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ASSESSING THE RELATIONSHIP BETWEEN SPECIATION AND EVOLUTIONARY CHANGE

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Recently we proposed and implemented a test of the potential role of speciation in accelerating evolutionary divergence based on comparing amounts of discrete character change between sister lineages relative to an outgroup (Mindell et al., 1989). Sanderson (1990) commented on our test in regard to detecting homoplastic change. We share his concern with making this test as definitive as possible; however, we feel his criticisms and "correction" are inappropriate, being based on misunderstandings or unsupported assumptions regarding sources of homoplasy, detection of homoplasy, effects of data removal on reconstruction of character evolution, and the actual extent of homoplasy in our data set.

Review of Model

Our model and test (Fig. 1) may be outlined as follows. If evolutionary divergence occurs gradually and continuously over time, with little or no contribution from speciation events, then amounts of discrete character change will be similar in any two species relative to an outgroup, regardless of differences in number of speciation events experienced. As long as the two species compared are sisters relative to the outgroup, they are, by definition, of equal age, and character change (in our case allozymes) will have had equal time to accrue in both. If speciation contributes significantly to divergence, the species which has experienced more splitting events in its past (species 3 relative to species 4 in Fig. 1) will show more character change, relative to the outgroup. Amounts of character change, including homoplastic change, are determined directly from branch lengths of a phylogenetic tree.

Detection of Homoplasy

Sanderson's criticism of our test is that clades with more taxa capture a larger proportion of the actual homoplastic change, and that this is a bias in favor of finding a correlation between branching events and divergence. Sanderson (1990: fig. 1) illustrates the fact that in some instances a lineage with more branching events will show more homoplastic character change than a clade with fewer branching events. He incorrectly assumes, however, that this will always be the case. There are other instances in which a speciose lineage on a phylogenetic tree will show a smaller proportion of the actual character change that has occurred, compared to a lineage with fewer branching

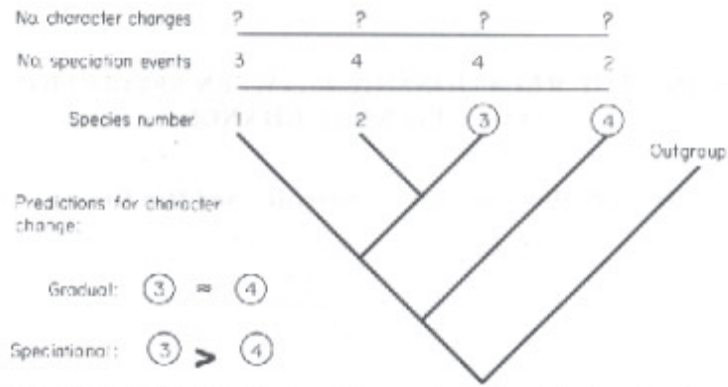

Fig. 1. Diagram outlining a phylogenetic test of the potential role of speciation in accelerating evolutionary divergence, after Mindell et al. (1989). The branching diagram represents a phylogenetic hypothesis based on shared derived characters. For each of four species shown, the number of speciation events experienced is indicated. Number of discrete character changes (? for each species) provides the empirical basis for the test, and is determined by summing branch lengths on a phylogenetic tree for each species. If character divergence is gradual, and not accelerated by speciation, amounts of change relative to an outgroup will be similar between species pairs differing in number of speciation events experienced (i.e., species 3 and 4). If speciation does contribute significantly to divergence, the species having experienced more splitting events will show more discrete character change relative to the outgroup.

events. For each of his [fig. 1(A–C)] examples, we provide an equally plausible counter example in which this is the case. In each of the hypothetical cladograms in our Fig. 2, three character state changes have occurred. However, the most parsimonious explanations of character states found (0 or 1) in the study taxa involve one or no character state changes, depending on the nature (gain or loss) and distribution of the actual character state changes. Thus, one cannot assume that adding taxa to a lineage will increase the proportion of homoplastic change *detected*, because in some instances, it can have the reverse effect.

Extent of Homoplasy

Sanderson's comments are also based on the premise that homoplasies due to multiple changes in one character on a branch (multiple hits) are common. If they were not, any bias in detecting them would have no net effect. Let us consider, then, whether homoplasies in our data set are common enough to pose a bias. Under a Poisson model of evolutionary change with two character states, there must be a 15% or greater chance of change in a character on a given branch for there to be a 1% chance of an undetected electrophoretic mobility change. Our character data show an approximately 6.6% chance of change per branch (165 character changes/36 branches/69 characters), and correspondingly less than a 0.5% chance for undetected changes. On this basis, multiple hits are insufficiently common in our data set for any potential difference in detection rates to have a net effect, and the reduction in character change seen after Sanderson's removal of taxa is an artifact of the omission of pertinent data and redistribution of character changes over the tree (see below).

Despite the reduction in character change following the removal of taxa, change was greater in the speciose lineage in 11 of 11 of Sanderson's (1990; table 1) comparisons using the ACCTRAN option and 7 of 11 comparisons using the DELTRAN option.

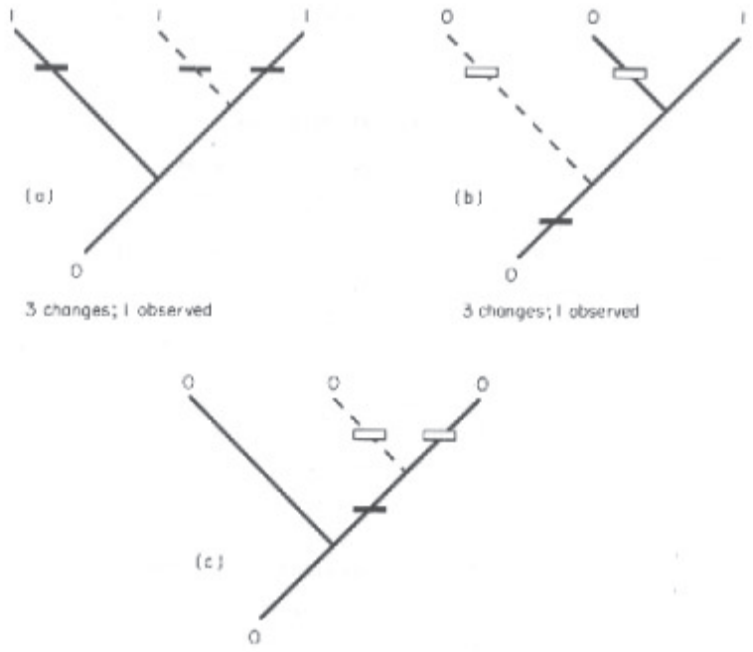

Fig. 2. Three instances are shown in which inclusion of additional taxa (dashed lines) results in a lower percentage of the actual change being observed (detected) in parsimony analyses. 0 and 1 represent character states for taxa. Solid bars represent character state origination, and open bars denote reversals or losses. In (a) a particular state for one character has originated independently in the three lineages as shown; however, parsimony analysis would detect (hypothesize) a single origin for the particular character state in this clade, occurring on the lowest branch prior to the first splitting event. In (b) one origin and two independent losses of a particular character state have occurred, yet the most parsimonious explanation of the observed character distribution is a single autapomorphic origin along the branch in the upper right portion of the clade. In (c) one origin and two independent losses of the particular character state have occurred, yet none of these changes will be detected. These examples correspond to Sanderson's [1990: fig. 1(A–C)] and demonstrate that speciose clades may not be assumed to detect a greater proportion of homoplastic change than does a clade with fewer taxa.

These comparisons are not entirely independent, however, the trend toward increased divergence in speciose sceloporine lineages is still apparent.

An earlier molecular model (Avise and Ayala, 1975) tests predictions that speciose and species poor clades of the same age will show: (1) similar mean genetic distances within clades if splitting events contribute little to divergence; or (2) greater mean genetic distance within the speciose clade if splitting contributes significantly to divergence. This model includes an assumption of equal amounts of homoplasy among lineages compared (being based on distance measures unable to discern homoplastic change), and provides an alternative assessment in which the potential bias of different homoplasy detection rates has been "corrected for" as by Sanderson in removing taxa from the species rich clade. We have also reported analyses using this approach for genera of sceloporine lizards of presumed similar ages, and the most species rich genus (*Sceloporus*) was found to have a larger mean genetic distance (0.877) than each of four genera having fewer constituent species (average = 0.329) (Mindell et al., 1990). So, with an alternative (and in our view less appropriate) model in which amounts of

homoplasy are presumed equal in different clades, a positive correlation between species richness and allozyme divergence in a larger set of sceloporine lizards is also observed.

Sources of Homoplasy

Based on the problematic assumption that addition of taxa results consistently in detection of more homoplastic change, Sanderson advocates factoring out all change associated with those "additional" taxa, by removing them all from the species-rich clade as a "correction". Not only does this presuppose that *all* change revealed by inclusion of the full data set in species-rich clades is matched by undetected change in species-poor groups (problematic for reasons discussed above), it also overlooks the fact that the homoplastic change removed may be, in fact, divergence associated with speciation. Homoplastic change is evolutionary change, and, therefore, constitutes divergence germane to the test. The removal of taxa is Procrustean in unduly restricting the body of evidence available for the assessment of the potential role of speciation in contributing to evolutionary divergence, and biases against finding any association between divergence and splitting events.

One History of Character Evolution

Following Sanderson's removal of taxa, character changes are redistributed on the original tree. This results in redefining the historical path of character change that supported the original tree. Synapomorphies may become autapomorphies and vice versa. This undermines the test, which requires comparison of amounts of character change as distributed on the original tree, providing the best possible estimate of the actual historical pattern of change. Further, this second distribution onto the first tree may no longer be the most parsimonious one, and, in turn, may support a different set of sister relationships. For example, consider evolutionary change of a single allozyme character (locus) taken from our original data set [character 7, S-Mc-A h (malic enzyme) in table 2 of Mindell et al. (1989) shown here in Fig. 3. Accepting the most parsimonious tree in Sanderson (1990: fig. 3), this particular character originally was hypothesized to have changed once and provided a synapomorphy for taxa 4–8 [Fig. 3(a)]. After removal of taxa 3 and 6, a new most parsimonious tree (majority rule consensus using PAUP 3.0h) places taxa 4 and 5 as sister to 2 rather than to 7 and 8, and two changes rather than one are hypothesized for the given character [Fig. 3(b)]. This provides only one example of what potentially can occur in numerous characters within a data set. Thus, the removal of taxa may have significant undesirable consequences beyond removal of homoplasy.

A related philosophical point is that removal of taxa, which is removal of data, compromises the basis for deciding among competing theories. This argument, that theory should conform to observation, without disposal of observations, provides the basic rationale for parsimony analyses of phylogenetic relationships as well (Farris, 1983).

Although we support our discrete character test (Fig. 1) as appropriate in assessing a potential relationship between lineage splitting and character divergence of any type (morphological or molecular), our initial application has certain technical limitations. Our allozyme data set included only 19 electrophoretic loci, whereas as many as 57 have been resolved in a recent phylogenetic study of *Cnemidophorus* lizards (Sites et al., 1990).

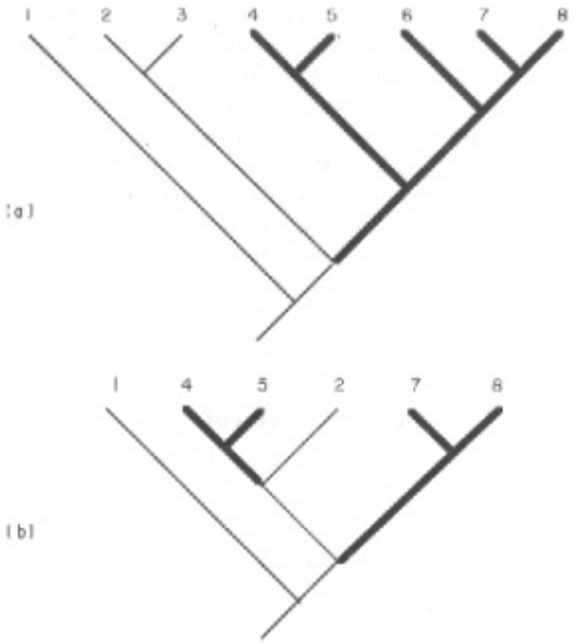

Fig. 3. Evolutionary history for an allozyme character S-Me-A b (character 7 from table 2 in Mindell et al. [1989]) before, (a), and after, (b), removal of data for species 3 and 6. Thin and thick lines represent alternative states for the single character shown. Removal of species alters history of character change as well as most parsimonious relationships (based on all characters) among species. Species codes are: 1 *Scelioporus clarki*, 2 *S. smiferus*, 3 *S. chrysostictis*, 4 *S. variabilis*, 5 *S. cozumelae*, 6 *S. occidentalis*, 7 *S. virgatus*, 8 *S. undulatus*.

In this light, our data set provides only a subset of the variation potentially available in allozyme characters. Not all electrophoretic charge differences among allozymes are readily detectable, although some of this "hidden heterogeneity" may be seen by using a series of different buffers, pHs, and heat treatments in various electrophoretic experiments (see Coyne, 1982; Murphy et al., 1990).

Regardless of this potential for greater resolution of variability, allozymes are inherently separated from some change occuring at lower levels of molecular organization. Only 15 to 30% of amino acid changes result in electrophoretic charge (allozyme) differences (Nei and Chakraborty, 1973; Marshall and Brown, 1975; Graur, 1986), and, due to redundancy in the genetic code, approximately 30% of the mutations occurring at the nucleotide level are undetected when examining amino acid changes (Nei, 1975). In this light, it would be informative to apply our discrete character test to nucleotide sequences in seeking corroboration among findings based on data from different organizational levels. Application of the test at any level of organization, including nucleotide sequences, would benefit from careful assessment of rates of evolutionary change for the characters to be used, and efforts designed to include data sets with relatively low amounts of homoplasy.

Summary

We share Sanderson's concern (1990) with making our original test of the potential role of speciation in contributing to evolutionary divergence as definitive as possible;

however, we feel his criticisms and "correction" are inappropriate. The assumption that clades with more taxa will always capture more of the existing homoplastic change is refuted by counter examples. Under a Poisson model of evolutionary change, our original allozyme character data show less than a 0.5% chance of change per character per branch, which is below the level at which any potential differences in homoplasy detection pose a problem. Removal of taxa as a "correction" has the undesirable consequences of removing character change potentially associated with speciation events, and redefining the historical path of character change supporting the original tree and required by the test. We can recommend that applications of the test include consideration of the relative rates of change for different character sets, with the goal of using data sets having relatively little homoplasy, as is done in many phylogenetic studies.

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