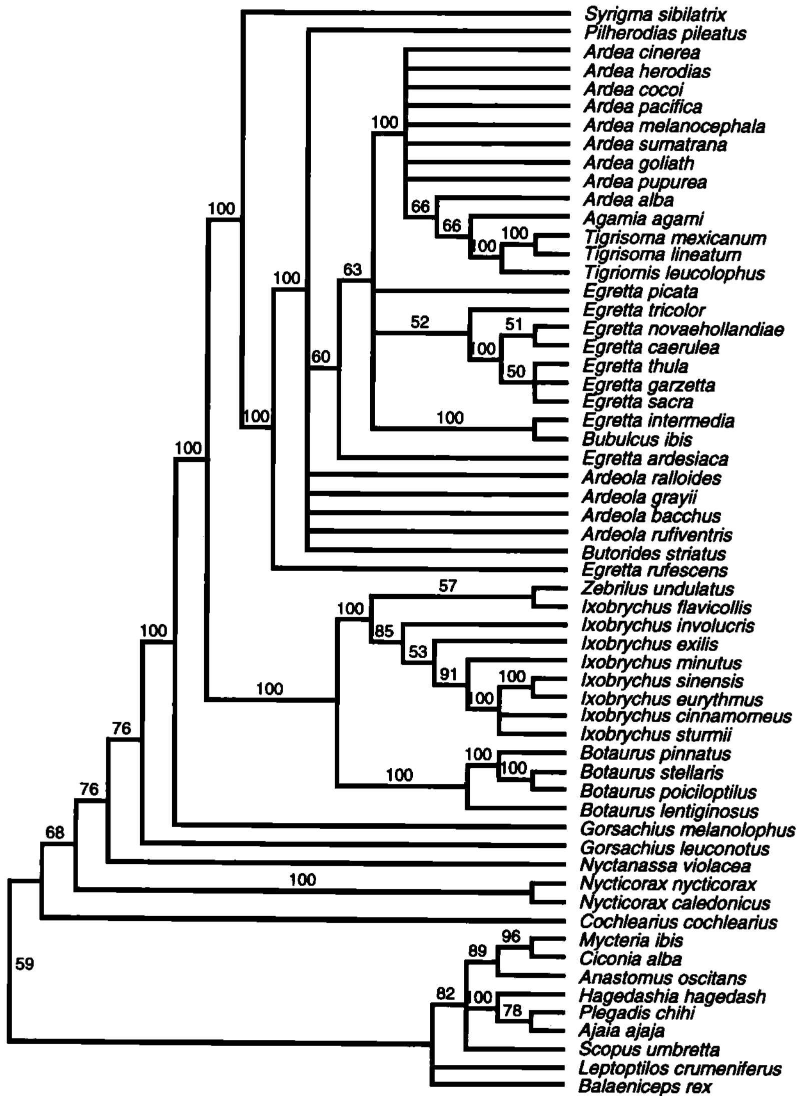

FIG. 3. Osteological estimate of heron phylogeny using 33 skeletal characters and 9 non-heron ciconiiforms as the outgroup. Fifty-percent majority-rule consensus (Rohlf's CI[1] = 0.684) of 2,500 most-parsimonious trees (length = 179, CI = 0.285, RI = 0.782).

PAUP to examine congruence between Figures 1 and 3 (Figs. 4 and 5). DNA-DNA hybridization estimates used the Glossy Ibis (*Plegadis falcinellus*) as the outgroup. To control for the effects of different outgroups, we attached our non-heron ciconiiform outgroup tree to the ingroup of the DNA-DNA hybridization tree using MacClade (Fig. 5). When the morphological characters were optimized over the DNA-DNA hybridization tree (Fig. 5), 26 more steps were required than when the same characters were optimized over the skeletal tree (Fig. 4). If the DNA tree is correct, the skeletal characters possess substantial homoplasy; parallel and reverse events occur more frequently in the DNA-DNA hybridization tree than in the skeletal tree. Repositioning the tiger-herons plus Boat-billed Heron as sister taxa to the rest of the herons requires seven additional steps (length = 144, CI = 0.333, RI = 0.622). Further placing the bitterns as the sister group to the day-herons and night-herons requires an additional seven

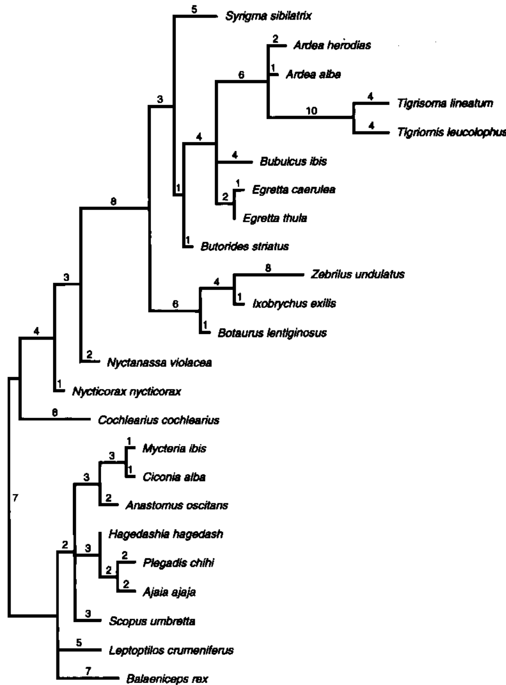

FIG. 4. Osteological estimate of heron phylogeny including all species included in Sheldon (1987a) and Sheldon et al. (1995). Branching topology is identical to that of Figure 3. Branch lengths were calculated by mapping 31 informative skeletal characters using PAUP (length = 137, CI = 0.350, RI = 0.637).

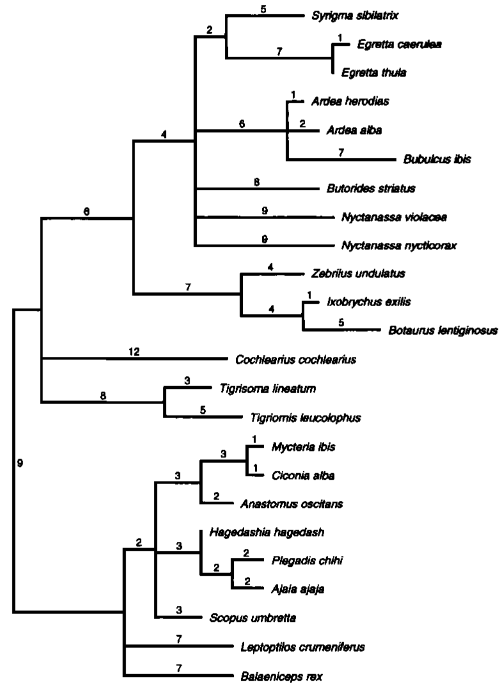

FIG. 5. DNA-DNA hybridization estimate of heron phylogeny (Sheldon et al. 1995) with attached non-heron ciconiiform outgroup. Branch lengths were calculated by mapping 31 skeletal characters using PAUP (length = 163, CI = 0.294, RI = 0.531).

steps, yielding a total of 14 steps (length = 151, CI = 0.318, RI = 0.594).

Skeletal character homoplasy.—A winning-sites test revealed that the total number of homoplastic steps (i.e. parallelisms and reversals) is greater for the DNA-DNA hybridization topology than for the skeletal tree ($n = 18$, $P < 0.031$). Separate winning-sites tests for parallelisms and reversals revealed that the total number of parallel events is greater for the DNA-DNA hybridization tree than for the skeletal tree ($n = 17$, $P < 0.013$), but the number of reversal events does not differ significantly between trees ($n = 20$, $P < 1.000$). If the DNA-DNA hybridization tree is correct, then the majority of skeletal-character homoplasy is accounted for by the unusually high rates of parallel evolution.

Homoplastic skeletal-character evolution is not randomly distributed among all of the skeletal characters. Our analyses of cranial and postcranial characters indicate that the total number of parallel and reverse events is greater in the DNA-DNA hybridization tree for cranial characters ($n = 10$, $P < 0.022$) but not for postcranial characters ($n = 8$, $P < 0.73$). Parallel events are significantly greater in the DNA-DNA hybridization tree for cranial characters ($n = 11$, $P < 0.012$), but not for postcranial characters ($n = 6$, $P < 0.69$). Reverse state-change events do not differ significantly between trees, but the trend in probability values also suggests that the frequency of reversals is greater for cranial characters ($n = 10$, $P < 0.36$) than for postcranial characters ($n = 10$, $P < 0.62$).

Frequency of homoplastic evolution.—Given that cranial character evolution is more homoplastic than postcranial character evolution in the DNA-DNA hybridization tree, we tested whether frequencies of parallelisms and reversals differed among the three heron lineages: (1) tiger-herons and the Boat-billed Heron, (2) bitterns, and (3) day-herons and night-herons. A contingency analysis of the total number of non-unique state changes revealed that cranial

TABLE 2. Mean number of homoplastic steps per character for cranial (n = 13) and postcranial (n = 18) skeletal characters in heron lineages as estimated by DNA-DNA hybridization (Fig. 5).

Lineage	Cranial	Post-cranial
Tiger-herons, Boat-billed Heron	0.769	0.667
Bitterns	0.462	0.667
Day-herons, night-herons	2.000	1.833

and postcranial parallelisms and reversals are not distributed homogeneously among the three heron lineages (χ^2 = 56.81, df = 2, $P <$ 0.001). Bittern DNA has evolved ~25% faster than in day-herons and night-herons and ~44% faster than in tiger-herons and the Boat-billed Heron. Evolution of bittern cranial parallelisms and reversals, however, has been ~78% less frequent than in day-herons and night-herons and ~40% less frequent than in tiger-herons and the Boat-billed Heron (Table 2). The evolution of bittern postcranial parallelisms and reversals has been ~64% less frequent than in day-herons and night-herons and approximately equal to that in tiger-herons and the Boat-billed Heron.

DISCUSSION

Phylogenetic estimation and skeletal character plasticity.—Since the time of Richard Owen (1866), homology has been regarded as the key to discovering the natural hierarchy of life. Thus, the correct identification of homologous characters and exclusion of homoplastic characters from phylogenetic analyses is one of the greatest challenges facing systematists. Darwin (1859) recognized that the reliability of taxonomic characters is inversely related to the degree to which characters have evolved for specialized habits. Features such as relative position, function, correspondence, and complexity of characters generally have been regarded as the hallmarks of adaptive specialization (Remane 1952). Bock (1967) further developed the criteria for recognizing useful taxonomic characters. He noted that systematists must distinguish between evolutionary forces associated with the origin and modification of characters and those associated with natural selection (i.e. differential survival and reproduction), because different suites of characters may be evolving under demonstrably different forces (see Bull et al. 1993, Miyamoto and Fitch 1995). The most useful taxonomic characters, i.e. characters that are most likely to be homologous, are those for which the probability of unique historical origin is high.

Our findings support traditional views about the usefulness of taxonomic characters. Given that herons use their bills and palatal structures in different ways to obtain a variety of prey, and that they use their eyes to detect prey, their cranial structures must be under considerable selective pressure and would be expected to evolve plastically. As such, it is not surprising that we found that cranial structures are not useful taxonomic characters in herons. Largely because of the influence of cranial characters, analysis of osteological characters places tiger-herons among the day-herons, even though day-herons are more closely related to night-herons. In contrast, heron postcranial skeletal evolution appears to have been less plastic. Although there have been significant changes in leg and wing length, particularly among day-herons and night-herons (e.g. *Agamia, Bubulcus, Ardeola, Nycticorax, Nyctanassa*), associated pelvic and pectoral structures appear to have evolved without the loss of phylogenetic information.

Herons traditionally have been divided into five groups: day-herons, night-herons, bitterns, tiger-herons, and the Boat-billed Heron (Bock 1956, Hancock and Elliot 1978). Some of these groups differ substantially in cranial morphology, apparently in response to ecological forces. This is particularly true of Boat-billed Heron and night-herons. The Boat-billed Heron is so different from other herons in bill morphology that it shows remarkable convergence (e.g. bill and palate characters 1 to 4) with the Shoebill (*Balaeniceps rex*). Apparently rapid, convergent, development of scotopic vision and subsequent modifications of orbital and bill structure for night feeding in night-herons and Boat-billed Heron (as well as plumage similarities) explain why taxonomists generally have lumped the Boat-billed Heron with the night-herons, and why they also have removed night-herons from the vicinity of day-herons in classifications (Bock 1956, Payne and Risley 1976, Hancock and Elliot 1978). Compared with night-herons, other groups are remarkably similar in cranial morphology, particularly

day-herons and tiger-herons, and to a lesser extent, bitterns.

For cladistic and other methods of analysis, the problem in estimating the correct phylogeny derives from the fact that groups with different cranial morphologies are interleaved within the heron phylogeny. Thus, highly similar day-herons and tiger-herons are separated by night-herons and bitterns, and night-herons and the Boat-billed Heron are separated by day-herons and bitterns. When cranial characters are optimized onto the phylogeny (Fig. 5), many convergent changes are required to account for the similarity in the separated groups, particularly the day-herons and tiger-herons.

Although homoplastic cranial characters are largely responsible for this result, we note that these characters would not necessarily have misled osteological analyses to the extent that they did had: (1) the data matrix been larger, or (2) the heron phylogeny been shaped differently. If there were more than 33 characters in the data matrix, the influence of the homoplastic cranial characters might have been reduced. If night-herons and Boat-billed Herons or day-herons and tiger-herons were sister taxa, much of the convergent evolution would have been obviated. However, despite these factors, this case in not likely to be an unusual or isolated incident in which the most-parsimonious morphological tree is suboptimal. Congruence analysis of crocodiles (Poe 1996), for example, demonstrated that the most-parsimonious morphological tree is suboptimal. Conversely, a circumstance in which a less-than-most-parsimonious molecular tree might better describe evolutionary history occurs when the stochastic accumulation of nucleotide substitutions causes long-branch attraction (Felsenstein 1978, Hendy and Penny 1989).

In the case of herons, a simpler explanation for cranial character evolution is that tiger-herons and day-herons share ancestral morphological features rather than convergent ones. This interpretation is intuitively appealing because it provides a hypothesis for the adaptive specialization and radiation of heron lineages into new feeding zones in the early history of the group. Assuming a primitive heron *Bauplan* similar to day-herons, tiger-herons, and bitterns, it is the night-herons and Boat-billed Heron that have diverged most dramatically in cranial morphology as they adapted to night feeding. Such an explanation, although not adhering strictly to parsimony, is consistent with the logic of Hennig's (1966) auxiliary principle, which holds that convergence should not be assumed in the absence of contrary evidence (Wiley 1981). Thus, cranial characters presented a problem at the outset of our analysis, but upon closer inspection, they actually helped us to formulate a more reasonable explanation of heron evolution.

Molecular and morphological rates of evolution.—The relationship between molecular and morphological rates of evolution has been discussed since the 1960s (Zuckerkandl and Pauling 1965), with the general conclusion that the two rates are not related (Wilson et al. 1977). Nonetheless, various authors have concluded that when nucleotide sequences evolve rapidly, morphological characters also evolve rapidly (Bosquet et al. 1992, Larson and Dimmick 1993, Omland 1994). We found no strong evidence supporting a general relationship between rates of molecular and morphological evolution in herons (Table 2). If cranial osteology in day-herons, tiger-herons, and bitterns actually has converged, then bitterns exhibit the least amount of change in cranial characters, which are the most labile characters, even though they have by far the fastest rate of single-copy DNA evolution. If day-herons and tiger-herons have retained their ancestral morphology, which is more likely the case, then high rates of cranial evolution have occurred in night-herons and the Boat-billed Heron. Neither hypothesis is compatible with a causal model linking rates of molecular and morphological evolution.

Analysis of incongruence.—We have documented one example in which an analysis of incongruence and explication of character-state changes can be used to identify correlated characters, partition homoplasy among lineages, and highlight basic evolutionary concepts and processes. Such analyses should be conducted whenever trees disagree. Particular effort should be placed on identifying characters responsible for incongruence, establishing their distribution and patterns of evolution in different parts of the tree, and thus explaining the overall effect these characters have on tree topology. In this case, we found that cranial characters introduce bias into the morphological data by virtue of their shared primitive states,

convergence, and possibly nonindependence as adaptive suites. As such, they should not have been used to infer phylogeny. Had we tried to solve the problem of incongruence by combining these cranial characters in a character-congruence assessment (Kluge 1989), as opposed to a taxonomic-congruence assessment (e.g. Bledsoe and Raikow 1990), the problem may have gone undiscovered. But such an option was not available to us because some of the data were DNA-DNA hybridization distances. Ardent proponents of the maxim of total evidence simply would have thrown out the DNA-DNA hybridization data, because they are viewed as nonphylogenetic (e.g. Kluge and Wolfe 1993). However, because overwhelming evidence indicates that DNA-DNA hybridization is an effective method of phylogenetic inference (Caccone and Powell 1989, Bledsoe and Sheldon 1990, Powell 1991, Sheldon 1994), not using its data would have been a violation of total evidence (de Queiroz 1993). Our findings support the argument that the practice of combining all available character data into a single phylogenetic analysis is ill advised when some characters are biased or, in the case of gene versus species phylogenies, when different suites of characters are tracking different histories (Bull et al. 1993, Miyamoto and Fitch 1995, Page 1996).

Acknowledgments

We thank Allan Baker, Anthony Bledsoe, Joel Cracraft, John Harshman, Brad Livezey, Therese Markow, Thomas Martin, Robert Payne, Robert Raikow, Jack Sites, Mike Stine, and two anonymous reviewers for their helpful comments on the manuscript; Clare Jones for her sequencing efforts and support in the lab; Mike Stine for providing a computer that facilitated analyses of large data sets; and J. V. Remsen, Steve Cardiff, and Dave Willard for access to specimens at the Louisiana State University Museum of Natural Science and the Chicago Field Museum. Institutional and financial support for this project was provided by the Louisiana State University Museum of Natural Science, School of Forestry, Wildlife, and Fisheries, College of Agriculture, Agricultural Center, Louisiana Board of Regents, and NSF grants BSR-8806890, BSR-9020183, and BSR-9207991.

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Associate Editor: A. J. Baker

APPENDIX 1. Heron species included in the analyses.[a]

Species	
Cochlearius cochlearius	Boat-billed Heron*†
Tigriornis leucolophus	White-crested Tiger-Heron*
Tigrisoma lineatum	Rufescent Tiger-Heron*†
Tigrisoma mexicanum	Mexican Tiger-Heron
Zebrilus undulatus	Zigzag Heron*†
Botaurus stellaris	Eurasian Bittern*
Botaurus poiciloptilus	Australian Bittern
Botaurus pinnatus	South American Bittern
Botaurus lentiginosus	American Bittern*†
Ixobrychus flavicollis	Black Bittern
Ixobrychus cinnamomeus	Cinnamon Bittern*
Ixobrychus sturmii	African Dwarf Bittern
Ixobrychus sinensis	Yellow Bittern
Ixobrychus eurythmus	Schrenck's Bittern
Ixobrychus involucris	Streaked Bittern
Ixobrychus exilis	Least Bittern*†
Ixobrychus minutus	Little Bittern*
Nyctanassa violacea	Yellow-crowned Night-Heron*†
Nycticorax nycticorax	Black-crowned Night-Heron*†
Nycticorax caledonicus	Nankeen Night-Heron*
Gorsachius melanolophus	Malay Night-Heron
Gorsachius leuconotus	White-backed Night-Heron
Butorides striatus	Green Heron*†
Ardeola ralloides	Squacco Heron
Ardeola grayii	Indian Pond-Heron*
Ardeola bacchus	Chinese Pond-Heron
Ardeola rufiventris	Rufous-bellied Heron
Syrigma sibilatrix	Whistling Heron*†
Pilherodius pileatus	Capped Heron
Agamia agami	Agami Heron
Egretta picata	Pied Heron
Egretta tricolor	Tricolored Heron*
Egretta caerulea	Little Blue Heron*†
Egretta thula	Snowy Egret*†
Egretta garzetta	Little Egret*
Egretta sacra	Eastern Reef Egret*
Egretta ardesiaca	Black Egret
Egretta rufescens	Reddish Egret
Egretta intermedia	Intermediate Egret*
Bubulcus ibis	Cattle Egret*†
Egretta novaehollandiae	White-faced Heron*
Ardea alba	Great Egret*†
Ardea cinerea	Grey Heron
Ardea herodias	Great Blue Heron*†
Ardea melanocephala	Black-headed Heron*
Ardea sumatrana	Great-billed Heron*
Ardea cocoi	Cocoi Heron*
Ardea pacifica	White-necked Heron*
Ardea goliath	Goliath Heron
Ardea purpurea	Purple Heron
Balaeniceps rex	Shoebill
Leptoptilos crumeniferus	Marabou Stork
Scopus umbretta	Hamerkop
Mycteria ibis	Yellow-billed Stork
Ciconia alba	White Stork
Anastomus oscitans	Asian Openbill Stork
Hagedashia hagedash	Hadada
Plegadis chihi	White-faced Ibis
Plegadis falcinellus	Glossy Ibis*†
Ajaia ajaja	Roseate Spoonbill
Phoeniconaias minor	Lesser Flamingo

[a] Asterisks indicate species with DNA-DNA hybridization data (Sheldon 1987a, Sheldon et al. 1995); crosses indicate species with vocalization data (McCracken and Sheldon 1997).

APPENDIX 2. Winning-sites test scores. Values are number of parallel and reverse steps for each possible state change of Payne and Risley's (1976) skeletal characters when optimized most parsimoniously onto DNA-DNA hybridization tree (Figs. 1A and 5) and skeletal-character tree (Fig. 4).

	DNA tree		Skeletal tree		Step difference	
State change	Parallel	Reverse	Parallel	Reverse	Parallel	Reverse
			Bill shape			
0 → 1	2	0	0	0	2	0
			Palatine shape			
0 → 1	3	0	0	1	3	−1
			Palatine emargination			
2 → 1	3	1	3	3	2	0
1 → 0	3	3	4	1	−1	2
2 → 3	2	0	0	0	2	0
			Palatine lateral process			
1 → 0	5	0	4	0	1	0
			Interorbital foramen			
2 → 0	4	0	0	0	4	0
2 → 1	2	0	0	1	2	−1
2 → 3	2	1	0	2	2	−1
			Supraorbital foramen			
2 → 1	3	1	2	1	1	0
1 → 0	3	2	2	1	1	1
			Lacrimal size			
1 → 0	3	1	3	0	0	1
1 → 2	0	0	0	0	0	0
			Lacrimal ventral projection			
2 → 3	0	0	0	0	0	0
1 → 2	3	1	4	0	−1	1
0 → 1	0	0	2	0	−2	0
			Lacrimal lateral groove			
0 → 1	5	1	4	2	1	−1
1 → 2	0	0	0	0	0	0
			Ectethmoid			
1 → 0	4	0	3	0	1	0
			Ectethmoid tubercle			
0 → 1	6	0	3	3	3	−3
1 → 2	0	0	0	0	0	0
			Ectethmoid ridge			
0 → 1	4	1	3	0	1	1
			Basitemporal ridge			
0 → 1	0	3	0	0	0	3
			Axis shape			
2 → 1	0	3	0	0	0	3
1 → 0	2	2	2	0	0	2
			Lateral canal			
0 → 1	6	0	2	2	4	−2
			Rib facets			
0 → 1	2	4	2	1	0	3
			Notches			
0 → 1	2	0	2	0	0	0

APPENDIX 2. Continued.

State change	DNA tree		Skeletal tree		Step difference	
	Parallel	Reverse	Parallel	Reverse	Parallel	Reverse
Manubrial length						
0 → 1	0	1	2	1	−2	0
1 → 2	5	1	3	2	2	−1
Sternocoracoidal process						
0 → 1	2	2	2	1	0	1
Sternal facet						
0 → 1	0	0	0	0	0	0
External spine						
0 → 1	3	1	2	2	1	−1
1 → 2	3	0	2	2	1	−2
2 → 3	3	0	2	2	1	−2
Internal spine						
0 → 1	3	1	2	3	1	−2
1 → 2	2	1	2	0	0	1
2 → 3	0	0	0	0	0	0
Deltoid crest shape						
0 → 1	2	0	2	0	0	0
Deltoid crest height						
0 → 1	4	0	3	1	1	−1
Pneumatic fossa						
0 → 1	5	1	5	1	0	0
1 → 2	0	0	0	0	0	0
Ligamental furrow						
0 → 1	2	0	2	0	0	0
Iliac crest shape						
0 → 1	3	2	4	0	−1	−2
Posterior iliac crest						
ni[a]	—	—	—	—	—	—
Paraphyses fusion						
0 → 1	4	0	4	0	0	0
Iliac recess						
0 → 1	4	3	5	1	−1	2
Ischiopubic symphysis						
0 → 1	0	0	0	0	0	0
Intercotylar prominence						
1 → 0	0	0	0	0	0	0
Metatarsal foramen						
ni	—	—	—	—	—	—

[a] Not informative.

APPENDIX 3. Parallel, reverse, and total homoplastic steps for each of Payne and Risley's (1976) skeletal characters in the DNA-DNA hybridization tree (Fig. 5).

Character	Tiger-herons, Boat-billed Heron			Bitterns			Day-herons, night-herons		
	Parallel	Reverse	Total	Parallel	Reverse	Total	Parallel	Reverse	Total
Total cranial	9	1	10	5	1	6	16	10	26
Bill shape	1	0	1	0	0	0	0	0	0
Palatine shape	1	0	1	0	0	0	0	0	0
Palatine emargination	1	0	1	1	0	1	1	4	5
Palatine lateral process	0	0	0	0	0	0	2	0	2
Interorbital foramen	1	0	1	1	0	1	5	0	5
Supraorbital foramen	2	0	2	0	0	0	0	3	3
Lacrimal size	1	0	1	0	0	0	0	0	0
Lacrimal ventral projection	0	0	0	1	0	1	1	0	1
Lacrimal lateral groove	0	0	0	0	0	0	2	1	3
Ectethmoid	0	0	0	0	0	0	0	0	0
Ectethmoid tubercle	1	0	1	1	0	1	4	0	4
Ectethmoid ridge	1	0	1	1	1	2	1	0	1
Basitemporal ridge	0	1	1	0	0	0	0	2	2
Total postcranial	9	3	12	8	3	11	21	12	33
Axis shape	0	1	1	1	1	2	1	3	4
Lateral canal	1	0	1	1	0	1	3	0	3
Rib facets	0	1	1	0	1	1	0	2	2
Notches	1	0	1	0	0	0	0	0	0
Manubrial length	1	0	1	1	1	2	3	1	4
Sternocoracoidal process	0	0	0	0	0	0	2	1	3
Sternal facet	0	0	0	0	0	0	0	0	0
External spine	1	0	1	1	0	1	4	1	5
Internal spine	0	0	0	1	0	1	3	2	5
Deltoid crest shape	0	0	0	1	0	1	0	0	0
Deltoid crest height	0	0	0	1	0	1	1	0	1
Pneumatic fossa	2	0	2	1	0	1	0	0	0
Ligamental furrow	1	0	1	0	0	0	0	0	0
Iliac crest shape	0	1	1	0	0	0	2	1	3
Posterior iliac crest	—[a]	—	—	—	—	—	—	—	—
Paraphyses fusion	1	0	1	0	0	0	0	0	0
Iliac recess	1	0	1	0	0	0	2	1	3
Ischiopubic symphysis	0	0	0	0	0	0	0	0	0
Intercotylar prominence	0	0	0	0	0	0	0	0	0
Metatarsal foramen	—[a]	—	—	—	—	—	—	—	—

[a] Not informative.