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Quantification of Homoplasy for Nucleotide Transitions and Transversions and a Reexamination of Assumptions in Weighted Phylogenetic Analysis

RICHARD E. BROUGHTON,[1,3] SCOTT E. STANLEY,[1,4] AND RICHARD T. DURRETT[2]

[1]*Department of Ecology and Evolutionary Biology and* [2]*Department of Mathematics, Cornell University, Ithaca, New York 14853-2701 , USA; E-mail: rtd1@cornell.edu*

[3]*Current address: Oklahoma Biological Survey and Department of Zoology, University of Oklahoma , Norman, OK 73019-0575 , USA; E-mail: rbroughton@ou.edu*

[4]*Current address: Genaissance Pharmaceuticals, Inc., Five Science Park, New Haven , CT 06511 , USA; E-mail: s.stanley@genaissance.com*

Abstract.—Nucleotide transitions are frequently down-weighted relative to transversions in phylogenetic analysis. This is based on the assumption that transitions, by virtue of their greater evolutionary rate, exhibit relatively more homoplasy and are therefore less reliable phylogenetic characters. Relative amounts of homoplastic and consistent transition and transversion changes in mitochondrial protein coding genes were determined from character-state reconstructions on a highly corroborated phylogeny of mammals. We found that although homoplasy was related to evolutionary rates and was greater for transitions, the absolute number of consistent transitions greatly exceeded the number of consistent transversions. Consequently, transitions provided substantially more useful phylogenetic information than transversions. These results suggest that down-weighting transitions may be unwarranted in many cases. This conclusion was supported by the fact that a range of transition: transversion weighting schemes applied to various mitochondrial genes and genomic partitions rarely provided improvement in phylogenetic estimates relative to equal weighting, and in some cases weighting transitions more heavily than transversions was most effective. [Character evolution; consistency; homoplasy; transitions; transversions; weighted parsimony.]

The ability to accurately reconstruct phylogenetic relationships is dependent on the amount and pattern of homoplasy present in the data. Because of its integral role in phylogenetic analysis, numerous indices have been developed to quantify homoplasy for characters or trees (reviewed by Archie, 1996), and several methodological approaches have been proposed to reduce the effects of homoplasy on tree reconstruction (e.g., Farris, 1969; Penny and Hendy, 1985a; Goloboff, 1993). Character or character-state weighting (e.g., Felsenstein, 1981; Wheeler, 1990; Williams and Fitch, 1990) is a common means of reducing the effect of misleading data in parsimony analysis. Weighting schemes are routinely used in molecular systematics, where homoplasy in nucleotide characters may be abundant because of the limited number of possible character states. However, determining appropriate weights for different types of changes is problematic. In practice, weights are frequently based on an explicit or implicit model of DNA sequence evolution incorporating generalized assumptions about the relative reliability of different types of character state changes. Yet the appropriateness of a model for a particular data set is never known a priori. Much of the problem in choosing a model or particular weights arises from substantial uncertainty about the rates and patterns by which homoplasy accumulates in DNA sequences.

A common element of models of sequence evolution is the premise that types of characters that evolve rapidly are more likely to be noisy or misleading than types of characters that evolve more slowly. It has long been recognized that nucleotide transitions occur more frequently and accumulate at a greater rate than transversions in nuclear (Fitch, 1967; Li, Wu, and Luo, 1984) as well as mitochondrial DNA (mtDNA) sequences (Brown, Prager, Wang, and Wilson, 1982; Aquadro and Greenberg, 1983). The elevated rate of transitions means that multiple changes per nucleotide site are more likely, increasing the opportunity for homoplasy. Thus, the relative reliability of transitions as phylogenetic characters is assumed to be lower than for transversions (Meyer, 1994; Simon, Frati, Beckenbach, Crespi, Liu, and Flook, 1994). Furthermore, at higher levels of divergence, the accumulation of transitions frequently exhibits a "plateau" effect; that is,

the number of transitions does not increase much beyond a certain level, even with increasing overall divergence (Irwin, Kocher, and Wilson, 1991; Kocher, Conroy, McKaye, Stauffer, and Lockwood, 1995). When additional transitions do not contribute to increased divergence, even as they continue to occur, they are said to be "saturated" (Brown, Prager, Wang, and Wilson, 1982).

A common solution to this problem in parsimony analysis is to assign higher weights to transversions via a cost-matrix so that potentially homoplastic transitions will have less influence on the topology of recovered tree(s). Many authors have advocated differential weighting schemes based on the ratio of transition to transversion frequencies (e.g., Irwin et al., 1991; Hillis, Huelsenbeck, and Cunningham, 1994; Kocher et al., 1995). Such weights are based on the ratio of "instantaneous" rates of transitions and transversions; that is, intrinsic substitution rates inferred by comparing closely related taxa (Brown et al., 1982). This approach can be extended by applying weight ratios specific to the different codon positions based on evolutionary constraints imposed by the genetic code and protein function (e.g., Simon et al., 1994; Mindell and Thacker, 1996; Martin and Bermingham, 1998).

Although these approaches lend some objectivity to devising weighting schemes, the extent to which transition:transversion frequency ratios reflect levels of homoplasy is not known. For example, if the observed transition:transversion ratio is 20:1, the necessary assumption would be that transitions are 20 times more homoplastic than transversions. Employing this assumption as weights in a parsimony analysis would require more than 20 transitions to override a single conflicting transversion. This places such a heavy weight on transversions that even rare homoplastic transversions may distort the topology of recovered trees. In addition, much of the information provided by transitions may be effectively lost, leading to reduced phylogenetic resolution. Therefore, it would be desirable to have a quantitative assessment of the relative amounts of homoplasy in transitions and transversions for taxa at various levels of divergence.

Studying the accumulation of homoplasy has been problematic because it can only be defined in the context of a phylogenetic tree and the true tree is not generally known. Although conflicting characters may be identified on any tree, determining which characters reflect historical relationships and which ones are homoplastic can only be determined on the correct tree. We have employed a phylogeny of mammalian relationships (Fig. 1) that has near universal support (Novacek, Wyss, and McKenna, 1988; Cummings, Otto, and Wakeley, 1995; Naylor and Brown, 1998) and is generally accepted as the true tree to the extent that any tree can actually be known. We used this tree to reconstruct character state changes for nucleotide sequences of all 13 mitochondrial protein genes in the included taxa. The distribution of homoplasy for transitions and transversions at different codon positions was examined and quantified relative to the pairwise divergence among taxa. This allowed inferences about the relative frequencies of homoplastic changes and their potential effects on phylogenetic reconstruction. We also examined the effectiveness of a range of transition–transversion weighting schemes for recovering the correct topology with individual genes and other subsets of the data.

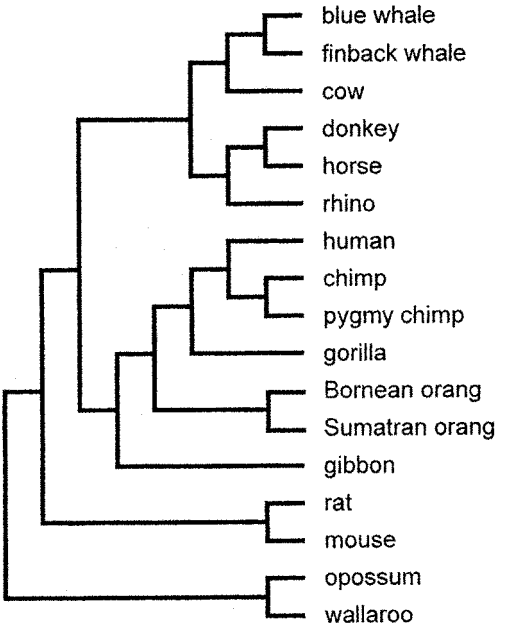

FIGURE 1. Phylogenetic relationships of mammal taxa used in this study.

MATERIALS AND METHODS

Complete mitochondrial genomes of 17 mammalian taxa were obtained from Genbank (Fig. 1). Genome sequences exist for

several additional mammalian taxa (e.g., carnivores) but we excluded these because their phylogenetic placement remains controversial (R. Honeycutt, April 1998, pers. comm.). Sequences were aligned using the Clustal method (implemented in the LaserGene package). Alignments were made using inferred amino acid sequences with gap and gap-length penalties each set to 10. Sequences of each of the 13 protein coding genes were aligned separately and then combined into a single data matrix. Because of their overlapping reading frames, 30 bp of overlap between ATPase 6 and ATPase 8 were excluded, as was a similar 7 bp segment between ND4 and ND4L. Regions with sequence for only one taxon in the alignment (gap for all others) were excluded, yielding a total of 11,343 nucleotides. The generally accepted phylogeny for these mammalian species (Fig. 1) was also obtained in heuristic parsimony searches with PAUP* 4.0b1 (Swofford, 1998) employing 100 random addition replicates, TBR branch swapping, and using opossum and wallaroo as outgroups.

Parsimony and maximum likelihood reconstructions of the characters on the accepted topology were performed using PAUP* (ver. 4d63; D. Swofford, March 1998, pers. comm.). Parsimony reconstructions employed ACCTRAN optimization and equal weighting of all character changes. In some cases, there was more than 1 equally most parsimonious reconstruction (MPR) for a character. For the present purposes we know of no justifiable criterion for preferring one MPR over another (see Swofford and Maddison, 1992). For unordered characters, PAUP arbitrarily assigns each a MPR (D. Swofford, April 1998, pers. comm.) that is reported in apomorphy lists, change lists, and other tree descriptions. We employed these reconstructions in our analysis, assuming that they are not subject to a systematic bias. For comparison we also examined MPRs based on DELTRAN optimization. Reconstructions under the maximum likelihood criterion employed the GTR model, 4-class gamma rate model ($\alpha = 0.5$), and empirical nucleotide frequencies.

Character reconstructions were used to count the total number of transition and transversion changes, as well as the number of homoplastic transitions and transversions on the tree paths between all pairs of taxa. Patristic differences were determined by summing the number of steps for each type of change across all branches separating taxa. Homoplasy (h) for each character was defined as the number of steps required on the tree (s) beyond the minimum steps necessary (m) to explain the number of character states present (i.e., $h = s - m$). Total homoplasy between taxa was taken as the sum of h values limited to changes on specific branch paths between taxa. With this method, homoplasies were recognized wherever a particular nucleotide state was derived more than once on the branches separating taxa, regardless of the character states found in terminal taxa.

To identify the type of homoplastic change (i.e., transition or transversion), we considered the identity of nucleotides involved in pairs of changes. Two changes are required to produce a single homoplasy, and each change was assigned a value of 0.5. For example, if two changes were both A ⟷ C, this was counted as (0.5 + 0.5 =) 1.0 transversion homoplasy; whereas, if two changes were A ⟷ G, 1.0 transition homoplasy was counted (Fig. 2a). Although this accounted for simple parallelisms and reversals, more complex situations are possible with convergences or multiple parallelisms and reversals (see Fig. 2b–d). For example, in the case of a convergence, if one change was C ⟷ T and the other was A ⟷ T, we identified this as "both" and counted 0.5 transition homoplasy and 0.5 transversion homoplasy (Fig. 2b). These operations were implemented with a computer program written by R.T.D. This approach allowed homoplasy to be quantified for pairwise comparisons and related to the total amount of divergence between taxa. Comparisons of the amount and type of homoplasy were made for each codon position separately and all positions combined.

To examine the effectiveness of character-state weighting, a variety of weighting schemes was employed on individual genes. Because the size of a gene (i.e., the number of characters) can influence results, we also divided the data into 10 equally sized (1,134 bp) partitions and subjected each of these to the various weighting schemes. Stepmatrices assigned relative weights to transversions and transitions in ratios of 2:1, 4:1, 10:1, 20:1, and 1:2, respectively. More weight was assigned to transitions than transversions with 1:2, but this is the maximum difference possible using a stepmatrix because any greater ratio would allow a "transition" change to be reconstructed with lower net cost by 2

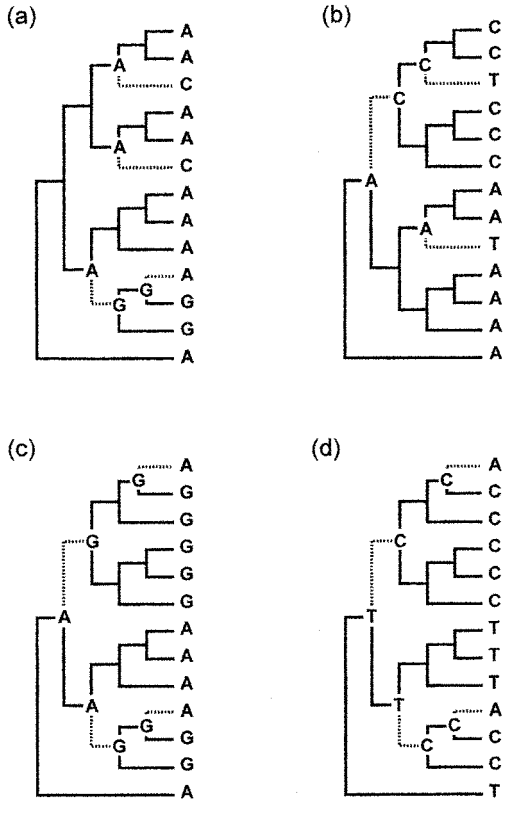

FIGURE 2. Homoplasy scoring. (a) examples of a transversion parallelism (upper) and a transition reversal (lower). (b) a convergence involving 0.5 transition (C ⟷ T) and 0.5 transversion (A ⟷ T). (c) multiple changes resulting in 2 transition homoplasies. (d) a double parallelism resulting in 1 transition homoplasy (T ⟷ C) and 1 transversion homoplasy (C ⟷ A).

transversions. Each weighting scheme was employed in heuristic searches with 10 random addition replicates. Results were evaluated by comparing the tree obtained with the correct tree. This involved counting the number of branches that would need to be collapsed and reconstructed in order to convert the obtained tree into the true tree (Robinson and Foulds, 1981; Penny and Hendy, 1985b).

RESULTS AND DISCUSSION

Patterns of Transition and Transversion Homoplasy

In Figure 3, the number of parsimony transition and transversion changes, the number of homoplasies, and the number of changes consistent with the tree are plotted versus total uncorrected divergence. In general, all values were greater for transitions than transversions, with the differences being most extreme at low divergence values and less at higher divergences. Here we report parsimony results only for ACCTRAN optimization. DELTRAN tends to push changes toward the tips of the tree so that homoplasies were involved in fewer pairwise comparisons. The result was that the homoplasy plots for transitions and transversions differed less than with ACCTRAN. Conversely, the maximum likelihood model favored transition changes resulting in greater homoplasy differences between transitions and transversions. However, results from all three optimization methods did not differ qualitatively from the ACCTRAN results shown (Fig. 3).

The number of pairwise transitions was greater than the number of pairwise transversions for divergences less than 25% (Fig. 3, left column). This is consistent with the observation that transitions are more frequent in mtDNA. In spite of their greater rate, transitions at all but 3rd positions appeared to accumulate in a more or less linear fashion over the range of divergence observed. Although the number of transitions and transversions converged at higher divergences (except for 2nd codon positions), this appears to be more a result of the increasing frequency of transversions than saturation of transitions.

The number of homoplasies (Fig. 3, middle column) was also greater for transitions than transversions. The greatest difference appeared at 2nd positions, and the least difference occurred at 3rd positions. Overall, the ratio of transition and transversion homoplasies is greatest at low divergences and least at elevated divergences. At greater divergences, where many homoplasies of both types have accumulated, the ratio becomes approximately 2:1. (Note that this ratio is heuristic, based on differences between regression lines for each type of change, but because the data points are not independent, the statistical variance around these lines cannot be addressed.) Convergence to a 2:1 homoplasy ratio may be a function of the number of alternative character states for transitions (1) and transversions (2). The character-state space for transitions is thus half that of transversions, with the expectation that transitions should be twice as likely to result in a homoplastic change, all else being equal. This suggests that at elevated

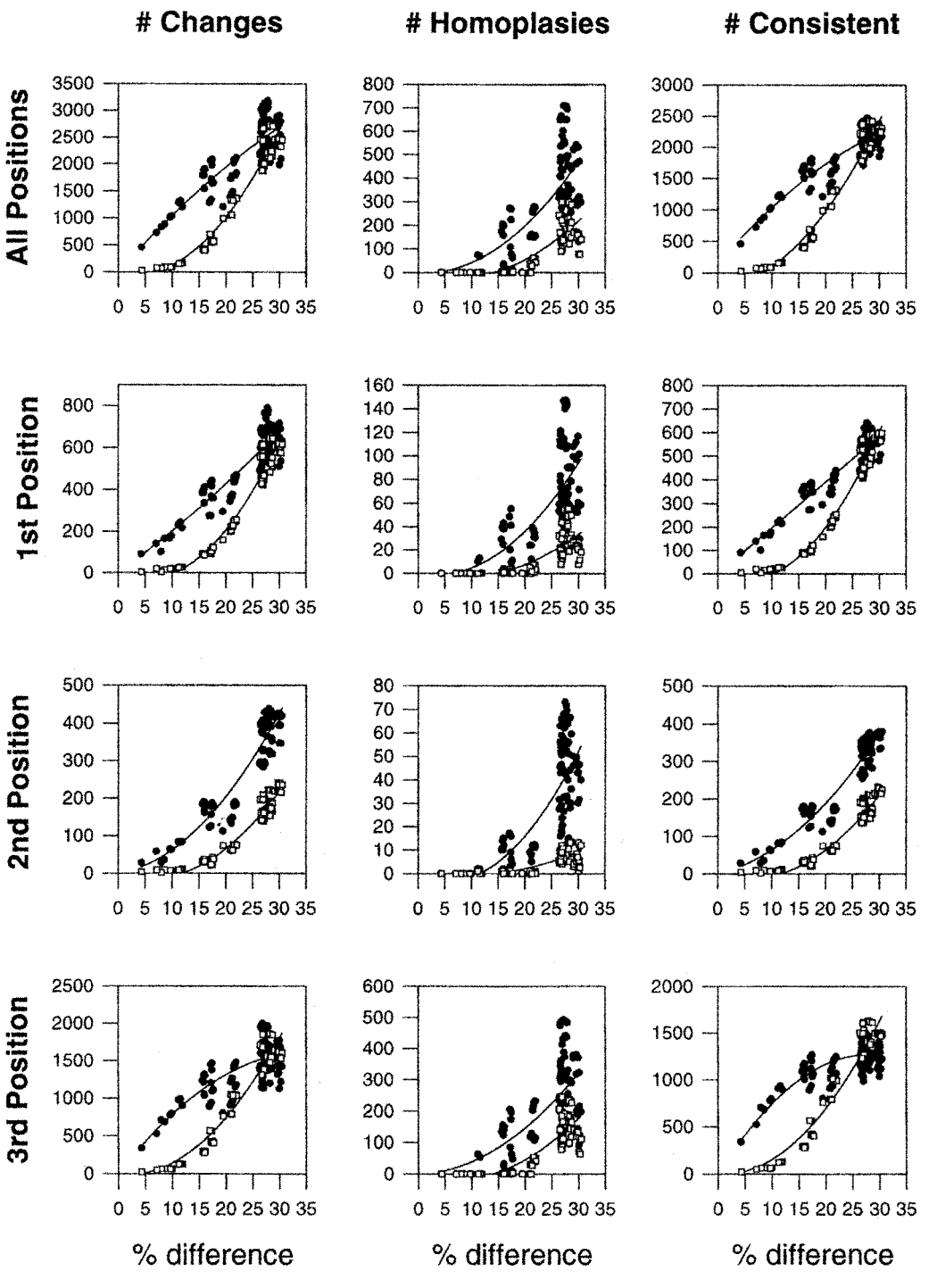

FIGURE 3. Transition (solid circles) and transversion (open squares) changes, homoplasies, and consistent changes from patristic pairwise comparisons. The horizontal axis for each graph is total uncorrected percent difference; the vertical axis is absolute number of changes. Curves are 2nd-order regression lines.

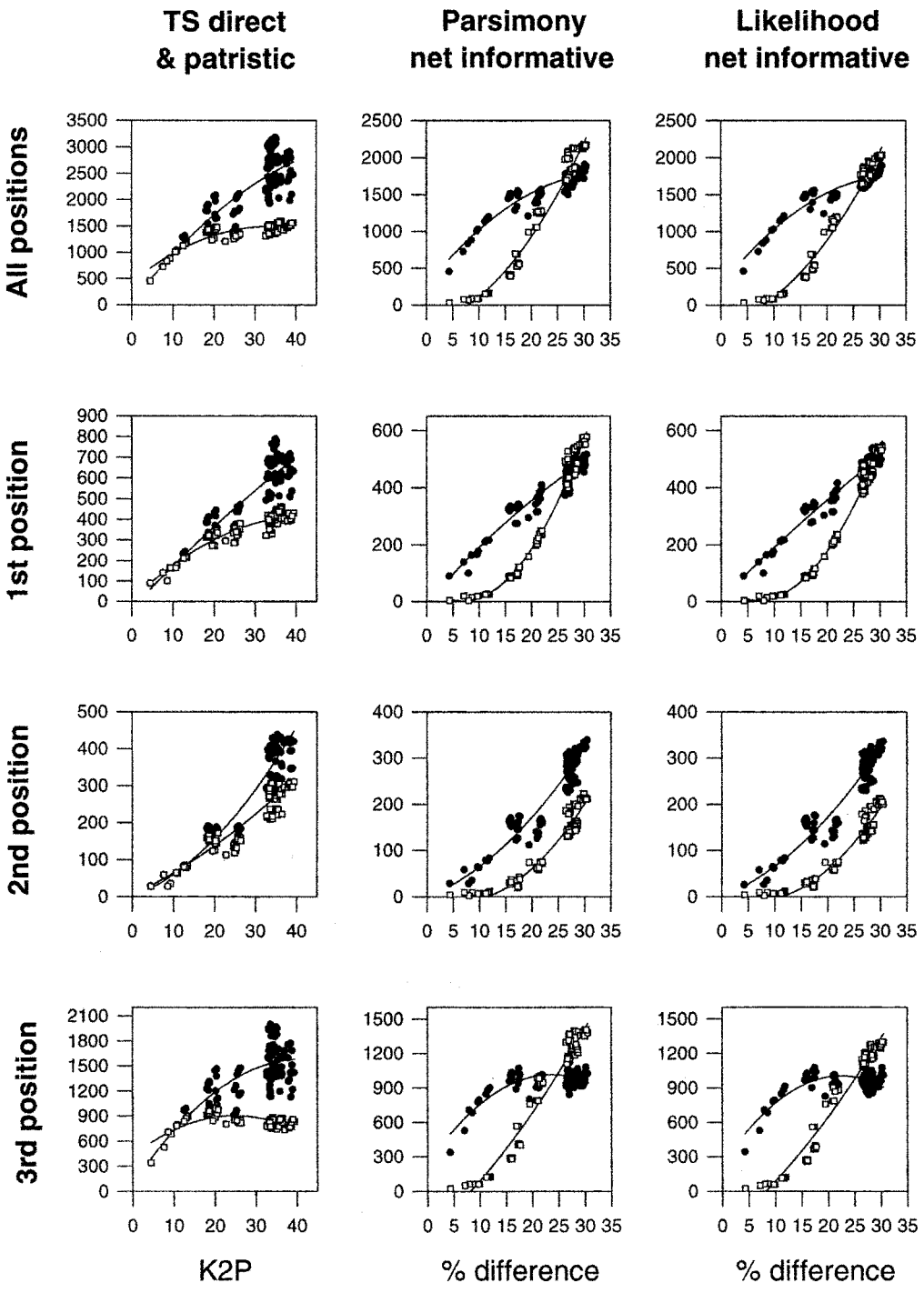

FIGURE 4. Direct and patristic transitions (left column), and net informative changes for parsimony reconstructions (middle column) and maximum likelihood reconstructions (right column). In the left column, the absolute number of transitions observed in direct pairwise comparisons (open squares) and patristic comparisons (solid circles) are plotted versus corrected (K2P) distance. In the middle and right columns, the horizontal axis is uncorrected percent difference and the vertical axis is net informativeness (# consistent − # homoplasies) based on parsimony and maximum likelihood reconstructions, plotted for transitions (solid circles) and transversions (open squares). Curves are 2nd-order regression lines.

divergence, observable homoplasy approaches an equilibrium-like condition that is governed more by the number of alternative states than the absolute rate of each type of change.

Although much discussion of character quality focuses on homoplasy, the number of character-state changes consistent with the tree is equally important in the context of tree construction. Consistent transitions outnumbered consistent transversions at all but the greatest divergence at 3rd positions (Fig. 3, right column). Plots of consistent changes are similar to plots of total changes, reflecting the fact that homoplasies made up a small proportion of the total change. Although transversions exhibited less homoplasy than transitions, particularly at low divergence, transversions also provided fewer useful characters for phylogenetic analysis. Because parsimony analysis is based on consistent changes, that is, synapomorphies, any measure of phylogenetic utility for different types of changes must account for relative amounts of both homoplasy and consistent changes.

As noted previously, the patristic comparisons provided little evidence for saturation of transitions except at 3rd positions. This observation holds even when the number of transitions is plotted versus a total distance measure that "corrects" for multiple hits (Kimura 2-parameter distance) (Fig. 4, left column). However, if the number of transitions is determined from direct sequence differences (i.e., not patristic differences), substantial plateau effects are evident. This difference between direct and patristic comparisons suggests that apparent saturation may often be an artifact of the way it is inferred. Direct pairwise comparisons can account for no more than one difference per site. However, patristic differences based on a tree can incorporate many such changes. Thus, in many cases where saturation might appear to be a problem, parsimony may correctly account for multiple changes per site. Furthermore, because transversions may obscure prior transitions at a site (e.g., Brown et al., 1982; Holmquist, 1983; DeSalle, Freedman, Prager, and Wilson, 1987), a substantial part of an observed transition plateau may, in fact, be due to the accumulation of transversions and does not necessarily indicate misleading phylogenetic information caused by multiple transitions at a single site. Therefore, it is important to assess saturation with a method that accurately measures the phenomenon as it pertains to phylogenetic analysis, and it appears that true saturation of transitions may require divergences greater than those investigated here.

Because transitions appear to provide a substantial amount of historically consistent information, we have attempted to account for amounts of both consistent and homoplastic change in an index called "net informativeness". This is the number of consistent changes minus the number of homoplastic changes for each pairwise comparison. Net informativeness describes the information present in a way most relevant to phylogenetic analysis; that is, by quantifying the amount of uncontested support for the tree provided by each type of change. These values are shown in Figure 4 for both parsimony reconstructions (middle column) and maximum likelihood reconstructions (right column). Transitions provided substantially more phylogenetic information than transversions up to about 25% divergence. Only beyond 25% divergence at 3rd positions were transversions better. These results illustrate clearly the importance of considering the number of consistent character-state changes as well as the amount of homoplasy when evaluating the phylogenetic utility of different types of character change. In general, it appears that transitions are much more reliable than has been assumed and suggests that downweighting transitions may lead one to ignore useful phylogenetic information.

This conclusion is corroborated by the fact that the correct mammalian tree was recovered in an analysis of only 3rd positions, which exhibit a transition plateau even with patristic differences. Previous simulation studies have reached similar conclusions. Fitch and Ye (1991) found that an average of at least 3 changes per site were required before parsimony began to fail. A study based on avian cytochrome *b* sequences indicated that removal of 3rd positions (where a majority of transitions occur) reduced the probability of finding the correct tree from 88% to 0 when genus level taxa were included, and from 55 to 32% at the family level (Hâstad and Björklund, 1998). Furthermore, Yang (1998) found that parsimony was robust to long branches and concluded that "... the problem of saturation may have been exaggerated."

TABLE 1. Results of transition:transversion weighting on mitochondrial genes and data partitions. Values are the number of rearrangements required to convert the tree obtained into the correct tree. Multiple values per cell apply to multiple most parsimonious trees. Underscore indicates the lowest weight ratio producing the best result for each gene or segment. Where results of 2:1 (transition) weighting were equal to the best result from other weights, both are indicated.

	= weight	1:2	1:4	1:10	1:20	2:1
ND1	6	6	6	4	6	2
ND2	0	0	0	0	0	4
COI	2,4	2	0	0	0	8
COII	2,2	2	2	2	2	2,4
ATP8	6,10	6,8,8,8	6,8	6,8	6,8	6
ATP6	4	4	4	4,8	10	8
COIII	4	4	2	2	2	6,8,10,10
ND3	6	6	6	6	6	8
ND4L	6	8	8	10	8	10
ND4	4	0	0	0	0	4
ND5	2	0	0	0	0	2
ND6	4	2	2	2	2	4
Cyt b	2,4,6	2	2	2	2	8
1–1134	6	6	6	4	4	4
1135–2268	0	0	0	0	0	2
2269–3402	2,4,6	2,4	4	4	6	10,10
3403–4536	2	2	2	2	2	0
4537–5670	4	4	0	0	0	8
5671–6804	2	2	4	4	4	0
6805–7938	0,4	0	0	0	0	2
7939–9072	4	4	0,4	0	0	2,4,4
9073–10206	0	0	2	2	2	0
10,207–11,340	2,4,6	2	2	2	2	8

Utility of Transition-Transversion Weighting

To test the generality of the previous results and to apply them to smaller data sets such as are usually available to practicing systematists, we applied a range of weighting schemes in parsimony analysis of subsets of the data (Table 1). Under equal weighting, the only mitochondrial gene to support the correct tree was ND2. Among equal-sized genome segments, Segment 2 (corresponding roughly to ND2) and Segment 9 (a portion of ND5 and all of ND6) supported the correct tree. For Segment 7 (parts of ND4 and ND5), one of the two equally parsimonious trees was correct. Other studies have also shown that ND2, ND4, and ND5 may outperform other mitochodrial genes in phylogenetic analysis (e.g., Cummings et al., 1995; Zardoya and Meyer, 1996).

For the 12 genes that did not support the correct tree, various transversion weights allowed recovery of the correct tree for three of them. However, in one case, weighting produced trees further away from the correct tree; and in another, transition weighting (2:1) produced the best result. Heavy weighting of transversions (i.e., 1:10 and 1:20) allowed improvement (over 1:2 and 1:4) in one case but produced a worse result in another. For most genes, differential weighting provided no improvement over equal weighting. Results for genomic segments were similar. In 2 cases, transversion weighting allowed recovery of the correct tree where equal weighting did not. Yet, in 2 cases, transversion weighting produced poorer results, and in 3 cases, weighting in favor of transitions lead to improved phylogenetic performance.

Overall, transversion weighting improved phylogenetic inferences in only a few cases, and transition weighting did so nearly as often. Furthermore, the extreme weighting schemes were not effective. The relatively poor performance of transversion weighting is consistent with several previous studies. For example, Kraus and Miyamoto (1991) reported that the antlered deer were not monophyletic when only transversions were considered, but they were monophyletic when all characters were included. In a study of phrynosomatid lizards, Reeder (1995) concluded that "... transitions were no less informative for phylogeny reconstruction than transversions. Therefore, transitions should not be down-weighted in phylogenetic

analysis, as is often done." Yang (1996) observed that among hominoids there were 3.7 substitutions per site at 3rd codon positions with a transition–transversion ratio of 52. Yet, 3rd position data alone produced the correct hominoid tree while 1st and 2nd positions failed to do so. Our results, along with these and other studies, suggest that differential weighting of transitions and transversions is of limited value in many cases and can result in poorer phylogenetic hypotheses than equal weighting.

Clearly, many factors other than relative evolutionary rates must be considered in models of DNA evolution. For example, we found a greater number of A ⟷ C transversions than A ⟷ G transitions in the mammal data and a corresponding greater amount of homoplasy. This would appear to result from the bias against G on the coding strand of all but the ND6 gene in mtDNA (Jermiin et al., 1995). In addition, 1st and 2nd positions may be less reliable than their lower evolutionary rates might suggest. Functional constraints may reduce allowable nucleotide states at 1st and 2nd positions, which may actually elevate levels of homoplasy relative to 3rd postions (Naylor, Collins, and Brown, 1995; Hassanin, Lecointre, and Tillier, 1998). Finally, higher variability does not necessarily reduce character quality. For both vertebrates (Philippe, Lecointre, Van Le, and Le Guyader, 1996) and plants (Kallersjo, Albert, and Farris, 1999), removal of the most variable (and most homoplastic) sites decreases more signal than noise, and the most variable sites may even be more consistent (based on CI) than less variable ones (Olmstead, Reeves, and Yen, 1998).

Because it is usually not possible to detect all changes that have occurred on each branch of a tree, our approach, no doubt, underestimated the absolute number of each kind of change. However, even when a likelihood model that preferentially infers transition changes was employed, the informative nature of transitions is still apparent. In fact, differences in net informativeness plots between maximum likelihood and parsimony reconstructions (Fig. 4) were minor and only detectable at the greatest divergences. Thus, our results appear to be robust and are not restricted to parsimony analysis. Indeed, we suggest that any method that assigns different costs or probabilities to transitions and transversions could underestimate the utility of transitions.

Conclusions

The assumption that transitions are categorically poor phylogenetic characters is common, based primarily on inferences of the relative rates of change of transitions and transversions. We find that this assumption is inaccurate and inferences based on it may be misleading in phylogenetic analysis. Our results suggest that although the ratio of transition and transversion homoplasies is related to the rate ratio, so is the ratio of consistent changes. Transitions exhibit more homoplasy, but this appears to be counteracted by their greater number of informative changes. Although differential weighting enhances the effect of transversions, which have generally lower homoplasy, it concomitantly increases the undesirable effect of homoplastic transversions and reduces the influence of the larger number of consistent transitions.

A pattern that emerges from our analysis is that the difference in relative amounts of homoplasy for transitions and transversions is greatest at divergence levels where the absolute number of homoplasies is least. Indeed, while homoplasy ratios at low divergences are extreme, the absolute number of homoplasies is so low that it is probably inconsequential for phylogenetic analysis. In contrast, where differential weighting might be expected to be most effective, that is, at divergences of 20 to 30% where the total amount of homoplasy is much greater, the homoplasy ratio for transitions and transversions is only about 2:1. One could conceivably make a case for using 1:2 weight ratios based on the 2:1 homoplasy ratio; however, this fails to account for consistent characters, and we found little benefit from a 1:2 ratio in our analysis of genomic partitions. Although additional work is necessary before we can fully appreciate patterns and rates of nucleotide evolution, it appears that previous assumptions about the poor phylogenetic utility of transitions were premature.

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