

Differing rates of macroevolutionary diversification in arboreal squirrels

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Current diversity is the result of macroevolutionary processes of origination and extinction of lineages through time. Here we make use of a fossil-calibrated molecular-clock phylogeny of modern squirrel genera to estimate both rates of ‘birth’ and ‘death’, and the net rate of accumulation of lineages since the origin of the squirrel family (Sciuridae) 36 Ma. As a family, the Sciuridae have exhibited modest rates of diversification in comparison with other mammalian clades. Within the Sciuridae, lineages of squirrels have accumulated at higher rates in geographically localized subclades in the tropics of different continents. The rate is strikingly high in the Sciurini of South America, which first entered and radiated within that continent comparatively recently (less than 3 Ma). It is noteworthy that the most rapidly diversifying groups are also relatively young. Because extinctions lag behind originations, the effects of extinction are not yet detectable in relatively recent radiations. The balance of origination and extinction is fragile, and is likely to become more so if increases in extinction due to habitat destruction, climate change and other human activity are not mitigated.

Keywords: Arboreal squirrels, diversification, molecular-clock phylogeny, macroevolutionary processes.

Introduction

ACCORDING to the *Ramayana*, a small squirrel endeared itself to Rama by bringing pebbles to build a bridge between India and the mythical Lanka (where his beloved Sita was captive). While evidence from phylogenetic and molecular-clock analyses suggests that, throughout their evolutionary history, members of the squirrel family have not readily dispersed across large bodies of water¹, squirrels do show potential for bridging our understanding of geological, ecological and genealogical processes. Among the advantages squirrels offer to the study of macroevolution are: (a) an earliest known fossil that is unusually well preserved, (b) a molecule with a substitution pattern that has been exceptionally clock-like, and (c) multiple dates for calibration. Using these tools we have inferred that arrival and diversification of squirrels on different land

masses have typically waited for a land corridor of suitable habitat to be established.

This article examines further the diversification of the Sciuridae in space and time as it may be understood from a molecular-clock phylogeny of modern genera. In a spatial context, phylogenetic trees have revealed that groups of genera that constitute major clades^{1,2} are geographically fairly coherent (Figures 1–3). Several subgroups that are well-defined geographically – such as, for instance, most squirrels of South America or of Sulawesi, the tree squirrels of Africa (Protoxerini), and African ground squirrels (Xerini) – are also monophyletic (Figure 1).

From the standpoint of species diversity, regions vary greatly. For example, tree squirrels (Sciurini) and flying squirrels (Pteromyini) span a wide swath of the northern Eurasian continent (Figures 2 and 3), but in each case they are represented there by a single wide-ranging species. In contrast, southeast Asia is home to about 100 species of tree or flying squirrels³. To explain this difference both spatial and temporal factors may be relevant: much of the area in northern Asia now occupied by squirrels was beneath a glacier until late in the Pleistocene. Meanwhile in tropical, insular southeast Asia, fluctuating sea levels and land areas for the duration of the late Cenozoic could have been a source of shifting barriers and connections accelerating allopatric speciation. Is the high species diversity of tropical squirrels a consequence of lengthy periods and rapid diversification?

In this article, we examine this question using the temporal framework provided by a molecular clock to assess rates and timing of diversification in several clades of squirrels.

Methods

The sources of data for this study are: (1) the molecular-clock phylogeny presented in ref. 1 (see Figure 1); (2) geographic distributions shown in Figures 2 and 3 (derived from multiple references and field guides, and with help from Giovanni Amori); and (3) tallies of species as listed and grouped taxonomically in ref. 3.

A clock-calibrated phylogenetic tree of modern taxa like the one we use here (Figure 1) depicts the divergence, through time and from a common ancestor, of lineages that have persisted to the present. It therefore represents

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what is known as a Yule, or 'pure birth' process⁴: the net accumulation of lineages throughout the history of a group, which is the product of the processes of both speciation and extinction. Here we focus mainly on accumulation, rather than separating originations and extinctions in modelling a 'birth-death' process, because rates obtained using a phylogeny of only modern (and no fossil) taxa do not directly incorporate the 'births' and 'deaths' of any lineages that are now extinct. Indirectly, this kind of a

'birth-death' process may be inferred from information gleaned from a comprehensive phylogeny of modern taxa through a close examination of the dynamics of diversification⁴. However, birth-death models are sensitive to errors in estimation, and uncertainties in taxonomy and phylogenetic resolution can be a source of spurious results; moreover, different groups of squirrels have received different levels of taxonomic attention, which in turn affects estimates of diversity and rates of diversification. For example, the number, timing and sequence of branching within Marmotini shown in Figure 1, is confounded by the facts that different genes suggest somewhat different positions for *Sciurotamias*^{1,2}, while *Spermophilus* is not only an unusually well-studied and species-rich genus, but is also paraphyletic⁵.

In basing our analyses on a comprehensive generic-level phylogeny and a listing of all modern species as they are currently understood, we are assuming, first, that the

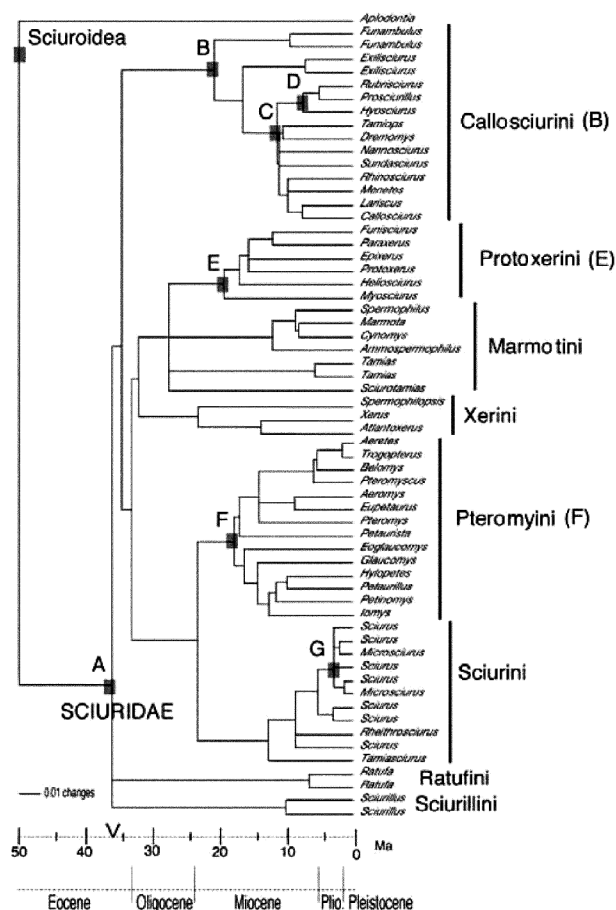


Figure 1. Maximum likelihood molecular-clock tree for squirrel genera, with *Aplodontia rufa* as outgroup. Topology was obtained from DNA sequences of 12S, 16S and IRBP; branch lengths are based on third codon-position substitutions in IRBP; calibration is based on several dated points. Two species were used to represent genera on long, deeply diverging branches and multiple species of *Sciurus* and *Microsciurus* were included to define the point of divergence of South American taxa. Two genera incorporated in phylogenetic analyses, but not shown here because they were represented by incomplete sequences are *Glyphotes*, which is placed as a sister group to *Callosciurus*, and *Syntheosciurus*, which diverged within the clade of *Sciurus* and *Microsciurus* subsequent to point G. See ref. 1 and its supplementary material (www.sciencemag.org/cgi/content/full/1079705/DC1) for methods and interpretation. Clades representing the eight sciurid tribes are indicated. The following crown-group nodes are labelled: A, Origin of Sciuridae; B, Origin of Callosciurini; C, Rapid divergence and origin of southeast Asian Callosciurini (excluding the earlier diverging *Exilisaurus*, in the Philippines, and *Funambulus*, from south Asia); D, Sulawesi genera; E, Origin of African tree squirrels; F, Origin of flying squirrels; G, Origin of main radiation of South American tree squirrels.

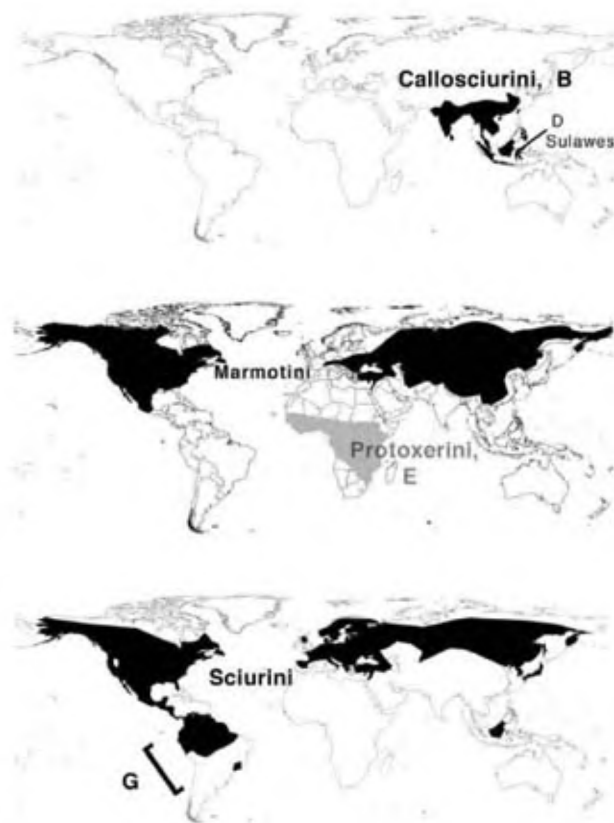


Figure 2. Approximate geographic distributions for four of the eight sciurid tribes. The ancestral nodes for the following groups of tree squirrels – Callosciurini (B), Sulawesi genera (D), Protoxerini (E), and South American Sciurini (G) – are marked in Figure 1. For the most widespread tribes, it should be noted that only four of the 37 species of Sciurini are native to Eurasia³, and the timing of their split from their New World relatives appears to coincide with a land connection across Beringia that was forested. Crossing of Beringia by terrestrial Marmotini is linked to a land bridge of more open habitat¹. (We thank Dr Giovanni Amori for plotting these distributions and those in Figure 3.)

phylogeny and divergence dates are essentially correct; second, either that each genus represented in the phylogeny is monophyletic, or that the pattern of branching leading to the exemplars chosen to represent each genus provides a good approximation of the divergence of lineages at the various points sampled in time; and last, that the distribution of terminal lineages across the phylogeny is well represented in the tallies of species. Confidence intervals and statistical comparisons will be applied in the future, as alpha taxonomy for the less well-studied groups improves and sampling becomes more detailed at the level of species.

In the 'pure birth' or Yule model we use here, tracking diversification by counting the number of lineages in the phylogeny reconstructed for extant genera, we take a conservative approach by sampling at reasonably even time intervals that represent several key points in history: (i) the point of divergence from the ischyromyid common ancestor with apodontids ('Sciuroidea': 50 Ma); (ii) the initial divergence of the Sciuridae (36 Ma); (iii) just after the divergence of the five subfamilial lineages (Sciurilli-

nae, Ratufinae, Callosciurinae, Xerinae [= Xerini + Marmotini + Protoxerini], and Sciurinae [= Sciurini + Pteromyini]: 30 Ma); (iv) after the divergences of *Funambulus* and *Spermophilopsis* within their respective tribes, but before divergence among other Callosciurine genera, or of Protoxerini, Pteromyini or Sciurini (20 Ma); (v) early in the diversification of these tribes (15 Ma); (vi) after the divergence of most of the extant genera (7.4 Ma), and (vii) the present¹. From a process of repeated splitting, the expected number of lineages increases exponentially, and so these numbers are logarithmically transformed. The slope of a curve for which $y = \ln(\text{number of lineages})$ and $x = \text{million years before present}$ is R , the exponential rate of net increase in number of lineages per million years⁴.

Here we estimate net rate of diversification using two approaches. If we assume that the relationship between $\ln(\text{number of lineages})$ and time is linear – i.e. that the net increase in lineages is exponential – we can use knowledge of extant diversity to calculate a rate simply by taking the increase in $\ln(\text{number of lineages})$ per unit time between the time of origin of a group and the present. For the squirrel family, Sciuridae, as a whole, we also took the slope of a least squares regression line fit to the six data-points (described above) spanning the time interval of its existence. The diversification of Sciuridae as represented by these six points (Figure 4a) does not in fact appear to be strictly linear, but may instead be approximated by three roughly linear phases with different slopes (Figure 4a, 1–3). The relationship for diversification of individual subclades can also be treated as linear. In this way we compare diversification for clades of tree or flying squirrels in three regions of the tropics – South America, Africa and southeast Asia (Figure 4b, B–G) – which experienced major radiations at different times in the past.

The relationship between the expected $\ln(\text{number of lineages})$ and time alternatively may be treated as a nonlinear by-product of 'births' and 'deaths'. In this case we calculate the net rate of diversification (R) by making use of eq. (2) in ref. 6 to obtain separate estimates of lineage 'birth' (λ) and 'death' (μ) rates, where $R = \lambda - \mu$, by fitting a nonlinear but continuous curve (Figure 4a) representing the expected number of lineages present at time t that survive to the present, and assuming both λ and μ are constant.

Results and interpretation

The 278 species of squirrels currently recognized³ descended from a common ancestor around 36 Ma, which in turn shared an ancestor with *Aplodontia*, the extant sister group of modern squirrels, around 50 Ma (see references cited in ref. 1). Working forward in time, Figure 4a shows the net increase of squirrel lineages represented in the modern fauna, from the initial bifurcation at 36 Ma and

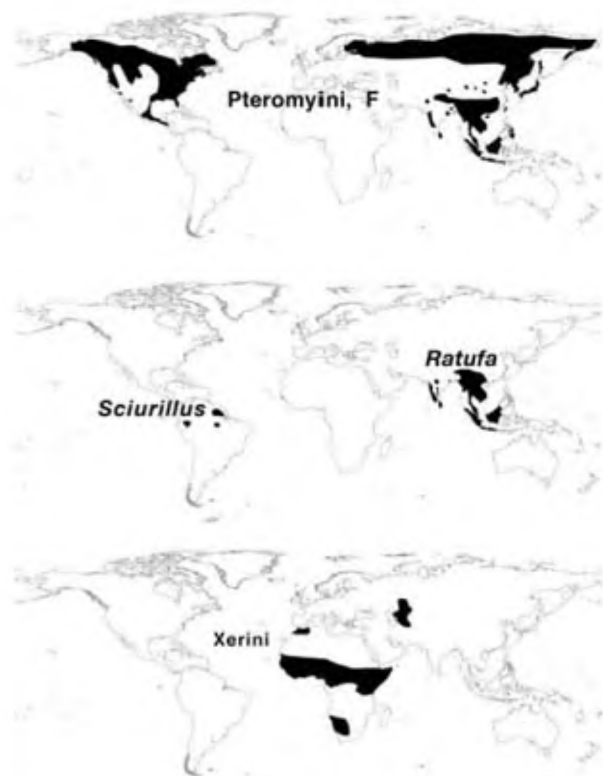


Figure 3. Approximate geographic distributions for the four remaining sciurid tribes. (Sciurillini and Ratufini are each represented by a single genus.) The ancestral node for the flying squirrels, Pteromyini (F), is labelled in Figure 1. With reference to the most widespread tribes, it should be noted that among the 44 species of Pteromyini, only two (in one genus) inhabit northern Eurasia, and the ancestors of *Glaucomys* in North America are hypothesized to have made use of a forested land connection to cross Beringia. Diversification of the African members of Xerini, like Protoxerini (Figure 2), followed establishment of a land bridge with Eurasia¹.

sampled at four intermediate time intervals to the present (see Methods, above).

We make several observations about the overall form of the relationship shown in Figure 4 *a*. As was noted above, the relationship does not appear to be strictly linear, and can be approximated either by a continuous curve representing the outcome of a 'birth-death' process, or, from

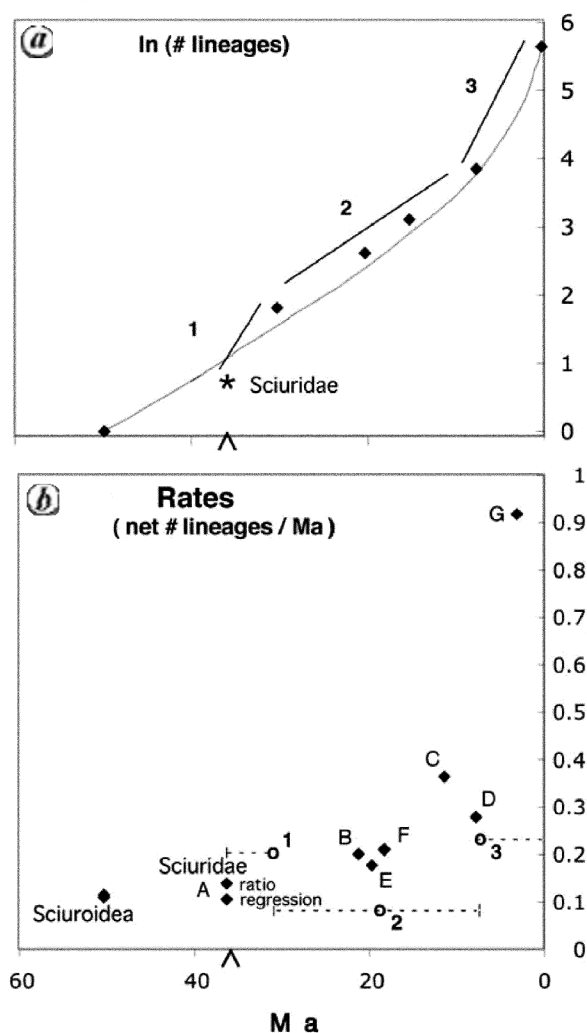


Figure 4. Log-transformed values of the number of lineages (*a*) and rates of diversification for various clades (*b*), are each plotted against their respective divergence times. The time of origin of the Sciuridae (36 Ma) is marked with a carat (^). The continuous curve in (*a*) is reconstructed from the seven datapoints using a nonlinear least-squares fit, and assuming lineage birth and death rates each to be constant. Alternatively, the diversification of squirrels can be described as taking place in three phases. Rates of diversification over each of these intervals (marked here as 1, 2 and 3) are plotted as open circles in (*b*), with the time intervals they represent shown with a dashed line; diversifications of each of the clades marked A–G in Figure 1 are also shown. The overall rate of diversification for Sciuridae (A) from its origin to the present is calculated in two different ways: Fitting a linear regression through the six points for Sciuridae shown in (*a*) yields a similar but slightly lower value than simply using the initial and final numbers of lineages over the entire time interval (which is the method applied in calculating diversification rates for each of the other clades).

the point of origin of the Sciuridae at 36 Ma, as three roughly linear phases. In either case, initially and during the most recent ~7 Ma or so (Figure 4 *a*, 1, 3), the net accumulation of lineages within Sciuridae appears to have been rapid, while during the intervening 23 Ma, its pace was more moderate (Figure 4 *a*, 2). In the first 'rapid' phase (labelled '1'), we can view the origin of the Sciuridae as either having been accompanied by an initial burst of diversification (coinciding with a climatic 'deterioration' in the Oligocene), or having produced lineages that have been unusually persistent (i.e. experiencing less extinction). In the time preceding that, the sciuroid lineage ancestral to the Sciuridae experienced comparatively higher extinction, lower rates of diversification, or both.

In the most recent time interval (Figure 4 *a*, 3), a curve representing the accumulated number of lineages reconstructed from a phylogeny of modern taxa, like this one, is expected to steepen, even if 'birth' and 'death' rates have been constant over time⁶. The reason is as follows: the net accumulation of lineages assessed for any point in time is the number of 'births' minus the number of 'deaths', and the origin of lineages must always precede their extinction. The level of diversity observed for the present day or the very recent past is enriched by the most recent 'births', but has been reduced only by those lineages of the very shortest duration, for which sufficient time has elapsed for extinction. With the passage of additional time, some of the lineages now or recently extant will go extinct and therefore fail to be represented in the new fauna. From a fauna in the future, the number of accumulated lineages that would be reconstructed for the present day will be less than what we currently count, because some modern lineages will have vanished with extinction. In the future, even if the rates of origination and extinction remain the same, the curve in the interval leading up to the present day will eventually straighten, while the subsequent portion of the curve will be steep.

Rates of net diversification are plotted in Figure 4 *b*. Points 1–3 represent the rates calculated for the intervals labelled on the graph above it (Figure 4 *a*) and for all other points, the overall rate of diversification between the origination of a clade and the present day is plotted against the time of origin of that group. Letters A–G correspond to labels of nodes in Figure 1 (so, for example, the rate of diversification since the arrival of squirrels on Sulawesi, D, is 0.28 per Ma.) For the squirrel family Sciuridae as a whole (A), two estimates of the slope were calculated assuming the relationship to be linear.

From the fitted curve in Figure 4 *a*, 'birth' (λ) and 'death' (μ) rates were also estimated for Sciuroidea. These values were $\lambda = 0.50$, $\mu = 0.43$, and it is noteworthy that their difference, 0.07, which would be the net rate of accumulation of lineages, corresponds well to the slope $R = 0.09$, which is calculated for the almost linear portion of the curve in Figure 4 *a* (labelled '2'). From this combination of birth and death rates we calculate that the

'extinction lag' (the most recent steep portion of the curve described in the previous paragraph) is negligible prior to $1/(\lambda - \mu)$, or approximately 14 Ma⁶.

Net rates of lineage accumulation for the Sciuridae throughout its duration – $R = 0.10$ or 0.14 , depending upon the method used for calculation – are modest in comparison to rates reported for other families of mammals, including not only younger taxa of rodents such as Hystriidae and Muridae (0.21 and 0.35 per Ma, assuming ages of 14 and 19 Ma respectively), but also families such as Bovidae (0.15) and Cricetidae (0.19), which are in excess of 30 Ma in age⁷. Vrba has hypothesized that rates of diversification should be high in clades of mammals that specialize on resources that may 'disappear during the recurrent environmental extremes that they encountered during their histories'⁸. The wide variety of food items many squirrels are capable of handling could suggest that their relatively low rates of diversification may be tied to being dietary generalists.

Subclades of squirrels (Figure 4 b, B–G) exhibit higher net rates of diversification. In part, this may be influenced by the 'extinction lag' described above, but even within the most recent 14 Ma, net rates of diversification are not perfectly correlated with time. For example, the rate we obtain for the pulse in diversification among callosciurine genera approximately 11 Ma (C) exceeds the net rate of increase on Sulawesi more recently (D), but this might be predicted by the much smaller land area of Sulawesi. Rates of diversification for the Callosciurini as a whole (B), for the arboreal squirrels of tropical Africa (E), and for Pteromyini – most of which are found not far from the tropics in Asia – (F) are similar to one another in magnitude (0.18–0.21). In fact, if taken from the same time point (19.4 Ma), the net rate of diversification of Protoxerini (E) from its time of origin in Africa, and the collective rate of diversification of all squirrel lineages in southern Asia (Pteromyini, Callosciurini, Ratufini, plus *Rheithrosciurus*, which in Borneo is the only representative of Sciurini; this collective rate is not plotted in the figure) are nearly identical (0.175 and 0.17 respectively).

Most noteworthy is the high rate obtained for Sciurini since its entry into South America (Figure 4 b, G): Although the total number of species there today (estimated as 13) is a fraction of the number of arboreal squirrel species in the tropics of Africa (30) or Asia³, the time interval in which the South American Sciurini arose – under 3 Ma – was remarkably brief. The net rate of 0.92 obtained for the diversification of this clade is high by any standard⁹ and is comparable to the rates obtained for Neogene horses as they diversified during a time of expanding grassland habitats and environmental change¹⁰. Yet despite their

relatively high rate of diversification, South American squirrels are at some risk: because of the 'extinction lag' phenomenon described above, this radiation into a new continent is too recent for steady-state rates of extinction yet to have made their mark. As a macroevolutionary perspective makes it clear, even if one assumes that such rates have been constant, the narrow margin between rates calculated for 'birth' and 'death' of squirrel lineages makes for a fragile balance, and has produced relatively low net rates of accumulation overall. With increasing rates of extinction from anthropogenic causes, the existing diversity of squirrels remains vulnerable.

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