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CONFOUNDING ASYMMETRIES IN EVOLUTIONARY DIVERSIFICATION AND CHARACTER CHANGE

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Abstract.—Studies of character evolution often assume that a phylogeny's shape is determined independently of the characters, which then evolve as mere passengers along the tree's branches. However, if the characters help shape the tree, but this is not considered, biased inferences can result. Simulations of asymmetrical speciation (i.e., one character state conferring a higher rate of speciation than another) result in data that are interpreted to show a higher rate of change toward the diversification-enhancing state, even though the rates to and from this state were in fact equal. Conversely, simulations of asymmetrical character change yield data that could be misinterpreted as showing asymmetrical rates of speciation. Studies of biased diversification and biased character change need to be unified by joint models and estimation methods, although how successfully the two processes can be teased apart remains to be seen.

Key words.—Diversification, evolutionary dead-end, extinction, irreversibility, phylogeny, speciation.

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The paradigmatic use of a phylogeny to study character evolution is by now familiar: an inferred phylogeny is taken as a “given”, and on it is interpreted the evolution of one or more characters of interest (e.g., Felsenstein 1985; Swoford and Maddison 1992; Sanderson 1993; Pagel 1994; Maddison 1994; Schluter et al. 1997; Cunningham et al. 1998). This approach, even if many alternative trees are examined (Huelsenbeck et al. 2000), treats the phylogeny as if it existed prior to the evolution of the character, like a series of branching paths along which the character was constrained to follow in its evolution. This may be a valid assumption if the character of interest is neutral. However, various factors affect speciation (Coyne and Orr 2004; Gavrillets 2004) and extinction (Erwin 2006), including features of the organisms themselves. If a character affects fitness and population sizes, fidelity to habitat, reproductive isolation, or migration, then the character may not have been a mere passenger along the phylogeny's branches. Depending on the state of the character, species lineages might be more or less likely to subdivide or go extinct, and thus the character will play an active role in shaping the phylogeny. If we treat a character that drives the shape of the tree as if it were merely a passenger on a pre-existing tree, what mistakes of inference could we make?

To examine interpretation of characters that shape the tree, characters and phylogenies were simulated using special modules with Mesquite 1.1 (Maddison and Maddison 2006) that evolve simultaneously a binary character and a tree (modules and files available; see Supplementary Material available online only at <http://dx.doi.org/10.1554/05-666.1.s1>). The character began at the root of the tree with either state 0 or 1 equiprobably. Character state change was symmetrical: in each time interval of 0.001 branch length units, the character had a probability 5×10^{-5} of changing from state 0 to 1 or 1 to 0. The tree evolved by a pure birth process, with speciation rate depending on the character state. Various

combinations of speciation rates were investigated, ranging from equal (probability of speciation in each interval 0.0001 regardless of the character state) to biased (probability of speciation 0.0001 if the lineage has one state, 0.0005 for the other state). The simulation continued until 100 species were evolved. During these simulations, the total number of actual (not reconstructed) character state changes ranged from about 10 (when speciation was biased and the tree grew quickly) to 50 (when speciation rates were equal). An example replicate with biased speciation is shown in Figure 1. Trees simulated with a higher probability of speciation for state 1 tend to show (as expected) many more species with state 1. State 0 is rarer, and may appear in scattered single lineages or in a sparsely diverging basal region. 10001 such replicates were done.

For each replicate, the tree (with branch lengths) was used to interpret the evolution of the character evolved with the tree. In particular, the tree was used to infer whether character change was asymmetrical, that is, whether the rate of gains (0 to 1) is different from the rate of losses (1 to 0). Asymmetry of character state change is of interest for various evolutionary studies (e.g., Sanderson 1993; Mooers and Schluter 1999; Ree and Donoghue 1999; McShea and Venit 2002). The maximum likelihood estimate of the bias between gains and losses (the ratio of their respective rates) was calculated using Mesquite's AsymmMk model, which is nearly equivalent to the unconstrained one character model of Pagel's (1999, 2000) Discrete program using the global option. Mesquite 1.1 differs in having the option to assume the frequencies of states at the root are not necessarily equal, but rather consistent with the model's implied equilibrium frequencies. We estimated bias under both assumptions (root states at equilibrium versus equal frequencies). The results were qualitatively similar, and we report only the results with root states at equilibrium.

Although the character was actually evolved with a bias

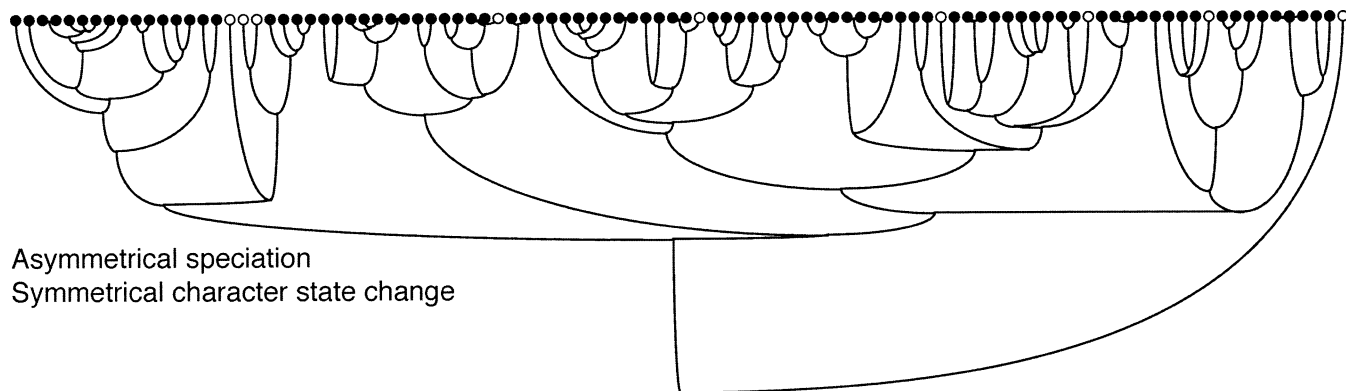


FIG. 1. An example tree and character distribution, simulated with biased speciation. Rate of speciation with state 1 (black) five times higher than rate of speciation with state 0 (white). States at internal nodes are parsimony reconstructions, not the actual ancestral states as simulated.

of 1.0 (i.e., no bias), the characters were interpreted to show biased state change when speciation rates differed (Table 1). For instance, when the speciation rate under state 1 is five times higher than the speciation rate under state 0, the median of the interpreted character state change bias among the 10001 replicates was 2.41. (Medians are presented instead of sample means, because the latter are strongly influenced by a few replicates of interpreted biases greater than 1000, probably due to flat likelihood surfaces causing problems for the likelihood estimator.) As expected, as the bias in speciation rates increases, the interpreted bias in character change increases. Thus, a pattern actually due to asymmetry in diversification (speciation more likely under state 1 than 0) is being misinterpreted as due to an asymmetry in character state change (change 0 to 1 at higher rate than 1 to 0). If the question of interest were whether there existed a bias in character state change, then biased diversification could mislead.

What about the converse? If a biologist were studying asymmetries in the rates of species diversification (speciation and extinction; Mitter et al. 1988; Slowinski and Guyer 1993; Barraclough and Nee 2001), and assumed character state change were symmetrical, what mistakes could be made if character state change were in fact biased? Phylogenies of 500 species were simulated with Mesquite's "Uniform Speciation (Yule)" module, in which each lineage has an equal probability of speciating at any time (a pure birth Yule process); the total

tree depth (sum of branch lengths from root to any tip) was 10.0. On each simulated tree, a single character was simulated with biased changes using the AsymmMk model with a rate of 0.4 for gains (0 to 1 changes) and 0.1 for losses (1 to 0 changes), that is, a bias or gain/loss ratio of 4.0. State 0 or 1 was assigned at the root in proportion to the equilibrium frequencies implied by the model. (Simulations were also done with the root state assigned equiprobably; the results were almost identical and are not reported.) A portion of an example replicate is shown in Figure 2. These simulations tend to produce trees with state 1 common and state 0 uncommon, scattered throughout the tree, presumably because whenever 0 evolves it tends to revert quickly to state 1.

The trees and their respective characters were then analyzed using a simple sister clade method for asymmetrical diversification (cf. Mitter et al. 1988). A special module of Mesquite was used to scan the tree for sister clades with uniform but differing states (i.e., one of the clades with uniform state 0, the other with uniform state 1). Only pairs of sister clades differing in number of species were examined, and were scored as to whether they favored state 1 (clade with state 1 more speciose) or state 0 (clade with state 0 more speciose). The number of pairs favoring states 0 and 1 were compared with a simple one-tailed sign test of the hypothesis that clades with state 0 are in general at least as large as those with state 1.

In 319 of the 1000 replicates the null hypothesis was rejected at the 0.05 level; that is, clades with state 1 were interpreted to have had greater diversification success. In contrast, when the bias of gains to losses was simulated as 1 (i.e., no bias) the null hypothesis was rejected in only 36 replicates. Thus, a pattern due to an asymmetry in character state change is being frequently misinterpreted as due to an asymmetry in diversification rates.

The explanation for these confusions appears simple. Asymmetries in both character change and diversification can leave one state rare. Seeing the rareness of that state, inference methods may assume that the state is relatively unstable. But is it unstable because it quickly reverts to the alternative (asymmetrical character change) or because its lineages quickly go extinct (asymmetrical diversification)? Alterna-

TABLE 1. Interpreted biases in character change (maximum likelihood estimate of ratio of gain/loss rates) for various probabilities of speciation for states 0 and 1 in each time interval of the simulation. A value of 1 indicates no bias. Medians for 10,001 replicates. Diagonal (bold) suggests no bias is interpreted when rates of speciation are equal. Above the diagonal the (mis)interpreted biases are in favor of changes to state 1; below the diagonal to state 0.

Probability of speciation with state 0	Probability of speciation with state 1				
	0.0001	0.0002	0.0003	0.0004	0.0005
0.0001	1.00	1.44	1.79	2.16	2.41
0.0002	0.70	0.99	1.18	1.35	1.52
0.0003	0.56	0.85	1.00	1.12	1.24
0.0004	0.46	0.76	0.88	1.00	1.11
0.0005	0.42	0.66	0.82	0.89	1.00

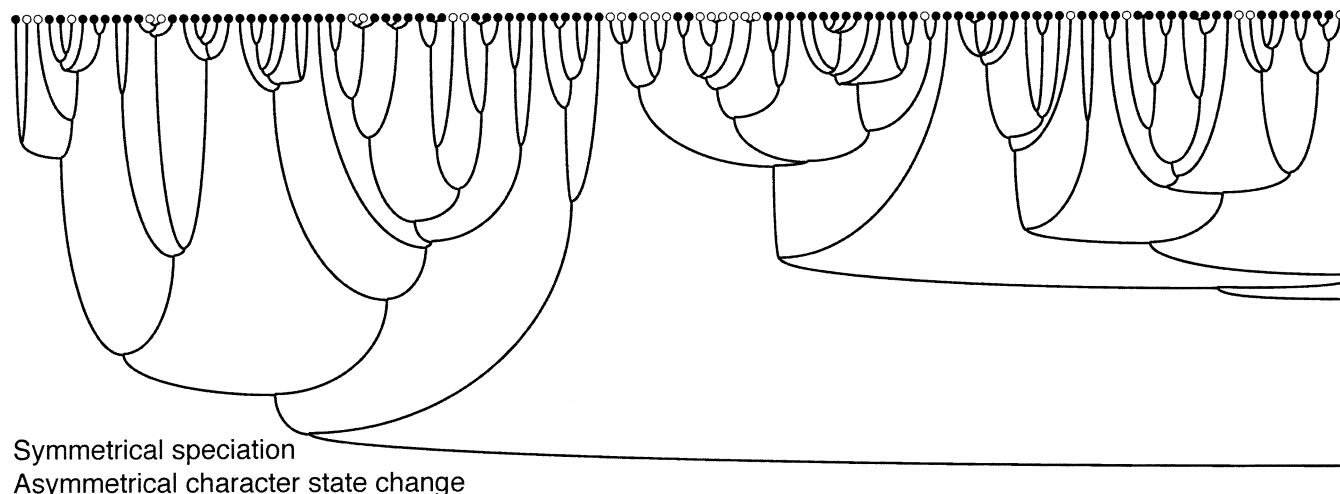


FIG. 2. An example tree with character distribution from the simulations of biased character change. Rate of 0 to 1 change is four times greater than rate of 1 to 0 change. States at internal nodes are parsimony reconstructions.

tively, is the other state more successful because it evolves frequently, or because its lineages speciate more quickly? A method considering only asymmetrical character state change would reasonably interpret the rarity of a state as indicating a low equilibrium frequency for it, which in the context of a two-parameter model is equivalent to inferring biased rates of change. This effect has been seen before: Nosil and Mooers (2005) have shown that asymmetrical character evolution tends to be inferred when one state is rare. On the other hand, a method comparing diversification rates examining a similar pattern would assume that lineages with the rare state simply fail to diversify, or go extinct more frequently.

Many of the characters we study comparatively may affect diversification rates through ecology or reproductive isolation, and their gains may be more or less likely than losses. Given this, methods that consider jointly the biases in diversification and character change are clearly needed. Although some empirical studies consider both biases together (e.g., Takebayashi and Morrell 2001), theoretical studies developing methods have focused on either diversification, or character change, but not both.

However, it is unclear whether a joint method could tease apart asymmetry in diversification from asymmetry in character change. In the best-case scenario, a fuller consideration of the data might permit us to distinguish these processes using phylogeny. For instance, asymmetrical diversification may leave a signature in the pattern of branch lengths that is distinct from the patterns left by asymmetrical character change. In Figure 1, the long branches leading to isolated terminals with state 0 may be a sufficient clue that differential diversification is involved. In the worst-case scenario, however, we may discover that the two processes are always confounded. Perhaps we can estimate only a single parameter representing the product of the two processes, much as θ , a product of population size and mutation rate, is estimated in population genetics. To untangle asymmetries in diversification from asymmetries in character change may require additional biological information, which might, for instance,

suggest that widespread reversal of a character state is unlikely.

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