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Impacts of the Cretaceous Terrestrial Revolution and KPg Extinction on Mammal Diversification

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Previous analyses of relations, divergence times, and diversification patterns among extant mammalian families have relied on supertree methods and local molecular clocks. We constructed a molecular supermatrix for mammalian families and analyzed these data with likelihood-based methods and relaxed molecular clocks. Phylogenetic analyses resulted in a robust phylogeny with better resolution than phylogenies from supertree methods. Relaxed clock analyses support the long-fuse model of diversification and highlight the importance of including multiple fossil calibrations that are spread across the tree. Molecular time trees and diversification analyses suggest important roles for the Cretaceous Terrestrial Revolution and Cretaceous-Paleogene (KPg) mass extinction in opening up ecospace that promoted interordinal and intraordinal diversification, respectively. By contrast, diversification analyses provide no support for the hypothesis concerning the delayed rise of present-day mammals during the Eocene Period.

The ~5400 described species of living mammals evolved to occupy diverse ecological niches and include arboreal, fossorial, volant, aquatic, and terrestrial forms—some of which exhibit 100 million-fold differences in body mass (1, 2). Mammals exhibit striking examples of ecomorphological convergence that has led to contentious debates in modern systematics (3–5). The diversity of living and extinct mammalian species is documented by the fossil record of ~220 million years and has evolved against the backdrop of radical alterations in terrestrial floras during the Cretaceous Terrestrial Revolution (KTR),

the Cretaceous-Paleogene (KPg) mass extinction, continental rearrangements, and changes in key environmental parameters, such as average global temperature. However, the impact of these drivers on taxonomic diversification, particularly near the KPg boundary, remains controversial (6–8).

Previous molecular studies have elucidated mammalian interordinal relations (9–11). One study (8) that examined relations and divergence times among all living mammalian families used matrix representation with parsimony (MRP) supertrees and was compromised by including numerous source phylogenies with overlapping data (12, 13). The supertree study (8) proposed that there was a dramatic upturn in diversification rates in the Eocene ~55 to 50 million years ago (Ma), but this hypothesis was inferred from a topology that contained numerous polytomies and was dated with a combination of local molecular clocks and pure birth interpolation for internal nodes. Even with these limitations, this time tree (8) underpins numerous studies in comparative biology (14–17). Here, we report an analysis of relations, divergence times, and diversification patterns among 97 to 99% of mammalian families (1, 2) on the basis of a molecular supermatrix that includes 164 mammals, five outgroups, and 26 gene fragments (tables S1 and S2). The resulting DNA and protein alignments comprise 35,603 base pairs (bp) and 11,010 amino acids, respectively. Divergence time estimates from molecular data used a large assemblage of fossil calibrations (table S3).

Phylogenetic relations from maximum likelihood (ML) and Bayesian methods are well resolved across the mammalian tree. More than 90% of the nodes have bootstrap (BS) support of $\geq 90\%$ and Bayesian posterior probabilities

(BPP) of ≥ 0.95 (Fig. 1, figs. S1 to S4, and table S4). Amino acid and DNA ML trees are in agreement for 163 out of 168 internal nodes (figs. S1 to S4). The MRP supertree (8) failed to recover ~30% of our well-supported nodes (Fig. 1). These disagreements occur in some of the most speciose mammalian clades, including bats, rodents, and carnivorans, and may potentially affect the conclusions of numerous studies that have relied on the MRP topology. Our phylogeny improves upon previous resolution (8) and provides a character matrix-based framework for reevaluating early mammalian divergence times.

Results derived from coalescence methods (18, 19) were broadly similar to ML and Bayesian supermatrix methods but, in some cases, failed to recover well-substantiated clades such as Amniota, Haplorhini, and Odontoceti (13) (figs. S5 to S8). Coalescence methods assume that discrepancies between individual gene trees and the species tree are solely the result of incomplete lineage sorting, but our results suggest otherwise and highlight difficulties of applying coalescence methods to deep-level phylogenetic problems where differences between individual gene trees often result from problems such as long branch attraction (13).

Rates of molecular evolution range over an order of magnitude for mammalian lineages (20, 21) and present an exceptional challenge for estimating divergence times. Mammals also have a fossil record that provides numerous constraints for calibrating relaxed clocks (22). Accordingly, we selected minimum and maximum constraints for 82 different nodes (table S3). Unlike previous studies (8–11), outgroup representation in our analyses provided well-constrained fossil calibrations that precede mammalian diversification and allowed us to bracket controversial interordinal divergences with both older and younger calibrated nodes. Further, we used relaxed clock molecular dating methods that utilized eight different combinations of molecule type (DNA versus amino acids), evolutionary rate (autocorrelated versus independent rates), and hard- versus soft-bounded constraints.

Molecular time-tree analyses that used subsets of constraints that were either temporally restricted (deep versus shallow nodes) or topologically confined to groups with fast (rodents) or slow (cetaceans) rates of molecular evolution resulted in poor estimates of divergence times that are in direct conflict with the fossil record (13) (table S5). For example, the fossil record provides robust support for the origin of crown-group mysticetes (baleen whales) no later than 20.4 Ma (23), but soft-bounded analyses with only rodent constraints suggested an age as young as 4 million years for Mysticeti. These results demonstrate that lineage-specific rate variation can have severe effects on resulting divergence dates when fossil calibrations are sparse and/or unevenly distributed throughout the tree and further suggest that appropriate caution should accompany molecular time-tree analyses for taxonomic groups

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with extensive rate variation and a poor fossil record.

By contrast, all eight relaxed clock analyses that used the full suite of fossil constraints yielded divergence time estimates that are largely consistent with each other and with the fossil record (tables S6 and S7). The mean date for the split between placentals and marsupials is ~190 Ma (table S6) and accords well with the discovery of a stem eutherian from the Jurassic Period (24). Our analyses suggest that only 29 to 32 mammalian lineages, nearly all of which are stem branches leading to extant orders, may have crossed the KPg boundary (Fig. 1 and table S8). Several orders have point estimates of basal diversification that precede the KPg, but only in the case of Eulipotyphla are the composite credibility intervals entirely within the Cretaceous (Table 1 and Fig. 2A). This inference is consistent with the long-fuse model of mammalian diversification (10, 25), which postulates interordinal diversification in the Cretaceous, followed by intraordinal diversification that is mostly restricted to the Cenozoic (Fig. 2A), although conflicts do remain with the Cretaceous eutherian fossil record (7). By contrast, the MRP supertree analysis (8) estimated that ~50% more placental lineages with extant descendants survived the mass extinction at the end of the Cretaceous and also recovered confidence intervals for basal cladogenic events in seven placental orders that are entirely in the Cretaceous.

Taxon sampling in our data set was chosen to index deeper nodes in Mammalia and, with minor augmentation, is complete or nearly so for lineages with extant descendants that diversified in the Cretaceous, Paleocene, and Eocene up through the end of the Lutetian (40.4 Ma, middle Eocene) (13) (table S9). Lineage-through-time (LTT) plots from molecular time trees were analyzed with two statistical methods to detect diversification rate shifts (Fig. 2, B and C) (13, 17, 26).

Analyses with both DNA and amino acid time trees identified rate increases in the Cretaceous, either at ~100 Ma and/or ~83 Ma, followed by a rate decrease at ~78 Ma [Akaike information criterion (AIC) score for the best-fit rate-constant model of diversification (ΔAIC_{RC}) test, table S10]. This increase in the mammalian diversification rate corresponds to interordinal cladogenesis and is apparent in diversification plots (Fig. 2D). This is consistent with Benton's hypothesis that the KTR (125 to 80 Ma), during which the angiosperm component of floras increased from 0 to 80%, was a key event in the diversification of mammals and birds (27).

In contrast, there was no statistical support for a rate increase at or near the KPg boundary. However, the findings that basal cladogenesis for the majority of orders occurred in the Cenozoic and that only one order has a 95% credibility interval that is entirely restricted to the Cretaceous support the hypothesis (28) that modern mammalian orders originated or only acquired their definitive ordinal characteristics after the KPg mass extinction, which resulted in the final demise of nonavian dinosaurs. Whereas previous molecular studies have suggested intraordinal divergences as far back as the early Cretaceous, our results are in better agreement with the fossil record (7) and suggest that we are unlikely to find crown rodents or primates in rocks that are much older than the latest Cretaceous (base of the Maastrichtian, 70.6 Ma).

The delayed rise of present-day mammals hypothesis (8) suggests that diversification rates in the direct ancestors of extant mammals were relatively low in the Paleocene before increasing in the Eocene, possibly in response to abiotic drivers (Paleocene-Eocene thermal maximum, early Eocene climatic optimum) and/or the extinction of Paleocene species that had previously inhibited the ancestors of present-day mammals (8). Our results contradict this hypothesis. No significant rate increases were detected during the early or middle Eocene (Fig. 2). Note that diversification rates in the ancestors of present-day mammals were not repressed by Paleocene lineages that subsequently went extinct (8).

The results reported here provide a robust molecular phylogeny for mammalian families and a solid foundation for resolving the remainder of the mammalian hierarchy below the family level. Molecular time-tree analyses based on this phylogeny and a comprehensive set of fossil constraints resulted in divergence time estimates that contradict the hypothesis of the delayed rise of placental mammals. Rather, our results are consistent with the hypothesis that both the KTR and the KPg mass extinction played important roles in the early diversification and adaptive radiation of mammals. The KTR increased ecospace diversity, possibly precipitating interordinal diversification, whereas the KPg mass extinction made more of this ecospace available for mammals, promoting the emergence of crown-group orders with their distinctive morphological adaptations.

Table 1. Divergence times estimated in this study for major ordinal and superordinal groups and comparison to the results of the MRP supertree (8). The 95% composite credibility intervals are listed in parentheses. Asterisks denote orders. Indentations in taxon names denote hierarchical relations. Mean difference is of absolute values.

| Taxon | Supermatrix mean div. time | MRP (8) div. time | Difference |
|-----------------------------------|----------------------------|---------------------|-------------|
| Mammalia | 217.8 (203.3–238.2) | 166.2 (Fixed) | –51.6 |
| Monotremata* | 36.7 (22.4–103.1) | 63.6 (52.2–75.0) | 26.9 |
| Theria | 190.0 (167.2–215.3) | 147.4 (141.8–153.1) | –42.6 |
| Marsupialia | 81.8 (67.9–97.2) | 82.5 (71.4–93.7) | 0.7 |
| Paucituberculata* | 11.7 (7.2–16.2) | 33.2 (26.3–40.1) | 21.5 |
| Didelphimorphia* | 31.4 (23.0–38.4) | 56.2 (45.9–67.7) | 24.8 |
| Peramelemorphia* | 28.0 (21.1–37.1) | 36.2 (30.6–41.8) | 8.2 |
| Dasyuromorphia* | 30.0 (22.1–41.7) | 31.3 (16.2–55.8) | 1.3 |
| Diprotodontia* | 52.8 (42.4–64.0) | 54.1 (52.4–55.8) | 1.3 |
| Placentalia | 101.3 (92.1–116.8) | 98.5 (93.2–108.1) | –2.8 |
| Xenarthra* | 65.4 (58.4–71.5) | 70.5 (65.6–75.4) | 5.1 |
| Afrotheria | 80.9 (74.4–96.5) | 90.4 (87.3–93.6) | 9.5 |
| Afrosoricida* | 68.2 (56.8–88.0) | 82.4 (78.3–86.4) | 14.2 |
| Macroscelidea* | 49.1 (37.7–57.2) | 47.5 (39.9–55.6) | –1.6 |
| Paenungulata | 64.3 (56.0–70.6) | 75.8 (72.4–79.2) | 11.5 |
| Hyracoidea* | 6.1 (3.9–8.3) | 18.6 (17.8–19.5) | 12.5 |
| Proboscidea* | 5.3 (1.8–8.0) | 19.5 (7.6–31.4) | 14.2 |
| Sirenia* | 31.4 (25.0–34.4) | 52.2 (37.9–66.5) | 20.8 |
| Boreoeutheria | 92.0 (82.9–107.6) | 96.1 (92.9–98.4) | 4.1 |
| Laurasiatheria | 84.6 (78.5–93.0) | 87.8 (85.0–90.5) | 3.2 |
| Eulipotyphla* | 77.3 (70.7–85.8) | 82.5 (79.8–85.3) | 5.2 |
| Chiroptera* | 66.5 (62.3–71.3) | 71.2 (68.0–74.3) | 4.7 |
| Perissodactyla* | 56.8 (55.1–61.0) | 55.8 (51.1–60.5) | –1.0 |
| Pholidota* | 25.3 (16.9–35.7) | 19.1 (7.3–46.9) | –6.2 |
| Carnivora* | 54.7 (47.4–60.6) | 63.4 (59.8–67.1) | 8.7 |
| Cetartiodactyla* | 65.4 (62.3–68.5) | 70.7 (67.6–73.7) | 5.3 |
| Euarchontoglires | 83.3 (74.1–97.8) | 91.8 (90.0–93.8) | 8.5 |
| Primateomorpha | 82.0 (73.7–97.4) | 88.5 (85.9–91.0) | 6.5 |
| Primates* | 71.5 (64.3–78.4) | 84.5 (81.9–87.1) | 13.0 |
| Dermoptera* | 7.4 (4.5–13.2) | 15.0 (10.2–19.9) | 7.6 |
| Scandentia* | 55.9 (45.0–63.9) | 31.7 (29.9–34.7) | –24.2 |
| Glires | 79.5 (71.5–94.1) | 88.9 (87.8–90.1) | 9.4 |
| Rodentia* | 69.0 (64.1–74.8) | 82.8 (80.2–85.4) | 13.8 |
| Lagomorpha* | 50.2 (47.4–56.9) | 64.3 (60.0–68.7) | 14.1 |
| Mean difference for orders | | | 11.1 |

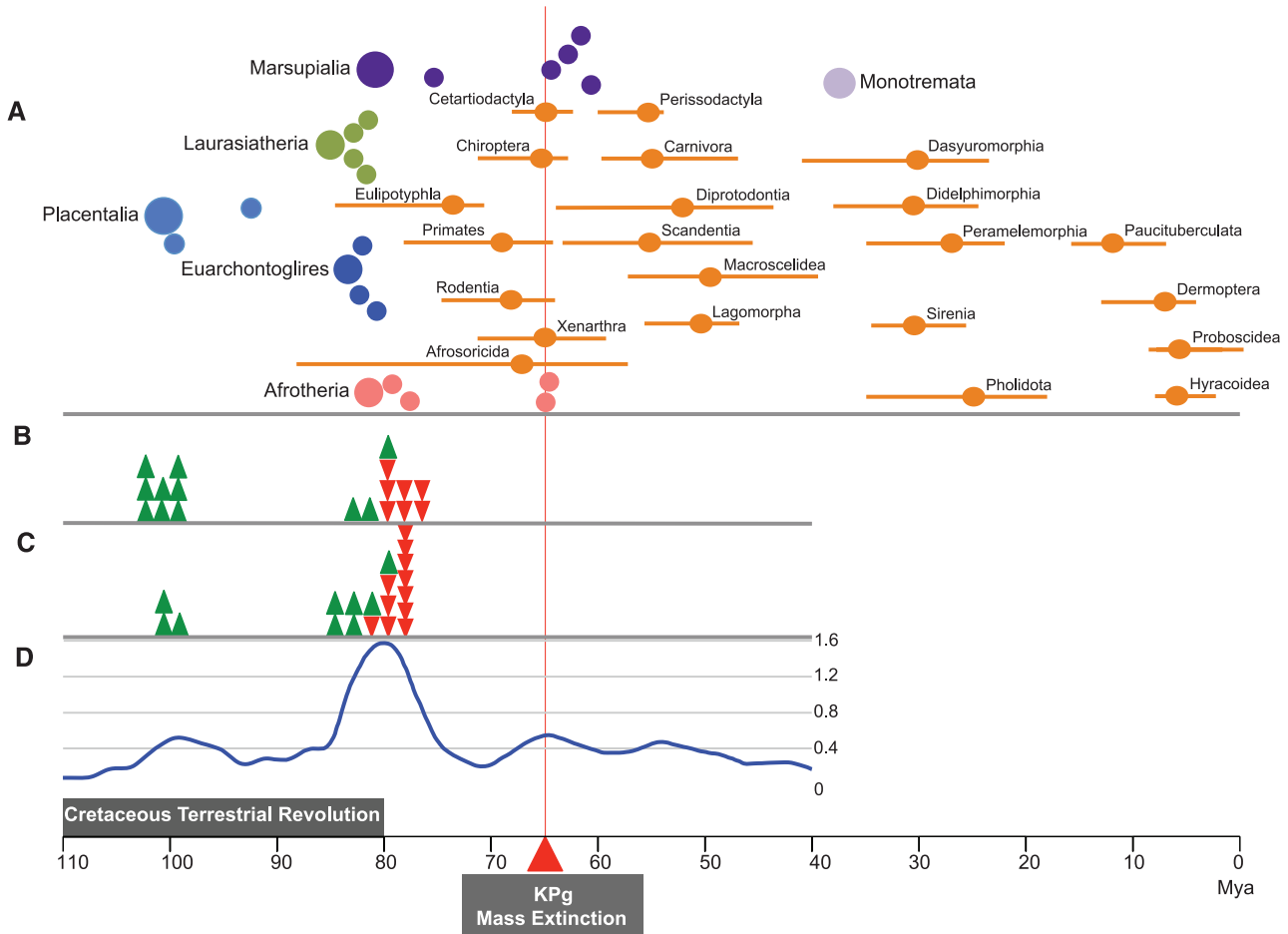


Fig. 2. Time scale of major mammalian divergence events during the past 110 My. **(A)** Colored circles represent cladogenic events leading to the four major groups of placental mammals (light blue) and interordinal splits within Euarchontoglires (dark blue), Laurasiatheria (green), Afrotheria (pink), and Marsupialia (dark purple). The split between ornithorhynchids and tachyglyssids is shown in light purple (Monotremata). Basal divergences within crown-group placental and marsupial orders are shown in orange (also see Table 1). For ordinal divergences, 95% composite credibility intervals are shown as hori-

zontal bars. Basal cladogenesis occurred near or after the KPg mass extinction event, ~65.5 Ma, for almost all orders. Diversification rate shifts detected by **(B)** TreePar and **(C)** likelihood analysis of speciation and extinction rates (LASER) are denoted with green (rate increase) and red (rate decrease) arrows for rate shifts that were identified in analyses with both amino acid and DNA time trees (table S10). **(D)** Sliding window analysis of the net diversification rate (originations, lineage, and window) is based on mean divergence estimates from eight different analyses.

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Acknowledgments: Supported by NSF (EF0629860 to M.S.S. and J.G.; EF0629849 to W.J.M., R.L.H. and T.L.W.), Science Foundation Ireland PIYRA 06/Y13/B932 to E.C.T., CNPq-Brazil to E.E., and South African National Research Foundation to T.J.R. New GenBank accession numbers: JN413803 to JN415086 and JN632710 to JN633870. TreeBase ID: S11872. The Zoological Society of San Diego provided samples for this project under materials transfer agreements as indicated in Materials and Methods. Requests for materials should be directed to authors and/or institutions as stipulated in Materials and Methods.

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 12 July 2011; accepted 9 September 2011
 Published online 22 September 2011;
 10.1126/science.1211028