Mode of allozyme evolution: Increased genetic distance associated with speciation events

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Introduction

The relative contribution to evolutionary change of anagenesis (phyletic evolution) versus that of cladogenesis (lineage splitting, speciation) has been a long-standing issue. Two opposing views have been outlined by Eldredge and Gould (1972). Phyletic gradualism holds that divergence among taxa accumulates gradually over time. Punctuated equilibria, on the other hand, proposes a more rectangular view, whereby most divergence arises in coincidence with speciation events, which occur rapidly on a geological time scale relative to prolonged periods of stasis. Although the original models concerned morphological change and were introduced to clarify paleontological controversies, molecular parallels were soon sought. Most notably, Avise and Ayala (1975) introduced a method for examining the logical consequences of both modes of evolution on the distribution of genetic distances among living species. Their test was based on comparing mean genetic distances between species-rich and species-poor clades. If the view of phyletic gradualism is true, genetic distances are predicted to be strictly a function of time, and would be equal in species-rich versus species-poor clades of equivalent evolutionary age. In contrast, if the rate of evolutionary change increases during speciation events, i.e., punctuated equilibria prevails, genetic distances should be higher in species-rich clades, with larger genetic divergence resulting from the greater number of speciation events within speciose clades.

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As pointed out by Avise (1977) and by Mayden (1986), however, there is a potential asymmetry in Avise and Ayala's test. Most importantly, if genetic changes accumulate in a gradual manner, Avise and Ayala's test will be extremely sensitive to age inequalities among the compared taxa. If, on the other hand, a large portion of the genetic divergence accumulates in coincidence with speciation events, inequalities in the ages of the compared clades will not affect the results as much. As there are usually many uncertainties regarding the dating of speciation events, the gradualists mode cannot, as a rule, be rejected by this method. The punctuated mode, on the other hand, is falsifiable. Results obtained by using Avise and Ayala's (1975) test in both protein electrophoretic and morphological data were generally taken to be inconsistent with a punctuated mode of evolution (Avise and Ayala, 1975; Avise, 1977; Douglas and Avise, 1982). Tests based on chromosomal characters, on the other hand, were equivocal (e. g. Gold, 1980; Bengtsson, 1980).

We present here an extensive survey of genetic distances based on electrophoretic data from 511 species belonging to 111 tetrapod genera. Using this set of data we test whether or not increased evolutionary changes at the level of protein electrophoretic mobility are associated with the number of speciation events, as predicted by the punctuated equilibria model. We also introduce a simple model, with which one can assess the relative contribution of cladogenesis and anagenesis to the process of genetic divergence.

Data

Genetic distances or identity values were gathered from the literature and converted or recalculated, as necessary, so that all comparisons reported are of Nei's (1972, 1978) distance. In each class, D_r and D_p represent the mean interspecific genetic distances for genera with number of species above or below the class mean, respectively. Figures for number of species in each genus frequently vary between sources. We have used either consensus figures (Frost, 1985; Peters et al., 1931, 1987; Clements, 1978; Anderson and Jones, 1984) or recommendations from the most recent analysis available. A compilation of species and a list of references will be provided on request. Sceloporine electrophoretic data are from Mindell et al. (1989) and from D.P.M., J.W.S., and D.G. (unpublished results).

Results

We find that in all four tetrapod classes, Amphibia, Reptilia, Aves and Mammalis, the mean interspecific genetic distance, D, is greater in species-rich genera than in species-poor ones, whether the criterion for delineating species-rich or species-poor status is the mean ($p < 0.005$) or the median ($0.01 < p < 0.025$) number of species within genera (Table 1). For individual classes the differences are statistically significant in amphibians and reptiles. This is not surprising, since in our

Table 1. Comparisons of mean genetic distances, D, within species-rich (D_r) and species-poor (D_p) genera.

	Amphibia	Aves	Mammalia	Reptilia
No. of genera	16	35	37	23
Mean no. of species per genus	56.1	11.0	18.7	25.9
Mean interspecific D	0.809	0.118	0.330	0.438
Mean no. of species per species-rich genus (N_r)	242.3	25.1	45.0	93.2
Mean no. of species per species-poor genus (N_p)	13.2	5.7	7.5	7.2
N_r/N_p	18.4	4.4	6.0	12.9
D_r	1.149	0.131	0.395	0.637
D_p	0.730	0.113	0.303	0.343
D_r/D_p	1.574	1.159	1.304	1.867
$p(D_r/D_p) = 1*$	0.017	0.694	0.357	0.006

* p = probability.

compilation of data, the difference in the mean number of species between species-rich (N_r) and species-poor (N_p) is larger in amphibians and reptiles than in birds and mammals. The ratio N_p/N_r is 18.4 and 12.9 in amphibians and reptiles, respectively, while in birds and mammals it is only 4.4 and 6.0, respectively. Obviously, not all the genera within each of the tetrapod classes are of equal age. However, as potential inequalities in age are critical for testing phyletic gradualism, but not punctuated equilibria, we conclude that the results are qualitatively consistent with the supposition that cladogenesis increases the rate of allozymic divergence.

A more rigorous implementation of Avise and Ayala's test would require that several conditions be met (Mindell et al., 1989). First, the clades under comparison should be monophyletic, of equal age, and should experience equal rates of anagenesis (evolution along each lineage) and homoplasy (parallel or convergent evolution). This is required so that the amount of genetic divergence due to the gradual accumulation of genetic changes with time would be equal in both clades. Second, the clades should experience equal rates of extinction so that the number of extant species within each clade would reflect accurately the number of speciation events. While none of the groups in our sample of genera meet all these requirements, one group, the sceloporine lizards, provides us with the opportunity to address most of them.

The sceloporine lizards are a subfamily of the Iguanidae with 9–10 North and Central American genera (Hall, 1983). Our compilation includes four sceloporine genera, *Phrynosoma*, *Holbrookia*, *Petrosurus*, and the unusually speciose *Sceloporus*. A fifth closely related genus, *Crotaphytus*, was also included. Monophyly for the five genera is well established, based on osteology and external morphology (Etheridge and de Queiroz, 1988). Monophyly for each of these genera was also indicated, albeit preliminarily, from electrophoretic data (D.P.M., J.W.S. and D.G.,

unpublished results). If *Sator*, *Uta* and *Urosaurus*, none of which are included in the present study, are considered as subgroups or sister groups of *Sceloporus*, the assumption of monophyly will still hold, although the number of species within *Sceloporus* would be slightly higher. Questions of age cannot presently be answered directly using paleontological data. Nevertheless, morphological and biogeographic evidence indicates that the sceloporine lizard radiation followed the evolution of the North American deserts during the Miocene (Axelrod, 1950, 1958). Thus, the sceloporines are much younger than any of the species used in previous studies and as such have had less time to accumulate mutations subsequent to speciation events that may obscure the genetic differentiation associated with cladogenesis. The fish species used by Avise and Ayala (1975, 1976), for instance, date back to the Cretaceous, and hence any excess genetic divergence due to speciation may have been subsequently obliterated. In addition, Avise and Ayala's studies involved interspecific distance comparisons among families, thus including generic level differentiation that has occurred independently of speciation. Finally, the five genera used here are monophyletic, while in the case of Avise and Ayala's studies the assumption of monophyly has been found to be incorrect (Mayden, 1986). As to anagenesis, recent results suggest that rates of molecular evolution may be influenced by generation time (Li and Tanimura, 1987; Li et al., 1987; Li and Wu, 1987). Because there are no differences in generation time among the sceloporines, we do not expect differences in anagenesis. Similarly, because all the sceloporines occur in xeric habitats from the western United States to just south of Panama, they should have been exposed to similar biogeographic and climatic factors. To the extent that extinction patterns are influenced by such factors, we can infer similarity in extinction rates among the sceloporines.

In the sceloporine lizard species, since we compare mean genetic distances in more than two genera, we extend the predictions of Avise and Ayala's test to a correlation analysis. The predictions are that species diversity and mean interspecific genetic distance are not correlated if the primary mode of allozyme evolution is gradual, whereas a positive correlation should be obtained if the mode of evolution is primarily rectangular. From Table 2 we see that mean genetic distances show a perfect correlation with the number of constituent species in the sceloporine lizard genera ($p = 0.02$). The mean genetic distance for the species-rich genus *Sceloporus* was larger than that for all four species-poor genera combined ($D_p = 0.403$, $D_r/D_p = 2.176$), as well as for each of the species-poor genera individually. Thus, the sceloporine results are also consistent with rectangular evolution.

Model for estimating the contribution of cladogenesis to genetic divergence

In the following we attempt to estimate the contribution of cladogenic events to genetic divergence. Given a genus with N constituent species, the total number of speciation events (S) required to produce the species is $N - 1$, regardless of the topology of the phylogenetic tree. On the other hand, the mean number of speciation events separating two congeneric species ($\bar{S}$), depends on the topology of

the phylogenetic tree. Nonetheless, the difference in the values of S between two topologically distinct trees having an equal number of constituent species is quite insignificant. Thus, assuming that the trees for all the sceloporine genera contain no internal nodes (Fig. 1a), it is possible to calculate accurately the values of S for each of them (Table 2).

We assume that the total amount of genetic divergence or genetic distance (D) represents the cumulative contributions of anagenetic divergence and cladogenetic divergence. We further assume that the effects of anagenetic divergence are linearly proportional to the time of divergence, while the effects of cladogenesis are proportional to the number of speciation events. Thus,

$$D = tD_a + nD_c$$

where D_a is the rate of anagenetic divergence per year, t is the time since divergence, D_c is the rate of cladogenic divergence per speciation event, and n is the number of such events.

The model makes several simplified assumptions. For instance, it assumes the existence of an approximate molecular clock, i. e., it assumes that the anagenetic rate of change of electrophoretic characters with time is equal among all lineages. The model also assumes that all speciation events contribute equally to genetic change. We do not know whether these assumptions are met in nature. However, the assumptions are justified for reasons of parsimony. To put it in another way, as long as the evolutionary dynamics of change in electrophoretic characters is not known, the simplest possible mode is the one assuming rate equalities among evolutionary lineages.

Since we have reasons to believe that t is the same in all the five iguanid genera, the first term in the equation reduces to a constant. Consequently, we obtain a
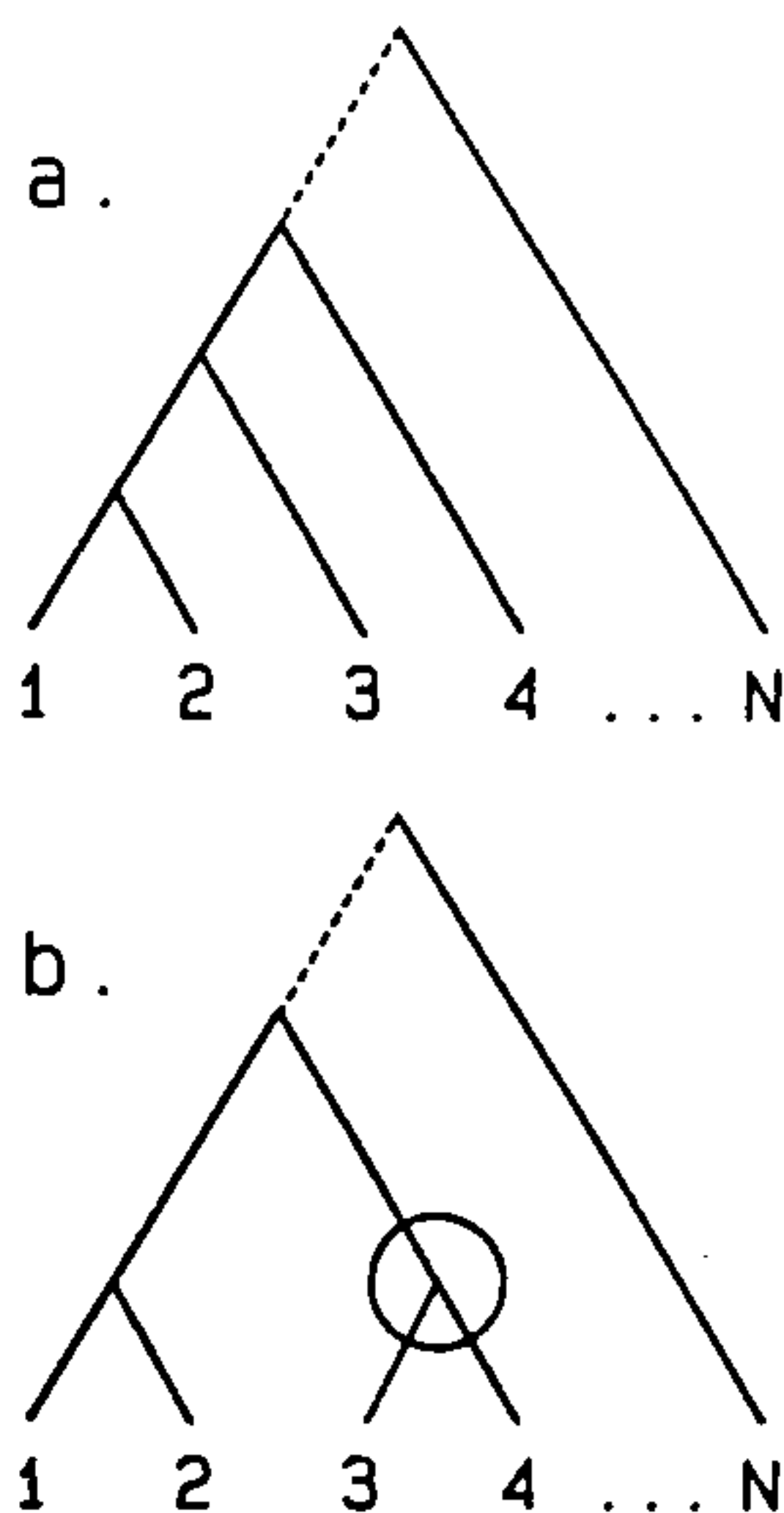

Fig. 1. Schematic representation of two types of phylogenetic trees. a. tree with no internal nodes; b. tree with an internal node (marked).

Table 2. Species diversity, mean genetic distance (D), D_r/D_p ratios, and mean number of speciation events (S) separating two species belonging to a sceloporine lizard genus.

Genus	No. of species in genus	No. of species compared	No. of loci resolved	D	D_r/D_p	S
Sceloporus	65	28	26	0.877	—	22.97
Phrynosoma	14	4	25	0.512	1.713	5.86
Holbrookia	4	3	25	0.373	2.351	2.16
Crotaphytus	3	3	25	0.312	2.811	1.67
Petrosaurus	2	2	34	0.118	7.432	1.00

linear regression equation in which D is the dependent variable, n is the independent variable, D_c is the slope, and the product tD_a is the intercept.

The observed slope of the regression equation of D over the mean number of speciation events ($\bar{S}$) is approximately 3%. Consequently, in *Sceloporus*, in which two species are separated on the average by about 23 speciation events, cladogenic events account for almost 80% of the interspecific genetic divergence. In *Phrynosoma* 35% of the total genetic divergence can be attributed to speciation events, and in the species-poor *Holbrookia*, *Crotaphytus* and *Petrosaurus* the contribution of cladogenesis to genetic divergence ranges from about 15 to 25%

Discussion

The issue at hand is a long-standing one, and the terminology involved has become quite convoluted. However, we can reduce the controversy between phyletic gradualism and punctuated equilibria to a simple question. What are the relative contributions to evolutionary divergence of anagenesis versus that of cladogenesis? By phrasing the question in such a way, we recognize that gradualism and rectangularity are not mutually exclusive modes of evolution. Indeed, the gradual accumulation of genetic changes during anagenesis is a well established phenomenon. Obviously, we are aware of the fact that rapid anagenesis, for instance, may in some cases lead to an increase in cladogenetic events. Nevertheless, at this stage, blurring the distinction between these two modes of evolution seems to serve no useful purpose.

The qualitative study presented here indicates that cladogenesis contributes significantly to genetic divergence independently of anagenesis. Indeed, substantial allozymic divergence was found to be associated with speciation events. It is thus possible that no clear linear relationship exists between the time of divergence and genetic distance, as proposed for instance by Nei (1975, p. 193). To estimate the time of divergence from genetic distances, one may need to take into account the number of speciation events separating the two taxonomic units under consideration.

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