J. Paleont., 78(1), 2004, pp. 45–50

0022-3360/04/0078-45$03.00

THE EFFECT OF EXTERNAL AND INTERNAL FOSSIL CALIBRATIONS ON THE AVIAN EVOLUTIONARY TIMESCALE

MARCEL VAN TUINEN[1] AND S. BLAIR HEDGES[2]

[1]Department of Biological Sciences, Stanford University, California 94305 and [2]NASA Astrobiology Institute and Department of Biology, 208 Mueller Laboratory, The Pennsylvania State University, University Park 16802-5301

ABSTRACT—Molecular clocks can provide insights into the evolutionary timescale of groups with unusually biased or fragmentary fossil records, such as birds. In those cases, it is advantageous to establish internal anchor points—molecular time estimates—using the best external fossil calibrations. In turn, those anchor points can be used as calibrations for more detailed time estimation within the group under study. This method also avoids the inherent problems in drawing conclusions about the evolution of a group based on data tied to the poor fossil record of that same group. The galliform-anseriform divergence (~90 million years ago) is an example of such an ideal anchor point for molecular clock analyses in birds.

THE TIMING of the origin and diversification of modern birds (Neornithes) has been based on the fossil record (Feduccia, 1980, 1995, 1999; Olson, 1985; Dyke, 2001), biogeography (Cracraft, 1973, 2001) and molecular clocks (Wilson, 1986; Sibley and Ahlquist, 1990; Hedges et al., 1996; Cooper and Penny, 1997; Waddell et al., 1999; van Tuinen and Hedges, 2001). Some studies have suggested that modern avian orders arose in the early Tertiary, possibly from a shorebird ancestor (Wyles et al., 1983; Feduccia, 1995), based on the fossil record (Benton, 1993; Feduccia, 1999). Under this hypothesis, even superordinal divergences occurred in the Tertiary. However, others have suggested a Cretaceous origin for crown ratites, Anseriformes, Pelecaniformes, and Gruiformes (Dyke, 2001; Hope, 2002). Yet another interpretation, based on continental breakup, suggests a Cretaceous origin of those basal bird lineages but includes the derived crown and stem Passeriformes and stem Caprimulgiformes (Cracraft, 2001).

Prior to these investigations, the modern bird timescale has been mostly in a state of flux (Padian and Chiappe, 1998). A major rearrangement occurred when several Cretaceous fossils, originally assigned to modern groups, were placed in a more basal position after discovery and description of the opposite birds (Enantiornithes) and the separation of the toothed ichthyornithine and hesperornithine birds from modern birds (Chiappe, 1995, 2001). Additional fossil evidence (Chiappe, 1996; Stidham, 1998a, 1998b; Hope, 2002) points to a Cretaceous origin of some modern bird orders, but the fragmentary nature of several Cretaceous fossils makes it difficult to draw conclusions (Kurochkin, 1995; Stidham, 1998a; Dyke, 2001; Norell and Clarke, 2001; Hope, 2002).

Molecular clock studies have indicated that superordinal and at least some ordinal lineages arose in the Cretaceous (Prager and Wilson, 1974; Prager et al., 1976; Wyles et al., 1983; Wilson, 1986; Sibley and Ahlquist, 1990; Hedges et al., 1996; Cooper and Penny, 1997; Waddell et al., 1999; van Tuinen and Hedges, 2001) (Table 1). These studies have differed in details, but that is not surprising because they involved indirect and direct measures of genetic divergence, different calibrations, and different numbers of genes. Here, we discuss the usefulness and limitations of molecular clocks in studying avian evolution, with emphasis on the use of an accurate calibration point.

CALIBRATING THE AVIAN MOLECULAR CLOCK

The accuracy of molecular time estimates depends on the establishment of correct phylogenetic relationships and the selection of a reliable fossil anchor point for clock calibration. Considering the condition of the current avian fossil record, most internal calibrations used in molecular studies are problematic, and in some cases they are substantial underestimates. However, precise calibrations are required for dating particular events such as the K-T impact on modern bird origins and radiations. In these cases, calibration-related phylogenetic errors should be precluded or minimized. Secondly, the age of chosen fossils should closely approximate the age of the calibration node (the node on which the evolutionary rate is based). With a complete fossil record, nodal age can be constrained by the age of the oldest fossils on a lineage leading from that node (minimum age) and the age of the oldest fossils leading to that node (maximum age). In modern birds, this information is seldom available because of several factors affecting probability of fossilization, such as lack of teeth, fragile skeletons, hollow bones, and ecological habits.

Despite this dilemma on how to calibrate avian molecular clocks from within birds, several internal calibrations (frequently ratite, galliform, or anseriform divergences) have been applied to molecular investigations (Table 2). An advantage of calibrating avian molecular clocks internally is the reduction of statistical errors due to large extrapolation of time. However, significant underestimates of divergence time are perhaps more likely for the bird fossil record than for most other vertebrate groups. In addition, if the question being investigated involves the timing of modern bird evolution, calibration within modern birds will influence the results. External calibrations can provide a reasonable alternative because they do not rely on the fossil record of the group in question, although external calibrations also carry intrinsic errors (larger extrapolation may lead to greater statistical error).

The majority of the fossils used for calibration of avian molecular clocks have uncertain phylogenetic relationships. Incorrect phylogenetic placement may lead to older divergence times, especially when used in junction with quartet analyses (Cooper and Penny, 1997). Furthermore, the assumption of well-constrained fossil calibrations is not always clearly met. For example, a study on parrot biogeography (Miyaki et al., 1998) chose a calibration age based on a midpoint between molecular estimates from Hedges et al. (1996) and Cooper and Penny (1997). While it represents a compromise, using an average of multiple ages may increase calibration error and compounding of other errors. Another example involves ratites, where Haddrath and Baker (2001) used a calibration age of 35 Myr for the emu-cassowary divergence to time several other ratite divergences. This age is based on *Emuarius gidju* (Boles, 1992) at 25 Myr, a fossil clearly on the emu line. To account for uncertainty in calibration point, the authors added 10 million years to the minimum age to approximate the divergence of emus and cassowaries. Similarly, Waddell et al. (1999) used the gamefowl-waterfowl divergence (or stem anseriform) at 68 Myr to set their mitochondrial protein clock. They reasoned that the currently oldest waterfowl fossils were 55 Myr

TABLE 1—Avian superordinal divergence times estimated from available molecular data.

Divergence between	Divergence time and SE (Myr)	Calibration point (Myr)	References
Multiple orders	97 ± 12	Bird-Mammal (310)	Hedges et al., 1996
Multiple orders	>100	Quartet analyses (40–70)	Cooper and Penny, 1997
Struthioniformes-Galliformes	73–98	Bird-Mammal (300); Artiodactyl-Cetacean (60)	Härlid et al., 1997
Struthioniformes-Galliformes	92	Bird-Crocodile (254)	Härlid et al., 1998
Galliformes-Anseriformes	112 ± 11.7	Bird-Mammal (310)	Kumar and Hedges, 1998
Passeriform-ratite/Galliformes	110–114	Bird-Mammal (310); Bird-Crocodile (254)	Härlid and Arnason, 1999
Palaeognathae-Galloanserae, Passeriformes-five bird orders	78.3 ± 5.2 116 ± 14.0	Galliform-Anseriform (68)	Waddell et al., 1999
Multiple orders (see text for explanation)	118–90 ± 17.2–13.4	Galliform-Anseriform (90)	van Tuinen and Hedges, 2001

old, applied this calibration and estimated the time of stem Anseriformes to be around 78 Myr. Their subsequent 68 Myr estimate is based on an average (plus three Myr) of the minimum crown age (55 Myr) and this molecular stem estimate (78 Myr). Yet, no clear evidence exists from either genetics or fossils that 68 Myr closely approximates the age of stem Anseriformes. To the contrary, recent fossil evidence exists that pushes even the crown Anseriformes into the late Cretaceous (Livezey, 1997; Stidham, 1998b; Cracraft, 2001; Hope, 2002) while nuclear estimates of stem Anseriformes points to a 90 Myr age (van Tuinen and Hedges, 2001; van Tuinen and Dyke, 2003).

CALIBRATION WITH CRETACEOUS LOONS

An example involving fossil ''loons'' demonstrates that different phylogenetic interpretations can have major effects on time estimation. *Neogaeornis* and *Polarornis* are two supposed modern loons that have been described from the late Cretaceous (~70 Myr) of South America and Antarctica (Lambrecht, 1929; Chatterjee, 1989). *Neogaeornis* is based on tarsometatarsus material that shows the presence of a hypotarsus as seen in modern foot-propelled diving birds (loons and grebes). Originally considered to be part of a larger hesperornithid-loon-grebe grouping, hesperornithids were later removed from this group. In 1992, Olson redescribed *Neogaeornis* as a modern foot-propelled diving bird because it lacked the defining characters of hesperornithids. Although he thought it conceivable that some other group could have given rise to *Neogaeornis* during the Mesozoic, he did not consider it the most parsimonious hypothesis. A close affinity with modern loons was based on the shape of the trochlea (Olson, 1992; Hope, 2002) and the placement of the distal foramina similar to that seen in Miocene loons (*Colymboides*). *Polarornis* is based on more extensive material (including cranial) apparently showing several defining characteristics of loons and a neognathous palate (Olson, 1992). However, the material has not been formally described (Chatterjee, 1989, 1997). Despite limited descriptions and the lack of thorough phylogenetic analyses on both fossil ''loons,'' these taxa are almost universally interpreted as modern loons (e.g., Chiappe, 1996; Dingus and Rowe, 1998; Padian and Chiappe, 1998; Hope, 2002) and have even served as molecular clock calibrations (Cooper and Penny, 1997).

However, three interpretations can be ascribed to the phylogenetic position of these fossils, assuming a sister group relationship

TABLE 2—Summary of avian molecular clock studies and type of calibrations used.

Reference:	Calibration used: (Myr)	Study describing
	Internal Calibration	
Helm-Bychowski and Wilson, 1986	Several galliform dates (3–40)	Divergence among: Fowl
Shields and Wilson, 1987	*Anser-Branta* (4–5)	Mitochondrial rate of birds (2%/Myr)
Sibley and Ahlquist, 1990	Rhea-Ostrich (80) OW-NW Cuckoos (80)	Modern birds
Krajewski and King, 1996	Crane-Crowned Crane (10–20)	Cranes
Cooper and Penny, 1997	Loon (70); Tropicbird (60); Rhea (60); Ostrich (43); Penguin (58); Charadriiformes (60); Procellariiformes (60); Cracidae (50); Numididae (40)	Modern birds
Miyaki et al., 1998	Gamefowl-parrots (100)	Parrots
Waddell et al., 1999	Stem Anseriformes (68)	Modern birds
Garcia-Moreno and Mindell, 2000	Phasianidae-Numididae (40); Galliformes-Anseriformes (68)	Modern birds
Cooper et al., 2001	Moa-other ratites (82–85)	Ratites
Haddrath and Baker, 2001	Emu-Cassowary (35)	Ratites
	External Calibration	
Prager et al., 1974	Birds-crocodiles (213); *Alligator-Caiman* (50); *Alligator*-Crocodile (70); Origin placental mammals (~75); Average modern bird (100); Megapodes-other Galliformes (70); Chicken-other phasianoids (40)	Modern birds
Hedges et al., 1996	Bird-mammal (310)	Modern birds
Harlid, Janke and Arnason, 1997	Bird-mammal (300); Artiodactyla-Cetacea (60)	Paleognath-Neognath
Kumar and Hedges, 1998	Bird-Mammal (310)	Vertebrates
Harlid et al., 1998	Bird-crocodile (254)	Paleognath-Neognath
Harlid and Arnason, 1999	Bird-Mammal (310)	Paleognath-Neognath
Groth and Barrowclough, 1999	Birds-crocodiles (250); *Gavialis-Alligator* (~80); Oscine-Suboscine (50)	Modern birds
van Tuinen and Hedges, 2001	Bird-Mammal (310) Primate-Rodent (110) Galliformes-Anseriformes (90)	Modern birds

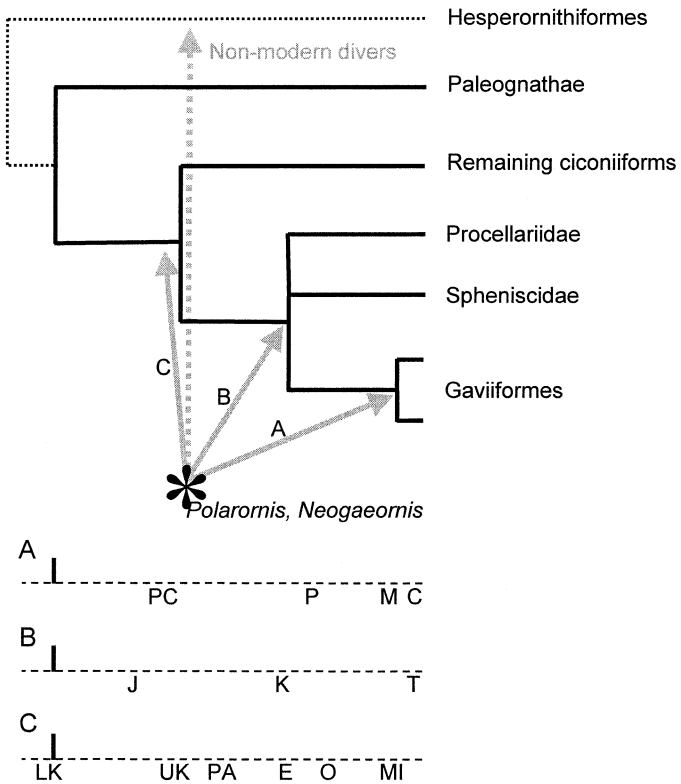

FIGURE *1*—Effect of three alternative phylogenetic interpretations concerning the Cretaceous ''loon'' fossils *Polarornis* and *Neogaeornis* on the modern bird timescale when their fossil age (~70 MYR) is used for calibration of avian molecular clocks. A fourth interpretation is shown by the dashed arrow, but would not allow internal calibration. The phylogenetic relationship of loons (Gaviiformes) within modern birds is shown, and Hesperornithiformes was used as outgroup. Branch lengths among modern birds were derived from Sibley and Ahlquist (1990) and van Tuinen et al. (2001). The three legends below the figure show: A. the timescale based on calibrating crown Gaviiformes, B. the timescale based on calibrating stem Gaviiformes, and C. the timescale based on calibrating crown Ciconiiformes (sensu Sibley and Ahlquist, 1990). Abbreviations refer to the following time periods: PC = Precambrian, P = Paleozoic, M = Mesozoic, C = Cenozoic, J = Jurassic, K = Cretaceous, T = Tertiary, LK = Lower Cretaceous, UK = Upper Cretaceous, PA = Paleocene, E = Eocene, O = Oligocene, MI = Miocene. The thick vertical line on each timescale denotes crown origin of Neornithes. See text for discussion.

between *Polarornis* and *Neogaeornis* (Fig. 1). First, a minimum age of 70 Myr can be applied to the stem of modern loons. This interpretation was used to calibrate portions of a nuclear and a mitochondrial gene resulting in deep Cretaceous estimates for the origins of several modern bird orders (Cooper and Penny, 1997). Even deeper divergence times (up to 200 Myr) would be obtained if such a calibration were applied to DNA hybridization or immunological data. If, instead, the 70 Myr ''loons'' represent the crown loon family Gaviidae (sensu Chatterjee, 1997), reanalyses of those molecular data would indicate a Precambrian origin for modern orders of birds. Alternatively, some diagnostic characters of Gaviiformes may have evolved repeatedly in other neognathous birds. This is the most likely explanation for two reasons: 1) convergent evolution is a frequent phenomenon within the crown of modern aquatic groups (van Tuinen et al., 2001) and 2) more consistent time estimates (e.g., 80 Myr for the Galliformes-Anseriformes divergence) are obtained when assuming that these ''loons'' were neognathous foot-propelled diving birds near the base of the stem of Ciconiiformes (the modern clade that includes nearly all aquatic radiations within modern birds). If true, this hypothesis would again imply that crown Gaviiformes is restricted to the Northern Hemisphere. Thus, *Polarornis* and *Neogaeornis* could be viewed as Southern Hemisphere analogs of modern day loons.

GENERATING A MOLECULAR-BASED INTERNAL CALIBRATION

We recently generated a molecular anchor point and applied it as an internal calibration in other more taxon-rich molecular data sets of birds (van Tuinen and Hedges, 2001). We analyzed all available protein sequences shared by Galliformes and Anseriformes and applied an external calibration (bird-mammal: 310 Myr; Kumar and Hedges, 1998). An average divergence time estimate of 90 ± 7.0 Myr was obtained from 21 proteins and used to time additional neornithine divergences in the three most taxon (ordinal) rich data sets: i) a mitochondrial DNA data set containing the complete sequences of the 12S rRNA, tRNA-Valine, and 16S rRNA genes (approximately 3-kilobase region) in 54 taxa representing all avian orders (van Tuinen et al., 2000), ii) a DNA-DNA hybridization distance data set with approximately 1,700 taxa (Sibley and Ahlquist, 1990), also representing all avian orders and most extant families, and iii) a transferrin immunological distance data set with 21 avian taxa representing 13 avian orders (Prager et al., 1974, 1976; Ho et al., 1976). A close relationship between galliforms and anseriforms is widely supported and represents a deep divergence within birds (Cracraft, 1988; Sibley and Ahlquist, 1990; Livezey, 1997; Groth and Barrowclough, 1999; van Tuinen et al., 1999, 2000; Cracraft and Clarke, 2001). This is fortunate because nuclear protein sequences of other orders of birds are poorly represented in the public sequence databases (Table 3 and Fig. 2).

Variation in the rate of amino acid substitution among proteins is well known and expected based on natural selection on protein function. Additionally, some variation in substitution rate among lineages is not unexpected given the relatively small number of variable sites in typical proteins. However, if rate heterogeneity among taxa is significant, it can pose a problem for time estimation. In that case, either the particular gene, protein, or lineage can be eliminated from analysis (Takezaki et al., 1995; van Tuinen and Hedges, 2001) or local clock methods can be used. Examples of various local clock methods include using a lineage-specific rate (Schubart et al., 1998), applying different rates depending on the taxa and calibration (Cooper and Penny, 1997; Cooper et al., 2001), and distorting or smoothing the heterogeneous rate (Haddrath and Baker, 2001). More study is needed to evaluate the advantages and disadvantages of the various methods. In our case, we chose simply to eliminate the rate-variable proteins from the analysis, although it has been shown that when large numbers of proteins are examined, time estimates from rate-constant and rate-variable proteins are nearly identical (Kumar and Hedges, 1998; van Tuinen and Hedges, 2001).

DISCUSSION

Because genetic differentiation begins when two lineages split, molecular time estimates always should be earlier than the earliest fossils of two lineages considered. In groups with relatively poor fossil records, such as birds, an even greater difference between molecular and fossil time estimates should be expected. Bromham et al. (2000) suggested that overestimation of divergence time may occur because of the insensitivity of relative rate tests to detect departures from rate constancy. However, departures from rate constancy also can result in underestimates, so assuming that such biases occur, and that they will always be directional, is unfounded. In fact, an earlier study involving 658 nuclear proteins tested this suggestion by increasing the stringency of the relative

TABLE 3—Avian protein sequence entries in Genbank by order based on searches performed in 1999 (left) and October 2001 (right). Note the bias toward Galliformes and Anseriformes and the preponderance of mitochondrial sequences, especially of Cytochrome *b* (nuc = nuclear, mt = mitochondrial). Ordinal classification according to Genbank taxonomy.

	Total entries (nuc+mt)	Total cytochrome *b*	Frequency Cytochrome *b*	Total Nuclear genes
Paleognathae				
Struthioniformes	61	6	0.10	9/34
Casuariformes	24	3	0.13	6/12
Tinamiformes	20	2	0.10	3/5
Apterygiformes	34	24	0.71	2/3
Rheiformes	17	5	0.29	1/6
Neognathae				
Galliformes	10,447	104	0.01	6,444/7,689[1]
Anseriformes	460	156	0.34	52/71
Columbiformes	119	0	0.00	23/37
Passeriformes	2,415	1,054	0.44	20/45
Psittaciformes	113	45	0.40	7/12
Strigiformes	117	16	0.14	5/6
Gruiformes	197	71	0.36	5/9
Charadriiformes	301	194	0.64	4/17
Sphenisciformes	21	5	0.24	3/12
Opisthocomiformes	24	4	0.17	3/3
Cuculiformes	122	52	0.43	3/5
Turniciformes	9	1	0.11	2/2
Gaviiformes	14	3	0.21	2/3
Coraciiformes	22	11	0.50	2/4
Piciformes	53	29	0.55	2/3
Falconiformes	126	62	0.49	2/7
Procellariiformes	225	154	0.68	2/4
Phoenicopteriformes	5	2	0.40	1/4
Coliiformes	14	4	0.29	1/2
Musophagiformes	30	6	0.20	1/2
Caprimulgiformes	33	21	0.64	1/4
Apodiformes	68	60	0.88	1/5
Pelecaniformes	75	29	0.39	1/7
Ciconiiformes	84	49	0.58	1/10
Trochiliformes	109	56	0.51	1/4
Upupiformes	2	1	0.50	0/2
Bucerotiformes	4	2	0.50	0/2
Ardeiformes	5	3	0.60	0/5
Podicipediformes	14	2	0.14	0/2
Trogoniformes	42	20	0.48	0/2
Craciformes	33	14	0.42	0/3

[1] Total number of Galliformes protein sequences, not gene amount, are shown.

rate tests and did not find a directional bias (Kumar and Hedges, 1998). This suggests that the rate differences, at least in those diverse comparisons, were not directionally biased.

An additional source of error could arise from the use of an external fossil calibration time that is an overestimate. In our case, we used the bird-mammal split at 310 Myr to yield a galliform-anseriform divergence of 90 Myr. In order to obtain an early Tertiary or latest Cretaceous origin of Anseriformes and Galliformes, the bird-mammal divergence would have to have taken place between 220 and 230 Myr, an hypothesis clearly disproved by the rich Permian synapsid and diapsid fossil records (Benton, 1993). These records show that the earliest synapsid and diapsid representatives are similar in morphology and known from comparable stratigraphic ages, indicative of a robust age of the bird-mammal divergence. Furthermore, the pre-amniote paleontological record indicates that this divergence is likely not a significant underestimate (Hedges, 2003). Although fossil-based calibrations are never completely without error we have assumed that the size of error is negligible to the age of the bird-mammal divergence. If the error in a calibration time is known, it could be incorporated in a propagated error when computing each single-gene time estimate (van Tuinen et al., 2001). An investigation is currently in progress on estimating a confidence limit around the bird-mammal divergence based on geological and phylogenetic errors (van Tuinen et al., 2001) so it may be accounted for in future molecular clock studies if significant.

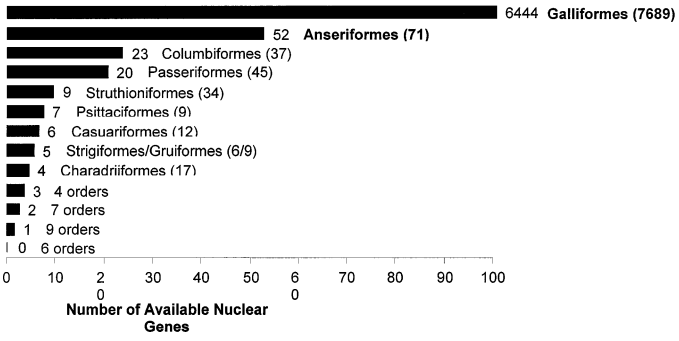

FIGURE 2—Biased taxonomic representation in molecular databases for birds. The abundance of anseriform and galliform nuclear data was the basis for establishing a Galliformes-Anseriformes anchorpoint (van Tuinen et al., 2001). A recent increase in number of columbiform, passeriform and struthioniform sequence data suggests that additional molecular anchorpoints will be available in the future.

Although it would be possible to use other external calibrations closer to the origin of birds (within Testudines or Crocodylia, both of which have well-sampled fossil records) instead of extrapolating to the divergence with mammals, the taxa required are not

yet strongly represented in the molecular databases. For this reason we also utilized the primate-rodent divergence (~110 MYR) as a secondary calibration. Although this is a molecular calibration dependent on the bird-mammal calibration (Kumar and Hedges, 1998; Smith and Peterson, 2002) it was chosen to maximize gene acquisition and increase the precision of the time estimates.

Another suggestion that has been made to explain the older time estimates is that molecular rates underwent an initial acceleration in the Paleocene (''early fast rate'') in all modern groups radiating at that time after which rates slowed down in unison (Benton, 1999). If true, relative rate tests cannot identify these patterns causing overestimated divergence times. However, divergence time estimates before or after the Cretaceous do not seem to be affected (Kumar and Hedges, 1998; Easteal, 1999). Interestingly, the disparity between fossil and molecular divergence times can be constrained mostly to the Late Cretaceous. This period likely involves the existence of stem groups leading to modern birds (and mammals). It is perhaps the difficulty in diagnosing those stems that has created these gaps. In the case of mammals, the early origin (Cretaceous) of ordinal stem lineages now is supported by fossils of 85–90 Myr old placental mammals (Archibald, 1996; Archibald et al., 2001).

CONCLUSION

An accurate timescale for modern birds will require more fossil discoveries and considerably more sequence data from diverse lineages other than galliforms and anseriforms. At present, the relationships of the majority of living avian orders are not well established and the phylogenetic position of many early fossils remains unclear. These factors pose great problems for using internal calibrations within birds in molecular clock studies, beyond the issue of circularity. Considerably more robust non-avian fossil calibration points exist and can be used to estimate one or a few key divergences within birds for use as better internal calibrations. We suggest that this two-step calibration is likely to yield a more accurate timescale for birds than direct calibration with the current avian fossil record.

ACKNOWLEDGMENTS

We thank C. Brochu, J. Theodor and C. Sumrall for organizing this molecular clock workshop at a paleontology venue and for inviting us to participate. L. Hadly, C. Brochu, G. Dyke, and two anononymous reviewers provided valuable comments on the manuscript. SBH was supported by the NASA Astrobiology Institute and by NSF.

REFERENCES

ARCHIBALD, J. D. 1996. Fossil evidence for a Late Cretaceous origin of ''hoofed'' mammals. Science, 272:1150–1153.

ARCHIBALD, J. D., A. O. AVERIANOV, AND E. G. EKDALE. 2001. Late Cretaceous relatives of rabbits, rodents and other extant eutherian mammals. Nature, 414:62–65.

BENTON, M. J. 1993. The Fossil Record 2. Chapman and Hall, London.

BENTON, M. J. 1999. Early origins of modern birds and mammals: molecules vs. morphology. Bioessays, 21:1043–1051.

BOLES, W. E. 1992. Revision of *Dromaius Gidju* Patterson and Rich 1987 from Riversleigh, northwestern Queensland, Australia, with a reassessment of its generic position. Natural History Museum of LA County Science Series, 36:195–208.

BROMHAM, L. D., A. RAMBAUT, M. D. HENDY, AND D. PENNY. 2000. The power of relative rate tests depends on the data. Journal of Molecular Evolution, 50:296–301.

CHATTERJEE, S. 1989. The oldest antarctic bird. Journal of Vertebrate Paleontology, 16a.

CHATTERJEE, S. 1997. The Rise of Birds. The Johns Hopkins University Press, Washington, D.C., 312 pp.

CHIAPPE, L. M. 1995. The first 85 Million years of avian evolution. Naturem, 378:349–355.

CHIAPPE, L. M. 1996. Early avian evolution in the Southern Hemisphere: the fossil record of birds in the Mesozoic of Gondwana. Memoirs of the Queensland Museum, 39:533–556.

CHIAPPE, L. M. 2001. Phylogenetic relationships among basal birds, p. 125–142. *In* J. Gauthier, and L. F. Gall (eds.), New Perspectives on the Origin and Early Evolution of Birds. Proceedings of the international symposium in honor of John H. Ostrom, Yale University, 1999, Special Publication Peabody Museum of Natural History, New Haven.

COOPER, A., AND D. PENNY. 1997. Mass survival of birds across the K-T boundary: molecular evidence. Science, 275:1109–1113.

COOPER, A., C. LALUEZA-FOX, S. ANDERSON, A. RAMBAUT, J. AUSTIN, AND R. WARD. 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. Nature, 409:704–707.

CRACRAFT, J. 1973. Continental drift, paleoclimatology, and the evolution and biogeography of birds. Journal of Zoology London, 169:455–545.

CRACRAFT, J. 1988. The major clades of birds, p. 339–361. *In* M. J. Benton (ed.), The Phylogeny and Classification of the Tetrapods. Clarendon Press, Oxford.

CRACRAFT, J. 2001. Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. Proceedings of the Royal Society London B. Biological Sciences, 268:459–69.

CRACRAFT, J., AND J. A. CLARKE. 2001. The basal clades of modern birds, p. 143–156. *In* J. Gauthier, and L. F. Gall (eds.), New Perspectives on the Origin and Early Evolution of Birds. Proceedings of the international symposium in honor of John H. Ostrom, Yale University, 1999, Special Publication Peabody Museum of Natural History, New Haven.

DINGUS, L., AND T. ROWE. 1998. The Mistaken Extinction. W. H. Freeman, New York, 332 p.

DYKE, G. J. 2001. The evolutionary radiations of modern birds: systematics and patterns of diversification. Geological Journal, 36:305–315.

EASTEAL, S. 1999. Molecular evidence for the early divergence of placental mammals. Bioessays, 21:1052–1058.

FEDUCCIA, A. 1980. The Age of Birds. Harvard University Press, Cambridge.

FEDUCCIA, A. 1995. Explosive evolution in tertiary birds and mammals. Science, 267:637–638.

FEDUCCIA, A. 1999. The Origin and Evolution of Birds (second edition). Yale University Press, New Haven.

GARCIA-MORENO, J., AND D. P. MINDELL. 2000. Rooting a phylogeny with homologous genes on opposite sex chromosomes (gametologs): a case study using avian CHD. Molecular Biology and Evolution, 17: 1826–32.

GROTH, J. G., AND G. F. BARROWCLOUGH. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Molecular Phylogenetics and Evolution, 12:115–123.

HADDRATH, O., AND A. J. BAKER. 2001. Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis. Proceedings of the Royal Society London B. Biological Sciences, 268:939–45.

HÄRLID, A., AND U. ARNASON. 1999. Analyses of mitochondrial DNA nest ratite birds within the Neognathae: supporting a neotenous origin of ratite morphological characters. Proceedings of the Royal Society London B. Biological Sciences, 266:305–309.

HÄRLID, A., A. JANKE, AND U. ARNASON. 1997. The mtDNA sequence of the ostrich and the divergence between paleognathous and neognathous birds. Molecular Biology and Evolution, 14:754–76.

HÄRLID, A., A. JANKE, AND U. ARNASON. 1998. The complete mitochondrial genome of *Rhea americana* and early avian divergences. Journal of Molecular Evolution, 46:669–679.

HEDGES, S. B. 2003. Molecular clocks and a biological trigger for the Neoproterozoic snowball earths and Cambrian explosion, *In* P. Donoghue, and P. Smith, (eds.), Telling Evolutionary Time: Molecular Clocks and the Fossil Record. Taylor & Francis, London.

HEDGES, S. B., P. H. PARKER, C. G. SIBLEY, AND S. KUMAR. 1996. Continental breakup and the ordinal diversification of birds and mammals. Nature, 381:226–229.

HELM-BYCHOWSKI, K. M., AND A. C. WILSON. 1986. Rates of nuclear-DNA evolution in pheasant-like birds: evidence from restriction maps, Proceedings of the National Academy of Sciences USA, 83:688–692.

HO, C. Y.-K., E. PRAGER, A. C. WILSON, D. T. OSUGA, AND R. E. FEENEY. 1976. Penguin evolution: comparisons demonstrate phylogenetic relationship to flying aquatic birds. Journal of Molecular Evolution, 8: 271–282.

HOPE, S. 2002. The Mesozoic record of Neornithes (modern birds). *In* L. M. Chiappe, and L. Witmer (eds.), Over the Heads of the Dinosaurs. University of California Press, Berkeley.

KRAJEWSKI, C., AND D. G. KING. 1996. Molecular divergence and phylogeny: rates and patterns of cytochrome b evolution in cranes. Molecular Biology and Evolution, 13:21–30.

KUMAR, S., AND S. B. HEDGES. 1998. A molecular timescale for vertebrate evolution. Nature, 392:917–920.

KUROCHKIN, E. N. 1995. Synopsis of Mesozoic birds and early evolution of class Aves. Archaeopteryx, 13:47–66.

LAMBRECHT, K. 1929. *Neogaeornis wetzeli* n. g. n. sp., der erste Kreidevogel der suedlichen Hemisphaere. Palaeontologische Zeitschrift, 11: 121–129.

LIVEZEY, B. C. 1997. A phylogenetic analysis of basal Anseriformes, the fossil *Presbyornis*, and the interordinal relationships of waterfowl. Zoological Journal of the Linnean Society, 121:361–428.

MIYAKI, C. Y., S. R. MATIOLI, T. BURKE, AND A. WAJNTAL. 1998. Parrot evolution and paleogeographical events: mitochondrial DNA evidence. Molecular Biology and Evolution, 15:544–551.

NORELL, M. A., AND J. A. CLARKE. 2001. Fossil that fills a critical gap in avian evolution. Nature, 409:181–184.

OLSON, S. 1985. The fossil record of birds, p. 80–238. *In* D. Farner, J. R. King, and K. C. Parkes (eds.), Avian Biology. Academic Press, London.

OLSON, S. 1992. *Neogaeornis wetzeli* Lambrecht, a Cretaceous loon from Chile (Aves: Gaviidae). Journal of Vertebrate Paleontology, 12:122–124.

PADIAN, K., AND L. M. CHIAPPE. 1998. The origin and early evolution of birds. Biological Reviews, 73:1–42.

PRAGER, E. M., AND A. C. WILSON. 1976. Congruency of phylogenies derived from different proteins: a molecular analysis of the phylogenetic position of cracid birds. Journal of Molecular Evolution, 9:45–57.

PRAGER, E. M., AND A. C. WILSON. 1980. Phylogenetic relationships and rates of evolution in birds. Proceedings of the 17th International Ornithological Congress, 1209–1214.

PRAGER, E. M., A. C. WILSON, D. T. OSUGA, AND R. E. FEENEY. 1976. Evolution of flightless land birds on southern continents: transferrin comparison shows monophyletic origin of ratites. Journal of Molecular Evolution, 8:283–294.

PRAGER, E. M., A. H. BRUSH, R. A. NOLAN, M. NAKANISHI, AND A. C. WILSON. 1974. Slow evolution of transferrin and albumin in birds according to micro-complement fixation analysis. Journal of Molecular Evolution, 3:243–262.

SCHUBART, C. D., R. DIESEL, AND S. B. HEDGES. 1998. Rapid evolution to terrestrial life in Jamaican crabs. Nature, 393:363–365.

SHIELDS, G. F., AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. Journal of Molecular Evolution, 24:212–217.

SIBLEY, C. G., AND J. AHLQUIST. 1990. Phylogeny and Classification of Birds. Yale University Press, New Haven, Connecticut.

SMITH, A. B., AND K. J. PETERSON. 2002. Dating the time of origin of major clades: molecular clocks and the fossil record. Annual Review of Earth and Planetary Sciences, 30:65–88.

STIDHAM T. A. 1998a. A lower jaw from a Cretaceous parrot. Nature, 39:29–30.

STIDHAM T. A. 1998b. Phylogenetic and ecological diversification of waterfowl (Anseriformes) in the Late Cretaceous and Paleogene. Journal of Vertebrate Paleontology, 80A.

TAKEZAKI, N., A. RZHETSKY, AND M. NEI. 1995. Phylogenetic test of the molecular clock and linearized trees. Molecular Biology and Evolution, 12:823–33.

VAN TUINEN, M., AND G. J. DYKE. 2003. Calibration of galliform molecular clocks using multiple fossils and genetic partitions. Molecular Phylogenetics and Evolution.

VAN TUINEN, M., AND S. B. HEDGES. 2001. Calibration of avian molecular clocks. Molecular Biology and Evolution, 18:206–213.

VAN TUINEN, M., C. G. SIBLEY, AND S. B. HEDGES. 1999. Molecular evidence for the early history of modern birds. Journal of Vertebrate Paleontology, 19:81A–82A.

VAN TUINEN, M., C. G. SIBLEY, AND S. B. HEDGES. 2000. The early history of modern birds inferred from DNA sequence of mitochondrial and nuclear ribosomal genes. Molecular Biology and Evolution, 17: 451–457.

VAN TUINEN, M., D. B. BUTVILL, J. A. W. KIRSCH, AND S. B. HEDGES. 2001. Convergence and divergence in the evolution of aquatic birds. Proceedings of the Royal Society London B. Biological Sciences, 268: 1345–50.

WADDELL, P. J., Y. CAO, M. HASEGAWA, AND D. P. MINDELL. 1999. Assessing the Cretaceous superordinal divergence times within birds and placental mammals by using whole mitochondrial protein sequences and an extended statistical framework. Systematic Biology, 48:119–137.

WILSON, A. C. 1986. Timescale for bird evolution. Proceedings of the Nineteenth International Ornithological Congress, 1912–1917.

WYLES, J. S., J. G. KUNKEL, AND A. C. WILSON. 1983. Birds, behavior, and anatomical evolution. Proceedings of the National Academy of Sciences USA, 80:4394–4397.

ACCEPTED 15 AUGUST 2003