

THE RECONSTRUCTION OF ANCESTRAL CHARACTER STATES

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Abstract.—The problem of error in the phylogenetic reconstruction of ancestral character states is explored by developing the model of Frumhoff and Reeve (1994). Information about the evolutionary rate of change within a character is inferred from the distribution of its character states on a known phylogeny, and this information is used to impose confidence limits on the error associated with ancestral state inference. Ancestral state inference is found to be remarkably robust under the model assumptions for a wide range of parameter values; however, the probability of error increases when the number of species within a clade is small and/or state-transition probabilities are strongly skewed in favor of the non-ancestral state. The rationale for expecting such a skew, a hypothesis of parallelism, is shown to rely on assumptions of low rates of change in at least two phylogenetically inherited characters: the tendency to occupy a particular ecological niche and the tendency to respond in a particular way to selection. A means for judging the relative likelihoods of parallelism vs. straightforward homology as explanations for a given character-state distribution is suggested. General problems with the model are discussed, as are methods for making it more realistic.

Key words.—Adaptation, character mapping, homology, homoplasy, parallelism, phylogeny.

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Uncovering the path of evolution through the distribution of features in extant species has been a primary tool of evolutionary biology since its inception (Darwin 1859). This comparative, historical approach is especially important for the study of adaptive traits, because only within a phylogenetic framework do such pivotal concepts as convergence, parallelism, and adaptive radiation become meaningful. Early evolutionary studies of behavioral and ecological traits were explicitly phylogenetic (e.g., Whitman 1899; Heinroth 1911; Chapin 1917; Lorenz 1941; Lack 1947; Tinbergen 1951), and during the past decade evolutionary biology has seen a resurgence of interest in this approach (e.g., Ridley 1983; Codrington 1988; Carpenter 1989; Brooks and McLennan 1991; Baum and Larson 1991; Harvey and Pagel 1991). This increased appreciation for the role of historical processes can be attributed at least in part to recent major conceptual and methodological advances in the field of systematics (Hennig 1966; Eldredge and Cracraft 1980; Nelson and Platnick 1981; Wiley 1981; Felsenstein 1982; Farris 1983; Ax 1987; Sober 1988; Hillis et al. 1994).

Responding to this trend, Frumhoff and Reeve (1994) examine both the usefulness and reliability of phylogenetic information in the study of evolutionary adaptation. In addition to scrutinizing specific methodological proposals, these authors conclude that the inference of ancestral character states from the distribution of character states present in descendant species is highly error prone. Because the inference of ancestral character states is central to the phylogenetic study of evolutionary patterns, this criticism, if accurate, has important negative consequences for historical studies of behavioral, ecological, and morphological evolution.

Here we focus on the issue of whether the distribution of character states within extant species provides information about ancestral states. We begin by arguing that evolutionary

homology provides the central concept for understanding this issue. We extend the model contributed by Frumhoff and Reeve (1994) for the neglected problem of error in ancestral character-state inference, focusing on the two evolutionary mechanisms identified by those authors: random and concerted homoplasy. By imposing confidence levels, we demonstrate that Frumhoff and Reeve's reported error rates are unduly pessimistic. We discuss the parameters that must be quantified in order to judge the likelihood of the general mechanism for parallel evolution proposed by those authors, and conclude with a discussion of how the model might be made more realistic.

Homology

The difference between homology and homoplasy lies at the heart of the problem of reconstructing ancestral character states. In phylogenetic reconstruction, the assignment of the same character state to two or more species constitutes a hypothesis of homology; that is, the character states are hypothesized to be similar owing to an unbroken chain of inheritance from a common ancestor that also possessed that state. When a number of such presumed homologies are combined into a phylogenetic analysis, a cladogram is identified in which the number of evolutionary origins of hypothesized homologies is minimized or in which their joint probability is maximized (as the sole criterion in "maximum parsimony" methods, or under additional a priori constraints in various other methods).

When most a priori homology assessments are correct, distributional congruence among characters will be high. This is because, owing simply to Darwinian "descent with modification" (Darwin 1859:123), we expect homologous characters to be correlated in accord with the same phylogenetic pattern. But even in cases where the proportion of mistaken homology assessments (homoplasies, i.e., unrecognized convergences, parallelisms, and reversals) is relatively high, we do not expect to be systematically misled by such characters

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as a general rule of evolution, because the distribution of homoplastic characters in extant species is predicted to be, on the average, random with respect to one another and with respect to the distribution of homologous characters. In other words, we expect homologous characters to produce a phylogenetic “signal” against a background of homoplastic “noise” (Hillis 1991; de Pinna 1991; Patterson 1982). (A possible exception arises under the parsimony criterion in the case of “long branch attraction” [Felsenstein 1978; Hendy and Penny 1989], which requires conditions that depart from the equal branch lengths modeled by Frumhoff and Reeve [1994], and that at any rate are unlikely to apply to the complex morphological, behavioral, and ecological characters discussed and modeled here.)

Contrary to these assumptions, Frumhoff and Reeve (1994) propose that homoplastic characters can be expected with high probability to be distributed in pseudophylogenetic patterns that are positively correlated with the distributions of genuine homologies. They identify two mechanisms responsible for generating this phenomenon: random homoplasy and concerted homoplasy (parallel evolution), which we discuss in turn below.

Pseudophylogenetic Patterns Produced by Random Homoplasy

In phylogenetic reconstruction it is generally assumed that, when all members of a monophyletic group of species possess the same character state, the unobserved ancestor(s) imbedded within that clade also possessed that state. (The algorithm for inferring ancestral states under the parsimony criterion, known as character state optimization, is formalized in Farris [1970], Fitch [1971], Sankoff and Rousseau [1975], Sankoff and Cedergren [1983], and Swofford and Maddison [1987], among others.) For example, the consistent presence of a vertebral column in 39,500 extant vertebrate species leads us to hypothesize that their common ancestor, as well as all subsequent ancestors, also possessed this anatomical feature; that is, that the vertebral column had a single evolutionary origin rather than up to 39,500 separate ones.

Challenging this logic, Frumhoff and Reeve (1994:174) suggest that the inference of an ancestral character state can be “extremely error-prone” and that “even as the number of sister taxa sharing a common character state increases, there may remain a substantial probability that their common ancestor possessed an alternative character state.” In particular, using a Markov model (with specific values reproduced in our Matrix 1; see the Appendix) for a two-state character in which one state is found to be fixed for all the species in a polytomy (e.g., state A in Fig. 1), they demonstrate that the expected error can be very high when one or more changes per branch are expected to have occurred. For instance, when the expected number of transitions within each descendant lineage is 4, error rates remain as high as nearly 0.5, even when there are as many as 20 descendants and all are fixed for the same character state. When the expected number of transitions is 1, error rates do not decrease to the 0.05 level until approximately 11 descendant species are present (Frumhoff and Reeve 1994:174; Fig. 2). This argument makes intuitive sense. Rapidly evolving characters retain little infor-

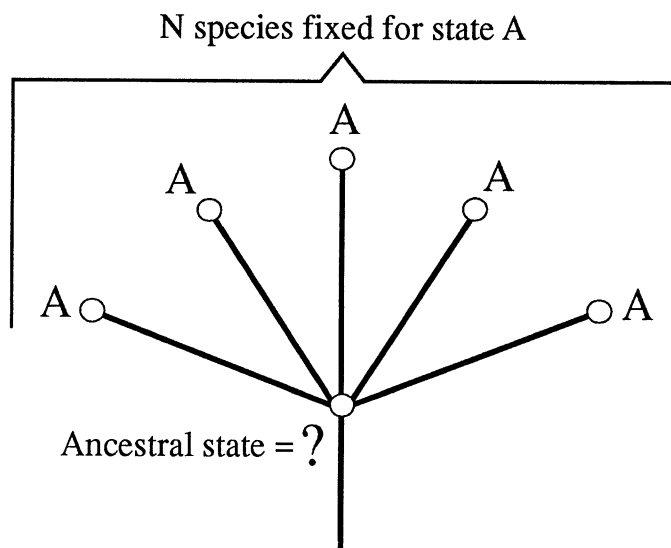


FIG. 1. The polytomous phylogenetic tree (“star phylogeny”) is modeled here. N species arise simultaneously from a common ancestor and all are subsequently observed to be fixed for character state A.

mation on history, because only a single transition is required to obliterate homology.

The critical concept that must be added to Frumhoff and Reeve’s (1994) analysis is that rapidly evolving characters governed by the Markov process specified in Matrix 1 are unlikely to generate a pattern of character-state fixation for a single state (rather than a mosaic of both states) in all N descendant species, particularly when N is large (Fig. 2). When a pattern of fixation is encountered (e.g., character state A in Fig. 1), it provides strong evidence that the character does not fit this model of rapid evolution. In fact, when any amount of accumulated change approaching 0.5 or more transitions per branch has occurred, the probability of fixation for the observed state is low when a clade contains more than four species. It is clear that, when such a pattern is encountered, it is unlikely that this amount of change has taken place. This additional information about the rate of evolution must be taken into account when calculating the probability of expected error in reconstructing the ancestral state (see the Appendix).

It seems reasonable to consider only the values for the expected number of transitions per branch (positively correlated with “time,” or t ; see the Appendix) that are consistent with the observation of fixation in all N species. The approach used here for estimating the probability of error in ancestral character-state reconstruction is first to place an upper bound on the expected number of transitions per branch and, second, to calculate the error probability corresponding to that value. Since error probability increases as expected number of transitions increases, this approach provides a conservative upper bound within the context of the model’s assumptions. For instance, if we choose to consider only those expected numbers of transitions per branch that could generate a pattern of fixation in five species (Fig. 1) with a probability greater than 0.05, this translates into an upper bound of 0.179 expected transitions per branch. The corre-

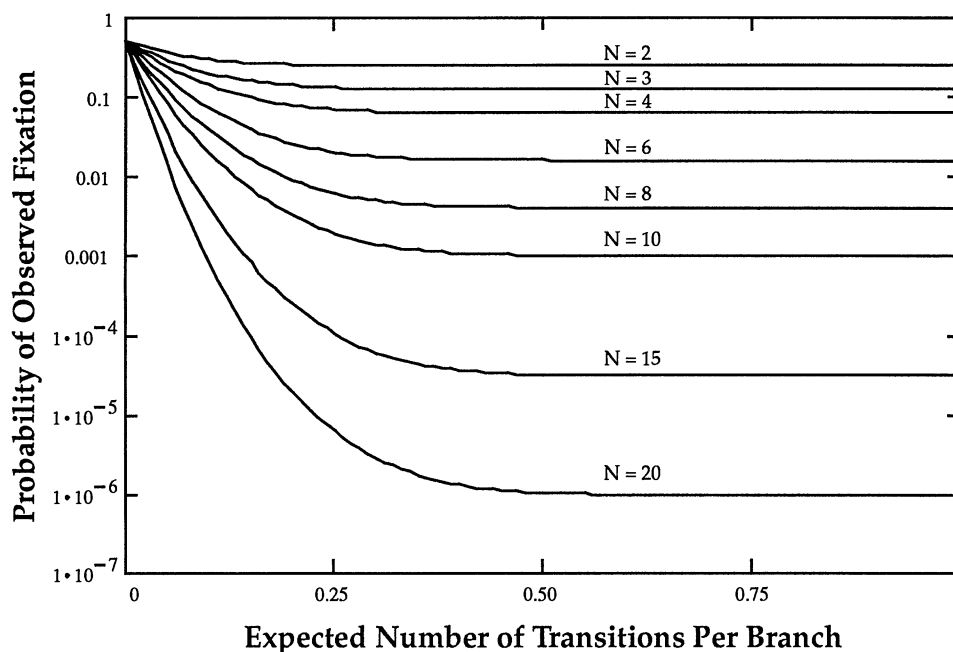


FIG. 2. The probability of fixation $F(t)$ for one state of a two-state character in N species, as a function of expected number of transitions per branch (ranging from 0 to 1), calculated using Equation A2 and assuming the topology in Figure 1, an ancestral-state probability of 0.5, and the state-transition probabilities in Matrix 1 (Appendix). When the expected number of transitions is low, the probability of fixation is high, with fixation resulting from homology (i.e., no change) comprising the vast majority of possible outcomes. As the expected number of transitions increases, the probability of fixation decreases, and outcomes resulting from homoplasy predominate. As the number of species N increases, the probability of fixation resulting from homoplasy decreases logarithmically.

sponding maximum error associated with this number of expected transitions is 0.081 (Fig. 3, at right).

By this reasoning, the range of evolutionary rates considered by Frumhoff and Reeve (1994) in their Figure 2 (from one to four expected transitions per branch) is irrelevant for all but the smallest clades. For example, Frumhoff and Reeve (1994:180) deduce that, for the case in which four transitions per branch are expected to have occurred, an inference of an ancestral character state with an error probability of 0.20 or less would require a clade containing a minimum of 2241 species fixed for the same state. However, the probability of 2241 species becoming fixed for the same state given four expected changes per branch is 6.2×10^{-675} . To put this into perspective, let us assume that the earth's current biota contains on the order of 5.0×10^7 species. Even if the history of life were to be reiterated 1,000,000,000 times, the chance of observing even one clade of 2241 species fixed for the same character state given these conditions is approximately 1 in 10^{661} .

The only expected numbers of transitions per branch that are likely to produce fixation in a large clade are values close to zero, when the case of homology predominates. (Exceptions can occur in special cases where the character-state transition probabilities are strongly biased in favor of one state; see below.) Figure 2 illustrates the probability of the observed fixation of a character state, as a function of the expected number of transitions per branch, for 2–20 species. Figure 3 illustrates the maximum probability of erroneously inferring an ancestral state, given that all N descendants are found fixed for one state, evaluated at $100(1 - c)\%$ confidence bounds for expected number of transitions ($c = 0.05$,

0.10, 0.20). These results demonstrate that the phylogenetic inference of ancestral character states is robust under a Markov chain model with conditions identical to those specified by Frumhoff and Reeve (1994:180, Fig. 2).

Pseudophylogenetic Patterns Produced by Concerted Homoplasy

We have shown that the circumstance of multiple transitions per branch, considered by itself, is unlikely to lead to error in ancestral-state reconstruction in all but the smallest clades. However, Frumhoff and Reeve (1994) discuss an additional circumstance that, when combined with multiple transitions per branch, will increase the probability of error: that in which conditions strongly favor the nonancestral state in the descendants. Under the Markov model, this condition is a function of two variables: (1) the probability that the ancestor possessed a particular character state, and (2) an asymmetry in the state-transition probabilities that favors a derived state that was improbable in the ancestor. In Figure 4, we present error curves for the particular case of eight species in which both quantities are varied (see also Table 1, and the Appendix for derivation). Again, when the probability of homoplasious character-state fixation for eight species is taken into account, a large portion of the calculated error curves (corresponding to probabilities of fixation less than 0.05) are likely to be irrelevant.

It is true, however, that when N is sufficiently small and when the probabilities strongly favor the nonancestral state in descendant species, the minimum probability of fixation for the nonancestral state can exceed 0.05. In these cases,

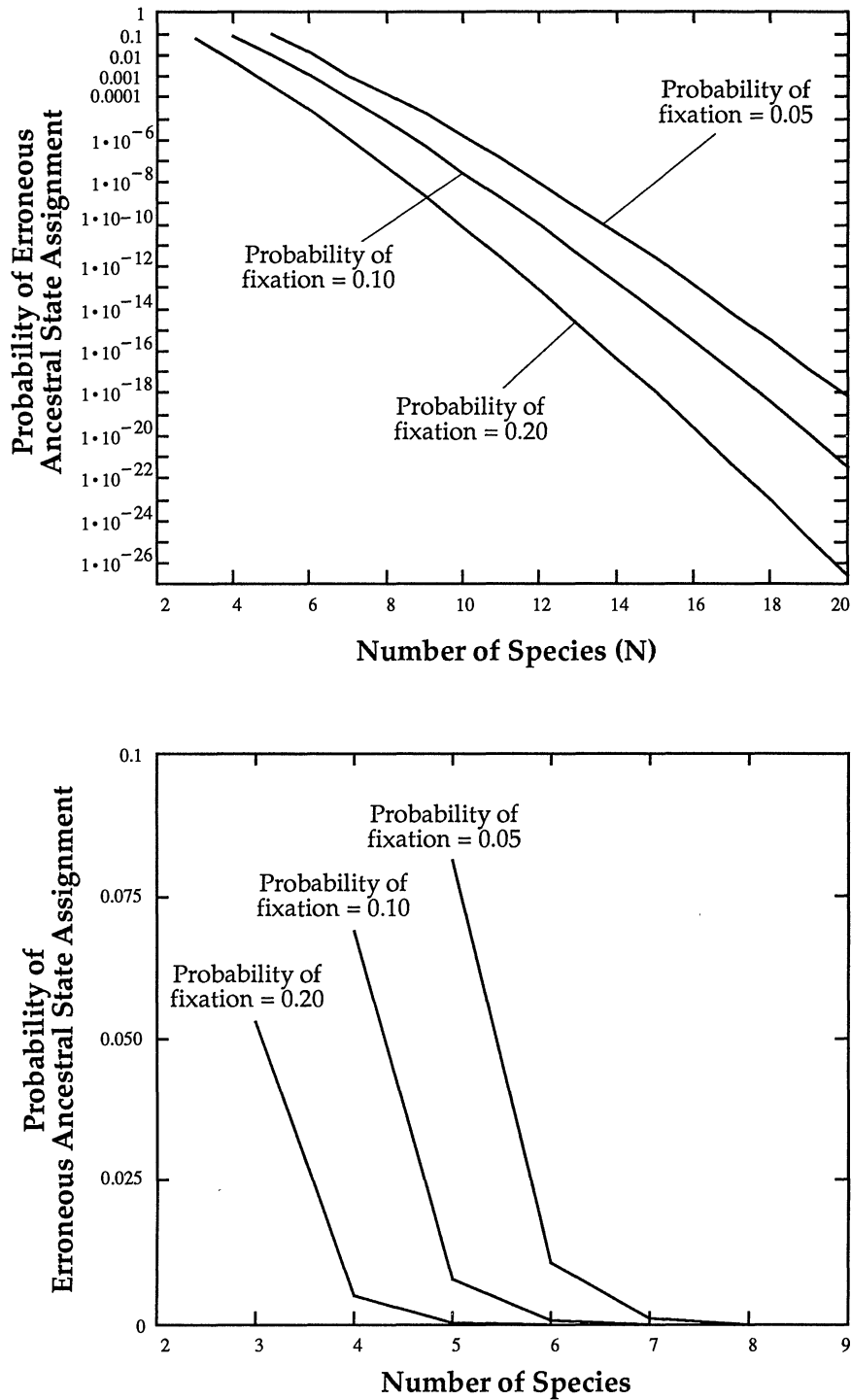


FIG. 3. The maximum probability of erroneously assigning an ancestral character state (expressed on a log scale, upper, and on an arithmetic scale, lower), given that all N species in a monophyletic group of species are observed fixed for one state of a two-state character. These curves correspond to upper confidence bounds (80%, 90%, 95%) on t (time elapsed since divergence), based on the observation of fixation in N species; that is, each curve corresponds to the maximum value of t that could have produced the observed fixation with a probability of 0.20, 0.10, and 0.05. When the probability of random fixation in N species is low, an observation of fixation indicates homology (i.e., that no transitions have occurred on the branches connecting the ancestor with the descendants). As the number of species increases, the maximum probability of error decreases logarithmically. Assumptions are as in Figure 1; derivation is in the Appendix.

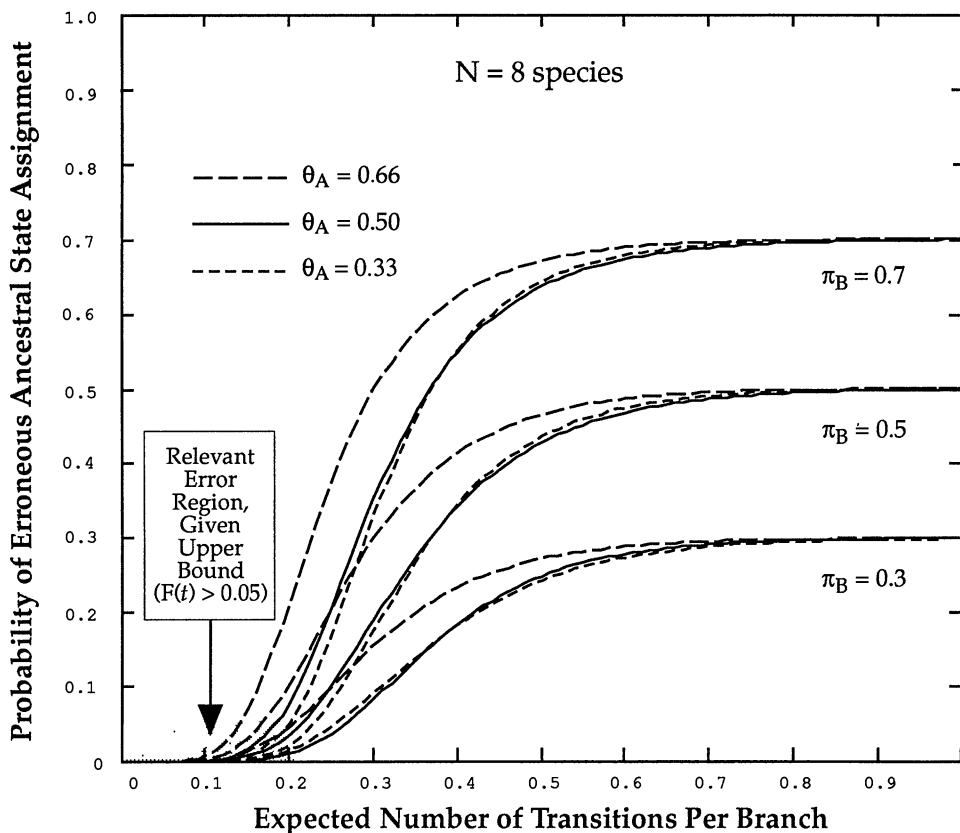


FIG. 4. The probability of erroneously assigning an ancestral character state for the case of a clade containing eight species, given that all are observed to be fixed for state *A* of a two-state character. Each of the three families of curves corresponds to a different prior probability ($\pi_B = 0.7, 0.5, 0.3$) that the ancestor possessed the opposite state, state *B*. Each curve within a given family corresponds to a different value of θ_A such that transitions favor state *A* ($\theta_A = 0.66$), transitions favor state *B* ($\theta_A = 0.33$), or transitions to either state are equiprobable ($\theta_A = 0.5$) (see the Appendix). However, only the shaded portions of these curves are relevant at the $P < 0.05$ confidence level because of the improbability that the number of expected changes (*x*-axis) in the unshaded region could have generated the observed pattern of fixation. See the Appendix for derivation and Table 1 for relevant error values for each curve.

the method employed here is unable to identify a reasonable upper bound on error probabilities for ancestral-state assignments. However, for every hypothetical case of this kind there is a symmetrical case in which the probabilities strongly favor the same state in the descendants as in the ancestor, and in which we would not be in error. Extrapolating from either case to character evolution in general requires that, whenever a cladogenetic event occurs, character states that are opposite to those in the ancestral species are either universally favored

(consistently producing erroneous inferences) or universally disfavored (consistently producing correct inferences) in the descendants, and that this trend continues only until the next cladogenetic event, so that equilibrium conditions (consistently producing correct inferences) are never achieved.

Frumhoff and Reeve (1994:173) suggest that the same state might be universally favored in all the descendants of a common ancestor “if closely related species have also experienced a similar series of selective environments and undergone, therefore, a positively correlated sequence of character-state transitions.” This is the circumstance of parallelism, widely discussed as a problem for phylogenetic studies (e.g., Hennig 1966; Eldredge and Cracraft 1980; Mayr and Ashlock 1991). All known cases of parallelism have been recognized precisely because the patterns produced by the parallel characters were in some way incongruent with the overriding phylogenetic pattern produced by other characters (e.g., trilobites [Kauffmann 1933], stalk-eyed flies [Hennig 1966]). However, Frumhoff and Reeve (1994:173) suggest a general mechanism of character evolution that is expected to generate parallelisms that exactly mirror phylogenetic patterns. Such undetected parallelisms “might be expected to be fairly common since closely related species tend to share similar niche

TABLE 1. The values of t' (expected number of transitions per branch) and z (error in assigning an ancestral state) corresponding to a probability of fixation $F = 0.05$ for the nine curves in Figure 4. The portions of the error curves exceeding these values of z are unlikely to obtain ($P < 0.05$) when a clade of eight species is found to be fixed for a given character state.

	$\theta_A = 0.66$	$\theta_A = 0.5$	$\theta_A = 0.33$
$\pi_B = 0.3$	$t' = 0.208$	$t' = 0.103$	$t' = 0.06$
	$z = 0.056$	$z = 2.301 \times 10^{-4}$	$z = 7.961 \times 10^{-7}$
$\pi_B = 0.5$	$t' = 0.154$	$t' = 0.087$	$t' = 0.052$
	$z = 0.0035$	$z = 1.571 \times 10^{-4}$	$z = 4.843 \times 10^{-7}$
$\pi_B = 0.7$	$t' = 0.1$	$t' = 0.064$	$t' = 0.039$
	$z = 0.007$	$z = 3.623 \times 10^{-5}$	$z = 1.228 \times 10^{-7}$

preferences (Harvey and Pagel 1991) and responses to selection. Thus, particular character states may be maintained by selection and be widely shared among related species, but be uniquely derived in each of them."

It must be emphasized that, as a substitute for hypothesizing homology for a single character state observed to be shared by a monophyletic group of species, this proposed mechanism for parallelism requires hypothesizing homology for multiple unobserved character states. That is, particular states of two characters rather than one are assumed to be faithfully inherited by each descendant species: the tendency to occupy a similar ecological niche (which we will call E_A) and the tendency to respond in a similar way to selection (which we will call S_A). In addition, an independent, homoplasious (parallel) origin of the observed character state (e.g., state A in Fig. 1) is required in each of the daughter species, as the result of a universal directional selection event acting across all such niches.

Given that a priori information about the rate of character evolution is unavailable, and given that the data consist solely of an observed, uniformly fixed character-state distribution within a clade, we might choose between a hypothesis of homology and a hypothesis of parallelism by comparing their likelihoods, that is, by comparing the probabilities that they confer on the data. A hypothesis of homology posits that the rate of evolution of the observed character is low enough that it is improbable that any change has taken place in that character on any of the branches connecting the species within the clade. (As long as the state-transition probabilities are noninteger numbers between zero and one, this probability will always be greater than zero because there is always the chance, however small, that at least one of the observed character states is the product of multiple transitions, e.g., $A \rightarrow B \rightarrow A$.) Thus, a hypothesis of homology confers on the data (consisting of N species fixed for state A) a probability that is close to 1.0.

A hypothesis of parallelism posits that the rate of evolution in the observed character has been rapid enough that it is probable that at least one transition has occurred on each branch, and that the state-transition probabilities favor the observed state. A hypothesis of parallelism further posits that this bias in transition probabilities applies to all N species because of their uniform inheritance of two characters, E_A and S_A , in which the rate of evolution is low enough that change on any of the branches is improbable. Thus, a hypothesis of parallelism confers on the data (consisting of N species fixed for state A) a joint probability that is the product of, minimally, two quantities: the probability of no change in E_A and the probability of no change in S_A .

For a hypothesis of parallelism to have a higher likelihood than a hypothesis of homology for a given set of data, the joint probability of two independent character-state distributions must exceed the probability of the single character-state distribution associated with homology. For example, if for N species the probability of fixation for each of the character states E_A and S_A is 0.90, and if this is also the probability of fixation for the observed character state (Fig. 1), then the likelihood of a hypothesis of parallelism ($0.90 \times 0.90 = 0.81$) is lower than that of a hypothesis of homology (0.90) in accounting for the observed distribution.

In any case, the existence of a correlation between phy-

logeny and both the tendency to occupy a similar ecological niche and the tendency to respond in a similar way to selection is open to empirical investigation. Also open to investigation is the general claim that entire classes of characters, for example, "most traits typically studied by evolutionary ecologists" (Frumhoff and Reeve 1994, Pp. 175–176), are especially prone to parallelism. This is at least partly contradicted by the surveys of de Queiroz and Wimberger (1993) and Wenzel (1992), which demonstrate that, across a wide range of studies, the kinds of behavioral characters utilized by systematists have proven no more homoplasious than morphological characters.

Problems with the Model of Character Evolution Employed Here

Although we have shown that ancestral character-state optimization performs well under the usual Markov-process assumptions, we caution against generalizing these error probabilities to character evolution as it actually occurs in nature. It is possible that the evolution of some characters may resemble this model (e.g., neutral-region DNA sites). However, the Markov process-based model used here requires assumptions and simplifications that are unlikely to be realistic for many characters, particularly complex behavioral and morphological ones. Shortcomings of this model include the following:

1. It requires the specification of state-transition probabilities that apply identically and independently to all branches of a phylogeny. As for all probabilities pertaining to biological processes, such probabilities must be obtained empirically (Maynard Smith 1971:59). Given a phylogeny, it is possible to retrospectively average the number of transitions between states that occurred in evolution, but it is not obvious how to interpret a probability thus obtained for any particular character. For a character-state transition that occurs more than once on a phylogenetic tree, the empirically obtained state-transition probability may be simply an a posteriori summary of unconnected events. Alternatively, it may be an indicator of an underlying mechanism, for example, of some innate "quality" of the character that allows us to predict its behavior in independent evolutionary lineages, even though parallel character-state transitions are not homologous. It seems more likely that character-state transitions, when they occur, are driven by a multiplicity of processes that vary radically between and within lineages at different points in time.

2. Character states present in outgroup species are not incorporated; however, they also provide important information on rates of evolution within that character, and hence on the probability of error in ancestral-state inference. For instance, the potential error rate for the case of $N = 2$ species is high because it is not improbable for a rapidly evolving character to be fixed for the same state in two independently evolving species at a given time owing simply to chance. However, if M outgroup species are additionally observed to be fixed for the alternate state, and if M is sufficiently large, then it becomes correspondingly less likely that the character conforms to a rapidly evolving model. Essentially, inferences about the overall rate of evolution will be more accurate if the probability of fixation (Equation A2) includes information from both ingroup and outgroup species.

3. A two-state character provides the worst case for ancestral-state reconstruction, because as the number of possible states increases, the probability of fixation for a particular state in N species decreases even when the transition probabilities favor that state. Given that different species can respond to the same selection event in novel ways, this limitation is clearly unrealistic and inflates the error rate.

4. The polytomous topology (Fig. 1) represents the best case for ancestral character state reconstruction, because each observation is conditionally independent and yields maximum information about the ancestor. In the case of more resolved topologies, observations of states in closely related species will be positively correlated under the Markov model, effectively reducing the sample size of data points bearing on the ancestral state.

CONCLUSION

Despite these shortcomings, the Markov model has proven useful here in allowing us to further explore the probability of error associated with the reconstruction of ancestral states first proposed by Frumhoff and Reeve (1994). Given the model's assumptions, we have shown that the observation of fixation in a clade for a given character state provides information on the evolutionary rate of change for that character, and that this critical information can be used to place an upper bound on the error associated with ancestral-state inference. Calculation of error using this method demonstrates that, when the clade contains more than four species and the state-transition probabilities are not strongly biased in favoring a particular state, ancestral states can be reconstructed with a high degree of reliability.

We hope that additional modifications of the model will lead to increasingly realistic assessments of the error associated with ancestral-state reconstruction. In turn, reliable reconstructions of ancestral character states can provide essential information about the sequences of transitions that have occurred in characters that are of particular interest to behaviorists and ecologists, allowing the elucidation of general evolutionary principles and permitting the testing of particular evolutionary hypotheses.

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APPENDIX

Here we modify the Markov model of Frumhoff and Reeve (1994) to incorporate the information about the expected number of transitions per branch that is provided by the discovery of fixation for a particular character state within a clade. The method employed here for incorporating this information is only one of a number of possibilities, and we hope this issue will be pursued further. Like Frumhoff and Reeve (1994), we assume N species related by a polytomy (i.e., star phylogeny; Fig. 1), a single two-state character with states A and B and with state transitions modeled as a Markov process, and independent evolution in each branch leading from the common ancestor to a descendant species.

We will assume the usual continuous-time, time-homogeneous Markov-process model of evolution (e.g., Tavaré 1986), such that evolution occurs independently in each branch, with the state-transition probabilities after t time units given by

$$e^{Qt} = \begin{bmatrix} P_{AA}(t) & P_{AB}(t) \\ P_{BA}(t) & P_{BB}(t) \end{bmatrix},$$

where Q is the infinitesimal-rate matrix given by

$$Q = \begin{bmatrix} -\beta & \beta \\ \alpha & -\alpha \end{bmatrix},$$

and where the relative rates of exchange between state A and state B are

$$\begin{array}{c} \beta \\ A \rightarrow B \\ \alpha \\ A \leftarrow B \end{array}$$

Rather than assume that the process is in equilibrium at the time of divergence from the common ancestor, we define a prior probability that the ancestor was in state B as

$$\Pr(\text{ancestor} = B) = \pi_B$$

The prior probability that the ancestor was in state A is $1 - \pi_B$.

We wish to determine the probability that the ancestor possessed state B given that all N descendants possess state A. This probability (as given by Frumhoff and Reeve 1994) is

$$z(t) = \frac{\pi_B P_{BA}(t)^N}{\pi_B P_{BA}(t)^N + (1 - \pi_B) P_{AA}(t)^N} \quad (A1)$$

where $z(t)$ is a function of the prior probability π_B and the relative rates P_{AB} and P_{BA} , as well as of time t .

However, this is the error given that we have observed all N descendants fixed for state A, which can be an extremely improbable occurrence when the value of N and/or t is sufficiently large such that one or more transitions are expected to have occurred on some branches. In such a case, at least some of the descendants share state A because of random homoplasy. In contrast, the fixation of all N descendants for state A will have the highest probability when the ancestor also possessed state A and no changes have occurred on any of the branches. The probability of fixation for state A is

$$F(t) = \pi_B P_{BA}(t)^N + (1 - \pi_B) P_{AA}(t)^N$$

When confronted with a pattern of fixation in N species, it is unreasonable to consider that it was generated by values of t for which $F(t)$ is low (e.g., $F(t) < 0.05$). Given that there is a maximum value of t above which fixation is improbable, the observation of fixation in N species provides information on the maximum number of transitions that actually have occurred. This upper limit for t may be identified by setting Equation A2 to a predetermined level (e.g., 0.05) and solving for t . This approach provides a valid upper confidence bound (Casella and Berger, 1990), which can be calculated for given values of N , π_B , P_{BA} and P_{AB} . In the example of Frumhoff and Reeve (1994, p. 174; their fig. 2), $\pi_B = 0.5$ and the transition probability matrix at time $t = 1$ is

$$\begin{bmatrix} 0.99 & 0.01 \\ 0.01 & 0.99 \end{bmatrix}. \quad (\text{Matrix 1})$$

As indicated in our Figure 2, when we consider only those values of t that are reasonable at given confidence levels [$F(t) = 0.05, 0.10$, and 0.20], as N increases the expected error caused by homoplasy decreases exponentially, and is dramatically lower than that indicated by Frumhoff and Reeve (1994).

To model the effects of varying the state transition probabilities (P_{AA} , P_{AB} , P_{BA} , and P_{BB}) and the prior probability of ancestral state π_B , we consider the case of $N = 8$ descendant species (Fig. 4). For the continuous-time Markov process, the probability of a transition from state B to state A at time t is given by $P_{BA}(t) = [\alpha/(\alpha + \beta)] - [\alpha/(\alpha + \beta)]e^{-(\alpha+\beta)t}$, whereas the probability of a transition from state A to state B is given by $P_{AB}(t) = [\alpha/(\alpha + \beta)] + [\beta/(\alpha + \beta)]e^{-(\alpha+\beta)t}$ (Tavaré 1986). Thus, the model is specified in terms of four parameters: t , α , β , and π_B . However, in order to avoid confusion between time (t) and number of transitions, we will constrain the relationship between α and β in such a way that t is expressed in units that are equal to the expected number of transitions per branch.

When the process is in equilibrium, $\Pr(\text{ancestor} = B) = \pi_B = \beta/(\alpha + \beta)$ and $\Pr(\text{ancestor} = A) = \pi_A = \alpha/(\alpha + \beta)$. In this case, the expected number of transitions per branch is given by $K = t[\beta\pi_A + \alpha\pi_B] = t[2\alpha\beta/(\alpha + \beta)]$ (Tavaré 1986). Thus, by setting $t[2\alpha\beta/(\alpha + \beta)] = 1$, we can use the constraint $\beta = \alpha/(2\alpha - 1)$ to ensure that $t = K$, that is, time units are expressed in expected number of transitions, which we will refer to below as t' .

If we now define a new parameter

$$\theta_A = 1 - \frac{1}{2\alpha} \quad 0 \leq \theta_A \leq 1, \quad (A3)$$

the state-transition probabilities can be written as

$$P_{BA}(t') = \theta_A - \theta_A e^{-2t'/\theta_A(1-\theta_A)} \quad \text{and}$$

$$P_{AA}(t') = \theta_A + (1 - \theta_A) e^{-2t'/\theta_A(1-\theta_A)},$$

where θ_A describes the tendency of the process to move in the direction of state A, that is $\theta_A = 0.5$ corresponds to $P_{AB} = P_{BA}$, $\theta_A > 0.5$ corresponds to $P_{BA} > P_{AB}$, and $\theta_A < 0.5$ corresponds to $P_{BA} < P_{AB}$.

In addition to the case of symmetrical exchange between states, two other values of θ_A are modeled in Figure 4, one of which favors transitions to state A twice as strongly as the other. For each case, the value of π_B is also varied. Error curves are generated for each case based on Equation 1; for each curve, an upper bound corresponding to a probability of fixation = 0.05 is applied. The results demonstrate that, even when the state transition probabilities favor the state opposite to that possessed by the ancestor, fixation resulting from random homoplasy is improbable, and the error probability associated with ancestral-state inference is low. However, as the probabilities favoring the opposite state increase, the probability of fixation resulting from homoplasy also increases, and beyond certain bounds the minimum probability of fixation exceeds 0.05, requiring another method for evaluating relevant error. This result is essentially tautological: when processes strongly favor misleading evidence, we are likely to be misled. It bears noting, however, that for every such case there is a symmetrical case in which the probabilities favor the same state in both the ancestor and descendants, and in which the circumstance of fixation resulting from random homoplasy rather than homology is highly improbable.