THE STOCHASTIC MODE OF MOLECULAR EVOLUTION: WHAT CONSEQUENCES FOR SYSTEMATIC INVESTIGATIONS?

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ABSTRACT.—Biochemical characters have been proposed as ideal sources of phylogenetic information. The homologous characters are easily identified, the relationships between such characters and the underlying genetic code is well understood, and, most importantly, biochemical characters are thought to evolve in a stochastic, clock-like manner. I present a modified version of the Zuckerkandl and Pauling (1965) model of stochastic evolution which is used to explore the consequences of a stochastic mode of evolution. The probability of observing shared character states is determined as a function of the evolutionary rate of a character, the time of independent ancestry for two sister taxa, the time of shared ancestry for the sister taxa independent of their next closest relative, and the number of functionally equivalent, equally probable character states. I found that, while many branching patterns can be reliably reconstructed using stochastically evolving characters, a large subset of theoretically possible phylogenies (as defined by the duration of shared and independent ancestry) would not be derived correctly. The model simulates a "best-case scenario" in which the rate of character evolution is constant over time. Violation of this assumption complicates phylogeny reconstruction and further limits the types of phylogenies that can be addressed with stochastically evolving characters. I discuss the implications of these findings for data analysis and experimental design. *Received 14 October 1987, accepted 14 March 1988.*

SYSTEMATICS has undergone a period of revitalization as a result of innovations in data analysis (Hennig 1966, Sneath and Sokal 1973, Felsenstein 1982) and availability of new sets of characters obtained with biochemical techniques (e.g. amino acid sequencing [Goodman et al. 1979], mtDNA sequencing [Brown 1983], DNA × DNA hybridization [Sibley and Ahlquist 1983], electrophoresis [Brush 1976, Matson 1984]). Although the use of biochemical characters has not met with complete acceptance, the impact of biochemical approaches on systematics has been profound (for reviews see Thorpe 1982, Buth 1984). Gould (1985) stated, for example, that DNA × DNA hybridization solves the problem of how and why organisms are related. One reason that biochemical characters evoke such enthusiasm is that they are thought to evolve in an essentially time-dependent manner, as a result of stochastic processes (Wilson et al. 1977, Kimura 1983, Gillespie 1986). To understand why stochastically evolving characters are considered such promising sources of phylogenetic information, it is instructive to examine why traditional morphological characters have often proved unreliable for phylogeny reconstruction.

Under natural selection, morphological characters may evolve at different rates in different lineages (e.g. bill morphology in the Galápagos finches compared with other assemblages of similar age). Furthermore, the direction of morphological change may not be strictly divergent because direct selection may result in reversal, convergence, and parallelism, all of which confound phylogenetic inference. Recognition of these limitations has lead to development of techniques to minimize the problem (Hennig 1966). The derivation of transformation series is a difficult task (Stuessy and Crisci 1984), and it is likely that the phylogenies produced from analysis of traditional characters will contain some misinformation.

In contrast, if the evolution of biochemical characters can be characterized as time-dependent divergence as a result of stochastic processes, systematic studies that employ such characters would not be plagued by convergence and parallelism. The expected magnitude of change in sister lineages would be the same, and would depend solely on time of independent evolution and on rate of change for the characters involved. Sister taxa would theoretically have more character states in common

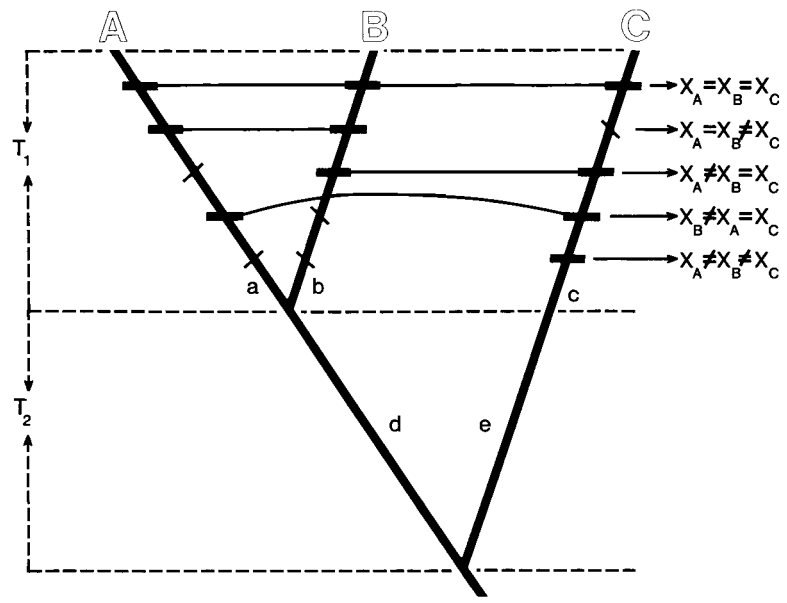

Fig. 1. Temporal definition for 3-taxon statements. T_1 is period of independent ancestry for sister taxa *A* and *B*, and T_2 is period of shared ancestry for sister taxa independent of their next closest relative (C). Lowercase letters identify 5 branch segments referred to in text. Symbols on branches *a*, *b*, and *c* illustrate 5 possible character-state distribution patterns for any character *X*.

than would nonsister taxa. Consequently, investigators could produce phylogenies through the application of algorithms that cluster taxa on the basis of overall similarity. It is this ease of analysis, in part, that causes investigators to suggest that biochemical characters are ideal sources of phylogenetic information.

Of course, if molecular evolution departs significantly from a stochastic mode of evolution, the hypothesized strong correlation between degree of overall biochemical similarity and phylogenetic relationship would not exist. The stochastic nature of molecular evolution has been a hotly contested topic (for reviews see Kimura 1983, Gillespie 1986). However, until the neutral theory is disproven, investigators will continue to invoke the concept of stochastic evolution when producing phylogenies from biochemical characters.

To ensure maximal extraction of reliable information, it is important to understand the limitations of biochemical characters with respect to phylogeny reconstruction. I present a simple model of molecular evolution to demonstrate how the nature of the true phylogeny under investigation might affect the probability of observing each of the alternative character-state distributions in stochastically evolving characters.

METHODS

For the remainder of this paper I shall discuss three taxon statements (Fig. 1). Each character (*X*) possessed by three taxa may be classified into one of 5 classes, depending on the character-state distribution that is expressed (X_A, X_B, X_C are the character states expressed by taxa *A*, *B*, and *C*, respectively). The 5 classes (Fig. 1) are: all taxa the same ($X_A = X_B = X_C$); all taxa different($X_A \neq X_B \neq X_C$); and three permutations in which two of the taxa are the same while the third demonstrates a unique condition ($X_A = X_B \neq X_C$, $X_A \neq X_B = X_C$, $X_B \neq X_A = X_C$). I modified the Zuckerkandl and Pauling model (1965) to produce equations to calculate the probability of observing each of the 5 alternative character-state distributions. The equations were based on a period of independent ancestry (T_1), a period of shared ancestry (T_2), the rate of character evolution (k), and the number of functionally equivalent and equally probable alternate character states (n).

To accomplish this goal, it is necessary to determine the probability of a character changing or remaining the same from the beginning to the end of each of the five branch segments (*a*–*e*, Fig. 1). These probabilities can then be used to calculate the conditional probability of occurrence of each of the n^5 possible evolutionary patterns. Because each pattern results in a single character-state distribution (e.g. stasis on branches *a*, *b*, *c*, *e*, and change to character state *z* on branch *d* results in pattern $X_A = X_B \neq X_C$), the probability of observing a particular character-state dis-

tribution is determined from the sum of the probabilities for each of the evolutionary patterns that result in that distribution.

Evolutionary change is a rare event in systematic characters, nucleotide substitutions in nuclear-DNA are thought to occur at a rate of 10^{-9} (Sibley and Ahlquist 1983). This led Zuckerkandl and Pauling (1965) to use a Poisson distribution to model molecular evolution. Gillespie (1984, 1986) raised doubts about the suitability of a Poisson distribution to represent molecular evolution. I employed a Poisson distribution because it permits the derivation of equations to calculate character-state distribution probabilities. The alternative models that have been proposed are more complex and do not lend themselves to this goal, but are more appropriate for simulations. The Poisson distribution is an infinite series that sums to 1 and provides the expected frequency of 0, 1, 2, 3, 4, . . . occurrences of a rare event:

$$\frac{1}{e^k}, \frac{k}{1!e^k}, \frac{k^2}{2!e^k}, \frac{k^3}{3!e^k}, \frac{k^4}{4!e^k}, \ldots, \quad (1)$$

where k is the mean expected number of changes in a biochemical character in 1 year. The probability of change over time (T) is, therefore, Poisson distributed with mean kT:

$$\frac{1}{e^k}, \frac{kT}{1!e^{kT}}, \frac{(kT)^2}{2!e^{kT}}, \frac{(kT)^3}{3!e^{kT}}, \frac{(kT)^4}{4!e^{kT}}, \ldots. \quad (2)$$

The probability that a character will, at the end of a period of evolutionary history of length T, exist in the same state it exhibited at the beginning of that interval of history, is equal to the probability of zero changes ($1/e^{kT}$) plus the probability that multiple changes have occurred and have resulted in a reversal. The frequency of reversals is dependent on the number of changes and on n. For simplicity, assume that changes to various alternate states are equally probable. Under this assumption, equations (3) and (4) provide, respectively, the probability of a character remaining the same at the end of a period of shared ancestry of length T_2, or of changing during the same period (see equations 3 and 4 below). The probability that a character will change during T_2 to a specific alternate character state (z) may be determined by dividing the probability of change during T_2, $P_{(\text{change } T_2)}$, by the number of alternate character states ($n - 1$).

$$P_{(\text{change } T_2 \text{ to state } z)} = \frac{P_{(\text{change } T_2)}}{(n-1)}. \quad (5)$$

It is possible to determine the conditional probability for the occurrence of each of the n^5 possible permutations of change and stasis across the five branch segments from equations (3) and (5), and their counterparts for the period of independent ancestry in which T_1 is substituted for T_2. For example, the probability (P) of stasis on branches a, b, c, and e, and of change to state z on branch d, is given by the product:

$$P = [P_{(\text{stasis } T_1)}][P_{(\text{stasis } T_1)}][P_{(\text{stasis } T_1)}] \cdot [P_{(\text{change } T_2 \text{ to state } z)}][P_{(\text{stasis } T_2)}]. \quad (6)$$

By classifying each of these patterns of change and stasis into one of the five character-state distribution patterns ($X_A = X_B = X_C, \ldots$) and summing these conditional probabilities across all n^5 patterns, it is possible to determine the probability of observing each of the five character-state distributions.

Results

The probability of observing each of the 5 character-state distributions at specific sites in nuclear DNA ($n = 4$ and $k = 10^{-9}$) as a function of T_1 and T_2 is presented in Fig. 2. The probability distribution for $X_A = X_B \neq X_C$ is characterized by four conceptual regions (Fig. 2b). The

$$P_{(\text{stasis } T_2)} = \frac{1}{e^{kT_2}} + \left[\frac{(n-1)}{(n-1)^2}\right]\left[\frac{(kT_2)^2}{2e^{kT_2}}\right] + \left[\frac{(n-1)^2-(n-1)}{(n-1)^3}\right]\left[\frac{(kT_2)^3}{6e^{kT_2}}\right] + \left[\frac{(n-1)^3-[(n-1)^2-(n-1)]}{(n-1)^4}\right]\left[\frac{(kT_2)^4}{24e^{kT_2}}\right] + \ldots. \quad (3)$$

$$P_{(\text{change } T_2)} = \left[\frac{(n-1)}{(n-1)}\right]\left[\frac{(kT_2)}{e^{kT_2}}\right] + \left[\frac{(n-1)^2-(n-1)}{(n-1)^2}\right]\left[\frac{(kT_2)^2}{2e^{kT_2}}\right] + \left[\frac{(n-1)^3-[(n-1)^2-(n-1)]}{(n-1)^3}\right]\left[\frac{(kT_2)^3}{6e^{kT_2}}\right] + \left[\frac{(n-1)^4-\{(n-1)^3-[(n-1)^2-(n-1)]\}}{(n-1)^4}\right]\left[\frac{(kT_2)^4}{24e^{kT_2}}\right] + \ldots. \quad (4)$$

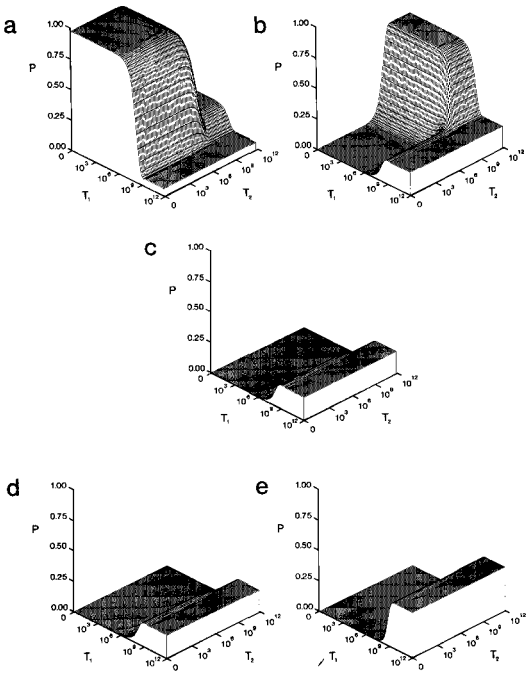

Fig. 2. Probability (P) of observing each of 5 character-state distributions for character X as function of T_1 and T_2 when $n = 4$ and $k = 10^{-9}$. (a) $X_A = X_B = X_C$, (b) $X_A = X_B \neq X_C$, (c) $X_A \neq X_B = X_C$, (d) $X_B \neq X_A = X_C$, and (e) $X_A \neq X_B \neq X_C$.

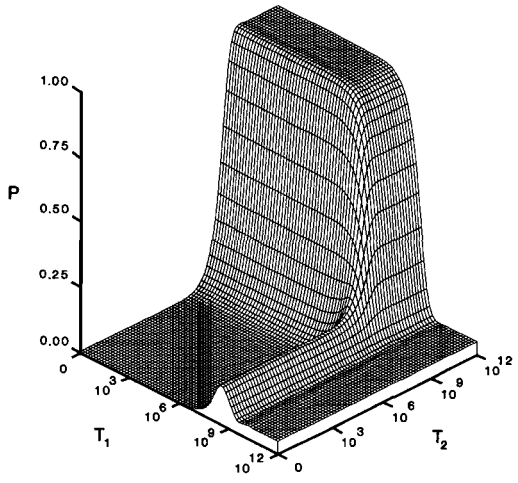

Fig. 3. Probability of observing $X_A = X_B \neq X_C$ as function of T_1 and T_2 when $n = 20$ and $k = 10^{-9}$.

remainder of this paper deals primarily with this character-state distribution because it alone supports the true branching sequence.

When T_1 and T_2 are less than 1 million years, there is virtually no chance that sister taxa A and B will share a character state independent of C. For this class of phylogenies, all three taxa are likely to share the same character state (Fig. 2a) because the characters evolve too slowly to have changed during these brief intervals.

For T_1 values above 1 billion years, there is a significant probability of observing a shared character state in 2 out of the 3 taxa. This character-state distribution results from random changes that lead to the independent evolution of the same character state in two different lineages. It is this pseudoconvergence that limits the probability of observing unique character states in all three lineages. The region affected by this pseudoconvergence may best be observed by comparing Fig. 2b and Fig. 3, between which the number of alternate character states differs. As n increases, the probability of observing pseudoconvergence decreases.

When the period of independent ancestry is between 1 million and 1 billion years, and T_2 is less than 10 million years, there is an increased probability of observing a shared character state in 2 out of the 3 taxa (this region is most evident in Fig. 3). However, the 3 character-state distributions, in which 2 taxa are the same, are essentially equally probable in this region because the shared state is actually a symplesiomorph (a shared primitive character state; compare Fig. 2b with 2c and 2d). Symplesiomorph probability is relatively high because for these combinations of T_1 and T_2 there is a high probability that 1 and only 1 of the 3 taxa will have evolved a new character state, leaving the remaining 2 to share the primitive state.

Finally, in the region bounded by $T_1 < 1$ million years and $T_2 > 10$ million years, the probability of observing $X_A = X_B \neq X_C$ is very high (Fig. 2b). The phylogenies in this region are characterized by relatively long periods of shared ancestry (T_2) and relatively short periods of independent ancestry (T_1). Large T_2 result in the evolution of many derived states in the common ancestor of A and B while small T_1 permit both A and B to retain some or all of these derived states.

The character-state distributions (Figs. 2, 3) do not differentiate synapomorphies (shared derived character states) from symplesiomorphies. Therefore, it is appropriate to ask whether a cladistic analysis would increase the number of phylogenies (as defined by T_1 and T_2) that could be reconstructed correctly using these characters. In theory, a cladistic analysis using

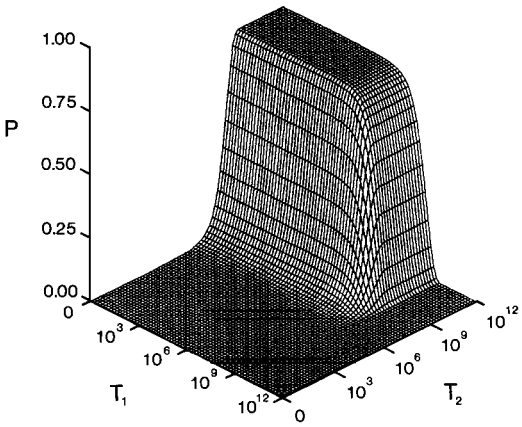

Fig. 4. Probability of observing synapomorphies for taxa A and B as function of T_1 and T_2 when $n = 4$ and $k = 10^{-9}$. Probability of observing $X_A = X_B \neq X_C$ presented in Fig. 2b differs because, in addition to synapomorphic probability, it includes the probability of observing a shared state for taxa A and B that is either a result of pseudo-convergence or is a symplesiomorphy.

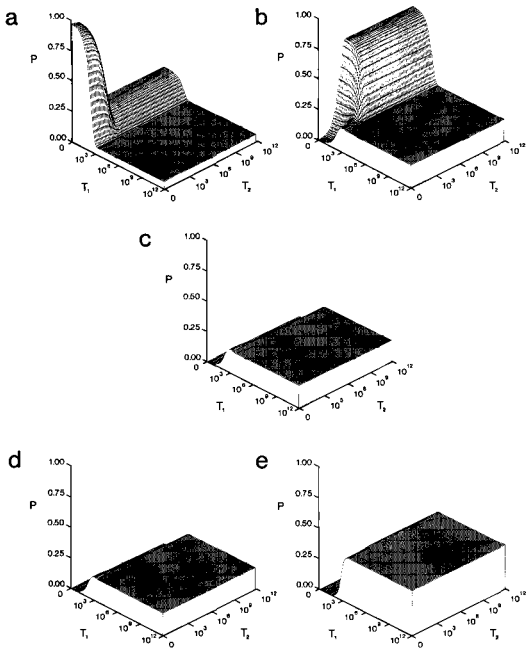

Fig. 5. Probability of observing each of 5 character-state distributions for character X as function of T_1 and T_2 when $n = 4$ and $k = 10^{-3}$. (a) $X_A = X_B = X_C$, (b) $X_A = X_B \neq X_C$, (c) $X_A \neq X_B = X_C$, (d) $X_B \neq X_A = X_C$, and (e) $X_A \neq X_B \neq X_C$.

a fourth outgroup taxon to identify primitive character states should be able to identify correctly the shared derived character states from equally frequent shared primitive character states. However, the model suggests that cladistic analyses would provide only a slight improvement over distance analyses. For many combinations of T_1 and T_2 the probability of observing a synapomorphy is effectively zero (Fig. 4). A cladistic analysis is ineffective when there are no shared derived states.

The previous discussion was restricted to characters that evolve at a rate of $k = 10^{-9}$. However, the implications remain the same regardless of the value of k (see Fig. 5; $k = 10^{-3}$, the rate of evolution for mtDNA; Brown 1983). The same four conceptual regions are evident, but with different temporal boundaries. When $k = 10^{-3}$, there is a reasonably high probability of observing $X_A = X_B \neq X_C$ only for phylogenies characterized by $T_1 < 1{,}000$ years and $T_2 > 1{,}000$ years. Thus, whether for nuclear DNA or mtDNA, a large subset of the universe of possible phylogenies cannot be reconstructed either because no shared character states are retained in sister taxa, or because the probability of shared character states in sister taxa does not significantly exceed the probability of shared states in nonsister taxa.

I suggest caution when interpreting biochemical data. To be properly appreciated, my findings should be related to the phylogenies being reconstructed by avian systematists. Sibley and Ahlquist have generated branch lengths for tyrannoid (Fig. 1 in 1985a) and corvine (Figs. 1–4 in 1985b) assemblages, as well as a calibration with which these lengths may be converted to T_1 and T_2 values. Their estimates of shared and independent ancestry for these taxa when viewed in the context of my results suggest that biochemical characters evolving at a rate of 10^{-9} will be uninformative about many phylogenies (Fig. 6a). This conclusion is not restricted to characters that evolve at a rate of 10^{-9}. The shadowed portion of Fig. 6b illustrates the combinations of T_1 and T_2 for which there exists a k between 10^0 and 10^{-12} that results in the probability of $X_A = X_B \neq X_C$ exceeding 0.19 (the maximum probability of observing $X_A \neq X_B = X_C$ or $X_B \neq X_A = X_C$). Note that a significant portion of the universe of possible phylogenies falls below the diagonal (Fig. 6b).

One major assumption of the model presented here is rate constancy, an assumption widely used in avian systematics (e.g. Gutiérrez et al. 1982; Sibley and Ahlquist 1985, and ref-

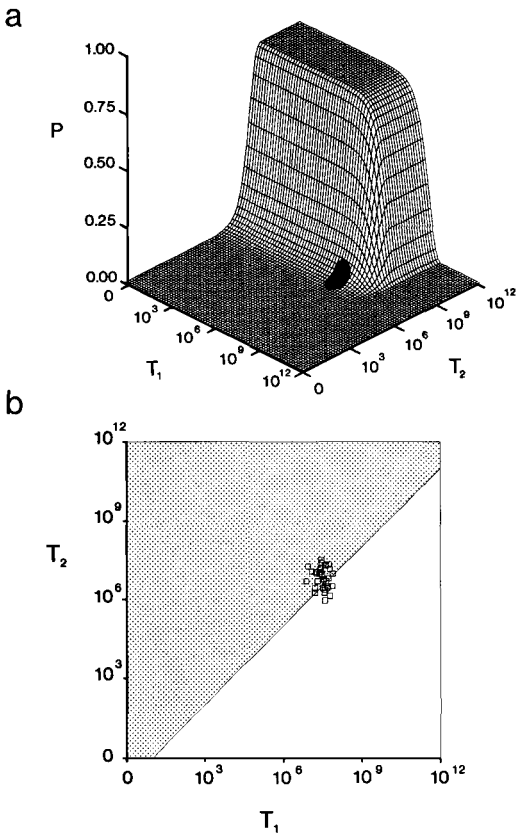

Fig. 6. (a) Probability of observing synapomorphies for taxa A and B as function of T_1 and T_2 when $n = 4$ and $k = 10^{-9}$. (b) In the universe of possible phylogenies (as defined by T_1 and T_2), the diagonal represents those for which the maximum probability of observing $X_A = X_B \neq X_C$ across all values of k is equal to 0.20, which is 5% greater than the maximum probability of observing $X_A \neq X_B = X_C$ or $X_B \neq X_A = X_C$ when $n = 4$ ($P = 0.19$). Ellipse in (a) and symbols in (b) represent nodes published in Sibley and Ahlquist (1985a, b).

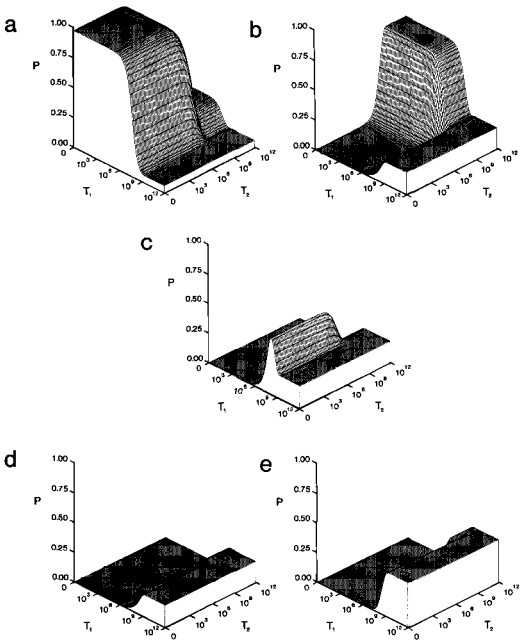

Fig. 7. Probability of observing each of 5 character-state distributions for character X as function of T_1 and T_2 when $n = 4$. (a) $X_A = X_B = X_C$, (b) $X_A = X_B \neq X_C$, (c) $X_A \neq X_B = X_C$, (d) $X_B \neq X_A = X_C$, and (e) $X_A \neq X_B \neq X_C$. Rate of character evolution is 10^{-9} on all branch segments illustrated in Fig. 1 except for branch a on which characters evolved at a rate of 10^{-8}.

erences therein; Shields and Wilson 1987). My intent in incorporating rate constancy in the model was to investigate the potential limitations imposed by this assumption. Furthermore, rate constancy represents a "best-case scenario." If biochemical characters depart significantly from this mode of evolution (e.g. Sheldon 1987), our ability to reconstruct phylogenies is even lower than I found. To examine the effect of unequal rates of change on different lineages, the probability distributions for an assemblage of three taxa were recalculated for characters evolving at a rate of 10^{-9} on all lineages except for the period of independent ancestry for taxon A, which evolved at 10^{-8} (branch a, Fig. 1). The results are illustrated in Fig. 7.

First, the probability of observing $X_A = X_B \neq X_C$ is lowered for $10^6 < T_1 < 10^{9.25}$ years (Figs. 2-b, 7-b). With a faster rate of change on lineage a, taxon A is unlikely to retain the shared derived state at these values of T_1. More seriously, with a faster rate of change in a, the probability of observing $X_A \neq X_B = X_C$ actually exceeds that of observing $X_A = X_B \neq X_C$ for some types of phylogenies (Figs. 7-b, 7-c). A distance analysis of data characterized by such T_1 and T_2 values would produce an incorrect branching sequence for the 3 taxa. By contrast, a cladistic analysis of such data would, in theory, identify the characters supporting the AC and BC clades as symplesiomorphs. A cladistic analysis would not produce an incorrect phylogeny, but would support a trichotomy. If branch c were the rapidly evolving lineage rather than branch a (Fig. 1), the result would have been an increased probability of correctly reconstructing the phylogeny. Thus, variable rates introduce a component of uncertainty into biochemical data that

reduces our ability to interpret observed patterns in a phylogenetic context.

Discussion

The actual probabilities generated in this study are dependent on the modified Zuckerkandl and Pauling (1969) model. I do not advocate that the model used in this study is the best representation of molecular evolution. The model simply was used to serve as a vehicle with which to explore how the length of shared ancestry might affect our ability to reconstruct phylogenies. Too many simplifying assumptions are included in this approach (e.g. that alternate character states are equally probable), and some authors have suggested that a Poisson distribution is not the best foundation for a model of molecular evolution (Gillespie 1986). Therefore, the probability distributions should not be interpreted literally. Instead, they represent rough approximations of the potential effects of four important factors: (1) the length of shared ancestry for 2 sister-taxa independent of their next closest relative; (2) the period of independent ancestry for the sister-taxa; (3) the number of functionally equivalent and equally probable alternative character states; and (4) the evolutionary rate of change for the systematic characters examined, on the probability of observing the various character-state distributions.

My results support the idea that biochemical characters can be excellent sources of phylogenetic information. It seems clear that many phylogenies could be reconstructed correctly through both distance and cladistic analyses of these characters. However, I believe that many phylogenies (as defined by T_1 and T_2) cannot be reliably reconstructed. This is of central importance because often investigators are overly optimistic about the informativeness of biochemical data sets. I suggest that there may be a significant subset of the universe of possible phylogenies for which biochemical characters are uninformative. This critical point may be responsible for some cases where a single study produces both correct and incorrect phylogenetic hypotheses.

Given that some phylogenies cannot be reconstructed from stochastically evolving characters, it should be clear that investigators must be extremely cautious when interpreting their data. If shared ancestry is very brief, fast-evolving characters are most likely to evolve derived states in the common ancestor of taxa *A* and *B*. However, if independent ancestry is long with respect to shared ancestry (low stemminess; Fiala and Sokal 1985), a fast rate of character change will virtually guarantee that one or both daughter lineages will develop autapomorphies for these characters. This would destroy our means of identifying *A* and *B* as sister taxa. Under these circumstances, it does not matter whether T_1 is actually 1,000 or 10 million years; if T_2 is short with respect to T_1, stochastically evolving characters will retain very little useful information.

Previous investigations of the effect of topology on phylogeny reconstruction have employed simulations and have examined the relative accuracy of various tree-generating algorithms (Felsenstein 1978, Fiala and Sokal 1985). My results are consistent with these findings. Some analytical approaches are expected to produce incorrect branching sequences for certain types of phylogenies. However, there may be a more fundamental problem. For many phylogenies, biochemical characters will retain no supporting synapomorphies.

If there is an expectation of uncertainty in biochemical data, it is important to resist the temptation to overinterpret data (as pointed out by Thorpe 1982). For example, forcing biochemical data to fit a dichotomously branching pattern is unwarranted if we suspect, a priori, that stochastically evolving characters will not retain information about every historic branching event. Instead, techniques such as bootstrapping (Felsenstein 1985) or jackknifing (Lanyon 1985a, 1987) should be used to identify poorly supported nodes and thereby permit investigators to concentrate on only those portions of the phylogeny for which the data set is particularly informative.

Furthermore, the inability to recreate a branching sequence from stochastically evolving characters may itself be useful information. If a given set of branching events is thought to have occurred relatively recently (i.e. T_1 is small), and if all attempts to recreate that pattern fail despite using a range of biochemical characters, then, according to the model presented here, the period of shared ancestry must be relatively short. For example, studies of DNA/DNA hybridization (Sibley and Ahlquist 1985) and electrophoretic characters (Lanyon 1985b) within the Tyrannoidea failed to identify reliably sup-

ported hypotheses concerning the relationships of the Cotingidae, Pipridae, and Tyrannidae. In both cases, jackknifing taxa revealed considerable instability due to inconsistencies within the data sets. Although by no means conclusive, these conflicts are consistent with the idea that the lineages arose within a short period of time, perhaps as a result of rapid speciation associated with an adaptive radiation. Such conflicts might provide a means for investigating the frequency of this mode of evolution.

Finally, if only a subset of the possible phylogenies can be addressed with molecular data, systematists must also examine characters that evolve in direct response to natural selection (i.e. use a multidisciplinary approach). For example, morphological characters may well retain synapomorphies under circumstances in which molecular characters would not, simply because of the nonstochastic nature of morphological evolution. Multidisciplinary investigations would maximize the chances of correctly reconstructing phylogenies.

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