0.2 m   Paleogene   Cretaceous

What caused the K-Pg mass extinction? The Cretaceous-Paleogene (K-Pg) boundary section in the Bottacione gorge northeast of Gubbio, Italy. Alvarez *et al*. first described elevated iridium concentrations in the red boundary clay (*3*). These results formed the basis of the impact hypothesis for the K-Pg mass extinction. Renne *et al*. now show that the Chicxulub event and K-Pg boundary coincided precisely.

argon isotope ratios in sanidine crystals found in volcanic ash layers. Recent studies have shown why the argon isotope method always yielded slightly younger ages than the U/Pb method (*6*, *7*). The results from the two methods can now be compared directly to improve accuracy and confidence.

Renne *et al*. show that combining multiple U/Pb and argon isotope system measurements from several stratigraphic horizons yields ages of 66.038 ± 0.049 million years for the Chicxulub impact tektites, and 66.043 ± 0.043 million years for the K-Pg boundary itself. These ages are indistinguishable from each other with a much reduced uncertainty of 32,000 years and suggest that the Chicxulub impact event coincides with the K-Pg boundary. The results also falsify the hypothesis that the Chicxulub impact predated the K-Pg boundary by ~300,000 years (*8*).

Renne *et al*.'s results also have important implications for another geological dating method that relies on matching time series of climatic reconstructions to variations in Earth's orbital motion (*9*). The orbital motions can be computed by astronomical theory and observation (*10*), but the precise orbital configuration becomes uncertain in the more distant past because of the chaotic nature of the solar system. Beyond 50 million years ago, only one orbital motion—the 405,000-year orbital eccentricity cycle—can be computed with enough accuracy for a reliable astronomical calibration of the geological record (*11*). Previous studies agree that the K-Pg boundary is situated near a minimum of a 405,000-year eccentricity cycle but disagree on whether it is placed at ~65.28, ~65.68, or ~66.08 million years ago (*7*, *12*–*15*). Renne *et al*.'s dating firmly supports the third option and could thus settle an important question about the astronomical calibration of Paleogene time.

Dating individual points along the sequence of events around the K-Pg boundary also provides further insights into the ecological and chemical evolution around this event. The dating suggests that in Montana, the vertebrate fauna recovered much faster after the mass extinction than previously thought, perhaps within 20,000 years. Renne *et al*. suggest that the faunal recovery occurred by migration from refugia rather than by evolutionary radiation.

Does this study finally close the debate about the cause of the demise of the dinosaurs? Not quite yet. Renne *et al*. suggest that brief cold snaps in the late Cretaceous were stressful to an ecology adapted to the long-lived preceding hothouse climate, and that the Chicxulub impact delivered a final coup de grâce to ecosystems, shifting the planet permanently into a new state. The next task will be to use the improved dating methodologies to precisely date the largest individual magmatic events during Deccan flood basalt formation. These dates will help to evaluate the role volcanism played in the initial onset of environmental and biotic change prior to the K-Pg boundary.

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EVOLUTION

Fossils Versus Clocks

Anne D. Yoder

An extensive morphological analysis supports the conclusion that ancestors of placental mammals evolved rapidly in the Cenozoic.

It's a great story, and one that most of us learned in grade school. Dinosaurs ruled Earth for eons, shaking the ground beneath them as their colossal forms roamed the dense tropical forests of the Mesozoic. Mammals were present but were minuscule by comparison, skulking about in the undergrowth as they foraged for insects. And so it went until a massive asteroid hit Earth about 66 million years ago, causing environmental havoc, climate change, and the worldwide extinction of non-avian dinosaurs [the Cretaceous-Paleogene (K-Pg) extinction event] (*1*). Only then did mammals begin to flourish and diversify into the myriad forms of today. It is a compelling tale, but one where timing is everything. On page 662 of this issue,

Department of Biology, Duke University, Durham, NC 27708, USA. E-mail: anne.yoder@duke.edu

O'Leary *et al*. (*2*) offer a fresh perspective on the pattern and timing of mammalian evolution drawn from a remarkable arsenal of morphological data from fossil and living mammals (see the figure).

The fossil record has always lent veracity to the classical account. Sediments from the Mesozoic (~250 million to 66 million years ago) are rich with the fossils of dinosaurs, both large and small, and less rich with mammals that were for the most part unlike those we see today (*3*). The record changes abruptly with the beginning of the Cenozoic (since ~66 million years ago), but it is not until about 55 million years ago that we begin to clearly see placental mammal lineages (*4*). Why did virtually all placental groups—such as primates, bats, ungulates, and whales—appear so abruptly in the fossil record? Where are the transitional forms that must link the diminu-

tive insectivores of the Mesozoic to today's multitude of mammals?

In the 1990s, molecular phylogenetic studies yielded divergence ages far older than the fossil evidence indicated (5). In the ensuing debate among molecular phylogeneticists and paleontologists, the classical Cenozoic rapid-radiation hypothesis was renamed the explosive model. Scenarios supported by the molecular divergence dates were termed short-fuse and long-fuse models, depending on whether they purported that extant clades began their evolutionary bursts long before (short-fuse) or just before (long-fuse) the start of the Cenozoic (6).

Given the notoriously incomplete nature of the fossil record, the revised and much older dates gained traction. The older dates were also attractive for their biogeographic implications. About 200 million years ago, the supercontinent Pangea began to split apart in a process that neared completion about 52 mil-lion years ago. With dates as old as 100 million years ago, hypothesized by the molecular clock studies, the placental radiation could be explained by continental breakup (7). Taxonomy followed suit, with major clades named for the landmasses on which they were postulated to have evolved (8).

O'Leary *et al.* now shed doubt on this sequence of events. They have used cladistic methods to reconstruct the characteristics of a hypothesized placental ancestor that evolved soon after the K-Pg event and after the complete sundering of the ancient supercontinents. This ancestral mammal is inferred to be the predecessor of the explosive radiation of placental mammals at ~60 million years ago, thus requiring long-distance dispersal, perhaps over large bodies of water, to explain the present-day distribution of placental species.

Today, sophisticated theoretical and computational methods are used to estimate and calibrate molecular phylogenetic branch lengths (which represent time) (9). Together with improved methods for integrating fossil and molecular data (10), dates derived from molecular phylogenies have inched closer to those implied by the fossil record and the explosive model, though in these studies, researchers have concluded that at least some modern placental groups evolved prior to the K-Pg extinction event (11, 12). Is the approach used in the O'Leary *et al.* study directly comparable to these recent molecular phylogenetic studies? Not really, as it turns out.

Owing to the very large morphological data set evaluated by O'Leary *et al.*, the authors devoted most of their analytical energy to scoring characteristics and estimating the shape of the tree rather than the length of its branches. For age estimation, they turned to fossil calibrations and a rarely used method, ghost lineage analysis. Typical molecular phylogenetic studies use fossils as the variable for solving the molecular rate equation (13). The ghost lineage approach instead uses the defining morphological characteristics that align fossils with living clades to calibrate the phylogeny as a whole. This it does by using direct fossil evidence for the earliest appear-
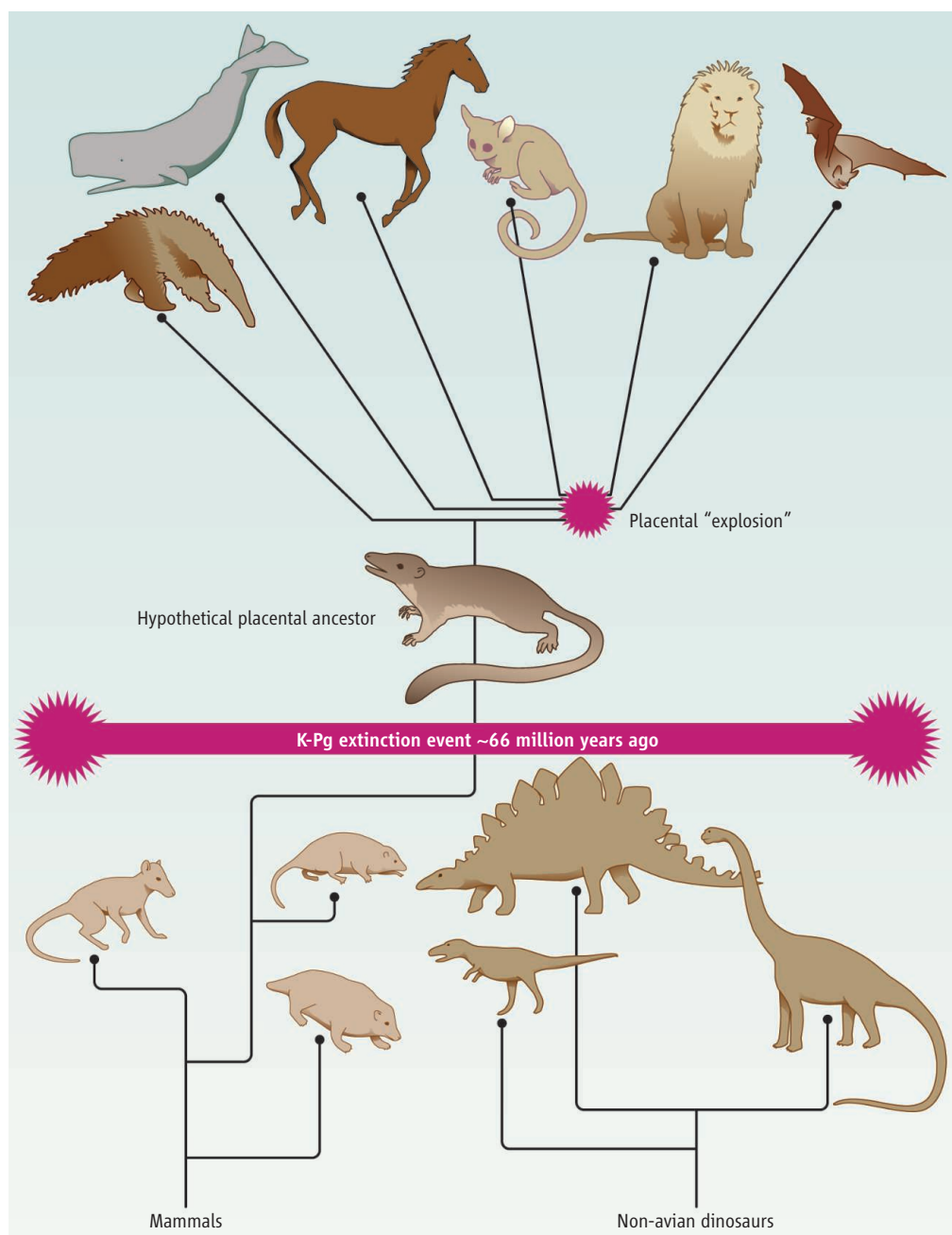
Support for a classical hypothesis. The fossil record has long reinforced the hypothesis that placental mammals did not begin to evolve and diverge until after the impact of a massive asteroid ~66 million years ago. A tour-de-force morphological phylogenetic analysis by O'Leary *et al.* supports this hypothesis, positing that the ancestral placental mammal was small and insectivorous. The authors further conclude that the first split in the phylogeny was between the xenarthran clade (anteaters, sloths, and armadillos) and all other placental mammals.

Labels in figure: Placental "explosion"; Hypothetical placental ancestor; K-Pg extinction event ~66 million years ago; Mammals; Non-avian dinosaurs

ance of lineages and by inferring the presence of "ghost lineages" not documented in the fossil record but implied by sister-group relationships.

To understand the method, consider the lemurs of Madagascar. There are no known fossil lemurs, only unfossilized bones of lemurs that went extinct in the past 12,000 years. A direct reading of this record would yield the conclusion that lemurs evolved a few thousand years ago (*14*). However, we know that the closest relatives of lemurs are the Lorisiformes of Africa and Asia. The fossil record reveals that within the Lorisiformes, lorises and galagos diverged from each other by 39 million years ago (*15*), thus establishing a minimum age for lorisiforms and—because lemurs must have split off before that divergence—for lemurs. Thus, we infer that the lemurs are the product of a ghost lineage that has persisted for at least 40 million years.

O'Leary *et al.*'s study offers a level of sophistication and meticulous analysis of morphological and paleontological data that is unprecedented. By analyzing a morphological data set an order of magnitude greater than prior analyses, and integrating fossils within the phylogenetic analysis, they conclude that the origin and divergence of placental mammals must have occurred entirely after the K-Pg event. Even so, the reliance of age estimation procedures on a single phylogeny and the disregard for the consequences of branch lengths leave us wanting more. Future studies are sure to benefit from probabilistic modeling and statistical methods such as those being developed in (*10*). These methods bring morphological and molecular data into the same statistical analytical framework, allowing the fossils to explicitly influence the estimation of branch lengths.

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CHEMISTRY

Copying Biology's Ways with Hydrogen

A model iron-nickel compound that mimics the core of hydrogenase enzymes provides insights into the formation of the H^- intermediate.

Fraser A. Armstrong

Hydrogen (H_2), produced from solar energy and water, offers the ultimate solution for generation and storage of renewable energy. The challenge is to find catalysts suitable for economic large-scale production (made from earth-abundant elements) that can interconvert H_2 and water rapidly without a large excess thermodynamic cost (which would waste available energy). The most efficient catalyst for electrolysis of water is the noble metal platinum, but microbes deal very well with H_2 by exploiting iron (Fe) and nickel (Ni), dressed up with special ligands and buried inside enzymes known as hydrogenases (*1*). Hydrogenases are extremely efficient catalysts for producing and oxidizing H_2 at rates exceeding thousands per second near the reversible potential, and establishing their mechanism is crucial for future H_2 technology (*2*). On page 682 of this issue, Ogo *et al.* (*3*) describe a synthetic analog of the active site of [NiFe]hydrogenases that oxidizes H_2 via a hydride-containing intermediate formally equivalent to the most reduced catalytic state of the enzyme.

Department of Chemistry, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, OX1 3QR, England, UK. E-mail: fraser.armstrong@chem.ox.ac.uk
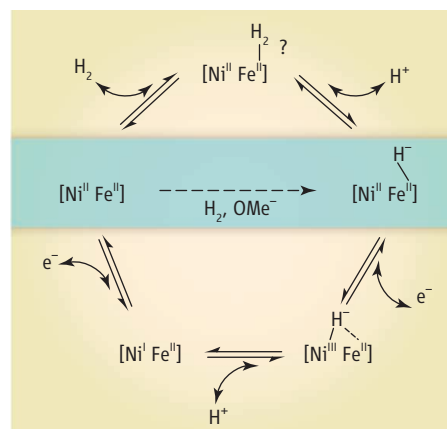
A minimal hydrogenase mechanism. The catalytic cycle shown for the core of [NiFe]hydrogenases is based on the constraints that only protons and electrons can enter or leave the active site, and do so singly. The reaction and species highlighted in blue is the equivalent chemical transformation reported by Ogo *et al.*; OMe^- is the methoxide ion.

In hydrogenases, the Ni atom is coordinated by four cysteine thiolates, two of which form bridges to the Fe atom, which is further coordinated by one carbon monoxide (CO) and two cyanide ligands. Model compounds are ultimately dictated by what is synthetically feasible and stable, and in the compounds described by Ogo *et al.*, an almost planar Ni(II) is coordinated by two N donors and two thiolate S donors—the latter bridging to an Fe(II) that is also coordinated by three triethylphosphites, which mimic the soft ligand environment of the Fe in the enzyme and provide steric stabilization.

Critics will argue that the analog lacks the outer shells of the enzyme's active site and that the best bio-inspired catalysts have a nitrogen base fixed at close range. Nonetheless, the stepwise details of H_2 oxidation described by Ogo *et al.* reveal intrinsic aspects of the $[Ni^{II}(SR)_2Fe^{II}]$ core (where SR is a thiol group) that have otherwise been elusive. In direct studies of the enzyme mechanism, locating H atoms in intermediates is challenging, as most are highly reactive and may contribute no signal in electron paramagnetic resonance (EPR) studies.

The mechanism of [NiFe]hydrogenases outlined in the figure arises by considering the constraints governing discrete steps rather than intrinsic properties of states. Isotope exchange experiments have shown that H−H bond cleavage is heterolytic, forming a metal-bound hydride and a proton (*1*). Further constraints arise from the isolated nature of the active site; hydrogen can only