

Amphibians as Indicators of Early Tertiary "Out-of-India" Dispersal of Vertebrates

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Sixty-five million years ago, massive volcanism produced on the India-Seychelles landmass the largest continental lava deposit (Deccan Traps) of the past 200 million years. Using a molecular clock-independent approach for inferring dating information from molecular phylogenies, we show that multiple lineages of frogs survived Deccan Traps volcanism after millions of years of isolation on drifting India. The collision between the Indian and Eurasian plates was followed by wide dispersal of several of these lineages. This "out-of-India" scenario reveals a zoogeographical pattern that might reconcile paleontological and molecular data in other vertebrate groups.

The mass extinction at the Cretaceous-Tertiary (K-T) boundary (~65 million years ago) coincided both with a well-documented asteroid or comet impact on the Yucatan coast of Mexico (1) and with massive flood basalt volcanism that produced on the India-Seychelles landmass the largest continental lava deposit (the "Deccan Traps") of the past 200 million years (2). It has been suggested that one or both of these events caused a global bottleneck of biodiversity, which was followed by a period of intense diversification through adaptive radiations (especially in birds and mammals) during the early Tertiary (3). However, some aspects of this theory have been seriously challenged by molecular evidence suggesting that many lineages of birds and mammals survived the K-T transition (4).

When the Madagascar-Seychelles-India continent rifted from the rest of Gondwana (Fig. 1A) (5), it probably held a diversity of life forms. The subsequent fragmentation of this landmass 88 million years ago (Ma) (6) into two elements (Madagascar and India-Seychelles) (Fig. 1B) resulted in the vicariant divergence of taxa on both islands. While the India-Seychelles landmass was drifting away from Madagascar toward Eurasia, large amounts (>2 million km³) of basaltic magmas erupted over a period of probably less than 1 million years encompassing the K-T boundary (7, 8), thereby covering a large fraction of the insular block. Hence, even if several vertebrate groups successfully survived the K-T boundary on a global scale, the Deccan Traps volcanism probably had a heavy impact on the fauna of the isolated Indian

subcontinent itself (9).

The Western Ghats of India (a mountain range that extends over part of the 0.5 million km² of lava still left after 65 million years of erosion) and the highlands of Sri Lanka are both known as current hotspots of biodiversity (10). In Amphibia, for example, southern India harbors endemism at higher taxonomic levels in both caecilians and anurans (11), and Sri Lanka is regarded as one of the global hot spots (12).

We estimated divergence times of major nodes in the phylogeny of ranid frogs (Ranidae) using about 3000 base pairs (bp) of mitochondrial (mt) and nuclear (nu) DNA sequences from representatives of the primary lineages from Madagascar and Asia (13). The family Ranidae, which represents about one-fifth of all living amphibian species, is a good model for assessing survival of vertebrates on the Indian subcontinent because (i) it includes several Indian endemic lineages; (ii) it is currently almost cosmopolitan; and (iii) amphibians have dispersal capacities that are low in general, and virtually nil over salt water.

Because the assumption of similar rates of evolution among different lineages is unrealistic, we extracted dating information from our data set with a Bayesian approach explicitly independent of any molecular clock (14). As a calibration point, we used the timing of the Madagascar versus India-Seychelles landmass fragmentation (87.6 ± 0.6 Ma) (15), a date reliably estimated from geological evidence (6). In the evolutionary history of Ranidae, this geological event is translated into the vicariant divergence of the Mantellinae clade (i.e., a monophyletic group including all native Madagascan ranid frogs) from the Rhacophorinae (Fig. 1) (16). We confirmed the validity of this calibration by re-computing dating estimates using the split between synapsids and diapsids as another calibration point (17). Consistency between the fossil- and paleogeographic-based cali-

brations dramatically raises confidence in our date estimates.

Our molecular phylogenetic analyses demonstrate that several lineages of ranid frogs survived the Deccan Traps volcanism. Given (i) our calibration point of ~88 million years (My) (indicated by an asterisk in Fig. 1), (ii) that the Madagascar-Seychelles-Indian landmass became isolated from Africa ~130 Ma (5), and (iii) that India connected to Eurasia 65 to 56 Ma (18), all lineages that originated between 130 and 65 Ma (Fig. 1) must have been present on isolated India during the volcanism. In other words, our dating estimates can be explained by plate tectonic events alone. An African origin for these lineages would require much less parsimonious scenarios such as the migration of the ancestor of the Madagascan clade across salt water, together with independent dispersal (across Eurasia or salt water) of a minimum of six frog lineages into India. Furthermore, the out-of-India hypothesis remains valid after constraining various clades as the sister group of the Madagascan lineage (i.e., the Mantellinae) (19). Hence, this conclusion does not depend on the exact topology of the true phylogenetic tree.

Frogs must have survived on the fringe of the drifting Indian subcontinent, i.e., in areas not covered by flood basalt. The Deccan volcanism consisted of several flows separated by short quiescent events, during which small lakes and swamps might have formed on the surface of the cooled lava, allowing colonization by survivors from refugia. Although there is no paleontological evidence for the presence of ranids in the Late Cretaceous of India, fossil remains from archaebatrachian frogs, and even from what must have been among the last dinosaurs, have indeed been found in the thin intertrappean sediments (20).

Our phylogenetic analyses and clock-independent dating estimates indicate (i) that several southern Indian endemic lineages (indicated by # in Fig. 1) diverged before the K-T boundary on an isolated landmass (Madagascar-India-Seychelles, or a portion of it) and probably never dispersed successfully out of the Indian subcontinent, but also (ii) that at least three ranid frog lineages dispersed out of India after its collision with Eurasia (21). As a first example, our results suggest that the rhacophorine treefrogs, which radiated almost exclusively in the Oriental realm into about 300 species (12, 22), dispersed out of India. The only African rhacophorine genus (*Chiromantis*, not included in our analyses) was shown to be nested well within this Asian clade, so it must have reached Africa over land (23). Second, Raninae (including the genus *Rana*) currently have a cosmopolitan distribution (except for southern South America and most of Australia) (Fig. 2). Our analyses indicate that this

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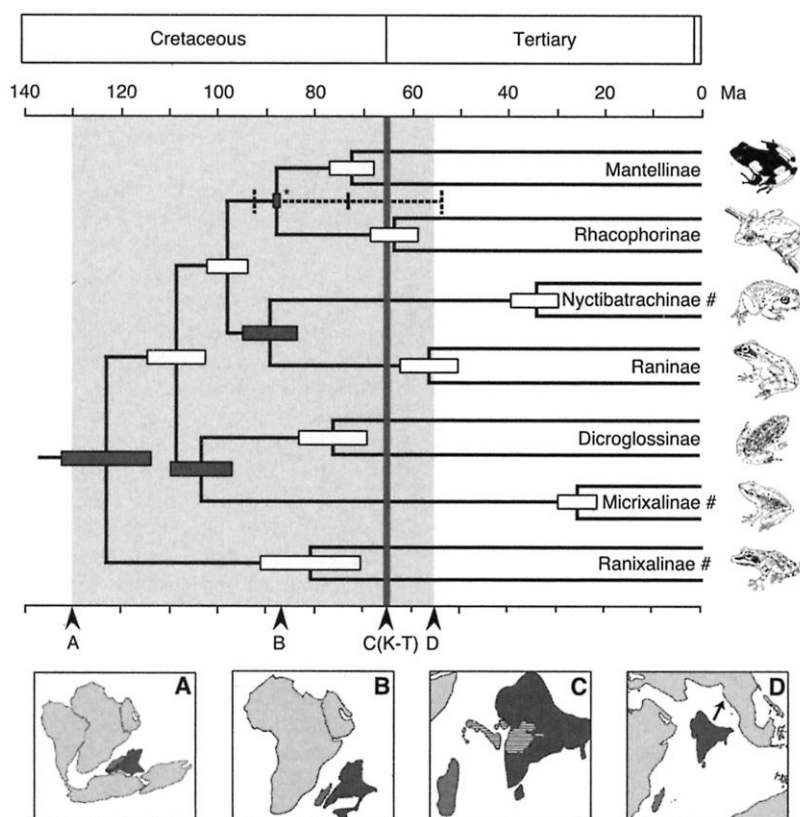
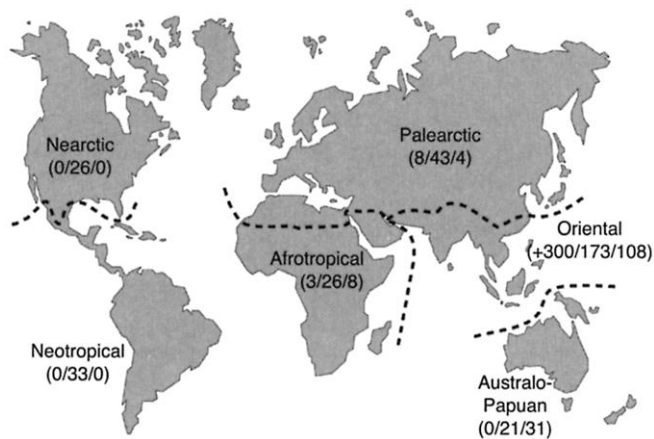


Fig. 1. ML tree topology ($-\ln L = 14,357.5641$; mt + nu data) combined with dating of the phylogenetic nodes. Divergence times are calibrated by dating the node indicated by an asterisk with the geological separation (87.6 ± 0.6 Ma) of Madagascar from the India-Seychelles landmass (B). Horizontal bars at the nodes indicate twice the value of the standard deviation for the age of the corresponding node. The dark horizontal bars are at the nodes corresponding to the origin of the Madagascar, the three southern Indian endemic (#), and the three "out-of-India" subfamilies. The shaded area represents the period between the isolation of the Madagascar-Seychelles-India block from Africa (A) (~ 130 Ma) (5) and the collision of India with Eurasia (D) (>56 Ma). We used 65 Ma as the earliest possible date of dispersal between the Indian plate and mainland Eurasia because of the postulated existence of a Late Cretaceous terrestrial connection between these two landmasses (20, 29). (C) The K-T boundary, and contemporaneous Deccan trap volcanism; the hatched area denotes land currently covered by volcanic basalts. Using the divergence between mammals and birds as a second calibration point (17) yields a date for the origin of Mantellinae/Rhacophorinae of 53.6 to 92.6 Ma (dotted line, mean \pm SD). This demonstrates the validity of our a priori hypothesis that the separation of Madagascar versus the India-Seychelles landmass (~ 88 Ma) (6) resulted in the vicariant divergence of Mantellinae versus Rhacophorinae.

Fig. 2. Current distribution across geographic realms of the three "out-of-India" lineages of ranid frogs shown in Fig. 1. Numbers shown are based on (22) and have been updated with (12) (for Rhacophorinae) and known phylogenetic relationships (16). Three lineages (Nyctibatrachinae, 10 species; Micrixalinae subfamily nov., 6 species; and Ranixalinae, 9 species) are endemic to southern India (# in Fig. 1). The three other groups clearly present the highest number of species in the Oriental realm (numbers correspond to Rhacophorinae/Raninae/Dicroglossinae), an observation compatible with the hypothesis that these lineages dispersed "out-of-India" after a long period of isolation.



lineage originated on the drifting insular block (Fig. 1) and radiated into more than 200 species, probably after its "out-of-India" dispersal (24). Consistent with this scenario, the earliest known European fossils of the genus *Rana* are only of Oligocene age (24 to 34 Ma) (22), whereas much older fossils would likely have been found if the lineage originated in Africa or Eurasia. The Dicroglossinae form a third out-of-India lineage. They experienced their main radiation on Asia, and only a limited number of members (e.g., *Hoplobatrachus*) reached Africa.

These findings contradict the "out-of-Africa" hypothesis (25), which postulates that contemporary Asian (including Indian) amphibian fauna resulted from terrestrial dispersal of mainly African forms. Zoogeographic data are consistent with the out-of-India scenario: In addition to the three lineages endemic to the Indian subcontinent, the three other subfamilies currently present a peak of biodiversity in the Oriental realm (Fig. 2). This observation is compatible with India being the center of dispersal from which ranid frogs colonized other parts of the world.

Because various lineages of ranid frogs originated between 130 and 88 Ma (Fig. 1), it is likely that several of them were present on Madagascar when it broke up from the India-Seychelles landmass. Although fossils of ranids have not been found from the Latest Cretaceous of Madagascar (26), the importance of this negative evidence is difficult to evaluate given that the Madagascar fossil record from that period is extremely poor and is restricted to a northwestern site of the island. Our divergence-time analyses here indicate that Mantellinae experienced their initial radiation on Madagascar around the K-T boundary (Fig. 1). Madagascar representatives of other lineages experienced either competitive take-overs or, more probably, extinction or opportunistic replacements associated with global (e.g., K-T) or local crises. The absence of extant native ranid frogs on the Seychelles is likely related to the break-up of these islands from India being caused by—and hence, coinciding with—the emergence of the mantle plume that produced the gigantic Deccan trap volcanism.

Our results have implications for interpreting paleontological data and inferring biogeographical patterns in other vertebrates. First, continuity between the Mesozoic and Cenozoic biota of Indo-Pakistan was not as disrupted as might have been predicted from the occurrence of the massive Deccan Traps volcanism. Second, the out-of-India hypothesis reveals a biogeographical pattern that also might help reconcile paleontological and molecular data in other vertebrates. Indeed, whereas paleontological data seem to indicate that extant birds and mammals originated from a few or perhaps, each, a single lineage surviving the K-T transition, molecular phylogenetic analyses (4) sug-

gest much earlier divergence events. One possible explanation for this discrepancy is that part of the early Tertiary burst in avian and mammalian diversity would reflect dispersal from previously isolated India to Laurasia, rather than actual phylogenetic radiation. Indeed, similarly to ranid frogs, several modern land vertebrate lineages may have originated and been isolated on the drifting Indian subcontinent until they could colonize the world after India collided with Eurasia. Analyses of molecular data in acrodont lizards (27) and ratite birds (28) are consistent with this biogeographical hypothesis.

Krause and Maas (29) suggested that "among early Tertiary large landmasses, the Indian subcontinent is unique in its combination of having been in the right places at the right times to provide for the development and the subsequent disembarking of several new higher taxa of mammals" (p. 96). Our analyses provide molecular evidence extending this zoogeographical perception to amphibians and suggest that the origin of other vertebrate lineages might need to be sought in India, despite extensive isolation and massive volcanism.

References and Notes

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13. The mtDNA fragments are (i) a 750-bp segment including portions of the 12S and 16S ribosomal RNA (rRNA) genes, as well as the tRNA_{VAL} gene; (ii) an additional 550-bp segment of the 16S rRNA gene; and (iii) a 580-bp segment of the cytochrome b gene. The nuDNA fragments are (i) a 316-bp segment of exon 1 and (ii) a 175-bp segment of exon 4 of the rhodopsin gene, and (iii) a 529- to 532-bp segment of exon 1 of the tyrosinase gene. The taxa used in our analyses were selected from a larger data set [GenBank accession numbers AF249001 to AF249191; (16)] first by excluding some taxa within tip clades very well supported in maximum parsimony analyses, then by choosing the combination of remaining taxa for which the rates of evolution were not significantly different under the 2DN Tajima test. The 14 species kept are (compare with the tree in Fig. 1; branches from top to bottom) *Mantella madagascariensis*, *Boophis tephraeomystax*, *Philautus charius*, *Polypedates cruciger*, *Nyctibatrachus major*, *Nyctibatrachus cf. aliciae*, *Rana curtipipes*, *Rana temporaria*, *Limnonectes kuhlii*, *Nannophrys ceylonensis*, *Micrixalus fuscus*, *Micrixalus kottigeharensis*, and *Indirana* spp. 1
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15. The separation of the Madagascar and India-Seychelles landmasses (Fig. 1B) was initiated by a mantle plume generating large quantities of basaltic magma that quickly formed an obstacle for faunal interchange. Furthermore, because of the high speed of the northward-moving India-Seychelles block (15 to 20 cm year⁻¹), an oceanic opening with Madagascar was quickly formed. Given the extremely low tolerance of amphibians to salty environments, the separation of these two islands (87.6 ± 0.6 Ma) can be regarded as an effective barrier to frog dispersal and thus as an accurate calibration point. Calibration was performed by constraining the split between the Madagascan clade and its sister group to between 87.0 and 88.2 Ma. We computed both the prior and posterior distributions to verify that the data provide significant information on the estimation of divergence time. We also computed the posterior distribution twice (i.e., using two different random seeds) to check for convergence of the results.
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Rising CO₂ Levels and the Fecundity of Forest Trees

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We determined the reproductive response of 19-year-old loblolly pine (*Pinus taeda*) to 4 years of carbon dioxide (CO₂) enrichment (ambient concentration plus 200 microliters per liter) in an intact forest. After 3 years of CO₂ fumigation, trees were twice as likely to be reproductively mature and produced three times as many cones and seeds as trees at ambient CO₂ concentration. A disproportionate carbon allocation to reproduction under CO₂ enrichment results in trees reaching maturity sooner and at a smaller size. This reproductive response to future increases in atmospheric CO₂ concentration is expected to change loblolly dispersal and recruitment patterns.

Under elevated atmospheric CO₂ concentrations, the biodiversity of forests will depend on both changes in growth rates that confer competitive advantage to some species over others and on reproductive success, a key

element of fitness. Greenhouse and growth-chamber studies demonstrate that although there is an average 31% growth enhancement of herbaceous plants and tree seedlings at doubled ambient CO₂ concentration, this response varies widely among species (1, 2). Tropical (3) and temperate (4-6) tree assemblages are "recruitment-limited." Tree population growth rates are constrained by low seed availability and low seedling establish-

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