

# PATTERNS OF CALIBRATION AGE SENSITIVITY WITH QUARTET DATING METHODS

CHRISTOPHER A. BROCHU

Department of Geoscience, University of Iowa, Iowa City 52242, <christopher-brochu@uiowa.edu>

**ABSTRACT**—Because more than one calibration is used, quartet dating (a molecular dating method) is thought to reduce error that might arise from a single calibration point. Within crocodylians, there is a strong correlation between calibration age and divergence estimate for five mitochondrial genes and one nuclear gene—estimates based on two Neogene calibrations are all younger than those based on two Paleogene calibrations, and estimates based on one Neogene and one Paleogene calibration are of intermediate age. Confidence limits on the youngest estimates exclude the oldest estimates, and in several cases they exclude known minimum divergences from fossil occurrences. Addition of time to the calibrations improves among-quartet and stratigraphic consistency, but not all kinds of modifications have the same impact; addition of uniform blocks of time to all calibrations efficiently increases among-quartet consistency, but with range extensions that more than double some of the Neogene calibrations. Modest increases in calibration age disproportionately impact divergence estimates based on later calibrations. Some among-quartet disparity might reflect calibration error, especially among caimans, but some range extensions necessary to improve consistency are unreasonably long. Quartet dating appears to systematically undercompensate branch length error with late calibrations and overcompensate it with early calibrations, but in all cases very reasonable results—alligatorid-crocodylid divergence in the Late Cretaceous and *Alligator*-caiman divergence at or near the Cretaceous-Tertiary boundary—are obtained when both a Neogene and a Paleogene calibration is used. This suggests that, given current likelihood models, the use of calibrations sampling different parts of a clade's history is the best strategy when using quartet dating.

## INTRODUCTION

**M**ETHODS FOR estimating divergence time between lineages using DNA sequence data rely on fixed calibration points to derive a molecular clock or to estimate evolutionary rates within a group of interest. These are usually in the form of fossil first appearance data for one or more branching points on the tree. But because of limitations with both the sequences and fossil calibrations, molecular dating methods can be sensitive to the fossil calibration chosen.

One important factor has long been recognized—multiple substitutions can occur at a single site over time, but we can only observe the last substitutions to have occurred. Consequently, measured molecular distances taken directly from sequences will underestimate the actual number of substitutions that have occurred since origination, and methods relying on uncorrected sequence distances will underestimate branch lengths and divergence times. The disparity between measured distance and actual number of substitutions is expected, and often observed, to increase with divergence age (Gingerich, 1986; Nei, 1987; Wayne et al., 1991; Arbogast et al., 2002).

These problems are usually addressed using model-based methods that can, in theory, account for our inability to observe multiple hits per site and correct underestimated branch lengths. Likelihood-based and Bayesian methods incorporating these models have rapidly grown more complex as various parameters are added, including rate heterogeneity among taxa, rate heterogeneity among sites, biases in sequence composition, and transition-transversion bias (Swofford et al., 1996; Felsenstein, 2003). These have in turn been applied to the question of time (e.g., Steel et al., 1996; Sanderson, 1997, 2002; Huelsenbeck et al., 2000; Thorne et al., 1998, 2002; Wray, 2001; Arbogast et al., 2002; Conroy and van Tuinen, 2003). Because contemporary molecular methods address branch length underestimation, anomalous divergence date estimates are often thought to reflect error with the calibrations themselves.

Fossils may be misidentified or misclassified, or paleontologists and neontologists may use the same taxon name in different ways (Marshall, 1990; Springer, 1995; Smith and Peterson, 2002). This kind of calibration error can translate into divergence time under- or overestimation, depending on the nature of the error. The root of this problem is twofold, neither of which is intrinsic to the

data: many groups of organisms have not been substantially revised in decades, and paleontologists and neontologists do not always communicate as they should (Lee, 1999; Brochu et al., this volume).

A second source of error springs from the fossil record. Calibration uncertainty aside (Marshall, 1990; Lee, 1999), fossils only provide minimum divergence times, and a gap of unknown length separates the first appearance datum from the true origination time. A fossil calibration significantly later than actual origination will cause likelihood-based methods to overestimate rates of evolution. This should translate into underestimated divergence times, in some cases to the degree that known fossils predate the divergence estimate supported by molecular data (Cooper and Penny, 1997; Bromham et al., 1998; Arnason et al., 1998; Lee, 1999; Huchon et al., 2000; Yoder and Yang, 2000; Conroy and van Tuinen, 2003).

But the more widely discussed conflicts between molecular and stratigraphic divergence estimates, such as the origins of meta-zoan phyla or mammalian and avian orders, involve what appear to some as extreme *overestimates* from molecules (e.g., Bromham et al., 1999; Waddell et al., 1999; Bromham and Hendy, 2000; Benton, 1998, 2001; Wray, 2001; Archibald and Deutschman, 2001; Dyke, 2001; Smith and Peterson, 2002; Springer et al., 2003). The problem might be methodological (Bromham et al., 1998; Yoder and Yang, 2000; Nei et al., 2001; Rodriguez-Trelles et al., 2002; Arbogast et al., 2002; Glazko and Nei, 2003), but it could also result if our fossil calibrations are too early. This can happen if we have mistaken a distantly related fossil for a member of the crown group under study (Marshall, 1990; Springer, 1995; Norman and Ashley, 2000)—the dating technique will attempt to stretch a given amount of evolutionary change over a longer period of time, deflating evolutionary rates.

Assuming we have accounted for rate heterogeneities or biases in the sequence data, are anomalous divergence time estimates necessarily the result of fossil calibration error? Do molecular dating methods account for sequence-based error as thoroughly as we would like? Some analyses based on different sources of data and methods recover congruent results, but an increasing number of studies is beginning to examine the potential weaknesses of the method as well as the calibrations (e.g., Norman and Ashley, 2000; Soltis et al., 2002; Adkins et al., 2003).

The present study is a preliminary empirical exploration of one

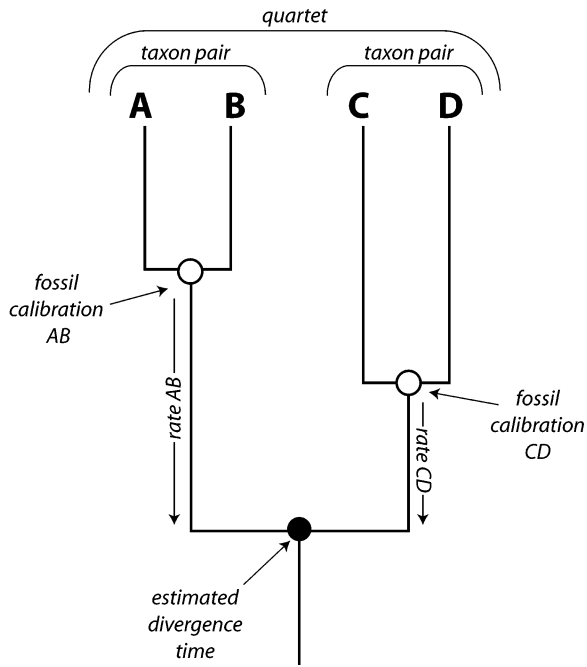


FIGURE 1—Diagram illustrating quartet dating procedure. The minimum divergences of taxon pairs A-B and C-D are used to estimate evolutionary rates for the branches descending from their nodes. These rates are then used to estimate the common divergence time for both taxon pairs.

molecular dating technique—quartet dating. Quartet-based methods estimate evolutionary rates for two pairs of related taxa, each calibrated with a separate fossil (Cooper and Penny, 1997; Rambaut and Bromham, 1998). These rates are used to estimate divergence time for the pairs (the quartet; Fig. 1). A single rate can be estimated for the whole quartet (one-rate model) or different rates can be estimated for each pair (two-rate model). Maximum likelihood-based applications of the quartet method (Rambaut and Bromham, 1998) allow the testing of these rate assumptions by comparing the one- or two-rate quartet tree against a quartet tree without such assumptions (i.e., each branch evolving at its own rate); significant difference between the constrained and unconstrained trees allows rejection of that particular rate scenario.

Quartet methods were designed to avoid the problem of assuming a single uniform rate throughout the group, but they are also thought to ameliorate problems associated with single fossil calibration points (Cooper and Penny, 1997; Rambaut and Bromham, 1998). They have been applied to a wide range of phylogenetic and molecular evolutionary problems (Bromham et al., 1998, 1999; Huchon et al., 2000, 2002; Delgado et al., 2001; Kawashita et al., 2001; Eizirik et al., 2001; Soltis et al., 2002; Corneli, 2003). They usually support estimates based on other molecular methods, including those that appear to conflict with the fossil record.

Thus far, most studies applying this method have been restricted by a relatively small number of fossil calibrations or by a narrow range of geological time from which calibrations may be drawn. But this limits our ability to test the degree to which quartet methods dampen error associated with individual calibrations. Some have asked about the impact of different calibration points on quartet methods (e.g., Huchon et al., 2000; Soltis et al., 2002), but the impact of different calibration age or changes to an individual calibration point are essentially unknown.

The ideal group for an empirical exploration of quartet methods

would have relatively low extant diversity to allow comprehensive sequence sampling, but also have a dense fossil record with possible calibrations covering a wide temporal range. It would also have a relatively noncontroversial phylogeny. Crocodylia is one such group: there are fewer than 25 living species, but crocodylians have robust skeletons and often live in their own depositional environments, resulting in a rich fossil record from their first appearance in the Campanian through the Cenozoic. There are some known biases in the crocodylian fossil record, but the preservation potentials of the animals themselves have arguably remained constant throughout the group's history. Recent phylogenetic analyses of both living and fossil crocodylians provide multiple nodes for large clades with fossil calibrations varying in age from 4 to 64 million years (m.y.) in age (Salisbury and Willis, 1996; Brochu, 2003).

Using two well-supported divergences within Crocodylia, this paper addresses three questions:

1. Are divergence time estimates from likelihood-based quartet dating consistent with the fossil record? Any molecular estimate older than its fossil first appearance datum is consistent with the fossil record, even if the molecular estimate is surprisingly old and implies long range extensions. We have minimum divergence ages based on fossils for some groups, placing an upper bound on any date estimate obtained from a molecular method. Assuming our fossil evidence is secure, molecular estimates significantly younger than fossils demonstrate can be rejected.
2. Are divergence time estimates for a particular node uniform for different calibration point ages? As with the first question, we are looking for consistency—in this case, internal consistency among quartets for the same evolutionary event. One should not expect the same *exact* divergence estimate from all calibration points, but assuming the likelihood models being used are adequately accounting for unobserved substitutions, and assuming biases in the fossil record are expressed more or less equally among all ingroup lineages, a range of dates within the confidence windows of all estimates should be recovered.
3. In the face of inconsistency in estimates, and assuming the problem is not methodological, how must the calibrations be manipulated to correct the problem? Groups like Crocodylia are ideal for a question like this, as we can compare the results of changes to calibrations covering a large proportion of the group's known history distributed through two large clades. We can thus see if changes to late and early calibration dates have the same influence on the results, and we can explore the impact of range extension and range contraction on calibrations.

#### METHODS

*Sequence data.*—Five mitochondrial genes were used (Table 1): cytochrome b (cytb; Gatesy et al., 2003); 12s and 16s rDNA (Gatesy et al., 1993, 2003); the mitochondrial control region (ctrl; Ray and Densmore, 2002, 2003); and a segment including a transfer RNA gene (tRNA<sup>glu</sup>) and adjoining portions of cytb and NADH-ubiquinone oxidoreductase chain 6 (nd6; White and Densmore, 2001; Ray et al., 2001). One set of nuclear sequences was also available—recombination activating gene 1 (rag1; Gatesy et al., 2003). Nuclear protein-coding genes may be more effective at estimating divergence time than mitochondrial genes (Glazko and Nei, 2003; Corneli, 2003), but the preponderance of mitochondrial information in this analysis reflects the information available. The phylogenetic hypotheses supported by these genes are largely congruent with each other and with those supported

TABLE 1—DNA data sets used in this study, showing crocodylian species sampled for each. \* for the ND6 data, sequence for *Paleosuchus* was based on both species.

	12s	cytb	ctrl	nd6	16s	rag1
length (bp)	267	240	456	313	430	1992
#taxa	13	12	16	20	12	12
#quartets possible	225	180	99	660	180	180
#quartets, 1-rate	93	23	17	129	62	86
#quartets, 2-rate	141	119	84	352	161	162
<i>Alligator mississippiensis</i> (Alligatorinae)	x	x	x	x	x	x
<i>Alligator sinensis</i> (Alligatorinae)	x	x	x	x	x	x
<i>Caiman crocodilus</i> (Caimaninae)				x		
<i>Caiman yacare</i> (Caimaninae)	x	x	x	x	x	x
<i>Caiman latirostris</i> (Caimaninae)	x	x		x	x	x
<i>Melanosuchus niger</i> (Caimaninae)	x	x		x	x	x
<i>Paleosuchus palpebrosus</i> (Caimaninae)	x	x		*	x	x
<i>Paleosuchus trigonatus</i> (Caimaninae)	x	x			x	x
<i>Tomistoma schlegelii</i> (Crocodylidae)	x	x	x	x	x	x
<i>Osteolaemus tetraspis</i> (Crocodylidae)	x	x	x	x	x	x
<i>Crocodylus cataphractus</i> (Crocodylidae)	x	x	x	x	x	x
<i>Crocodylus acutus</i> (Crocodylidae)			x	x		
<i>Crocodylus intermedius</i> (Crocodylidae)	x	x	x	x	x	x
<i>Crocodylus moreletii</i> (Crocodylidae)			x	x		
<i>Crocodylus rhombifer</i> (Crocodylidae)	x	x	x	x	x	x
<i>Crocodylus niloticus</i> (Crocodylidae)			x	x		
<i>Crocodylus palustris</i> (Crocodylidae)			x			
<i>Crocodylus siamensis</i> (Crocodylidae)			x	x		
<i>Crocodylus porosus</i> (Crocodylidae)			x	x		
<i>Crocodylus johnstoni</i> (Crocodylidae)			x	x		
<i>Crocodylus novaeguineae</i> (Crocodylidae)				x		
<i>Crocodylus mindorensis</i> (Crocodylidae)			x	x		

by morphology. Alignments follow those used provided by these authors.

**Quartet assembly.**—This analysis considers two divergence points. The first is between Alligatoridae (alligators and caimans) and Crocodylidae (“true” crocodiles and allies), which must have occurred by the early Campanian. We can sample a wide range of calibrations for both clades. The second is the divergence between Alligatorinae (today restricted to modern *Alligator*) and Caimaninae (caimans), which was at least an Early Paleocene event. The hypothesis of relationships used in this study is shown in Figure 2, with taxonomy following protocols in Brochu (2003).

The alligatorid-crocodylid quartets (Fig. 2) used two alligatorids (the two species of *Alligator*, two caimans, or an *Alligator* and a caiman) and two crocodylids (*Osteolaemus*, *Tomistoma*, and/or one or two species of *Crocodylus*). Alligatorine-caimanine quartets compared the two living species of *Alligator* with two caimans. These quartets share three desirable qualities: they are noncontroversial, with morphological and molecular data sets supporting essentially identical trees (Poe, 1996; Brochu and Densmore, 2001; Brochu, 2003; but see below); they are long-lived clades with fossil calibrations spread over a broad temporal range (Brochu, 1997); and in both cases we have robust first appearance data, with fossils fixing the alligatorid-crocodylid split at no earlier than the Campanian and the alligatorine-caimanine split at no earlier than the Early Paleocene. Our calibration sample for Alligatorinae is limited to one, and caimans have the most problematic fossil record of any crocodylian clade (and thus the most problematic fossil calibrations), but these can be modified by adding time to simulate range extensions from new discoveries.

The Indian gharial (*Gavialis gangeticus*) was excluded from this study. Based on morphology, *Gavialis* is the basalmost living crocodylian with a Late Cretaceous divergence for its lineage (Norell, 1989; Salisbury and Willis, 1996; Poe, 1996; Brochu, 1997; Hua and Jouve, in press). Molecular data instead support a close relationship and Cenozoic divergence between *Gavialis* and *Tomistoma* (Densmore, 1983; Densmore and Owen, 1989; Densmore and White, 1991; Hass et al., 1992; White and Densmore,

2001; Gatesy et al., 2003; Harshman et al., 2003). All available data put *Tomistoma* closer to *Osteolaemus* and *Crocodylus* than to the alligatorids, and the Early Eocene divergence between *Tomistoma* and other crocodylids is not dependent on fossil tomistomines (see below). Nearly all available data sets support the same set of relationships if *Gavialis* is disregarded.

Relationships within *Crocodylus* are currently poorly resolved, but a distant relationship between the African sharp-nosed crocodile (*Crocodylus cataphractus*) and all other living *Crocodylus* is supported by several data sets (Densmore, 1983; Brochu, 2000; White and Densmore, 2001). On this basis, species pairs consisting of *C. cataphractus* and another species of *Crocodylus* were applied where possible. Pairs of *Crocodylus* not including *C. cataphractus* were not considered.

Relationships among caimans are also poorly resolved, and fossil calibrations do not exist for every possible node. That the dwarf caimans (*Paleosuchus*) are basal to other caimans is robust, and pairs were used including either species of *Paleosuchus* with another caiman. A close relationship between the broad-snouted caiman (*Caiman latirostris*) and the black caiman (*Melanosuchus niger*) was accepted for purposes of this analysis because fossils close to these allow calibration of divergence points among derived caimans, but some data sets instead support *Caiman* monophyly excluding *Melanosuchus* (Poe, 1996; Brochu, 1999; Gatesy et al., 2003; Harshman et al., 2003). Pairs drawn from within this group were therefore limited; *C. latirostris* was paired with *Melanosuchus*, *C. latirostris* with either *C. yacare* or *C. crocodilus*, and *Melanosuchus* with *C. yacare* or *C. crocodilus*. The absence of fossils from the *Paleosuchus* lineage prevented us from using the two living species as a pair.

For both divergences, we can sample a wide range of calibration ages. Calibrations within Alligatoridae range from 4 m.y.a. to 64 m.y.a., and within Crocodylidae they range from 12 to 54 m.y.a. We thus have several Neogene and several Paleogene calibrations. For the alligatorid-crocodylid split we have quartets made up of two Neogene calibrations, two Paleogene calibrations, or one Paleogene and one Neogene calibration. Calibrations among caimans range from 4 m.y.a. to 54 m.y.a., but there are



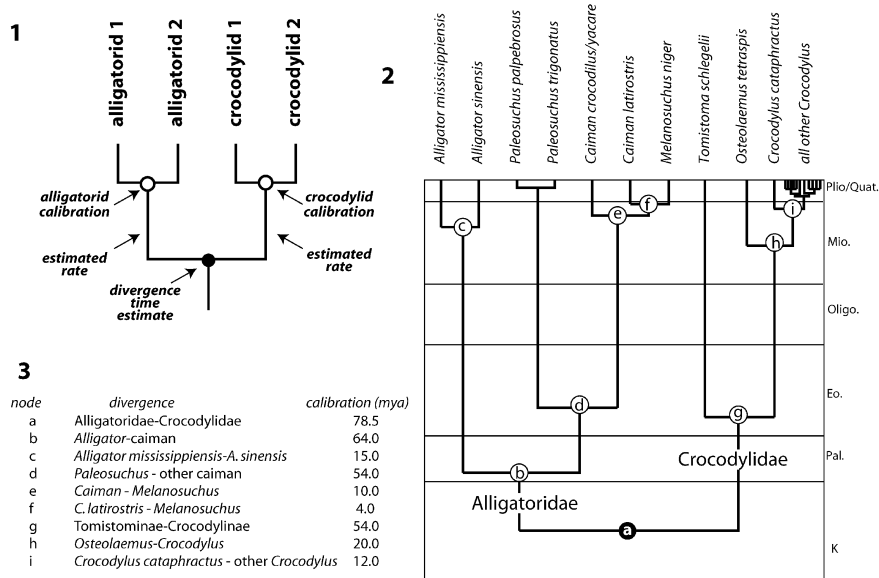


FIGURE 2—Calibration scheme for quartet dating of alligatorid-crocodylid divergence. 1, diagram showing how quartets were assembled. 2, cladogram for Crocodylia (*Gavialis* excluded) superimposed on a geological time scale, showing the specific nodes (labelled) used as calibrations. The point to be estimated with quartet dating is “a.” 3, calibration dates for nodes shown in 2.

only two living alligatorines—the two living species of *Alligator*. We are thus unable to use quartets with two Paleogene calibrations for the alligatorine-caimanine split.

**Calibrations.**—*Alligator* first appears in the Late Eocene, but the basalmost species of the group are outside a clade including *A. sinensis* and *A. mississippiensis*. For most analyses, the calibration for crown-genus *Alligator* is set at 15 million years ago (m.y.a.) based on the first appearance of fossils closer to *A. mississippiensis* than to *A. sinensis* in the Middle Miocene of Nebraska (Brochu, 1999 and in press). Alternative phylogenetic arrangements would put slightly older North American fossils closer to *A. mississippiensis* (e.g., Malone, 1979; Snyder, 2003), resulting in a within-*Alligator* calibration of 20 m.y.a.; this calibration is simulated as part of the range-extension experiments described below.

The split between *Paleosuchus* and other caimans at 54 m.y.a. is based on fossils from the Early Eocene of North America (Brochu, 1999). The first appearance datum for *Melanosuchus* is in the Pliocene (Medina, 1976), and fossils from the Early Miocene of Colombia indicate an age for the divergence of *C. latirostris* (and possibly *Melanosuchus*) from other *Caiman* at 9 m.y.a. (Langston, 1965; Gasparini, 1996; Brochu, 1999).

The split between alligatorines and caimanines is based on *Navajosuchus mooki* from the Early Paleocene (Puercan) of New Mexico. *Navajosuchus* is part of a basal polytomy at the base of Alligatorinae including *Ceratosuchus* from the Paleocene of North America and *Hassiacosuchus haupti* from the Early Eocene of Germany. Both *Navajosuchus* and *Hassiacosuchus* are often classified as “*Allognathosuchus*,” but the name *Allognathosuchus* is based on material more closely related to *Alligator* than to either *Navajosuchus* or *Hassiacosuchus* (Brochu, 1999 and in press). *Navajosuchus* allows calibration of species pairs using an *Alligator* and a caiman to 64 m.y.a.

This date also gives us a minimum age for the alligatorine-caimanine split when that is itself tested. *Navajosuchus* closely resembles most Late Cretaceous alligatoroids, all of which lie outside Alligatoridae, but shares a few synapomorphies with *Alligator* and its closest extinct relatives. Slightly older fossils from the Hell Creek Formation (Lancian) of Montana might represent

a caiman (Bryant, 1989), but the phylogenetic placement of this form is unclear; although it is not directly considered in this study, some of the range extensions applied in our analysis would approximate an alligatorine-caimanine split based on it.

Calibration within *Crocodylus* is difficult, both because of the unclear relationships among living species and the uncertain affinities of many fossils assigned to the group. Assuming a basal dichotomy between *C. cataphractus* and all other living *Crocodylus*, we can use the first appearance of crown *Crocodylus* in the Siwaliks sequence of Pakistan (12 m.y.a.) to calibrate pairs using *C. cataphractus* and another *Crocodylus* species.

Until recently, the extent of *Crocodylus* within the Siwaliks sequence was unclear—most curated material was collected in the nineteenth and early twentieth centuries, and locality and horizon information were minimal. Previous calibration for the species found in that unit, *Crocodylus palaendicus*, was a midpoint age for the sequence (Brochu, 1997, 2000). We now know *C. palaendicus* extends further down in the Siwaliks, allowing the earlier calibration (J. Head, personal commun.). Other fossil *Crocodylus*, including those closer to *C. cataphractus*, are no older than 5 m.y.a. (Brochu, 2000).

The 20 m.y.a. calibration for *Osteolaemus* and *Crocodylus* is based on the first appearance of *Euthecodon*—a bizarre longirostrine crocodylian closer to *Osteolaemus* than to *Crocodylus*—in the Lower Miocene. The 54 m.y.a. calibration for *Tomistoma* and other crocodylids is based on the geologically simultaneous appearance of early tomistomines in the Ypresian of Britain (Owen, 1850; Swinton, 1937) and mekosuchine crocodylids in the Ypresian of Australia (Willis et al., 1993; Salisbury and Willis, 1996).

Although alligatorids and crocodylids are only known from the Cenozoic, fossils close to both groups (alligatoroids and crocodylids) are known from the Late Cretaceous. The oldest crocodyloid is *Prodiplocynodon langi* from the Lance Formation (Maastriachian) of Wyoming, but Alligatoroidea is represented by several Campanian taxa (Williamson, 1996; Buscalioni et al., 1997; Wu et al., 2001; Schwimmer, 2002). The date applied here, 78 m.y.a., is a midpoint calibration for the stage. Some of these alligatoroids are from earlier in the Campanian, and so the actual

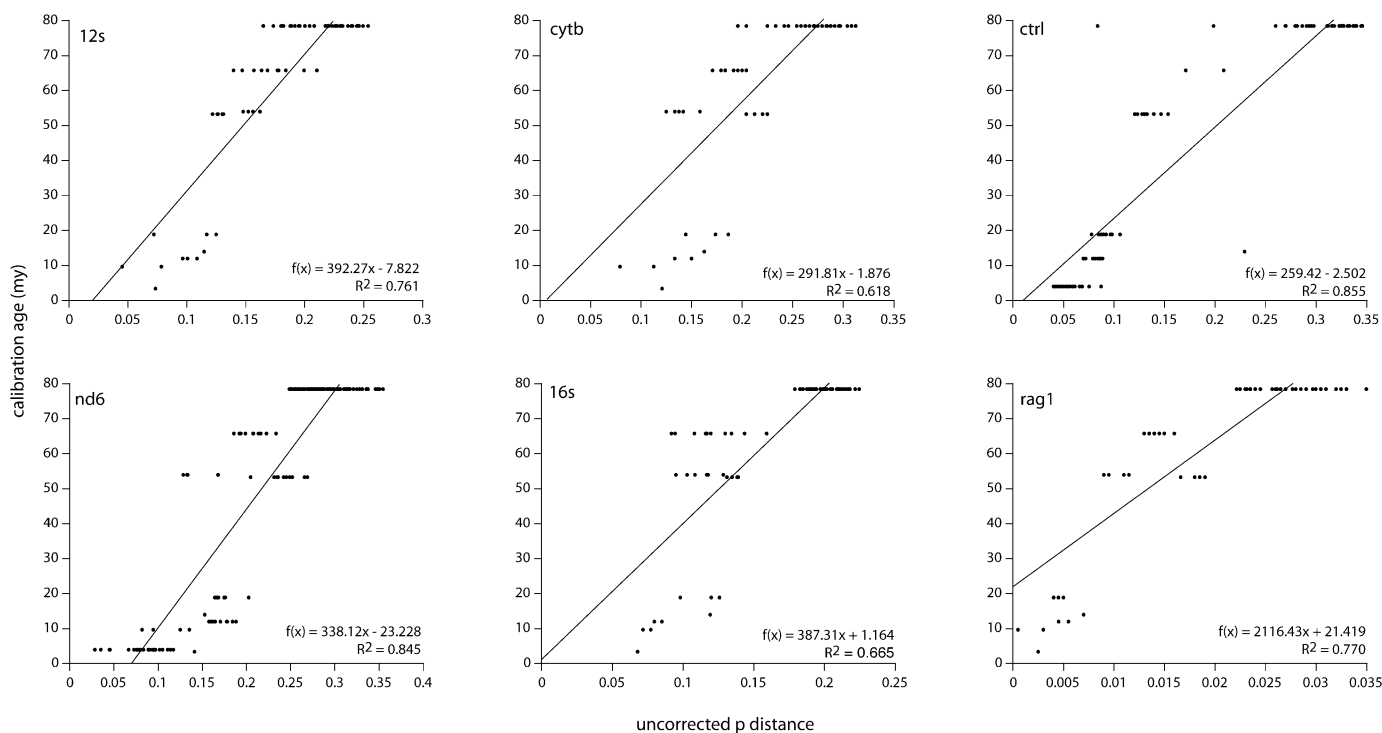


FIGURE 3—Relationship between crocodylian calibration age and uncorrected molecular (p) distance for the six data sets used in this study. Data points are pairwise distances between two crocodylian species. Note that although the square regression coefficients are all very high, the data points in the graphs are not independent.

minimum calibration is probably earlier than 78 m.y.a.; but because absolute dates have not been extracted for these fossils, the midpoint will be used throughout this analysis.

**Divergence time estimation.**—The computer program Qdate (Rambaut and Bromham, 1998) was used to estimate divergence dates using the quartet method. All dates considered in this paper are based on the two-parameter model of Hasegawa et al. (1985; HKY85) with among-site rate heterogeneity assumed to follow a gamma distribution. All analyses used a shape parameter for the gamma distribution ( $\alpha$ ) of 0.5, which is close to the estimated shape parameters for all data sets. Both one- and two-rate models were applied. Other models (e.g., general time-reversible) yield results very similar to those produced using HKY85 and are not discussed further.

Likelihood ratio tests were performed to test the rate homogeneity assumptions in each case. These compared the one- and two-rate results with a five-rate model. A significant difference between the one- or two-rate and five-rate results (with significance based on a chi-square distribution) forced rejection. Quartets not rejected were assumed to fit the applied rate model and used to estimate divergence times.

Calibrations might long postdate actual divergence times (and thus be too young), or they might reflect mistaken identifications (and be too young or too old). These sources of error are subject to future investigation as new fossils are discovered and our understanding of phylogeny improves, but we can simulate the changes these might prompt by either adding or subtracting time from the calibrations. In this study, calibration changes were restricted to the nd6 data set with the two-rate model, as this resulted in the largest number of quartets not rejected by a likelihood-ratio test when calibrations were unaltered.

To investigate the impact of calibration range extension, calibration ages were increased in two ways—by uniformly increasing calibrations by 5, 10, 15, or 20 m.y.; and by increasing all calibrations in 10-percent increments from 10 to 100 percent. Discrete blocks of time (5, 10, 15, 20, or 25 m.y.) were added to four of the youngest calibrations—within *Crocodylus*, within *Alligator*, *C. latirostris-Melanosuchus*, and *C. latirostris/Melanosuchus—C. crocodilus/C. yacare*. The earlier calibrations were left unchanged. Because the first set of range-extension experiments shows very little impact on divergence time estimation from extensions of this magnitude on earlier calibrations, converse experiments in which earlier calibrations were extended but more recent calibrations left unchanged, were not performed.

Some of these range extensions approximate the changes that might result from alternative phylogenetic arrangements. To mimic the impact of fossil misidentification and extreme range contraction, the current first appearance datum for *Crocodylus* (12 m.y.a.) was compared with pre-phylogenetic estimates that drew *Crocodylus* as far back as the Mesozoic. Most of these earlier applications did not regard the divergence among extant *Crocodylus* as an ancient event; rather, they applied the name *Crocodylus* as a form taxon regardless of how extinct species related to living *Crocodylus* (Brochu, 2000). A first appearance datum of 97 m.y.a. (Markwick, 1998) was used to simulate the earlier *Crocodylus* calibration.

## RESULTS

All of these genes show the expected strong correlation between uncorrected interspecific distance and calibration age (Fig. 3). The slopes of the lines in question differ, indicating (in part)



that these genes are evolving at different rates, but the more recently diverged pairs of species have uniformly smaller distances than those with older divergences.

A similar plot was shown for the 12s data by Brochu (1997), who interpreted the results as indicating highly variable evolutionary rates in crocodylians, with alligatorids evolving more rapidly than other groups. Indeed, measured rates seemed to vary by as many as two orders of magnitude, and no single rate could account for all pairwise comparisons. But in this case, the distances measured were uncorrected distances, and because all of the relatively recent divergences with high apparent rates were within-alligatorid comparisons, the results were interpreted as supporting high rates within Alligatoridae. The plots shown in Figure 3 include several relatively recent within-crocodylid comparisons not available in the earlier study, and the nature of the distribution has not changed. These graphs should thus be interpreted as showing decreasing apparent rates over time as a result of accumulated multiple substitutions per site.

*Alligatorid-crocodylid divergence, unmodified calibrations (mitochondrial).*—The relationship between quartet calibration and alligatorid-crocodylid divergence estimate is shown for the five mitochondrial genes in Figures 4 and 5. The average of the two calibrations is used in each quartet as a measure of relative calibration age. Plots using either of the actual calibrations yield similar results.

Figure 4 shows the alligatorid-crocodylid divergence estimates ordered by age, illustrating the wide variation in resulting within-gene divergence estimates. The oldest estimates in any given analysis are as much as 7.5 times older than the youngest (Table 2). This expresses differences of from 68 to 193 million years, depending on the analysis.

In all cases, there is a visible relationship between average calibration age and divergence time estimate (Fig. 5). Estimates based on Neogene calibrations are uniformly recent (within the past 50 million years, with the exception of the ctrl sequences), and the oldest divergence estimates are related to Paleogene calibrations. Regression lines through these scatters are all statistically significant, but because the data points are not independent, these are shown merely to reinforce the clarity of the trend.

*Differences between analyses.*—Models that account for more rate heterogeneity are generally a better fit than those that account for less. Hence, more quartets were rejected with the one-rate model than with the two-rate model for each gene. Rejection disproportionately impacted quartets with two Neogene calibrations, which is why the difference between oldest and youngest divergence estimate is always less in the one-rate analyses. With the control region sequences, the oldest estimate is only 1.92 times older than the youngest estimate with the one-rate model, but 4.64 times older with the two rate model. In this case, no quartet with an average calibration later than 40 million years was consistent with the one-rate model, but 21 quartets with average calibrations later than 20 million years were consistent with the two-rate model.

Differences in taxon sampling are another source of divergence variation between genes. For example, only two data sets—nd6 and ctrl—were sequenced for more than a handful of *Crocodylus* species (Table 1). This resulted in a larger number of calibrations involving *Crocodylus*, including Neogene calibrations between *C. cataphractus* and another *Crocodylus*, and hence a larger number of quartets supporting relatively young divergence time estimates.

The ctrl data set does genuinely seem to be telling a different story (though it was sequenced for fewer alligatorids, and hence there were fewer Neogene alligatorid calibrations available), but differences between nd6 and other mitochondrial genes reflect sampling differences as much as gene-specific differences. For these reasons, the numbers in Table 2 are not directly comparable between data sets or models.

Differences between data sets are more apparent if we compare the results for specific quartets (Table 3). Estimates from the ctrl data are consistently higher than for the other genes. This is not unexpected given the gene's intraspecific variability (Ray and Densmore, 2002; see below). The remaining mitochondrial data support similar divergence time estimates, with differences increasing in magnitude as average calibration age increases. This is also to be expected, as stochastic evolutionary effects should accumulate over time and cause the results from any two data sets to diverge. This is also why the error margins on older estimates are broader. The average estimates from the cytb data are the lowest for any mitochondrial gene, but specific estimates from cytb are consistently older than for any other mitochondrial data set except for ctrl, reflecting the larger number of estimates based on Neogene calibrations not rejected with the cytb data set.

*Among-quartet consistency.*—In all cases, the lower 95 percent confidence limit for the younger estimates would exclude the upper confidence limit for the older estimates, and there is no single range of dates on which all quartets would agree. The oldest and youngest estimates exclude each other. The difference between the youngest estimate's lower confidence limit and the oldest estimate's upper confidence limit (the error gap) is from 52 to 117 million years in the two-rate case. The closest any of these comes to finding a common age range for all quartets is with the control region sequences under a one-rate model, with an error gap of only 19.14 million years, but this is because of the rejection of all quartets with two Neogene calibrations; in the two-rate case, the gap expands to 117.14 million years.

*Consistency with fossil record.*—The minimum alligatorid-crocodylid divergence estimate from fossils is shown by a dashed line in Figure 4. Logically, all quartet-based divergence estimates should lie at or below this datum, and none should exclude it as too old. The proportion of quartets for a particular gene that are consistent with the fossil datum depends both on the number of quartets available at the outset and the number that are not rejected as being inconsistent with the one- or two-rate model. Still, for most genes, the majority of divergence estimates are consistent with this datum—they are either older or include it within their confidence limits. The same is true for the average divergence estimates obtained from all data sets (Table 2).

For all but one mitochondrial gene in this analysis, some of the estimates are impossibly young. Many estimates are post-Mesozoic, and some are even post-Oligocene. This is younger than the alligatorine-caimanine and *Tomistoma-Crocodylus* divergences, let alone the alligatorid-crocodylid divergence. The youngest divergence time estimates underestimate divergence time by at least 150 percent, and the maximum estimates based on 95 percent confidence intervals are underestimates by as much as 200 percent.

Impossibly young estimates invariably involve quartets with two Neogene calibrations—for example, using a calibration within derived caimans and one within *Crocodylus*, both of which lie within the past 15 million years. The mean divergence estimate

←

FIGURE 4—Alligatorid-crocodylid (G-C) quartet divergence time estimates from mitochondrial data. In each graph, the heavy line connects individual divergence time estimates; gray lines connect the upper and lower 95 percent confidence limits for the estimates. Estimates ordered by age. Dashed line with a superimposed crocodile skull is the minimum alligatorid-crocodylid divergence based on fossils.

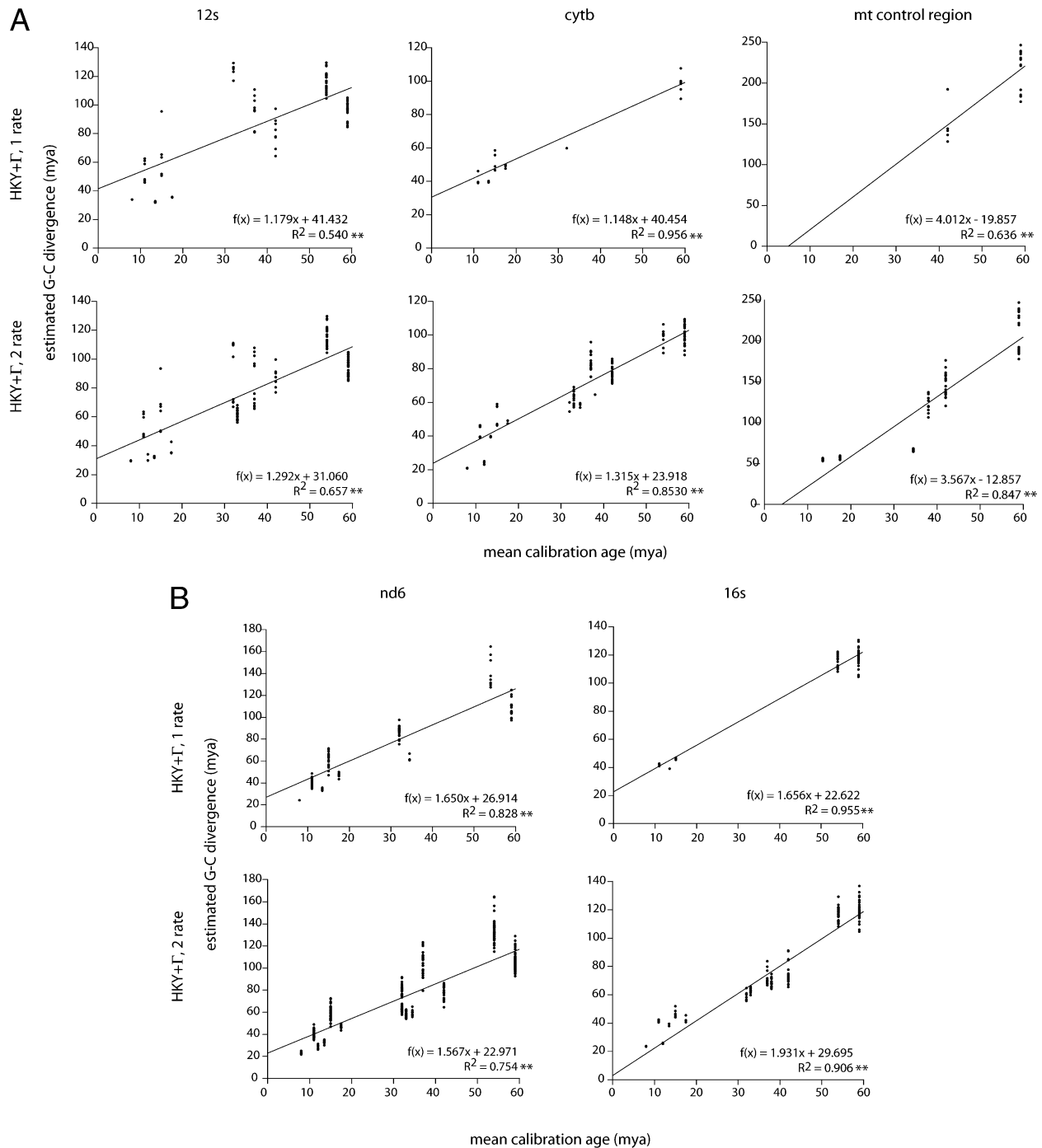


FIGURE 5—Relationship between alligatorid-crocodylid quartet divergence time estimate and average calibration age from mitochondrial data. \* = significant at  $P > 0.05$ ; \*\* = significant at  $P > 0.01$ ; but data points not independent.

derived from quartets using a Neogene alligatorid and Paleogene crocodylid is not statistically distinguishable from that derived from quartets using a Paleogene alligatorid and a Neogene crocodylid ( $P > 0.001$ ). Although this does not argue against incorrect calibration dates as a factor, it suggests that the source of underestimation is not taxon-specific and that if the calibrations are being misled, they are being misled in similar ways.

The confidence intervals for most of the older estimates include

the minimum age for crocodylid-alligatorid divergence in the fossil record. These generally involve quartets in which one of the calibrations is of Paleogene age—for example, the alligator-caiman split for alligatorids or the *Crocodylus-Tomistoma* split for crocodylids. But when both of these Paleogene calibrations are used, alligatorid-crocodylid divergence estimates become much older, supporting range extensions of at least 150 percent, and in extreme cases (2-rate model, ctrl), having upper confidence limits



in the Middle Jurassic and lower confidence limits extending into the Paleozoic.

The oldest estimates for most genes are in the Early Cretaceous with lower confidence limits in the Middle or Early Jurassic. Two sequences support somewhat older low-end estimates—within the Middle Jurassic with lower limits in the Middle Triassic for nd6, within the Early Triassic with lower limits in the Mississippian (Early Carboniferous) for the control region. The control region sequence is also the only one with no quartets rejecting a pre-Cenozoic divergence.

*Alligatorid-crocodylid divergence—results from a nuclear sequence.*—Like the mitochondrial genes, rag1 shows a significant relationship between uncorrected distance and age (Fig. 3), suggesting (as with the mitochondrial genes) that apparent rates diminish as age increases. But the slope of the line for rag1 is much steeper than for the mitochondrial genes, and the range of variation covers much smaller distances—from 0.0005 to 0.035 for rag1, as opposed to from 0.029 to 0.35 for nd6. This is not altogether surprising, as protein-coding nuclear genes are thought to evolve more slowly (and to thus accumulate fewer substitutions over time) than mitochondrial genes (Graybeal, 1994; Springer et al., 2001).

Very few quartets support mutually exclusive alligatorid-crocodylid divergence estimates, and only two would exclude the fossil datum as too old (Fig. 6a, b). Moreover, although the correlation between divergence estimate and average calibration age is still statistically significant, correlation coefficients are low and the relationship is not as robust as with the mitochondrial genes (Fig. 6c, d).

Three outliers are apparent for both rate models (Fig. 6c, d). These are quartets in which *Caiman latirostris* and *Caiman yacare* form the alligatorid pair. Comparisons involving *Melanosuchus* and *Caiman yacare* are also anomalously high, especially when the one-rate model was applied. Quartets with either *Melanosuchus* or *C. yacare* and a species of *Paleosuchus* also support old divergence dates compared with other quartets with an average calibration of between 30 and 35 million years. For both rate models, correlation improved when the *latirostris-yacare* comparisons were excluded, and in the case of the two-rate model, the correlation was not significant at  $\alpha = 0.01$  with the outliers, but significant without them.

*Alligatorine-caimanine divergence.*—In most cases, likelihood-ratio tests rejected the majority of quartets. For the mitochondrial genes, all quartets with a Paleogene calibration (i.e., one in which the caiman pair included a species of *Paleosuchus*, with a calibration age of 54 m.y.a.) were rejected. Only the rag1 data included some nonrejected quartets with a Paleogene calibration point. All 12s quartets were rejected under the one-rate model, and all ctrl quartets were rejected for both models (Fig. 7).

Even the sparse data available for the alligatorine-caimanine split is consistent with the results observed for the alligatorid-crocodylid split. Nonrejected quartets for the mitochondrial genes all had Neogene caiman calibrations, and in all, the lower confidence level excludes the minimum divergence date from fossils as too old.

The rag1 data preserve some quartets with a Paleogene caiman calibration, and in these cases, the estimated divergence time is at or near the Cretaceous-Tertiary boundary between the first appearances of caimans and alligatorines, with confidence windows consistent with the fossil record. Quartets based on a Neogene caiman calibration exclude the fossil minimum divergence estimate as too old.

#### IMPACT OF RANGE MODIFICATION ON DIVERGENCE TIME ESTIMATION

*Range extension on all calibrations.*—We would expect addition of an equal amount of time to all quartets to have a disproportionate impact on later calibrations. Addition of 5 million

years, for example, more than doubles the *Melanosuchus-Caiman latirostris* calibration but only increases the alligatorine-caimanine calibration by 7 percent. We would also expect a disproportionate impact on resulting divergence age estimates.

The results are consistent with this expectation. In general, increases in divergence estimate are proportional to the amount of time added to the calibrations (Figs. 8, 9). Addition of 5 million years increases the alligatorid-crocodylid divergence estimate by nearly 50 percent for most quartets with two Neogene calibrations, but by less than 10 percent for quartets with two Paleogene calibrations. Adding 20 million years expands the divergence by more than 75 percent for Neogene quartets, but less than 30 percent for Paleogene quartets (Fig. 9c). This means that, for example, the estimated divergence date based on *Melanosuchus-Caiman latirostris* (4 m.y.a.) and *Crocodylus cataphractus-Crocodylus moreletii* (12 m.y.a.) increases 93.6 million years (77.5 percent), from 25.00 m.y.a. to 111.01 m.y.a., by adding 20 million years. Conversely, the estimated divergence based on *Alligator mississippiensis-Caiman latirostris* (64 m.y.a.) and *Tomistoma-Crocodylus novaeguineae* (54 m.y.a.) increases by only 35.4 million years (25.9 percent), from 101.00 m.y.a. to 136.38 m.y.a.

Increasing calibration age by a uniform percentage also results in higher divergence time estimates (Fig. 9), but with a slightly greater impact on earlier calibrations, especially at higher percentages. This makes intuitive sense—increasing the *Melanosuchus-Caiman latirostris* divergence by 100 percent merely doubles it from four to eight million years, but the same increase would extend the alligatorine-caimanine calibration to 128 m.y.a.

Differences between addition of discrete and proportional blocks of time can be seen when the regression lines for various plots of the data are compared. Plotting mean calibration age against estimated divergence time results in parallel regressions for proportional added time, but in regression lines with progressively decreasing slope with added discrete time (Fig. 9a). Conversely, regression lines are negative when the difference between original and new divergence estimate is compared with mean calibration age when discrete time blocks are involved, in contrast to the positive and increasing slopes with proportional increases (Fig. 9b). Discrete blocks of time disproportionately impact later calibrations, whereas proportional blocks of time have a disproportionate absolute impact on earlier calibrations.

For the nd6 gene, divergence estimates later than 50 to 55 m.y. generally have lower confidence limits excluding the minimum date from fossils. Minimal changes to calibration age do not correct all impossibly young divergence time estimates from some of the later calibration quartets, but the number of estimates in this category decreases as time is added to the calibrations (Fig. 8). With no time added, 98 of 404 estimates (24.3 percent) are too young; this decreases to 27 of 447 (6.0 percent) with uniform addition of 5 m.y. and to one of 501 (0.002 percent) with uniform addition of 10 m.y. With the addition of 15 or 20 m.y. across the board, all estimates include the minimum fossil date within their confidence intervals. Addition of 100 percent across the board leaves only 2.5 percent (10 of 404) of estimates too young.

Although among-quartet inconsistency is evident in all cases of across-the-board range extension, among-quartet consistency does improve in two ways. First, addition of time increases the number of younger estimates that are consistent with the error window of the oldest estimate based on unmodified calibrations. 58.3 percent (235 of 403) are inconsistent with this benchmark when calibrations are unmodified, but only 1.6 percent (eight of 508) when 20 m.y. are added across the board. Addition of proportional time also improves this situation, but because the absolute amount of added time with the Neogene calibrations is usually less than what was added with discrete time blocks, it does so less dramatically; 25.0 percent (101 of 404) are inconsistent with the oldest estimate

TABLE 2—Summary of results from quartet dating of the alligatorid-crocodylid divergence with unmodified calibrations. Error gap = gap between lower bound of youngest estimate and upper bound of oldest estimate; error window = amount of time separating upper bound of youngest estimate from lower bound of oldest estimate. nn = estimates based on two Neogene calibrations; np = estimates based on one Neogene and one Paleogene calibration; pp = estimates based on two Paleogene calibrations. Dashes indicate cases in which no quartets with the indicated calibrations passed a likelihood ratio test for model consistency. \* = in this case, only a single quartet with a Neogene and Paleogene calibration fit the model. All numbers are millions of years (my).

Gene	Model	Mean estimate	Standard deviation	Mean upper limit	Mean lower limit	Youngest estimate	Youngest upper limit	Older estimate	Oldest lower limit
nd6	1 rate	70.16	32.07	52.33	98.58	24.20	17.67	164.52	230.78
nd6	2 rate	78.89	32.47	61.07	110.18	21.84	15.60	164.60	234.32
12s	1 rate	90.67	27.27	69.39	126.71	31.86	23.79	129.65	181.22
12s	2 rate	82.59	26.41	63.15	116.96	29.36	19.72	129.73	181.35
16s	1 rate	106.46	26.76	85.45	137.10	39.08	30.16	130.74	169.45
16s	2 rate	84.72	29.62	70.00	111.88	23.38	16.81	136.89	179.35
ctrl	1 rate	196.80	40.18	151.78	262.38	128.48	100.27	246.76	332.16
ctrl	2 rate	125.36	61.72	95.74	169.33	53.22	40.78	247.00	332.56
cytb	1 rate	65.72	25.24	50.16	91.74	39.04	27.85	107.72	146.01
cytb	2 rate	75.78	21.10	59.89	107.87	20.81	14.48	109.42	149.19
rag1	1 rate	112.06	27.00	80.68	166.36	50.39	35.39	180.15	376.15
rag1	2 rate	106.61	27.03	77.99	157.09	49.70	29.19	215.57	457.88

based on unmodified calibrations when 50 percent is added to all calibrations, and 12.6 percent (51 of 404) when 100 percent is added.

Second, among-quartet inconsistency diminishes for a given analysis when discrete blocks of time are added across the board. Inconsistency is 58.3 percent with unmodified calibrations, but 29.9 percent when 20 m.y. are added to all calibrations. The slope of the regression line comparing average calibration and divergence estimate decreases from 1.57 with unmodified calibrations to 0.699 with 20 m.y. added. Extrapolation of the relationship between slope and amount of added time indicates that the regression line would be 0, and we would have among-quartet consistency, with the addition of between 35 and 36 m.y.

This is true only when discrete time blocks are added. Addition of proportional time has no impact at all on among-quartet inconsistency: it is 58.6 percent for all proportional increases. This is another expression of the disproportionate impact on later calibrations when discrete time blocks are added—they increase estimates from later calibrations more than those from earlier calibrations, and the slope of the regression approaches horizontality as the magnitude of the discrete block increases.

*Range extension on Neogene calibrations.*—Increasing the age of a single calibration by a discrete amount increases estimate age and generally increases consistency with the fossil record (Fig. 10a, b, c). But unlike discrete-block increases to both calibrations, addition of time to these individual calibrations has a disproportionate impact on older estimates (Fig. 11), a pattern more similar to the addition of proportional blocks of time to both calibrations.

Among-quartet consistency remained unchanged with addition of time to the *Alligator* calibration—10 of 33 estimates using the *Alligator* calibration are inconsistent with the error window of the oldest estimate even if 25 m.y. is added to the *Alligator* calibration (Fig. 10a). But consistency with the fossil record does improve—23 (69.7 percent) were inconsistent with the fossil record with unmodified calibrations and 20 (60.1 percent) when 5 m.y. was added to the *Alligator* calibration, but more noticeably to 11 (33.3 percent) with the addition of 10 m.y.

These impossibly young estimates remain impossibly young even with the addition of 25 m.y. to the *Alligator* calibration, and in all of these cases the crocodylid calibration was within *Crocodylus*. Addition of 35 or 45 million years to the *Alligator* calibration removes this problem, but only because these quartets are rejected as inconsistent with the two-rate model.

Similar results were obtained when time was added to the *Crocodylus* calibration (Fig. 10b): 60 of 90 estimates (66.7 percent)

are inconsistent with the fossil record when the *Crocodylus* calibration is not changed, but this drops to 36 of 179 (20.1 percent) when 5 m.y. are added and two of 171 (1.2 percent) when 25 m.y. are added.

Addition of time to the Neogene caiman calibrations has a stronger impact on consistency with the fossil record (Fig. 10c). All estimates based on *C. latirostris-Melanosuchus* are inconsistent with the fossil record, but only 15 of 33 with the addition of 6 m.y. and one with the addition of 25 m.y. Likewise, 43 of 131 (32 percent) of estimates based on *C. latirostris-Melanosuchus—C. crocodiles/C. yacare* are inconsistent with the fossil record, compared with 34 (25.9 percent) with the addition of 5 m.y. and five (3.8 percent) with the addition of 25 m.y.

In this case, among-quartet consistency decreases with addition of time to the later caiman calibrations. Only 1 calibration is inconsistent with the error window of the oldest estimate when 6 or 10 m.y. is added to the *C. latirostris-Melanosuchus* calibration, but four are inconsistent with the addition of 15 m.y. and 9 with the addition of 20 or 25 m.y. There are 28 estimates inconsistent with the oldest estimate with no time added to the *C. latirostris-Melanosuchus—C. crocodiles/C. yacare* calibration, but 38 with the addition of 5 m.y. and 41 with the addition of 10 to 25 m.y.

An additional variable in these cases is the number of quartets not rejected by a likelihood ratio test. For both the *Crocodylus* and *C. latirostris-Melanosuchus* calibrations, the number of quartets consistent with a two-rate model increases with the addition of time, from 90 to 179 for *Crocodylus* and from 21 to 33 for the caimans, with the addition of 5 m.y. Addition of 5 to 20 m.y. does not change the number of consistent quartets using the *Alligator* calibration, but it drops by one, from 33 to 32, with the addition of 25 m.y. and more sharply with the addition of 35 or 45 m.y. For *Crocodylus*, the number of consistent quartets drops from a high of 181 with the addition of 10 m.y. to 171 with the addition of 20 or 25 m.y.

*Range contraction from taxonomic revision.*—The results of this experiment are not surprising. Clipping the *Crocodylus* calibration from 97 to 12 m.y.a. has a disproportionate impact on younger estimates, some of which are shortened by more than half (Fig. 10d). Taxonomic revision leading to a later calibration reduces divergence time estimates.

#### DISCUSSION

The majority of divergence time estimates obtained in this study predate the oldest known crown-group crocodylians (and are thus consistent with the fossil record), and they do not require

TABLE 2—Extended.

Estimate range	Error gap	Error window	NN Mean	NN Standard deviation	NP Mean	NP Standard deviation	PP Mean	PP Standard deviation
140.32	87.52	213.11	47.97	11.61	84.38	9.30	120.27	18.18
142.75	89.00	218.71	44.66	12.96	74.89	17.38	115.11	16.30
97.80	52.46	157.42	50.32	16.13	91.32	20.72	107.83	11.70
100.37	52.12	161.63	46.33	16.71	77.48	16.49	104.94	12.01
91.65	51.84	139.29	43.34	2.76	—	—	117.18	5.70
113.51	74.34	162.54	38.85	8.89	68.88	6.85	118.11	6.33
118.29	19.14	231.90	—	—	148.65	25.17	216.86	25.00
193.78	117.14	291.78	56.43	2.06	117.62	35.80	209.76	24.14
68.68	26.47	118.15	47.42	6.37	59.91*	—	98.47	5.10
88.61	53.46	134.71	41.27	2.84	74.33	9.58	99.60	5.60
129.76	23.46	340.76	93.65	37.89	116.93	21.10	—	—
165.87	24.68	428.69	94.92	49.61	106.73	26.41	111.00	9.34

unexpectedly long range extensions. But quartet analyses with unmodified calibrations still resulted in considerable inconsistency with the fossil record. Varying numbers of divergence estimates would have excluded the minimum divergence age based on fossils as too old. These invariably involve quartets with two Neogene calibrations.

In all cases, there is a significant relationship between average calibration age and divergence time estimate. None of the analyses in this study resulted in among-quartet consistency, and a single temporal range common to the divergence time estimates from all quartets was never recovered. For the two-rate model, the lower error margin for the youngest estimate is from 52 to 117 million years younger than the upper error margin for the oldest estimate (Table 2). This falsifies the hypothesis that quartets with disparate calibrations will yield consistent divergence estimates.

That quartet dating is sensitive to calibration choice is not a novel observation, but a relationship between calibration age and divergence estimate is harder to extract from the literature. Some studies do suggest such a relationship (Bromham et al., 1999; Huchon et al., 2002), but with relatively few calibration points or divergence estimates. Others do not indicate this pattern (Huchon et al., 2000; Soltis et al., 2002). In the study by Eizirik et al. (2001), earlier calibrations seem to support estimates ranging over wider stretches of time (and thus include some older estimates), but these also have more quartets fitting their model (and more chances for an older date to be estimated).

A striking case of calibration age sensitivity was described by Norman and Ashley (2002), who used two different calibrations to study divergences among perissodactyls: 3 m.y.a. for the horse-ass divergence and 50 m.y.a. for the ceratomorph-hippomorph split (Norman and Ashley, 2000). Divergence estimates between extant perissodactyl species were closely correlated with calibration age—the divergence between rhinos and tapirs, for example, differed by an order of magnitude depending on the calibration

applied. Relative rate tests did not reject a molecular clock model for their data sets, but likelihood ratio tests did.

Norman and Ashley’s study differed from the present analysis in a few important ways. First, theirs was not a quartet analysis—either of the two calibration points was used to estimate group-wide rates based on maximum likelihood. Second, although both calibrations were based on ingroup taxa (and were thus internal calibrations), one of them (the ceratomorph-hippomorph calibration) was at the base of the clade, and date estimates from that calibration are interpolations. The other calibration (horse-ass) resulted in extrapolative estimates like those provided by quartet dating.

Rate variation was thought to be partially responsible for some of the estimate variation observed in the above analyses. Is this the case with crocodylians? At least one relative rate test suggested higher evolutionary rates for alligatorids for the 12s data (Brochu, 1997). This might lead one to suspect rate variation as a factor in this study as well, but rate variation among alligatorids is not evident, and the two-rate model would presumably accommodate any variation between alligatorids and crocodylids. Moreover, patterns of overestimation and underestimation seem to be taxon-independent—dates are similar when one calibration is Neogene and the other Paleogene, and it does not matter which clade is Neogene or Paleogene.

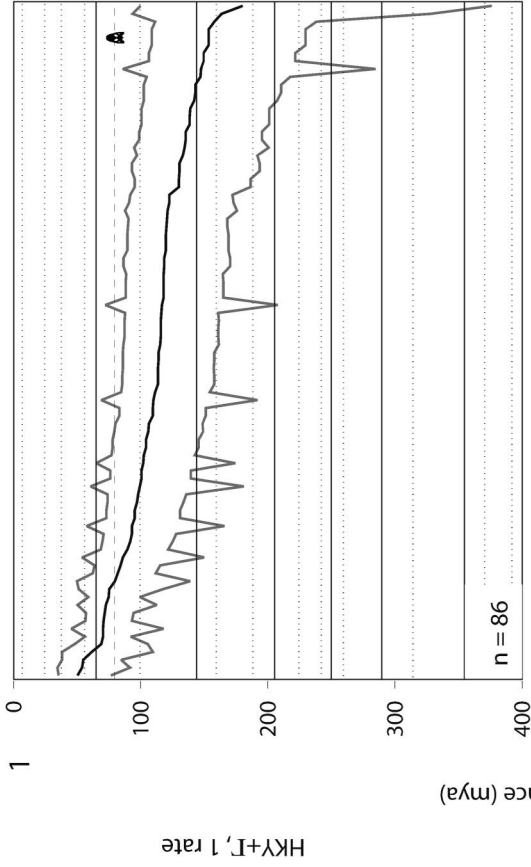
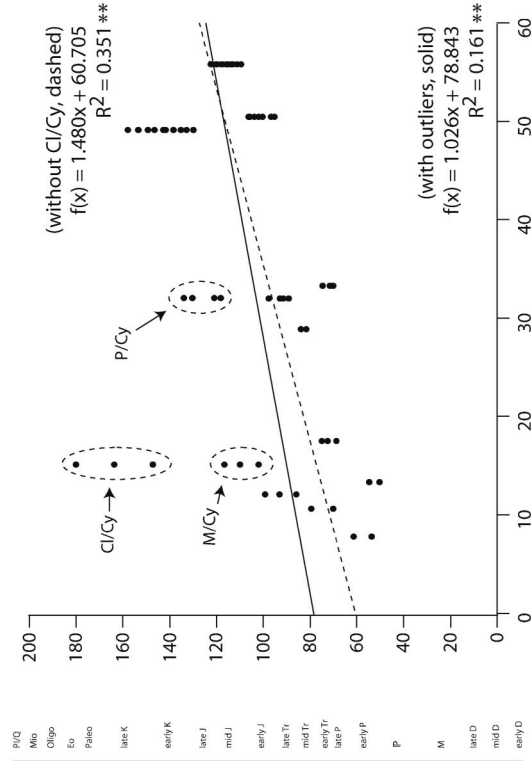
Some of the inconsistency can be ameliorated by increasing calibration ages, suggesting that fossil calibration error is part of the problem. Indeed, if small amounts of calibration underestimation disproportionately influence Neogene calibrations, then the use of two Neogene calibrations will compound this error. But not all kinds of calibration age increases have the same effect. Improvements in consistency are greatest when discrete blocks of time are added to both calibrations, as this has a heavier impact on younger estimates; this not only increases the proportion of divergence estimates consistent with the fossil record, it improves among-quartet consistency. Addition of proportional amounts of

TABLE 3—Results from quartet dating of the alligatorid-crocodylid divergence with unmodified calibrations for four specific quartets. AC, alligatorid calibration; CC, crocodylid calibration; AVE, average divergence estimate; mis, *Alligator mississippiensis*; sin, *Alligator sinensis*; yac, *Caiman yacare*; osteo, *Osteolaemus tetraspis*; rhom, *Crocodylus rhombifer*; cat, *Crocodylus cataphractus*. All numbers are millions of years (my).

Quartet	AC	CC	AVE	12s	cytb	ctrl	nd6	16s	rag1
((mis, yac), (osteo, cat))	64	20	42	80.46	81.02	130.69	79.93	90.86	114.62
((mis, yac), (tom, osteo))	64	54	59	87.55	99.01	192.52	98.89	126.93	100.19
((sin, mis), (osteo, rhom))	15	20	18	35.24	49.16	58.01	45.83	42.03	70.81
((sin, mis), (cat, rhom))	15	12	14	32.86	39.96	54.73	34.25	39.53	55.44



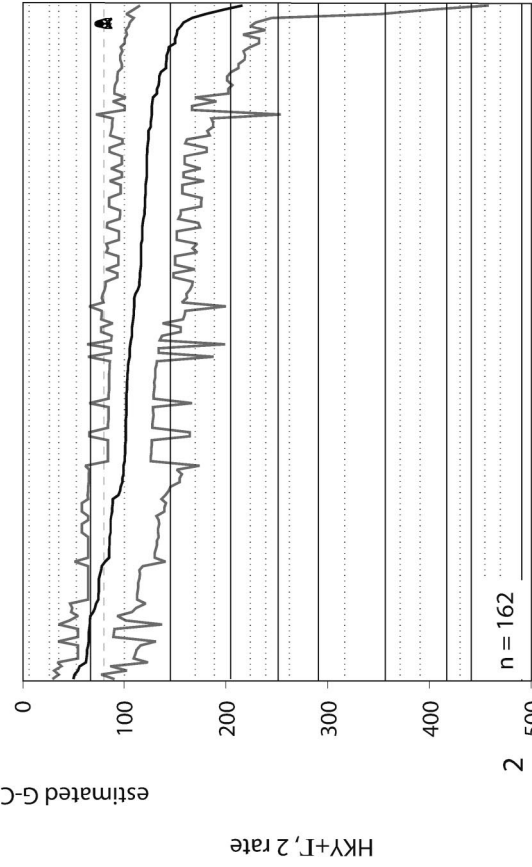
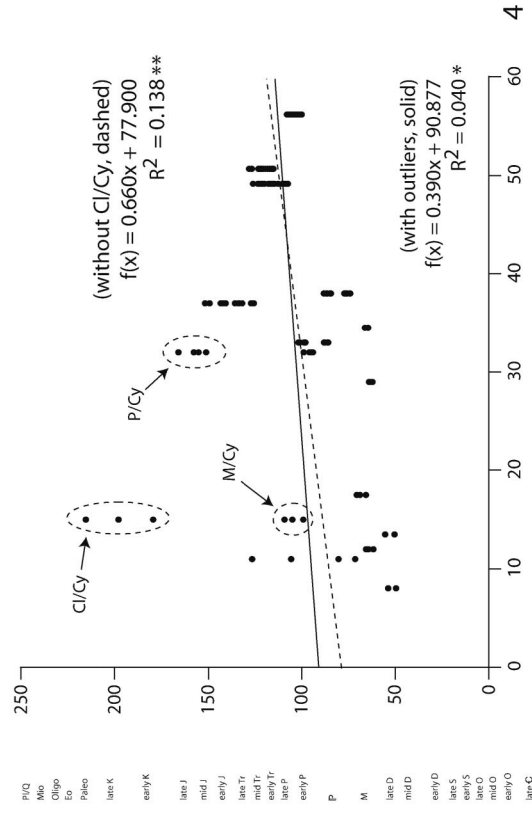
3



estimated G-C divergence (mya)

HKY+I, 1 rate

n = 86



HKY+I, 2 rate

n = 162

mean calibration age (mya)

FIGURE 6—Results of quartet dating of alligatorid-crocodylid divergence from rag1 nuclear DNA data. 1, 2, alligatorid-crocodylid quartet divergence estimates based on one-rate and two-rate models, respectively; in each graph, the heavy line connects individual divergence time estimates; the dashed line with a superimposed crocodile skull is the minimum alligatorid-crocodylid divergence based on fossils. 3, 4, relationship between alligatorid-crocodylid quartet divergence estimate and average calibration age from mitochondrial data. \* = significant at  $P > 0.05$ ; \*\* = significant at  $P > 0.01$ ; but data points not independent. CI/Cy, data points using *Caiman latirostris-Caiman yacare* calibration; M/Cy, data points using *Melanosuchus-Caiman yacare* calibration; P/Cy, data points using *Paleosuchus-Caiman yacare* calibration.



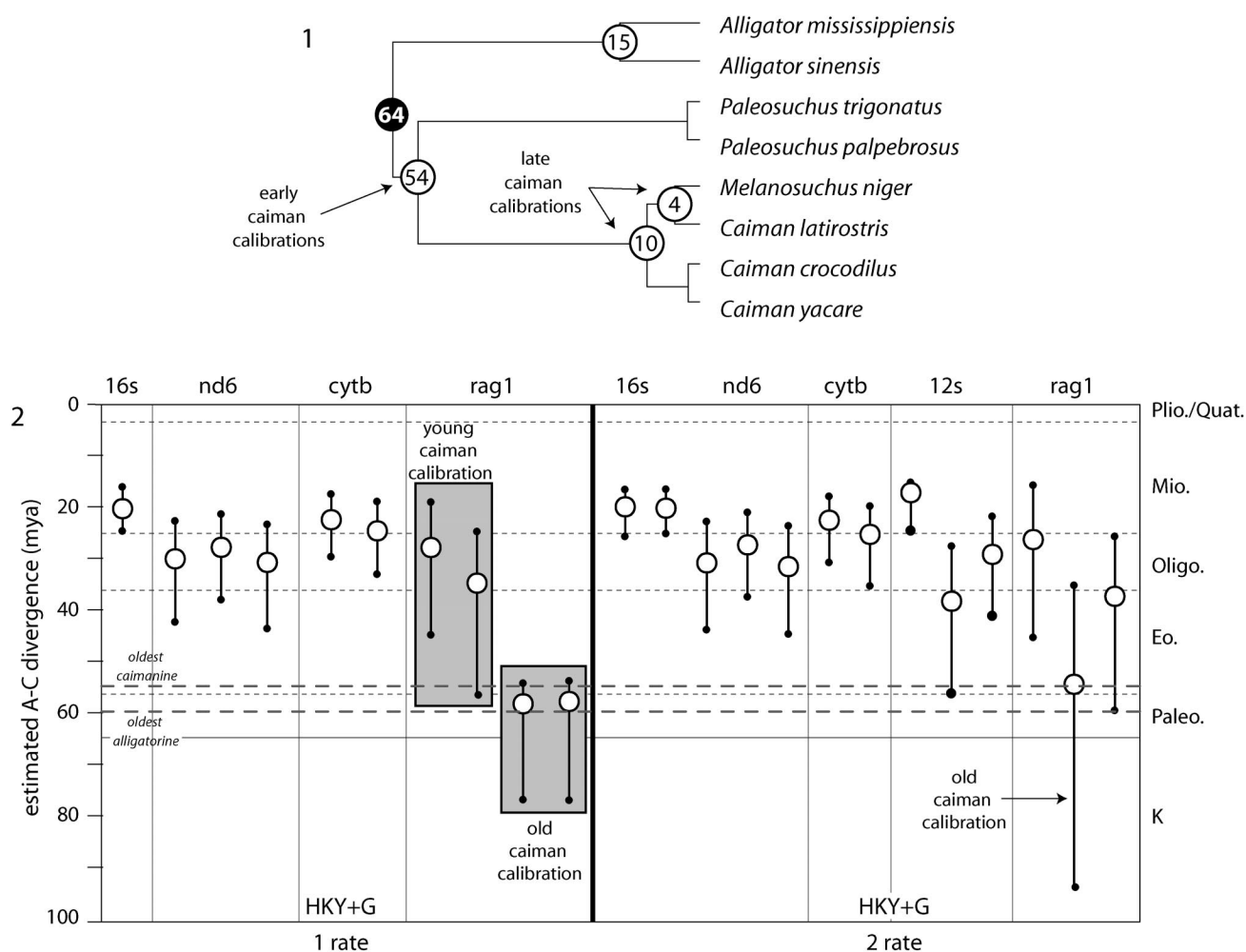


FIGURE 7—Quartet dating of alligatorine-crocodyline (A-C) divergence. 1, cladogram showing nodes used in quartet construction; calibration dates in circles. 2, alligatorine-caimanine quartet divergence estimates and 95 percent confidence intervals from mitochondrial and nuclear DNA data. Dashed lines are first appearance data for caimanines and alligatorines.

time increases estimate age, but for the range of increase explored here (up to 100 percent) it improves stratigraphic consistency less efficiently and has virtually no impact on among-quartet consistency. Addition of time to later calibrations in isolation increases estimate age and improves stratigraphic consistency, but among-quartet consistency is not improved and may actually decrease.

There are indications of this phenomenon in the literature, although interpretation has been influenced by scale. Huchon et al. (2000), for example, used two different calibration ages for a single rodent clade—9.7 m.y.a. and 13.0 m.y.a. The difference between calibrations is only 3.3 m.y., but some estimates using this calibration point increase by more than 40 m.y. But 3.3 m.y. increases a 9.7 m.y. calibration by 25 percent, and most resulting estimates increase by a similar proportion. Conversely, Soltis et al. (2002) added nearly twice as much time to the angiosperm calibration, increasing it from 125 m.y.a. to 131.8 m.y.a., and estimates increased by the same proportion as the calibration was increased (between seven and eight percent). The absolute increase was lower in the rodent example, but it was a proportionally much larger increase than in the botanical study.

#### WHAT DOES ESTIMATE VARIATION MEAN?

*Variation between data sets.*—Estimates vary somewhat between genes, but this probably reflects differences in how the

genes are evolving and different degrees of fit between the data and the model used in this study. That the control region sequences, for example, support older divergence dates and greater stratigraphic consistency might reflect a higher evolutionary rate. Portions of the control region are highly variable within crocodylian species, as they are in most amniotes (Ray and Densmore, 2002, 2003). Model choice may thus be partly responsible for some of the difference—the quartet methods used here are generally robust to some variables in simulation (Rambaut and Bromham, 1998), but the model or parameters used here may not adequately describe the actual evolutionary history of this set of sequences. This analysis did not separate the more conserved domain II from flanking domains I and III, both of which are more variable; later work might find that domain II by itself supports younger dates than the more rapidly evolving domains of that gene.

*Underestimation and stratigraphic incongruence.*—On the surface, estimate incongruence for a particular gene is more problematic. Quartets comparing two alligatorids and two crocodylids all express the divergence between Alligatoridae and Crocodylidae regardless of the specific alligatorids and crocodylids involved. The same is true for quartets comparing the two living species of *Alligator* with any two caimans—all express the divergence of alligatorines from caimanines. If these are reflecting

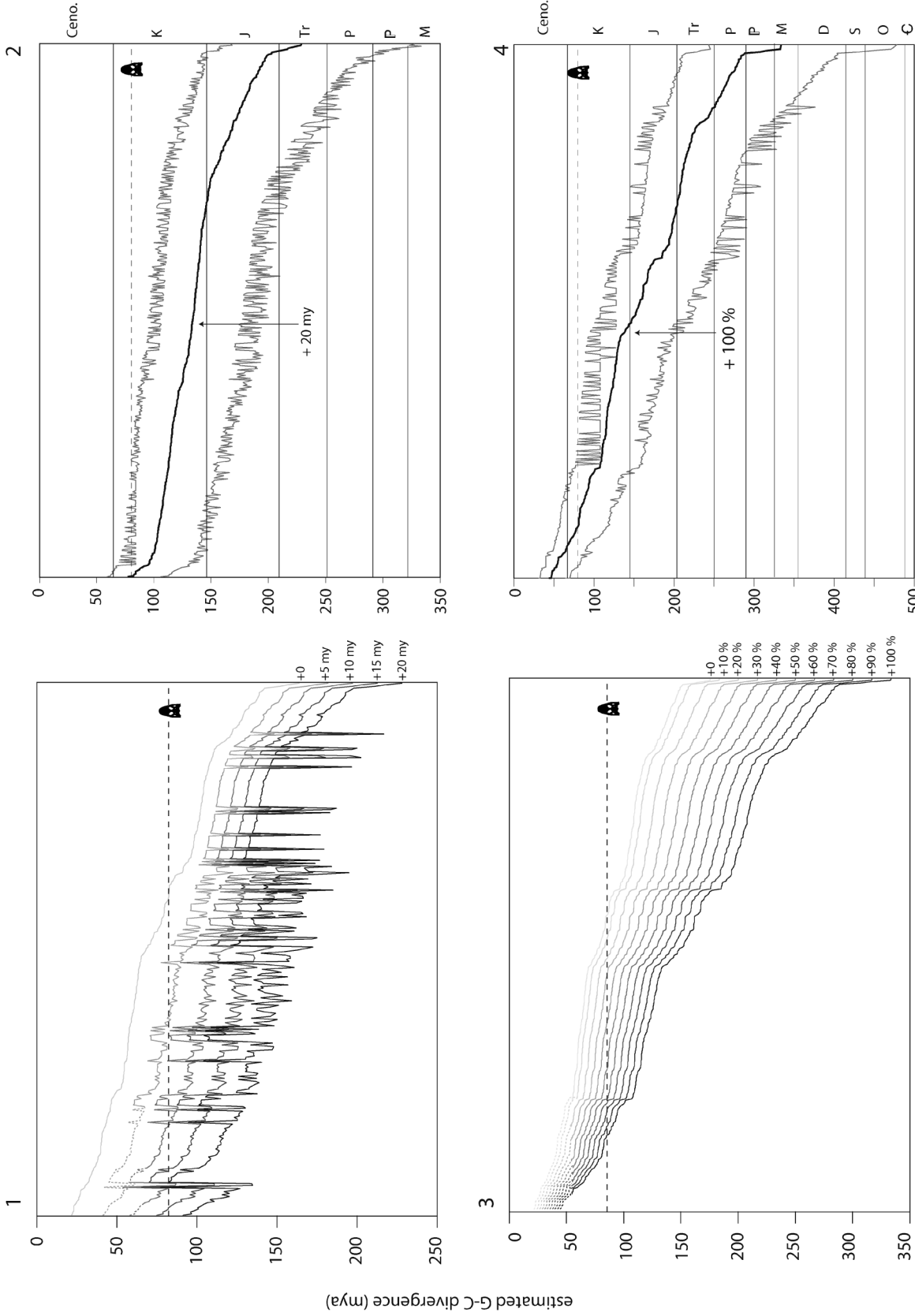


FIGURE 8—Alligatorid-crocodylid quartet divergence estimates including range extensions on calibrations. 1, 3, estimates ordered by unmodified estimate; lines are dashed when estimates are stratigraphically inconsistent; lines shown in different shadings to enhance readability. 2, 4, estimates with 95 percent confidence limits for maximum calibration modification (+20 m.y.; +100 percent, 4).

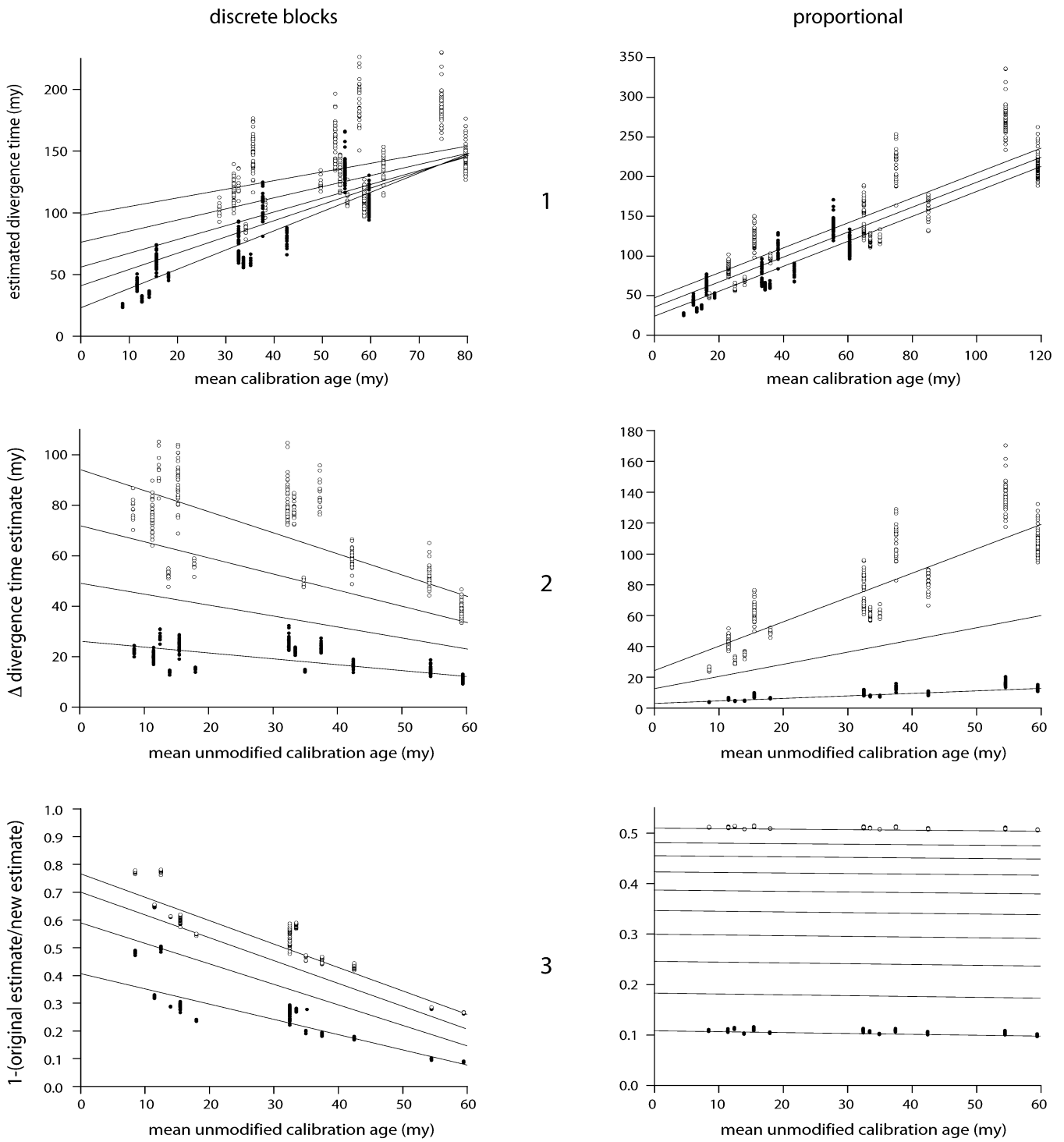


FIGURE 9—Impact of modification to both calibration points on quartet dating. Data based on nd6 data, HKY+ $\Gamma$  2-rate model. Time was added in discrete blocks (left) or proportionally (right). 1, estimated alligatorid-crocodylid divergence date plotted against average calibration age. 2, amount of estimate increase compared with average unmodified calibration age. 3, percent increase of divergence estimate compared with average unmodified calibration age. For 1, solid dots are from unmodified calibrations, open circles from calibrations with 20 m.y. (left) or 100 percent (right) added; for 2, and 3, solid dots from calibrations with 5 m.y. (left) or 10 percent (right) added, open circles from calibrations with 20 m.y. (left) or 100 percent (right) added.

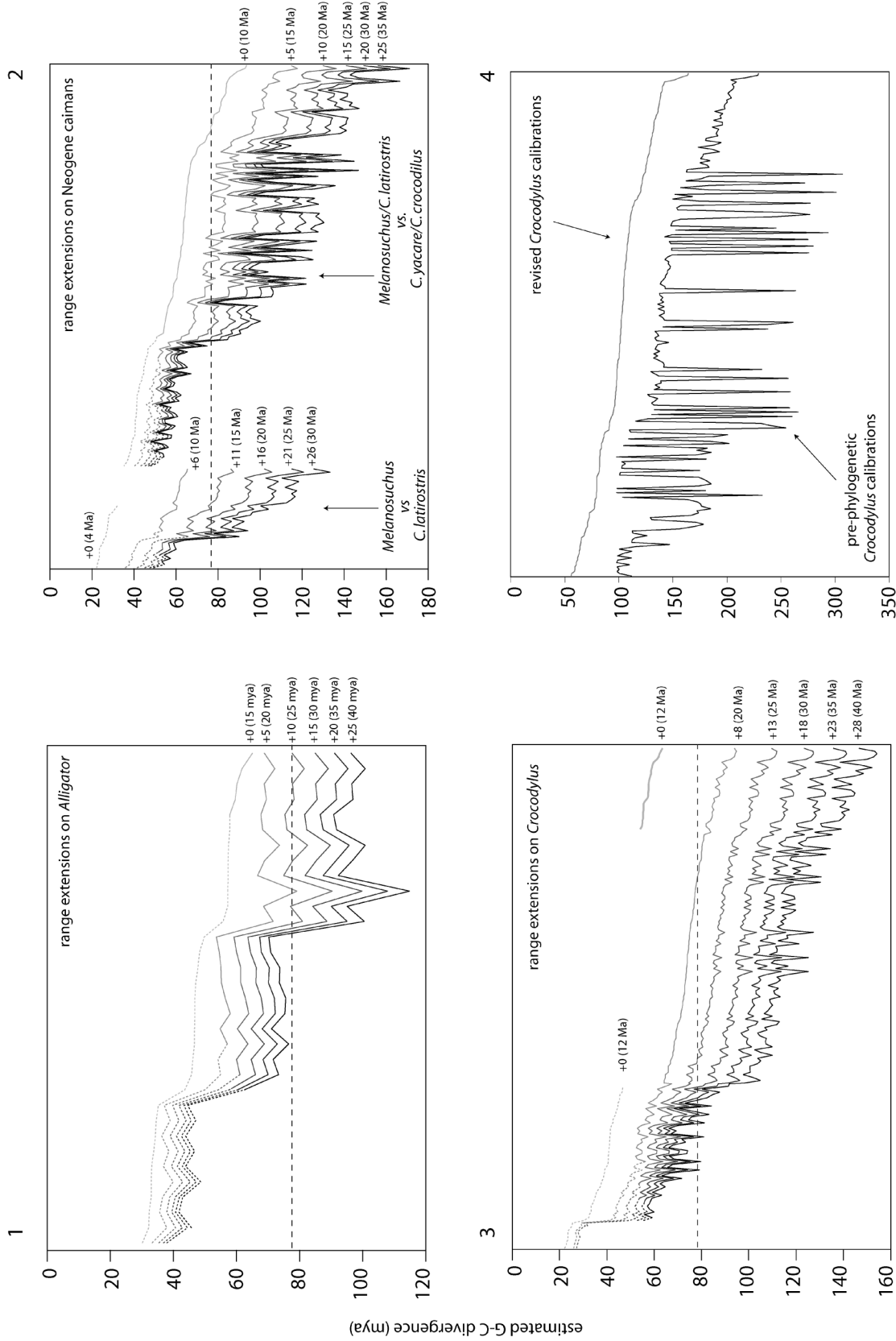


FIGURE 10—1, 2, 3, alligatorid-crocodylid quartet divergence estimates including range extensions on four late calibrations. Lines dashed when inconsistent with stratigraphy. 4, alligatorid-crocodylid quartet divergence estimates based on pre-phylogenetic calibration of Crocodylus at 97 m.y.a. Data points ordered by unmodified estimate.



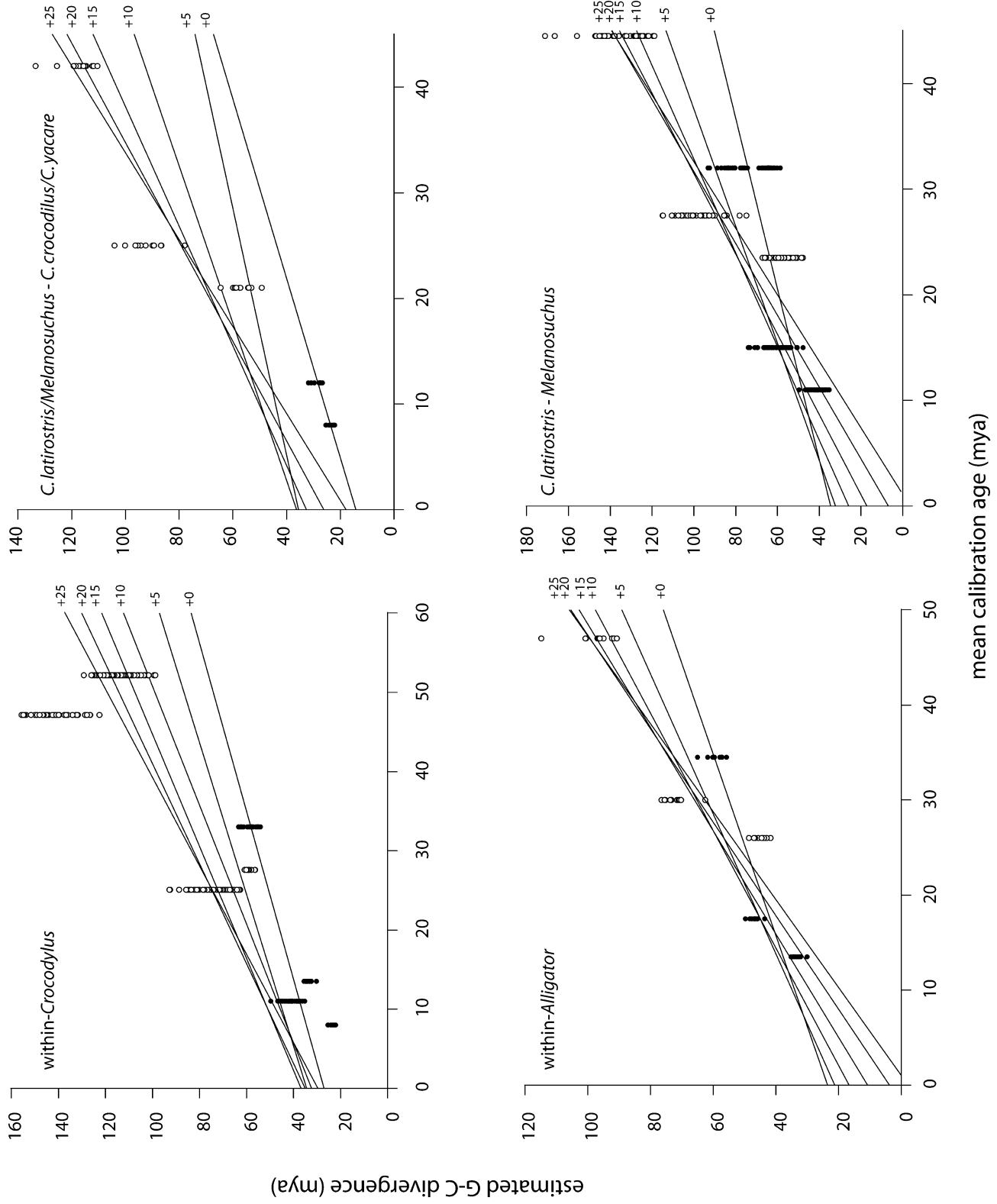


FIGURE 11—Relationship between alligatorid-crocodylid quartet divergence estimate and average calibration age, with different discrete amounts of time added to the indicated calibrations. Solid dots are based on unmodified calibrations; open circles based on calibrations with 25 m.y. added.

the same evolutionary event, why should they not all support similar divergence time estimates?

The obvious first approach to this explanation is a closer look at the calibrations involved. Underestimated calibrations should have a similar impact as calibrations based on rapidly evolving taxa—they should support higher rates of evolution and favor younger divergence estimates. Rapidly evolving lineages can also influence the root, which can further lead to divergence underestimation (Yoder and Yang, 2000). Some metazoan divergence estimates obtained by Bromham et al. (1999) are significantly younger than others, with nonoverlapping error intervals. The anomalously young divergences used Chordata as a calibration, and the basalmost known chordates (e.g., cephalochordates) are soft-bodied. The fossil record of early chordates prior to the evolution of a mineralized skeleton is predictably sparse and restricted to *lagerstätten*. It was therefore suggested that the chordate calibration was too much of an underestimate of actual origination time, leading to erroneously high rate estimates and young divergence estimates.

This is a reasonable explanation for the inconsistency encountered by Bromham et al. (1998)—there are legitimate reasons to believe preservation potential to have increased with in the calibration clade some time after it originated. But in the crocodylian situation, organismal preservation potential has remained constant. All crocodylians have bony internal skeletons, large numbers of enamel-covered teeth, and an extensive array of bony osteoderms, all of which can (and frequently do) enter the fossil record. Substantial gaps in the crocodylian record probably reflect the absence, nonpreservation, or nonexposure of appropriate depositional systems rather than widespread failure to be represented in a given unit (Markwick, 1998; Holland and Patzkowski, 2002). Is serious calibration error a reasonable explanation in this study?

In some cases, the answer is yes—especially with the Neogene calibrations among caimans. The continental record in South America, where caimans spent much of their history, is sporadic. Many fossils relevant to the problem of caiman phylogeny have not yet been analyzed phylogenetically. Caimans have an imperfectly known record, and range extensions further back into the Neogene are not unreasonable.

Another indication that at least one Neogene caiman calibration is too young is the proportion of quartets that are not rejected by a likelihood ratio test. As time is added to the *C. latirostris-Melanosuchus* calibration, more quartets are not rejected. This could reflect an unsampled history, but it could also reflect a form of misidentification. Many sequence-based analyses support a closer phylogenetic relationship between *C. latirostris* and other *Caiman* (e.g., Gatesy et al., 2003), which would minimally place the divergence between *C. latirostris* and *Melanosuchus* at 10 m.y.a. The fossils are not themselves misidentified, but a mistaken phylogenetic hypothesis leads to the calibration of a false node. A calibration of this age does not correct all stratigraphic inconsistency, but it does greatly increase the number of unrejected quartets.

But in general, we have no a priori basis for regarding all later calibrations as more or less reliable than earlier calibrations. Neogene and Paleogene calibrations were distributed among both alligatorids and crocodylids; if the problem is error associated with later calibrations, then all of the later calibrations are too recent. Moreover, there is no objective way to estimate the amount of missing record an underestimated calibration needs to correct for any bias it might introduce to the analysis (see below).

*Alligator* is very well sampled in the fossil record. Range extensions are likelier to occur through phylogenetic revision than new fossil discoveries (e.g., Snyder, in press), and even here they are unlikely to push the divergence between the two living *Alligator* species prior to the Miocene. Divergences within the past

20 to 25 million years are also consistent with several distance-based molecular estimates (Densmore, 1983; Hass et al., 1992). And yet range extensions of 20 million years, pushing the *Alligator* divergence into the Late Eocene, continue to result in younger estimates than when unmodified Paleogene calibrations are used. Such extensions would render the divergence between extant species of *Alligator* constrictigraphic with the earliest appearance of *Alligator*, but given the large samples we have for many early species of *Alligator*, this appears unlikely. Underestimates that include the within-*Alligator* calibration are thus problematic, because the error is less likely to arise from calibration underestimation.

Underestimation can arise if one does not account for enough among-site variation (Rambaut and Bromham, 1998). The gamma shape parameter used here is, if anything, a modest overestimate of among-site variation; nevertheless, one wonders if the models currently available systematically underestimate among-site variation when faced with very short distances between taxa. Increasing the age of the calibration may change the age of the divergence estimate, but as seen here, the proportional increase must sometimes be large to achieve stratigraphic consistency. It would be worth considering an approach in which among-site variation rates were themselves allowed to vary with divergence age.

*Consistency and overestimation.*—The above discussion focuses on clear cases of underestimation with respect to stratigraphic inconsistency. It does not entirely address the problem of among-quartet inconsistency. Under the two-rate model with the nd6 data, 22 percent of quartets are inconsistent with the fossil record—they support results that are directly contradicted by known fossil occurrences. The remaining 78 percent predate the minimum divergence date from fossils and are thus not falsified by the fossil record.

The words “consistency” and “compatibility” are often used to describe the fit between molecular and fossil divergence estimates, but these are sometimes used imprecisely. In principle, any molecular estimate that predates the first appearance datum in the fossil record is *consistent*—we know fossil calibrations are minimum estimates, and we would expect molecular data to place true originations before fossil appearances. What many mean when they remark on the “inconsistency” or “incompatibility” of their results with the fossil record is not consistency per se, but accuracy. The molecular estimate predates fossils by a very wide margin, placing the origin of a clade not only before the first appearance datum (which we know should be the case), but long before the first appearance datum.

In fact, this kind of “consistency” compares molecular data not with stratigraphy, but with a scenario—in this case a literal reading of the fossil record, often in the absence of a phylogenetic analysis. Bird order origination dates from molecules are deep in the Cretaceous (Cooper and Penny, 1996; Härlid et al., 1998; Waddell, 1999; van Tuinen et al., 2000; Haring et al., 2001), but the competing model, in which bird orders radiated explosively at the end of the Mesozoic, is a scenario based on the fossil record (Feduccia, 1995; Benton, 2001). The range extensions predicted by the molecular data are, logically, just as consistent with the fossil record as is the brief window of explosive radiation drawn directly from the fossil record—both regard first appearances as underestimates of actual divergences. The difference is in the magnitude of the gaps between originations and first appearances.

Assuming the minimum alligatorid-crocodylid divergence from fossils is a close approximation of true divergence age, we can regard the estimates from Neogene quartets as underestimates and those from Paleogene quartets as overestimates. Those from mixed quartets, without modification, are remarkably close to the fossil datum.

In the present case, adding 20 million years across the board

eliminates stratigraphic inconsistency and reduces among-quartet inconsistency. But assuming Crocodylia originated not long before it first appears in the fossil record, some of these range extensions are less reasonable than others—though not strictly impossible, it is unlikely that we will find close relatives of *Tomistoma* or *Paleosuchus* in the Late Cretaceous. And even here, we still face a few estimates that would be excluded as too young based on the oldest estimates from unmodified calibrations. Where do we draw the line between a reasonable estimate and an unacceptably large range extension implied by molecular data?

We could regard all stratigraphically consistent estimates as potentially correct and collectively revealing a stratigraphic range during which the divergence event of interest occurred (e.g., Huchon et al., 2000; Fig. 12). This would maximize the consistency of our results, as information from as many quartets as possible would be included. But this comes at the cost of precision. Some of the ranges implied in this case are rather broad—the distance between the latest upper bound and oldest lower bound (the confidence envelope) spans from 134.7 to 291.8 million years under the two-rate model (Table 2), spanning most of the Cenozoic, all of the Cretaceous, and in some cases the rest of the Mesozoic.

Do we accept the oldest estimates as the best estimate of divergence time? This would involve an objective criterion—whatever is oldest is best, since it cannot be falsified on stratigraphic grounds and all other estimates are stratigraphically consistent with it, even if their confidence limits do not intersect—and it would give us a clear set of hypotheses—if the oldest estimates are accurate, we would predict the discovery of fossils consistent with these estimates in the future. This enables direct falsification of the original fossil estimate. But in some cases, these oldest estimates may be much older than the fossil record would suggest, and if older fossils are unavailable for physical reasons (e.g., relevant units were never exposed and cannot be sampled), we may never be able to test these hypotheses, rendering them functionally unfalsifiable. Debates over the origins of mammalian and avian orders have been mired in such issues for many years, and without major new discoveries, they are likely to continue into the future.

Or do we take a “too cold, too hot, just right” approach, regarding the average or median estimates as best and disregard the end members as under- or overestimates? Breaking the results down by calibration age suggests that, for the mitochondrial data, estimates based on one Neogene and one Paleogene calibration are very close to the minimum divergence from fossils. Estimates based on two Neogene calibrations are too young, and those based on two Paleogene calibrations are much older, usually rejecting the minimum fossil datum as too young. If we accept the fossil datum as a reasonable approximation for actual divergence age (see below), we would prefer estimates based on a Neogene and a Paleogene calibration.

But where do we draw the lines between the “reasonable” middle range and the “unreasonable” outliers? Do we take the average of the error margins, or use a stated number of standard deviations from the average estimate? This approach might improve precision over a straight adoption of the entire confidence envelope, but the specific criteria would be subjective. Moreover, it might not address the problem of unexpectedly old estimates—the average estimate, like the oldest, might greatly predate the minimum divergence time from fossils.

We could also consider the role of calibration error, but while increasing the age of a calibration will improve the situation with underestimates, it will make the situation worse in cases where we suspect the method to be giving us an overestimate. Assuming the divergence estimate is too old, the only potential source of calibration error is taxonomic—either we have misidentified key fossils or our phylogenetic hypothesis is inaccurate. In this case,

care has been taken to apply the most rigorous calibrations available. Overestimation from taxonomic error can only be tested with further phylogenetic work and the addition of new sources of information, including new taxa. Ironically, the one case of genuine taxonomic revision tested in this study, that of *Crocodylus*, shortens divergence time estimates but increases stratigraphic inconsistency.

The problem thus extends beyond stratigraphic inconsistency. Given a wide range of divergence estimates, we are forced to either accept a very imprecise range of estimates or apply subjective criteria to narrow the range and improve precision, and any approach we adopt might be in conflict with the fossil record in perpetuity. Ultimately, they involve subjective views on what constitutes a “reasonable” estimate.

The stratigraphically consistent molecular estimates for the alligatorine-caimanine divergence are, in this author’s view, reasonable. The early record of caimans is spotty, but that of early alligatorines is excellent—much of the group’s early history took place in North America and Eurasia, both of which have been densely sampled for the Late Cretaceous and Paleogene. The earliest-known alligatorines in the Early Paleocene are just barely alligatorines—they share very few derived character states with later alligatorines such as *Alligator*, and the alligatoroids known from the Late Cretaceous are sister taxa to crown-group Alligatoridae (Williamson, 1996; Brochu, 1999 and in press). A shift in the alligatorine-caimanine divergence to the Maastrichtian is not unreasonable, but we would not expect to find alligatorines in pre-Maastrichtian deposits. In this case, the handful of divergence estimates consistent with the fossil record are right in the middle of this window (Fig. 7).

The alligatorid-crocodylid divergence is much less straightforward. At the present time, there is no objective way to quantify a “reasonable” divergence estimate directly from the fossil record for this event. Approaches have been proposed to place error margins on the stratigraphic range of a fossil group (e.g., Strauss and Sadler, 1989; Marshall, 1990, 1997; Solow, 2003), and these could potentially guide the improvement of calibration-based molecular dating methods. But for many groups, including Crocodylia, these approaches are not completely appropriate. They require information about sampling distribution through a given stratigraphic range. The ingroup crocodylians in current phylogenetic work are mostly species, and as most of these occur in a single unit, they are effectively point occurrences with infinite error margins. The larger clades have extensive stratigraphic ranges, but phylogenetic sampling has been applied to fossils that can be identified beyond the level of these clades, and we need a more thorough inventory of known material around the world to properly characterize the distribution of fossil occurrences. Present data bases (e.g., Markwick, 1998) are a good start and will ultimately permit calculation of stratigraphic error margins, but these require extensive taxonomic revision and the addition of information from new discoveries before this can be done.

Based on the derived nature of the earliest-known alligatoroids, the alligatorid-crocodylid split occurred prior to the Campanian. The continental record for the middle Cretaceous is depauperate (Smith, 2001; Smith et al., 2001), and eusuchian occurrences are very sporadic between their first appearance in the Barremian and the Campanian (Stromer, 1925, 1933; Persson, 1960; Clark and Norell, 1992; Buscalioni et al., 2001). But crocodyliforms are common in continental deposits of Late Jurassic and Early Cretaceous age (Markwick, 1998), and despite the wealth of fossil information from this temporal window, not a single crown-group crocodylian of that age has been found.

If we regard divergence times between 78 and 127 m.y.a. as

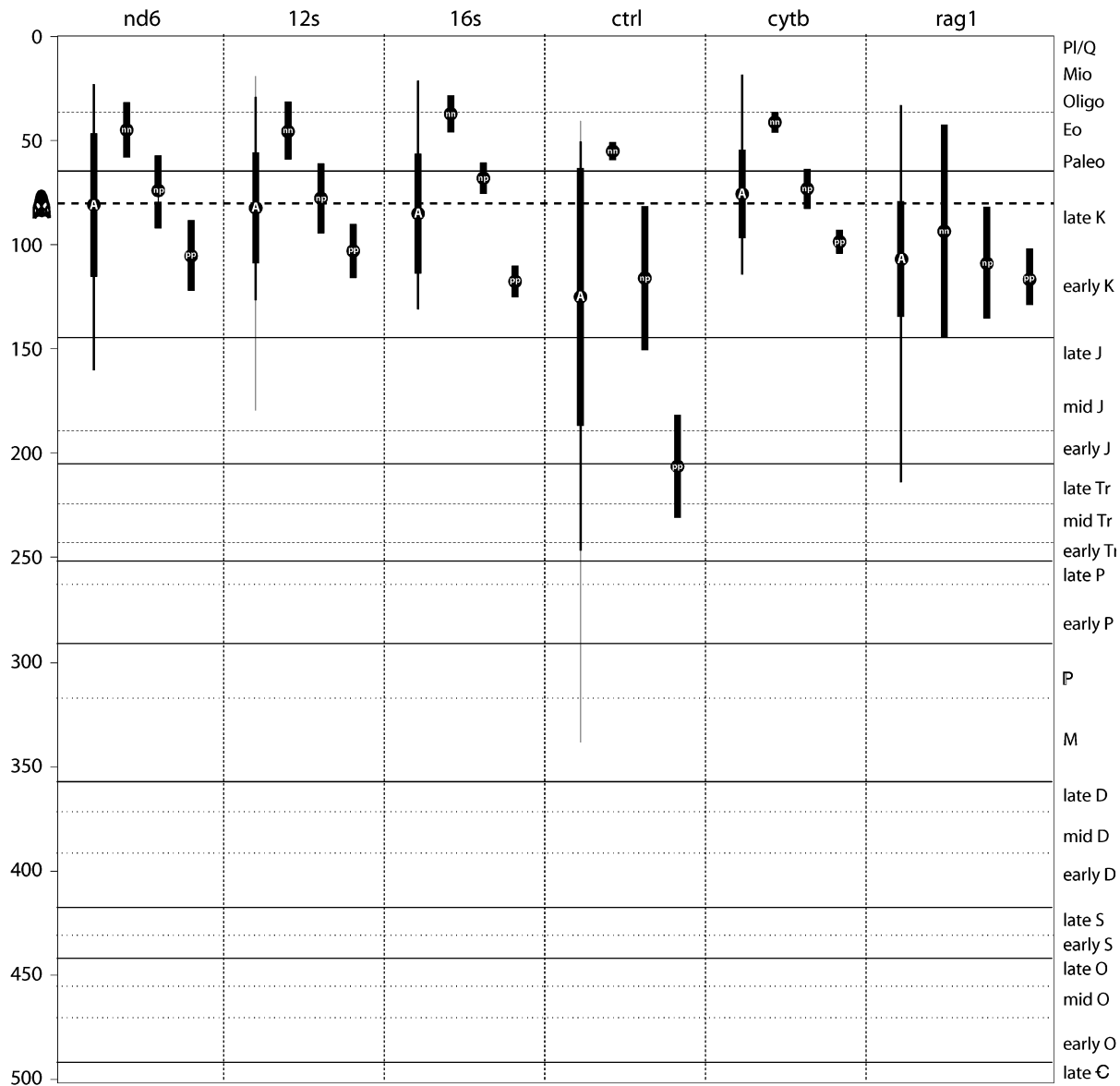


FIGURE 12—Mean divergence estimate (black circles), standard deviation (thickest lines), estimate range (midweight lines), and error window (fine lines) for five mitochondrial genes and one nuclear gene. Means and standard deviations are based on all quartets (A), quartets with two Neogene calibrations (nn), quartets with two Paleogene calibrations (pp), and quartets with one of each (np). Heavy dashed line is the first appearance datum for the quartet.

“reasonable,” most data sets considered in this study yield reasonable results. With two exceptions from the mitochondrial genome (nd6 and ctrl), the oldest divergence estimates are either no older than Barremian in age or have upper confidence limits extending into or beyond the Barremian. The same is true for rag1, although the magnitude of confidence intervals is quite large; the oldest divergence estimates under the two-rate model, for example, are unable to exclude a divergence as far back as the Ordovician. It is unclear how a confidence interval for a continental vertebrate clade including most of the Phanerozoic can be considered informative.

But consider the nd6 data set under the two-rate model. Some divergence estimates are as much as 164.5 m.y. in age. In all, 9.4 percent of the estimates for this data set under this model predate the Barremian (although the upper error margins all extend at least into the Aptian). This is consistent with the fossil record, but

given the crocodyliform penchant for living in depositional systems, a range extension into the Middle Jurassic is best interpreted as an overestimate. In these cases, the method seems to be overcompensating for unobserved substitutions and overestimating branch lengths.

For the ctrl sequences, 16 estimates under the two-rate model are inconsistent with the fossil record, and all of these have lower error margins within 7 million years of the fossil datum. But 22 of the 84 estimates under the two-rate model predate the Barremian, and the oldest of these would exclude any divergence timing later than the Toarcian (Early Jurassic) as too young. They would also fail to reject divergence times as old as the Mississippian. Falsified? No—but neither do I anticipate the discovery of alligatoroids in the Permian.

This approach accepts a wide “reasonable” window, but one could adopt a narrower one. For example, the continental record



during the middle Cretaceous is improving. On phylogenetic grounds, crown-group crocodylians are predicted to have had a Laurasian origin (Brochu, 2001), and the number of fossil localities in the pre-Campanian Cretaceous is increasing—and thus far, none of the crocodyliforms known from this period of time are crown-group crocodylians. For that matter, the Gondwanan record for this period of time is also improving, and thus far most crocodyliforms found in the region are even more distantly related to crown-group crocodylians than are those of the Northern continents (Clark, 1994; Pol, 1999; Carvalho and Bertini, 1999; Buckley et al., 2000; Sereno et al., 2003). On this basis, one could accept a narrower window as reasonable—perhaps the base of the Late Cretaceous (Cenomanian, ~100 m.y.a.). None of the estimates for the nd6 and 16s data sets (two-rate model) have upper confidence limits older than the Barremian, but 5.2 percent of nd6 estimates and 3.7 percent of 16s estimates have upper bounds older than the Cenomanian. Nearly half (44.0 percent) of estimates from the ctrl data set (two-rate model) completely predate the Cenomanian.

Because alligatoroids have a dense fossil record in the Campanian and Maastrichtian, Solow's method (Solow, 2003) would extend the first appearance of Alligatoroidea to no further back than 85 million years. If we accept this as a robust divergence estimate from fossils, then mitochondrial estimates based on two Paleogene calibrations can be rejected as supporting divergence overestimates (Table 2 and Fig. 12). The single nuclear gene used here yields the most consistent results when two Neogene calibrations are used, but the range of estimates is much broader than with most mitochondrial data sets, and like the control region data set, the standard deviation of estimates based on a Neogene and Paleogene calibration approaches the fossil datum more closely than that for the two-Paleogene quartets with other mitochondrial data sets (Fig. 12).

This narrower range of reasonable estimates has implications for how mitochondrial genes, at least, should best be sampled when using quartet methods. Quartets based on widely divergent calibrations, sampling different parts of a clade's history, yield results most in accord with the fossil record. We cannot reject estimates based on two Paleogene calibrations, but assuming the fossil record is preserving a close approximation of the alligatorid-crocodylid divergence, most such quartets are overestimating divergence age.

#### CONCLUSIONS

For many reasons, the results presented here are preliminary. The mitochondrial genes used in this study are very short, and the power of at least some tests for rate consistency is thought to increase as sequence length increases (Bromham et al., 2000). Only a single nuclear gene was used, and analyses were restricted to Crocodylia. It was also restricted to a single method for divergence estimation, and many such methods exist. Future investigations should include longer sequences (especially from the nuclear genome), concatenated sequences, and additional clades with good fossil records.

In all cases, quartets including both a Neogene and a Paleogene calibration resulted in divergence time estimates that are both consistent with the fossil record and reasonable. This is most apparent with the alligatorine-caimanine divergence, but is also true for the alligatorid-crocodylid divergence, depending on the gene being analyzed. The use of two very Neogene calibrations leads to unreasonably (and sometimes impossibly) young divergence estimates. The use of two Paleogene calibrations sometimes results in divergence estimates that are not impossibly old but appear unreasonable.

The optimal sampling strategy, based on the results of this

study, would pair two calibrations of very different age. This minimizes the error associated with later calibrations, but also prevents the analysis from overestimating branch lengths and divergence time estimates, as sometimes appears to be the case when two early calibrations are applied.

Some of the quartet-based analyses of more controversial clades, such as mammalian and avian orders, rely exclusively on calibrations within the Paleogene (e.g., Cooper and Penny, 1996; Eizirik et al., 2001). None have applied calibrations younger than the Eocene, and thus far all support divergence dates deep in the Mesozoic, long predating the abrupt first appearances of these groups in the Early Tertiary. Many explanations have been put forth to explain this disparity, including incompleteness of the fossil record; low diversity of stem lineages followed by adaptive radiations of crown clades, leading to the sudden appearance of clades long after they diverged; and simultaneous evolutionary rate increases at the beginning of the Cenozoic for multiple clades (Bromham et al., 1999; Benton, 2001; Eastal, 2001). If the quartet methods used in these analyses are overestimating branch lengths, the disparity between fossil and molecular divergence time estimates may simply be a reflection of the chosen calibrations. Reliance on early calibrations may lead to divergence overestimation, and future quartet analyses of these groups should consider a wider range of calibration ages.

The results of this analysis also suggest a strategy for increasing calibration age in the face of divergence time underestimation and the lack of information needed to place objective confidence limits on fossil ranges. Minimal changes to earlier calibrations are unlikely to alter the resulting divergence time estimate, but they can have a significant impact on late calibrations. If one believes the later calibrations to be problematic, addition of discrete blocks of time—provided both calibrations are increased—is the most efficient way to improve among-quartet consistency and resolve conflicts between younger divergence estimates and the fossil record. If one does not know which calibrations are misleading, addition of proportional blocks of time will increase the impact of range extension on the earlier calibrations.

At present, decisions to use or modify a particular calibration point are made subjectively. Paleontologists say a given point is "robust," and so it is used; the result from another calibration looks odd, so there must be something wrong with it. In this case, we have the luxury of calibrations of different ages. We can see what happens when we arbitrarily decide to change only the Neogene calibrations, or what happens when we change all calibrations in a certain way. We also have a temporally symmetrical tree, with differently-aged calibrations in both clades. Those working with groups of low diversity but ancient heritage (such as ratites) might not have this option—individual species might be distantly related to their sister species, and late calibrations may not exist. Or one might be working with clades that are themselves recent radiations, but which diverged from each other long ago (e.g., cichlids), eliminating any early calibrations one might want to use. Simply dropping the too-late or too-early calibration points may not always be an option.

Quartet methods, based on this study, are prone to divergence underestimation when later calibrations are too young, but they may also overestimate divergence time if very old calibrations are used. Underestimation is partly solvable by modifying the fossil calibrations, but the solution to overestimation is unlikely to lie in miscalibration—shortening of a calibration age can dramatically decrease divergence estimate age, but this kind of error is growing less likely as phylogenetic analyses progress. The likelihood models used here seem to be overcompensating for branch length underestimation.

The solution lies not only in the continued search for robust

phylogenetic hypotheses of fossils, but in the development of better molecular analytical methods. The results obtained in this study may not apply to some of the more recently developed methods that relax clock assumptions and permit the use of multiple calibration points. For quartet-based approaches, this will involve the development of better evolutionary models. The nature of these improvements will become clearer as more empirical surveys of the method, using different groups of organisms, are completed.

## ACKNOWLEDGMENTS

Many thanks to D. Ray, L. Densmore, and J. Gatesy for access to sequence data, much of which was unpublished when these analyses were underway. Discussions with J. Huelsenbeck, D. Swofford, C. Sumrall, and J. Theodor helped clarify the approaches and interpretations herein, and two anonymous reviewers commented on a previous version of this work.

## REFERENCES

- ADKINS, R. M., A. H. WALTON, AND R. HONEYCUTT. 2003. Higher-level systematics of rodents and divergence time estimates based on two congruent nuclear genes. *Molecular Phylogenetics and Evolution*, 26: 409–420.
- ARBOGAST, B. S., S. V. EDWARDS, J. WAKELEY, P. BEERLI, AND J. B. SLOWINSKI. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic time scales. *Annual Review of Ecology and Systematics*, 33:707–740.
- ARCHIBALD, J. D., AND D. H. DEUTSCHMAN. 2001. Quantitative analysis of the timing of the origin and diversification of extant placental orders. *Journal of Mammalian Evolution*, 8:107–124.
- ARNASON, U., A. GULLBERG, AND A. JANKE. 1998. Molecular timing of primate divergences as estimated by two nonprimate calibration points. *Journal of Molecular Evolution*, 47:718–727.
- BENTON, M. J. 1998. Molecular and morphological phylogenies of mammals: congruence with stratigraphic data. *Molecular Phylogenetics and Evolution*, 9:398–407.
- BENTON, M. J. 2001. Early origins of modern birds and mammals: molecules vs. morphology. *BioEssays*, 21:1043–1051.
- BROCHU, C. A. 1997. Morphology, fossils, divergence timing, and the phylogenetic relationships of *Gavialis*. *Systematic Biology*, 46:479–522.
- BROCHU, C. A. 1999. Phylogeny, systematics, and historical biogeography of Alligatoroidea. *Society of Vertebrate Paleontology Memoir*, 6: 9–100.
- BROCHU, C. A. 2000. Phylogenetic relationships and divergence timing of *Crocodylus* based on morphology and the fossil record. *Copeia*, 2000:657–673.
- BROCHU, C. A. 2001. Congruence between physiology, phylogenetics, and the fossil record on crocodylian historical biogeography, p. 9–28. *In* G. Grigg, F. Seebacher, and C. E. Franklin (eds.), *Crocodylian Biology and Evolution*. Surrey Beatty and Sons, Sydney.
- BROCHU, C. A. 2003. Phylogenetic approaches toward crocodylian history. *Annual Review of Earth and Planetary Sciences*, 31:357–397.
- BROCHU, C. A. In press. Alligatorine phylogeny and the status of *Allognathosuchus* Mook, 1921. *Journal of Vertebrate Paleontology*.
- BROCHU, C. A., AND L. D. DENSMORE. 2001. Crocodile phylogenetics: a review of current progress, p. 3–8. *In* G. Grigg, F. Seebacher, and C. E. Franklin (eds.), *Crocodylian Biology and Evolution*. Surrey Beatty and Sons, Sydney.
- BROCHU, C. A., J. M. THEODOR, AND C. D. SUMRALL. In review. When clocks collide: estimating divergence time from molecules and the fossil record. *Journal of Paleontology*.
- BROMHAM, L., AND M. D. HENDY. 2000. Can fast early rates reconcile molecular dates with the Cambrian explosion? *Proceedings of the Royal Society of London B*, 267:1041–1047.
- BROMHAM, L., M. J. PHILLIPS, AND D. PENNY. 1999. Growing up with dinosaurs: molecular dates and the mammalian radiation. *Trends in Ecology and Evolution*, 14:113–118.
- BROMHAM, L., D. PENNY, A. RAMBAUT, AND M. D. HENDY. 2000. The power of relative rate tests depends on the data. *Journal of Molecular Evolution*, 50:296–301.
- BROMHAM, L., A. RAMBAUT, R. FORTEY, A. COOPER, AND D. PENNY. 1998. Testing the Cambrian explosion hypothesis by using a molecular dating technique. *Proceedings of the National Academy of Sciences of the USA*, 95:12386–12389.
- BRYANT, L. J. 1989. Non-dinosaurian lower vertebrates across the Cretaceous-Tertiary Boundary in northeastern Montana. University of California Publications in Geological Sciences, 134:1–107.
- BUCKLEY, G. A., C. A. BROCHU, D. W. KRAUSE, AND D. POL. 2000. A pug-nosed crocodyliform from the Late Cretaceous of Madagascar. *Nature*, 405:941–944.
- BUSCALIONI, A. D., F. ORTEGA, AND D. VASSE. 1997. New crocodiles (Eusuchia: Alligatoroidea) from the Upper Cretaceous of southern Europe. *Comptes Rendus de l'Académie des Sciences de Paris, Sciences de la Terre et des Planètes*, 325:525–530.
- BUSCALIONI, A. D., F. ORTEGA, D. B. WEISHAMPEL, AND C. M. JIANU. 2001. A revision of the crocodyliform *Allodaposuchus precedens* from the Upper Cretaceous of the Hateg Basin, Romania. Its relevance in the phylogeny of Eusuchia. *Journal of Vertebrate Paleontology*, 21:74–86.
- CARVALHO, I. S., AND R. J. BERTINI. 1999. *Mariliasuchus*: um novo Crocodylomorpha (Notosuchia) do Cretáceo da Bacia Bauru, Brasil. *Geología Colombiana*, 24:83–105.
- CLARK, J. M. 1994. Patterns of evolution in Mesozoic Crocodyliformes, p. 84–97. *In* N. C. Fraser and H.-D. Sues (eds.), *In the Shadow of the Dinosaurs: Early Mesozoic Tetrapods*. Cambridge University Press, New York.
- CLARK, J. M., AND M. A. NORELL. 1992. The Early Cretaceous crocodylomorph *Hylaeochampsia vectiana* from the Wealden of the Isle of Wight. *American Museum Novitates*, 3032:1–19.
- CONROY, C. J., AND M. VAN TUINEN. 2003. Extracting time from phylogenies: positive interplay between fossil and genetic data. *Journal of Mammalogy*, 84:444–455.
- COOPER, A., AND D. PENNY. 1997. Mass survival of birds across the Cretaceous-Tertiary Boundary. *Science*, 275:1109–1113.
- CORNELI, P. S. 2003. Complete mitochondrial genomes and eutherian evolution. *Journal of Mammalian Evolution*, 9:281–305.
- DELGADO, S., D. CASANE, L. BONNAUD, M. LAURIN, J.-Y. SIRE, AND M. GIRONDOT. 2001. Molecular evidence for Precambrian origin of amelogenin, the major protein of vertebrate enamel. *Molecular Biology and Evolution*, 18:2146–2153.
- DENSMORE, L. D. 1983. Biochemical and immunological systematics of the order Crocodylia, p. 397–465. *In* M. K. Hecht, B. Wallace, and G. H. Prance (eds.), *Evolutionary Biology*. Volume 16. Plenum Press, New York.
- DENSMORE, L. D., AND R. D. OWEN. 1989. Molecular systematics of the order Crocodylia. *American Zoologist*, 29:831–841.
- DENSMORE, L. D., AND P. S. WHITE. 1991. The systematics and evolution of the Crocodylia as suggested by restriction endonuclease analysis of mitochondrial and nuclear ribosomal DNA. *Copeia*, 1991:602–615.
- DYKE, G. J. 2001. The evolutionary radiation of modern birds: systematics and patterns of diversification. *Geological Journal*, 36:305–315.
- EASTEAL, S. 2001. Molecular evidence for the early divergence of placental mammals. *BioEssays*, 21:1052–1058.
- EIZIRIK, E., W. J. MURPHY, AND S. J. O'BRIEN. 2001. Molecular dating and biogeography of the early placental mammal radiation. *Journal of Heredity*, 92:212–219.
- FEDUCCIA, A. 1995. Explosive radiation in Tertiary birds and mammals. *Science*, 267:637–638.
- FELSENSTEIN, J. 2003. *Inferring Phylogenies*. Sinauer Associates, Sunderland, Massachusetts, 580 p.
- GASPARINI, Z. 1996. Biogeographic evolution of the South American crocodylians. *Münchner Geowissenschaftliche Abhandlungen*, 30:159–184.
- GATESY, J., R. D. SALLE, AND W. WHEELER. 1993. Alignment-ambiguous nucleotide sites and the exclusion of systematic data. *Molecular Phylogenetics and Evolution*, 2:152–157.
- GATESY, J., G. AMATO, M. NORELL, R. DESALLE, AND C. HAYASHI. 2003. Combined support for wholesale taxic atavism in gavialine crocodylians. *Systematic Biology*, 52:403–422.
- GINGERICH, P. D. 1986. Temporal scaling of molecular evolution in primates and other mammals. *Molecular Biology and Evolution*, 3:205–221.
- GLAZKO, G. V., AND M. NEL. 2003. Estimation of divergence times for

- major lineages of primate species. *Molecular Biology and Evolution*, 20:424–434.
- GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Systematic Biology*, 47:9–17.
- HARING, E., L. KRUCKENHAUSER, A. GAMAUF, M. J. RIESING, AND W. PINSKER. 2001. The complete sequence of the mitochondrial genome of *Buteo buteo* (Aves, Accipitridae) indicates an early split in the phylogeny of raptors. *Molecular Biology and Evolution*, 18:1892–1904.
- 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.
- HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 21:160–174.
- HASS, C. A., M. A. HOFFMAN, L. D. DENSMORE, AND L. R. MAXSON. 1992. Crocodylian evolution: insights from immunological data. *Molecular Phylogenetics and Evolution*, 1:193–201.
- HOLLAND, S. M., AND M. E. PATZKOWSKY. 2002. Stratigraphic variation in the timing of first and last occurrences. *Palaios*, 17:134–146.
- HUA, S., AND S. JOUVE. In press. A primitive gavialoid from the Paleocene of Morocco. *Journal of Vertebrate Paleontology*.
- HUCHON, D., F. M. CATZEFLIS, AND E. J. P. DOUZERY. 2000. Variance of molecular datings, evolution of rodents and the phylogenetic affinities between Ctenodactylidae and Hystricognathi. *Proceedings of the Royal Society of London B*, 267:393–402.
- HUCHON, D., O. MADSEN, M. J. J. B. SIBBALD, K. AMENT, M. J. STANHOPE, F. M. CATZEFLIS, W. W. DE JONG, AND E. J. P. DOUZERY. 2002. Rodent phylogeny and a timescale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. *Molecular Biology and Evolution*, 19:1053–1065.
- HUELSENBECK, J. P., B. LARGET, AND D. L. SWOFFORD. 2000. A compound Poisson process for relaxing the molecular clock. *Genetics*, 154:1879–1892.
- KAWASHITA, S. Y., G. F. O. SANSON, O. FERNANDES, B. ZINGALES, AND M. R. S. BRIONES. 2001. Maximum-likelihood divergence date estimates based on rRNA gene sequences suggest two scenarios of *Trypanosoma cruzi* intraspecific evolution. *Molecular Biology and Evolution*, 18:2250–2259.
- LANGSTON, W. 1965. Fossil crocodylians from Colombia and the Cenozoic history of the Crocodylia in South America. University of California Publications in Geological Sciences, 52:1–152.
- LEE, M. S. Y. 1999. Molecular clock calibrations and metazoan divergence dates. *Journal of Molecular Evolution*, 49:385–391.
- MALONE, B. 1979. The systematics, phylogeny and paleobiology of the genus *Alligator*. Ph.D. dissertation, City University of New York, 159 p.
- MARKWICK, P. J. 1998. Crocodylian diversity in space and time: the role of climate in paleoecology and its implication for understanding K/T extinctions. *Paleobiology*, 24:470–497.
- MARSHALL, C. R. 1990a. Confidence intervals on stratigraphic ranges. *Paleobiology*, 16:1–10.
- MARSHALL, C. R. 1990b. The fossil record and estimating divergence times between lineages: maximum divergence times and the importance of reliable phylogenies. *Journal of Molecular Evolution*, 30:400–408.
- MARSHALL, C. R. 1997. Confidence intervals on stratigraphic ranges with nonrandom distributions of fossil horizons. *Paleobiology*, 23:165–173.
- MEDINA, C. J. 1976. Crocodylians from the Late Tertiary of northwestern Venezuela: *Melanosuchus fisheri* sp. nov. *Breviora*, 438:1–14.
- NEI, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- NEI, M., P. XU, AND G. V. GLAZKO. 2001. Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. *Proceedings of the National Academy of Sciences of the U. S. A.*, 98:2497–2502.
- NORELL, M. A. 1989. The higher level relationships of the extant Crocodylia. *Journal of Herpetology*, 23:325–335.
- NORMAN, J. E., AND M. V. ASHLEY. 2000. Phylogenetics of Perissodactyla and tests of the molecular clock. *Journal of Molecular Evolution*, 50:11–21.
- OWEN, R. 1850. Monograph on the Fossil Reptilia of the London Clay, and of the Bracklesham and Other Tertiary Beds, Part II: Crocodylia (*Crocodylus*, etc.). Paleontographical Society, London, 50 p.
- PERSSON, P. O. 1960. Reptiles from the Senonian (U. Cret.) of Scania (S. Sweden). *Arkiv för Mineralogi och Geologi*, 2:431–478.
- POE, S. 1997. Data set incongruence and the phylogeny of crocodylians. *Systematic Biology*, 45:393–414.
- POL, D. 1999. Basal mesoeucrocodylian relationships: new clues to old conflicts. *Journal of Vertebrate Paleontology*, 19:69A.
- RAMBAUT, A., AND L. BROMHAM. 1998. Estimating divergence data from molecular sequences. *Molecular Biology and Evolution*, 15:442–448.
- RAY, D. A., AND L. D. DENSMORE. 2002. The crocodylian mitochondrial control region: general structure, conserved sequences, and evolutionary implications. *Journal of Experimental Zoology*, 294:334–346.
- RAY, D. A., AND L. D. DENSMORE. 2003. Repetitive sequences in the crocodylian mitochondrial control region: Poly-A sequences and heteroplasmic tandem repeats. *Molecular Biology and Evolution*, 20:1006–1013.
- RAY, D. A., P. S. WHITE, H. V. DUONG, T. CULLEN, AND L. D. DENSMORE. 2001. High levels of genetic variability in West African dwarf crocodyles *Osteolaemus tetraspis tetraspis*, p. 58–63. In G. Grigg, F. Seebacher, and C. E. Franklin (eds.), *Crocodylian Biology and Evolution*. Surrey Beatty and Sons, Sydney.
- RODRIGUEZ-TRELLES, F., R. TARRIO, AND F. J. AYALA. 2002. A methodological bias toward overestimation of molecular evolutionary time scales. *Proceedings of the National Academy of Sciences of the U. S. A.*, 99:8112–8115.
- SALISBURY, S. W., AND P. M. A. WILLIS. 1996. A new crocodylian from the Early Eocene of southeastern Queensland and a preliminary investigation of the phylogenetic relationships of crocodyloids. *Alcheringa*, 20:179–227.
- SANDERSON, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution*, 14:1218–1231.
- SANDERSON, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, 19:101–109.
- SCHWIMMER, D. R. 2002. King of the Crocodylians: The Paleobiology of *Deinosuchus*. Indiana University Press, Bloomington, 220 p.
- SERENO, P. C., C. A. SIDOR, H. C. E. LARSSON, AND B. GADO. 2003. A new notosuchian from the Early Cretaceous of Niger. *Journal of Vertebrate Paleontology*, 23:477–482.
- SMITH, A. B. 2001. Large-scale heterogeneity of the fossil record: implications for Phanerozoic biodiversity studies. *Philosophical Transactions of the Royal Society of London*, 356:351–367.
- 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.
- SMITH, A. B., A. S. GALE, AND N. E. A. MONKS. 2001. Sea-level change and rock record bias in the Cretaceous: a problem for extinction and biodiversity studies. *Paleobiology*, 27:241–253.
- SNYDER, D. 2003. New *Alligator* remains from the Miocene of Florida and notes on *Alligator* phylogeny. *Journal of Vertebrate Paleontology*, 23:99A.
- SOLOW, A. R. 2003. Estimation of stratigraphic ranges when fossil finds are not randomly distributed. *Paleobiology*, 29:181–185.
- SOLTIS, P. S., D. E. SOLTIS, V. SAVOLAINEN, P. R. CRANE, AND T. G. BARRACLOUGH. 2002. Rate heterogeneity among lineages of tracheophytes: integration of molecular and fossil data and evidence for molecular living fossils. *Proceedings of the National Academy of Sciences of the U. S. A.*, 99:4430–4435.
- SPRINGER, M. S. 1995. Molecular clocks and the incompleteness of the fossil record. *Journal of Molecular Evolution*, 41:531–538.
- SPRINGER, M. S. 1997. Molecular clocks and the timing of the placental and marsupial radiations in relation to the Cretaceous-Tertiary boundary. *Journal of Mammalian Evolution*, 4:285–302.
- SPRINGER, M. S., W. J. MURPHY, E. EIZIRIK, AND S. J. O'BRIEN. 2003. Placental mammal diversification and the Cretaceous-Tertiary boundary. *Proceedings of the National Academy of Sciences of the U. S. A.*, 100:1056–1061.
- SPRINGER, M. S., R. W. DEBRY, C. DOUADY, H. M. AMRINE, O. MADSEN, W. W. DE JONG, AND M. J. STANHOPE. 2001. Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. *Molecular Biology and Evolution*, 18:132–143.
- STEEL, M. A., A. C. COOPER, AND D. PENNY. 1996. Confidence intervals



- for the divergence time of two clades. *Systematic Biology*, 45:127–134.
- STRAUSS, D., AND P. M. SADLER. 1989. Classical confidence intervals and Bayesian probability estimates for ends of local taxon ranges. *Mathematical Geology*, 21:411–427.
- STROMER, E. 1925. Ergebnisse der Forschungsreisen Prof. E. Stromers in den Wüsten Ägyptens, II: Wirbeltier-Reste der Baharije-Stufe (Unterestes Cenoman), 7: *Stomatosuchus inermis* Stromer, ein schwach bezahnter Krokodilier. *Abhandlungen der Bayerischen Akademie der Wissenschaften Mathematisch-naturwissenschaftliche Abteilung*, 30:1–9.
- STROMER, E. 1933. Ergebnisse der Forschungsreisen Prof. E. Stromers in den Wüsten Ägyptens. 12: Die Procölen Crocodilia. *Abhandlungen der Bayerischen Akademie der Wissenschaften Mathematisch-naturwissenschaftliche Abteilung*, N. F., 15:1–55.
- SWINTON, W. E. 1937. The crocodile of Maransart (*Dollosuchus dixonii* [Owen]). *Mémoires du Musée Royal d'Histoire Naturelle de Belgique*, 80:1–46.
- SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic inference, p. 407–514. *In* D. M. Hillis, C. Moritz, and B. K. Mable (eds.), *Molecular Systematics* (second edition). Sinauer Associates, Sunderland, Massachusetts.
- THORNE, J. L., AND H. KISHINO. 2002. Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, 51:689–702.
- THORNE, J. L., H. KISHINO, AND I. S. PAINTER. 1998. Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution*, 15:1647–1657.
- VAN TUINEN, M., C. G. SIBLEY, AND S. B. HEDGES. 2000. The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. *Molecular Biology and Evolution*, 17:451–457.
- 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.
- WAYNE, R. K., B. VAN VALKENBURGH, AND S. J. O'BRIEN. 1991. Molecular distance and divergence time in carnivores and primates. *Molecular Biology and Evolution*, 8:297–319.
- WHITE, P. S., AND L. D. DENSMORE. 2001. DNA sequence alignments and data analysis methods: their effect on the recovery of crocodylian relationships, p. 29–37. *In* G. Grigg, F. Seebacher, and C. E. Franklin (eds.), *Crocodylian Biology and Evolution*. Surrey Beatty and Sons, Sydney.
- WILLIAMSON, T. E. 1996. ?*Brachychampsia sealeyi*, sp. nov., (Crocodylia, Alligatoroidea) from the Upper Cretaceous (lower Campanian) Menefee Formation, northwestern New Mexico. *Journal of Vertebrate Paleontology*, 16:421–431.
- WILLIS, P. M. A., R. E. MOLNAR, AND J. D. SCANLON. 1993. An early Eocene crocodylian from Murgon, southeastern Queensland. *Kaupia*, 3: 27–33.
- WRAY, G. A. 2001. Dating branches on the Tree of Life using DNA. *Genome Biology*, 3:1–7.
- WU, X.-C., A. P. RUSSELL, AND D. B. BRINKMAN. 2001. A review of *Leidyosuchus canadensis* Lambe, 1907 (Archosauria: Crocodylia) and an assessment of cranial variation based upon new material. *Canadian Journal of Earth Sciences*, 38:1665–1687.
- YODER, A. D., AND Z. YANG. 2000. Estimation of primate speciation dates using local molecular clocks. *Molecular Biology and Evolution*, 17:1081–1090.

ACCEPTED 15 AUGUST 2003