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Speciation durations and Pleistocene effects on vertebrate phylogeography

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An approach applied previously to avian biotas is extended in this paper to other vertebrate classes to evaluate Pleistocene phylogeographic effects and to estimate temporal spans of the speciation process (speciation durations) from mitochondrial (mt) DNA data on extant taxa. Provisional molecular clocks are used to date population separations and to bracket estimates of speciation durations between minimum and maximum values inferred from genetic distances between, respectively, extant pairs of intraspecific phylogroups and sister species. Comparisons of genetic-distance trends across the vertebrate classes reveal the following: (i) speciation durations normally entail at least two million years on average; (ii) for mammals and birds, Pleistocene conditions played an important role in initiating phylogeographic differentiation among now-extant conspecific populations as well as in further sculpting pre-existing phylogeographic variety into many of today's sister species; and (iii) for herpetofauna and fishes, inferred Pleistocene biogeographic influences on present-day taxa differ depending on alternative but currently plausible mtDNA rate calibrations.

Keywords: population structure; phylogroups; sister species; molecular clocks; genetic distances; comparative molecular evolution

1. INTRODUCTION

This study was motivated by a recent resurgence of interest in Pleistocene biogeographic influences on speciation processes as deduced from genealogical data on extant organisms (Hewitt 1996; Klicka & Zink 1997). It follows an approach introduced by Avise & Walker (1998) for assessing durations of the speciation process in birds. The method involves contrasting sequence differences in mitochondrial (mt) DNA between extant sister species and between major matrilineal phylogroups within species.

Here we extend the Avise & Walker approach to mammals, amphibians, reptiles and fishes. The extensive literature on mtDNA sequence differences within and between closely related species will be reviewed to evaluate 'speciation durations', defined for current purposes as the time elapsed from the original separation of a pair of conspecific populations to their differentiation to a degree that has resulted in their classification as taxonomic species (the 'validity' of current species-level taxonomies is a separate matter, not addressed here). Results will be interpreted in the context of Pleistocene biogeographic impacts on recent genealogical divergences in the vertebrates.

2. MATERIALS AND METHODS

Analysis procedures generally follow those in the summary of avian taxa by Avise & Walker (1998), which should be consulted for details. In short, two criteria were met by the intraspecific phylogeographic studies included in this review: (i) more than 150 base pairs (bp) of mtDNA sequence per individual were assayed, either as the sum of recognition sequences of multiple enzymes in restriction-fragment length polymorphism (RFLP) studies or as direct sequences from particular mtDNA genes; and (ii) conspecific populations were sampled from multiple, widely spaced geographic locales. The literature search was conducted primarily during the autumn of 1997, and is not claimed to be exhaustive. Reptiles and amphibians were poorly represented in the literature relative to mammals and fishes, so these two vertebrate classes were pooled in the graphical and statistical summaries that follow.

Sequence divergence (p) values between mtDNA haplotypes were taken from the original papers. Where possible from the data provided, p values between major phylogroups (e.g. 'A' and 'B') were corrected for within-phylogroup variation according to the following formula: $p_{AB(net)} = p_{AB} - 0.5(p_A + p_B)$. Sequence divergence values were converted to provisional estimates of genealogical separation times using a standard mtDNA clock for birds and mammals: 2% sequence divergence between a pair of lineages per million years (Brown *et al*. 1979; see Klicka & Zink (1997) for a review for avian taxa). For some of the poikilothermic vertebrates in particular, clocks that are several-fold slower have been suspected (Kocher *et al*. 1989; Avise *et al*. 1992; Martin *et al*. 1992; Martin & Palumbi 1993; Canatore *et al*. 1994; Rand 1994; Mindell & Thacker 1996) and these too were employed as alternative calibrations in this summary.

Most available phylogeographic studies involved either RFLP analysis of the whole mtDNA genome, or evaluations of particular mtDNA loci via direct sequencing or by polymerase chain reaction (PCR) after RFLP. To lessen complications of pronounced rate heterogeneity across mtDNA gene regions (and also to avoid undue focus on shallow mtDNA phylogroups that

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are of little interest in the current context), studies that monitored the rapidly evolving control region (CR; Taberlet 1996; Baker & Marshall 1997; McMillan & Palumbi 1997) were disregarded. Exceptions involve a few instances where explicit divergence dates from CR sequences were proposed in the original papers.

Genetic distances for pairs of avian sister species were taken from Klicka & Zink (1997), and those for sister species of non-avian vertebrates from a recent review on sequence data from the mitochondrial cytochrome *b* (cyt*b*) gene (Johns & Avise 1998). Kimura's (1980) two-parameter method was used to calculate genetic distances from cyt*b* sequences (retrieved from GenBank) that were more than 200 bp in length per individual. Data from 242 presumed sister-species pairs (a small but relevant subset of the congeneric pairs analysed in Johns & Avise (1998)) are included here. Genetic distances from the cyt*b* studies also were converted to estimates of absolute divergence time using standard and alternative molecular-clock calibrations as mentioned above.

3. RESULTS

At the intraspecific level, we found published phylogeographic data that met the stated criteria on a total of 189 non-avian vertebrate species. Relevant data summarized from these studies, and literature citations, are provided in an electronic appendix on the Royal Society Web site at (`http://www.pubs.royalsoc.ac.uk/publish/pro_bs/sep98pb2/htm`). Comparable information for 63 avian species are presented in Avise & Walker (1998).

Approximately 44% of the vertebrate species displayed either shallow or no evident phylogeographic structure in the available assays, and these will not be considered further in an intraspecific context. The remaining 56% of species (140 out of 252) were subdivided into two or more highly distinctive mtDNA phylogroups as gauged, typically, by large genetic gaps between respective branches that received strong bootstrap support in the mtDNA gene trees. These strongly sundered species usually showed two major phylogroups apiece, although some were subdivided into as many as six deep matrilineal assemblages. Broader geographic surveys of course might uncover additional phylogroups in some species.

In most cases, the principal phylogroups within a taxonomic species displayed a strong geographic orientation. Indeed, 93% of the surveyed species with deep matrilineal sundering conformed to 'phylogeographic category I' as defined by Avise *et al*. (1987), in which distinct genealogical assemblages are regionalized geographically. Examples involving a mammal, a reptile and a fish are illustrated in figure 1.

For comparison against findings for the intraspecific phylogroups, genetic distances also were recovered for presumed sister-species pairs. For the mammals, herpetofauna and fishes (as well as birds), mean mtDNA genetic distances between sister species were significantly larger than those between the pairs of distinctive intraspecific phylogroups (Wilcoxon two-sample tests (Sokal & Rohlf 1995), $p < 0.02$ for fish, and $p < 0.001$ for the other groups).

(a) *Mammals*

Among more than 70 pairs of major intraspecific phylogroups identified in the mammals, estimates of

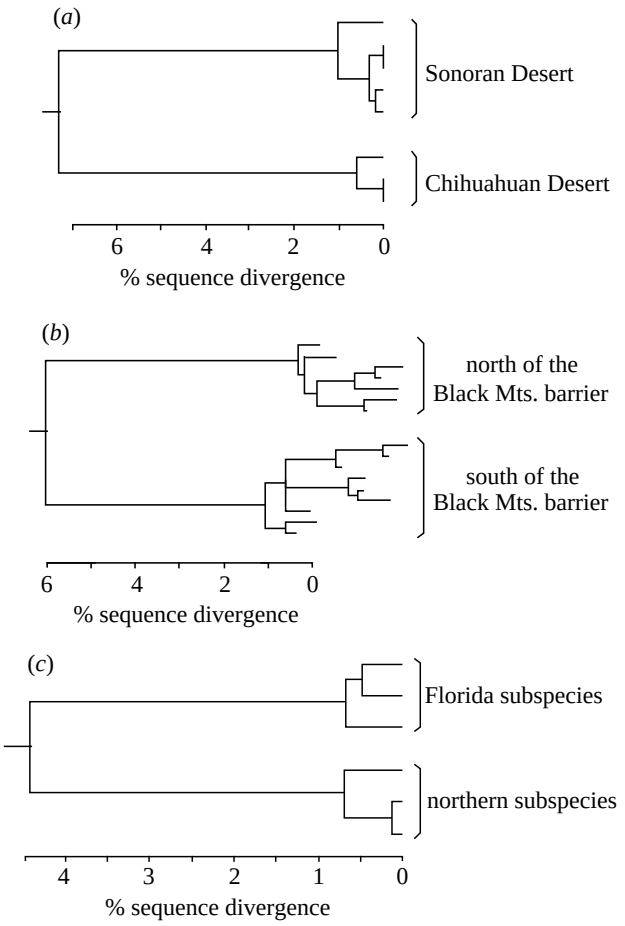

Figure 1. Examples of a common situation (phylogeographic category I) in which distinctive, geographically orientated mtDNA phylogroups have been reported within a vertebrate species. (*a*) UPGMA cluster phenogram for the pocket mouse, *Chaetodipus penicillatus* (after Lee *et al*. 1996); (*b*) neighbour-joining tree for the prickly skink, *Gnypetoscincus queenslandiae* (as drawn by Joseph *et al*. 1995); (*c*) UPGMA cluster phenogram for the largemouth bass, *Micropterus salmoides* (after Nedbal & Philipp 1994). Additional examples are summarized in Avise (1994).

mtDNA sequence divergence ranged from *ca*. $p = 0.004$ to $p = 0.130$ (figure 2*b*). Under a standard mtDNA clock, these translate into population divergence times ranging from 0.2 to 6.5 million years (Ma). Seventy-two per cent of these separation dates fall within the Pleistocene, i.e. within the last two Ma, whereas the remainder date mostly to the Pliocene. By contrast, at face value only 25% of more than 90 sister-species pairs of mammals similarly date to separation times in the Quaternary (table 1 and figure 2*a*). This difference between phylogroup pairs and sister-species pairs in the percentages of inferred Quaternary separations is highly significant (*G*-test of independence (Sokal & Rohlf 1995), $G = 37.6$, d.f. $= 1$, $p < 0.001$).

(b) *Herpetofauna*

Among *ca*. 50 pairs of major intraspecific phylogroups identified in amphibians and reptiles, comparable appraisals based on a standard mtDNA clock (2% sequence divergence per Ma) yield estimates of population

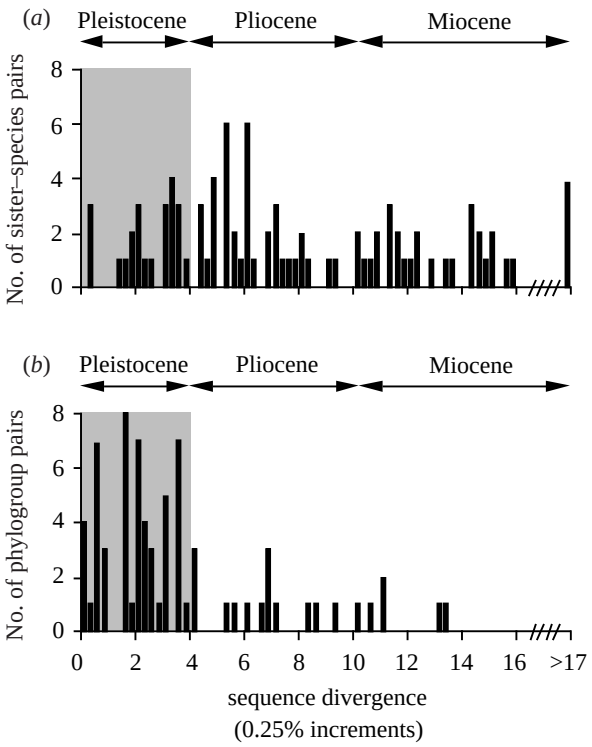

Figure 2. Histograms of mtDNA genetic distances plotted on a common scale for (*a*) pairs of mammalian sister species (data from Johns & Avise 1998) and (*b*) major intraspecific phylogroups (this paper). The shaded area covers Pleistocene separations under the 'standard' mtDNA-clock calibration.

divergence times ranging from less than 0.1 to 4.6 Ma. Fifty-seven per cent of these separation dates fall within the Pleistocene; the remainder date to the Pliocene (figure 3*b*). Under the same clock, at face value only 33% of the 42 herpetofaunal sister-species pairs similarly date to the Quaternary (table 1 and figure 3*a*). This difference in percentages of phylogroups versus sister-species that date to Quaternary separations is significant ($G=5.3$, d.f. $=1$, $p<0.05$).

A quite different picture emerges regarding absolute (although not relative) separation times for phylogroups and sister species under the several-fold slower mtDNA clocks that have been suggested for some poikilotherms. For example, figure 3 notes the pronounced shift to more ancient separation times that would be implied by a four-fold-slower mtDNA clock (i.e. 0.5% sequence divergence per Ma) for the amphibians and reptiles surveyed. Under that clock calibration, only 15% and 12% of intraspecific phylogroups and sister-species pairs, respectively, date to the Quaternary (table 1).

(c) *Fishes*

Among 26 pairs of major intraspecific phylogroups identified in fishes, appraisals based on a standard mtDNA-clock calibration (2% sequence divergence per Ma) yield estimates of population separation times ranging from 0.2 to 4.2 Ma. Seventy-three per cent of these dates occur within the Pleistocene; the remainder fall in the Pliocene (figure 4*b*). Under the same clock, at face value only 48% of the 108 sister species of fishes similarly date to the Quaternary (table 1 and figure 4*a*). This difference in percentages is significant ($G=5.4$, d.f. $=1$, $p<0.05$).

Again, a different picture emerges of absolute separation times under alternative mtDNA clocks (figure 4). For example, under a fourfold-slower clock calibration, only 31% and 17% of the piscine intraspecific phylogroups and sister-species pairs, respectively, date to the Quaternary (table 1).

4. DISCUSSION

(a) *Speciation durations*

In most vertebrate groups, where allopatric speciation undoubtedly is the norm (Mayr 1963), evolutionary divergence to species-level taxonomic recognition often must be preceded by differentiation into recognizable phylogeographic population units. If so, then separation dates for the major phylogroups highlighted in this summary provide provisional lower bounds on temporal durations of typical vertebrate speciations. Similarly, because sister species are the closest extant forms traditionally considered to warrant different Latin binomials, their separation dates provide upper bounds on estimated temporal durations of recent speciations. Inferred separation times for intraspecific phylogroups and sister species thus bracket speciation durations, and midpoints between these values provide estimates of the general evolutionary time-frame typically associated with the vertebrate speciation process.

Consider, for example, the mammals. Median estimates of mtDNA sequence divergence between phylogroup pairs and sister-species pairs are $p=0.024$ and $p=0.064$, respectively (table 1), which translate under the standard clock to about 1.2 and 3.2 Ma, respectively. The midpoint between these times, 2.2 Ma, provides a general sense of the temporal framework of a typical mammalian speciation. All of these estimates conform closely to those for birds. Median sequence divergences between avian phylogroup pairs and sister-species pairs are $p=0.022$ and $p=0.056$, respectively (table 1), which translate to about 1.1 and 2.8 Ma, respectively. The midpoint between these values suggests that avian speciations typically entail a time-frame of about 2.0 Ma.

Similar exercises can be applied to the poikilothermic vertebrates, with the added caveat that mtDNA rates perhaps are known less securely in many of these groups. If we assume the standard homeotherm clock and follow the same analysis procedures as above (using the data in table 1), estimates of typical speciation durations for herpetofauna and for fishes are about 2.3 Ma and 1.7 Ma, respectively. These estimates of speciation durations in the poikilotherms are close to those for mammals (2.2 Ma) and birds (2.0 Ma).

However, the use of lower mtDNA rates (as suggested for various poikilotherms) extends speciation-duration estimates accordingly. For example, estimates for herpetofaunal taxa and fishes are lengthened to about 9.2 Ma and 6.8 Ma, respectively, under a fourfold-slower clock.

(b) *Pleistocene phylogeographic effects*

With the general frameworks of speciation durations established from mtDNA data, the importance of Pleistocene biogeographic effects on extant vertebrate faunas can be re-examined in a new light.

Table 1. *Summary information relevant to Pleistocene phylogeographic effects and speciation durations for vertebrate taxa based on mtDNA appraisals*

	phylogroup pairs				sister-species pairs			
statistic	mammals	birds[a]	herpetofauna	fish	mammals	birds[a]	herpetofauna	fish
total number of comparisons	72	37	47	26	92	35	42	108
median sequence divergence (%)	2.4	2.2	3.1	2.6	6.4	5.6	6.1	4.1
% dating to Pleistocene (standard clock, 2% per Ma between lineages)								
uncorrected	72	76	57	73	25	31	33	48
corrected[b]	—	—	—	—	50	71	57	67
% dating to Pleistocene (fourfold-slower clock)								
uncorrected[c]	—	—	15	31	—	—	12	17
corrected[b]	—	—	—	—	—	—	29	31

[a] Phylogroup values for birds taken from Avise & Walker (1998), and for avian sister species from Klicka & Zink (1997).
[b] Times for sister species adjusted by subtracting a correction factor for mean estimated separation times between intraspecific phylogroups (see text).
[c] Values not given for homeotherms because the standard mtDNA clock generally is thought to apply.

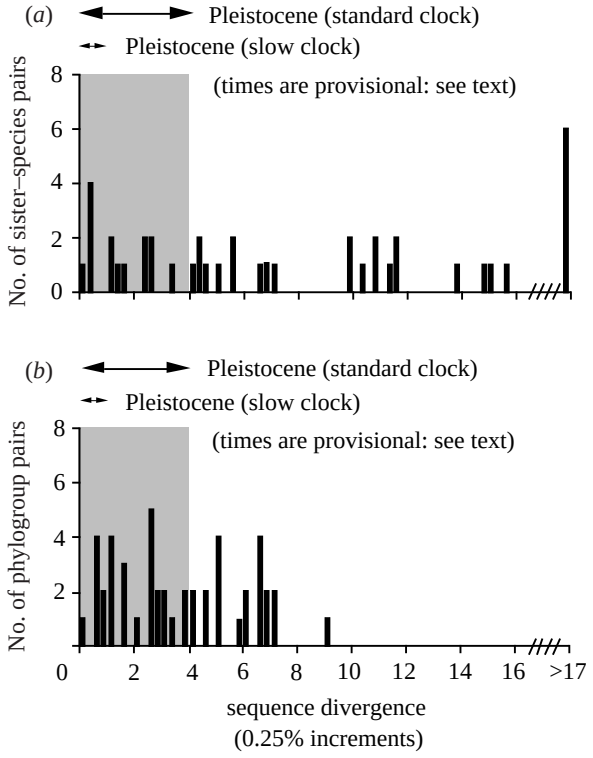

Figure 3. Histograms of mtDNA genetic distances plotted on a common scale for (*a*) pairs of sister species and (*b*) intraspecific phylogroups in herpetofauna. See legend to figure 2, and see the text for caveats and alternatives to the standard mtDNA-clock calibration.

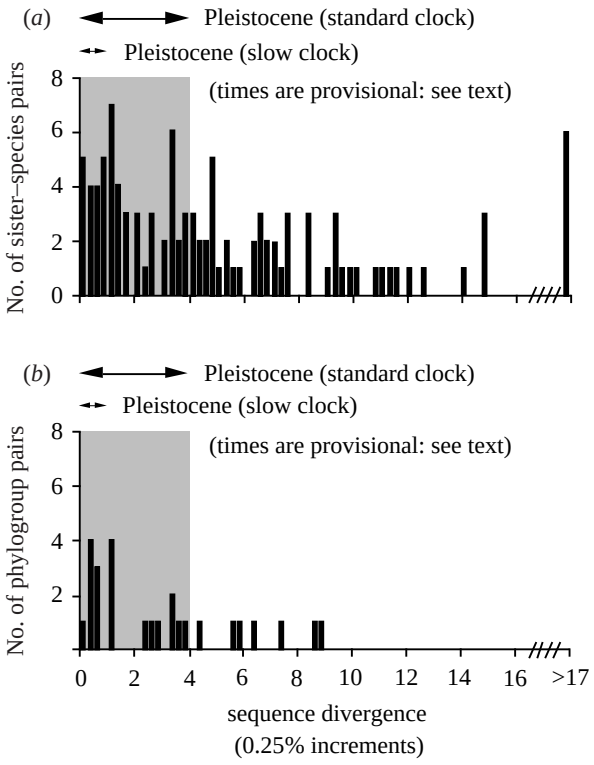

Figure 4. Histograms of mtDNA genetic distances plotted on a common scale for (*a*) pairs of sister species and (*b*) intraspecific phylogroups in fishes. See legend to figure 2, and see the text for caveats and alternatives to the standard mtDNA-clock calibration.

(i) *Homeotherms*

At face value, 23 pairs of mammalian sister species (25%) date to Pleistocene separations (figure 2 and table 1). However, separation times for sister species should be adjusted by a correction factor to account for times of phylogroup separation within species, and thereby to counteract a tendency to view speciation as a point event rather than a temporal process. A conservative correction involves median phylogroup separation times, because, as noted above, these provide a lower bound on typical speciation durations.

For pairs of mammalian phylogroups within species, median mtDNA sequence divergence is about 2.4% (clock translation 1.2 Ma). When this correction is subtracted from the between-species distance estimates, an additional 24 sister-species pairs are 'bumped' sufficiently to the left in figure 2*a* such that their inferred separations extend across the Tertiary/Quaternary boundary into the Pleistocene.

We interpret these results to mean that many mammalian species entering the Pleistocene would have already been separated into distinctive phylogeographic units (as are many mammalian species today; figure 2*b*). Many such phylogroups continued to diverge during the Quaternary, eventually achieving a level of differentiation recognizable as today's taxonomic species. Thus, altogether at least 50% of the mammalian speciations leading to extant sister species appear to have been associated in whole or part with the Pleistocene epoch.

These findings for mammals are similar to those reported previously by Avise & Walker (1998) for avian taxa, where the extended speciations leading to about 71% of the surveyed sister species appear to fall at least in part within the Pleistocene. Further documentation of Pleistocene phylogeographic influences on homeotherms comes from the *prima facie* evidence that about 72% and 76% of the major phylogroups within extant species of mammals and birds, respectively, also date to separations in that epoch (table 1).

(ii) *Poikilotherms*

Similar conclusions about an important role for Pleistocene events apply for assayed reptiles, amphibians and fishes if the standard mtDNA-clock calibration for homeotherms generally applies to these groups (table 1). For example, 57% and 73% of the phylogroups within herpetofaunal and fish species, respectively, then have separations dating to the Pleistocene; and 57% and 67% of the respective sister-species pairs had extended speciations falling at least in part within that epoch under the conservative correction for within-species phylogroup separation times.

However, these estimates are perhaps less secure than those for the homeotherms given current uncertainties about mtDNA evolutionary rates in poikilotherms. Plausibly slower clocks influence interpretations greatly. For example, under a fourfold-lower rate calibration for mtDNA, only some 20% of intraspecific phylogroups and 15% of sister-species pairs of the poikilotherms assayed have separation dates consistent with Pleistocene impacts (figures 3 and 4). If slower clocks apply, then speciation durations are extended and separation times made proportionately older on average.

(c) *Conclusions*

This comparative summary should not be interpreted to supersede or diminish focused appraisals of separation times and speciation durations for particular taxa in specific instances. The original literature clearly documents a considerable variance in tempos and modes of phylogeographic differentiation and speciation across vertebrate taxa. To cite just a few examples that depart radically from the vertebrate norm, the proliferation of cichlid fish species (and genera) in Lake Victoria appears from mtDNA and other evidence (Meyer *et al*. 1990) to have taken place over a time-frame (perhaps the last few thousand years) vastly shorter than what is characteristic for even modest phylogroup separations within many other vertebrate species. Species radiations within several other fish groups in closed lacustrine environments imply speciation durations often much less than 0.3 Ma, and speciation durations in some island birds (and arthropods) are reportedly about 0.6–1.0 Ma (McCune 1997). Near the other end of the scale, huge genetic distances between 'conspecific' phylogroups in the salamander *Ensatina eschscholtzii* indicate periods of population isolation far longer than most vertebrate speciation durations (Moritz *et al*. 1992; Wake 1997). Other such outlier examples can be seen in figures 2–4. In general, speciation durations in the vertebrates may be considerably longer under conventional allopatric circumstances than under the explosive and perhaps sympatric speciation scenarios that apply, for example, to species flocks in some freshwater fishes (McCune & Lovejoy 1997), or to other organismal groups undergoing rapid adaptive radiations (Givnish & Sytsma 1997).

The intent of this review has been to identify central tendencies, trends, and research challenges that otherwise might be less apparent. The current analysis documents that the Pleistocene had considerable impact on phylogeographic patterns within and among closely related species of many vertebrates. A remaining challenge is to ascertain molecular rates with greater confidence in various vertebrate groups and to extend such analyses to invertebrate animals and plants.

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