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Power of the Concentrated Changes Test for Correlated Evolution

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Abstract.— The concentrated changes test (CCT) calculates the probability that changes in a binary character are distributed randomly on the branches of a cladogram. This test is used to examine hypotheses of correlated evolution, especially cases where changes in the state of one character influence changes in the state of another character. The test may be sensitive to the addition of branches that lack either trait of interest (white branches). To examine the effects of the proportion of white branches and of tree topology on the CCT probability, we conducted a simulation analysis using a series of randomly generated 100-taxon trees, in addition to a nearly perfectly balanced (symmetrical) and a completely imbalanced (asymmetrical) 100-taxon tree. Using two models of evolution (gains only, or gains and losses), we evolved character pairs randomly onto these trees to simulate cases where (1) characters evolve independently (i.e., no correlation among the traits) or (2) all changes in the dependent character occur on branches containing the independent trait (i.e., a strong correlation among the traits). This allowed us to evaluate the sensitivity of the CCT to type I and type II errors, respectively. In the simulations, the CCT did not appear to be overly sensitive to the inclusion of white branches (low likelihood of type I error with both CCT probabilities < 0.05 and < 0.01). However, the CCT was susceptible to type II error when the proportion of white branches was $< 20\%$. The test was also sensitive to tree shape and was positively correlated to Colless's tree imbalance statistic I. Finally, the CCT responded differently for simulations where only gains were allowed and those where both gains and losses were permitted. These results indicate that the CCT is unlikely to detect a correlation between characters when no such correlation exists. However, when a trait can be gained but not lost, the CCT is conservative and may fail to detect true correlations among traits (increased type II error). Determination of the sampling universe (the taxa included in the comparative analysis) can strongly influence the probability of making such type II errors. We suggest guidelines to circumvent these limitations. [Character correlation; correlated evolution; power; taxon sampling; tree balance; tree topology; type I error; type II error.]

The comparative method remains one of the most powerful tools available in evolutionary biology (Brooks and McLennan, 1991; Harvey and Pagel, 1991; Maddison and Maddison, 1992). Recent developments in phylogenetic analysis have made it possible to study evolutionary patterns across diverse taxa while taking into account shared phylogenetic history. These developments have proven particularly valuable for researchers interested in testing hypotheses of correlated evolution between two or more characters (reviewed by Harvey and Pagel, 1991). Of the several techniques now available to test for patterns of correlated evolution between discrete characters (Ridley, 1983; Proctor, 1991; Sillén-Tullberg, 1993; Pagel, 1994a; Read and Nee, 1995), Maddison's concentrated changes test (hereafter referred to as the CCT; Maddison, 1990) has proven to be popular (Donoghue, 1989; Hunter, 1995; Höglund and Sillén-Tullberg, 1994; Janz and Nylin, 1998) because it is logical, powerful, and readily accessible in the program MacClade (Maddison and Maddison, 1992). The CCT is particularly well suited to testing hypotheses about whether the evolution of a trait is more likely when another character is in a particular state.

The CCT determines whether changes in one character (the dependent character) are concentrated on branches of a tree that have a particular state of a second character (the independent character). The test is performed by first reconstructing the evolution of two characters on a phylogenetic tree. The user then counts the number of gains (n) and losses (m) in the dependent character over the whole tree and also the number of these gains and losses that occur on branches reconstructed to have the derived state ("black" branches) of the

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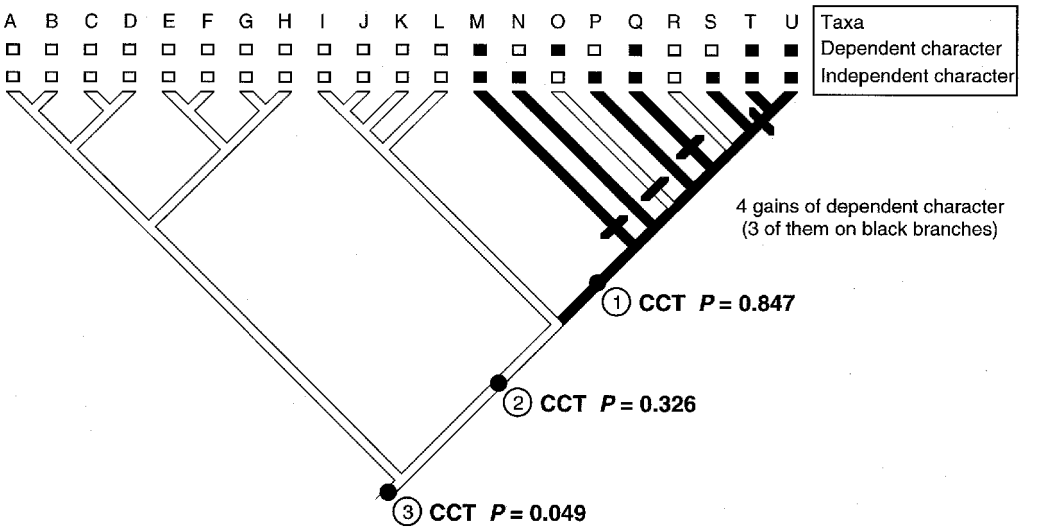

FIGURE 1. Demonstration of the effect on the concentrated changes test (CCT) of including clades with neither trait of interest (white branches). *P*-values indicate the test probability when the CCT is calculated at each point for four gains in the dependent character (three of them on black branches), given the distribution of the independent character shown. The part of the tree containing taxa A–H is completely balanced, but the part with M–U is completely imbalanced.

independent character (p gains and q losses on black branches). To calculate the CCT probability, the test calculates (a) the number of ways n gains and m losses can be distributed on the entire phylogeny, and (b) the number of ways the p gains and q losses can be distributed onto the black branches, given n gains and m losses on the whole tree. The ratio of b/a expresses the probability that the observed number of changes in the dependent character would occur by chance on black branches—the smaller the ratio, the more strongly the changes in the two traits are associated. The test assumes equal branch lengths and uses the number of gains and losses in the whole tree as a way of weighting how important these two kinds of changes are (a detailed description of how this test is performed was provided by Maddison [1990] and Maddison and Maddison [1992]).

The purpose of our paper is twofold. First, we consider whether the CCT is sensitive to the inclusion of clades composed of "white" branches, that is, branches of a phylogeny reconstructed to have the ancestral state of two characters (wherein neither trait of interest is present). Maddison (1990) recognized that the concentrated changes test would be sensitive to the inclusion and exclusion of taxa. For example, he noted that addition of taxa in which there has not been a change in any of the characters of interest could result in a situation where "a weak association between changes and black areas in the rest of the tree might become a strong association when many species ... are added, because there would then be many more white branches on which changes could have occurred but did not" (Maddison, 1990:554). The problem is illustrated in Figure 1. In this example, seven of nine taxa (M–U) possess a trait of interest (two have lost it). This so-called independent trait is optimized on the tree, as shown by the black branches. Suppose a second, dependent trait has arisen four times (in taxa M, O, and Q and at the base of clade T–U), as illustrated by crossbars on the branches at the point where these origins occurred. The CCT allows us to ask whether the dependent trait is more likely to evolve when the independent trait is present

(i.e., do gains of the second trait occur more often than would be expected by chance on the black branches, given the tree structure). In this example, the result of the CCT is highly dependent on the position in the tree at which the test is conducted. If we calculate at point 1 (Fig. 1) the CCT probability that three gains and no losses occurred on black branches by chance (given four gains overall), the test reveals no significant association of the two characters (CCT = 0.847). At point 2, however, the association is stronger (CCT = 0.326), and at point 3 the association is significant (CCT = 0.049). The only difference among these tests is which taxa we sampled. By we progressively adding clades (I–L and A–H) consisting of white branches, we see that the CCT is sensitive to the inclusion of taxa that lack either of the traits of interest.

One could argue that the addition of the white branches simply increases the power of the test to detect a true association. The newly added lineages could have had changes within them but did not, and as Maddison points out, all statistical methods should react to inclusion of new sample points. However, we became concerned that weak or even random associations between characters might become statistically significant simply through the addition of taxa with neither trait of interest. The relationship between the proportion of white branches and the likelihood of making type I error (the probability that an association will be detected when there is none) needs to be evaluated. We do so here by simulating the random evolution of characters on a series of trees and then asking whether a concentrated changes test for an association between characters is sensitive to the inclusion of white branches.

The problem of adding white branches leads to the broader question of "taxon sampling"—how to define the sampling universe to examine correlated evolution without influencing the chances of finding a significant correlation (Coddington, 1992; 1994; Sillén-Tullberg, 1993; Pagel, 1994b). Sillén-Tullberg (1993) considered the sensitivity of comparative tests to the inclusion and exclusion of taxa at length. However, taxon sampling involves changes in both the number of taxa and in the proportion of branches that are white, an issue that Sillén-Tullberg (1993) did not address. We attempt to separate these influences by examining variation in the proportion of white branches on 100-taxon trees and on 50- and 25-taxon subsets.

A second goal of our study is to examine the extent to which the CCT is affected by tree shape. The CCT explicitly incorporates tree topology in its calculation of the number of ways that changes in the dependent character could occur on a given tree. However, Maddison (1990) recognized that tree shape may nonetheless influence the value of the test statistic. For example, if a tree is highly asymmetrical or imbalanced (as in Heard [1992], here the part of tree in Fig. 1 that contains taxa M–U), some branches represent greater lengths of time than others. Accordingly, apparent correlations could arise between characters, not because of any causal connection, but simply because changes in both characters are more likely to occur on longer branches. If this were true, we would expect that the CCT would be more susceptible to type I error as trees become more imbalanced. The CCT may also be sensitive to tree shape because of potential effects on the number of black branches. Imbalanced clades can have a much wider range of black branches than balanced clades (Werdelin and Tullberg, 1995).

Partly in response to concerns about the effects of tree shape, Sillén-Tullberg (1993) designed a new test, the contingent states test (CST), which is less sensitive to tree topology. This test compares the relative frequency of gains and stasis in a dependent trait and asks whether these frequencies depend on the state of an independent trait. Werdelin and Tullberg (1995) recently compared the performance of the CCT and the CST on two extreme tree shapes—perfectly symmetrical and perfectly asymmetrical 64-taxon trees. They found that the tests yield different probabilities in some situations (e.g., for asymmetric trees with small numbers of black branches) and that the CCT is sensitive to tree shape, but they did not examine the power of either test in detail. Because

most phylogenies for real organisms are not as extreme as those used by Werdelin and Tullberg (1995), a further evaluation of the influence of tree shape on the CCT would be valuable. Here we use measures of tree balance (Colless, 1982; Heard, 1992; Kirkpatrick and Slatkin, 1993) to examine the sensitivity of the concentrated changes test to tree shape. We also examine whether the proportion of white branches (rather than the number of black branches) influence the likelihood of making type I and type II errors. Our results provide an assessment of the statistical power of one of the more accessible comparative techniques for testing hypotheses of the correlated evolution of discrete characters.

Methods

In setting up the simulations described below, we had to deal with several factors simultaneously. We wanted to test the effect of adding white branches and the effect of tree shape, using the same trees and characters so we could evaluate the importance of these effects relative to each other. Because tree shape statistics are sensitive to the number of taxa in the tree (Heard, 1992; Kirkpatrick and Slatkin, 1993; Rogers, 1994), we held the number of taxa constant at 100 for most of our analyses. We chose to use trees with large numbers of taxa and chose parameters (e.g., transition probabilities, DELTRAN resolving options) for character evolution that would give us a range of white and black branches. This allowed us to do two things. First, we could look at the effects of white branches by using variation in the proportion of white branches between characters without varying the number of taxa as we did in the example above (Fig. 1). Second, this approach provided sufficient variation in the number of black branches to enable a test for the effects of interest. Setting the probability of gains too high results in characters with large numbers of black branches. Setting the probability of gains too low leads to the opposite (small numbers of black branches). Either of these alternative approaches would limit the scope of our examination of the CCT (and the generality of our results) by constraining the range of probabilities possible (i.e., to be close to one when there are many independent black branches and close to zero when there are few). Likewise, we chose to emphasize trees with 100 terminal taxa rather than smaller trees because this allowed a larger range in the number of gains and losses possible for a trait of interest. For approaches such as the CCT that depend on convergence for power (Coddington, 1994), large numbers of taxa are desirable. The CCT has been used on trees ranging in size from 52 to several hundred (52 in Hunter [1995]; 64 in Werdelin and Tullberg[1995]; 72 in Höglund and Sillén-Tullberg [1994]; 80 in Donoghue [1989]; and 437 in Janz and Nylin [1998]).

To assess the effects of tree shape on the CCT, we needed a method of generating random trees with shapes similar to real trees. Trees generated by using real character matrices have been compared with various kinds of random trees of equivalent size through use of the shape statistic I (defined below), which indicates the average balance between the number of taxa in the descendent clades for each node in a tree. We generated 10 random trees containing 100 taxa, using the Equiprobable random trees option in MacClade 3.0 (Maddison and Maddison, 1992), which utilizes a model equivalent to the equal-probability model of Rogers (1994) or the proportional-to-distinguishable arrangements (PDA) model of Savage (1983) and Mooers and Heard (1997). This method randomly samples all possible rooted dichotomous trees. Another model for producing random trees is known as the equal-rates Markov (ERM) model (Rogers, 1994). (Although not used in this study, the random joining option in MacClade produces trees equivalent to the ERM model [Maddison and Slatkin, 1991].) Real trees tend to be less balanced than ERM trees and more balanced than PDA trees (Rogers, 1994; Mooers and Heard, 1997). In other words, mean I values of real trees tend to fall between mean I values for ERM- and PDA-generated trees (on average, ERM $I <$ "real" $I <$ PDA I), especially for large trees (Rogers, 1994). The expected value of I for ERM trees with 100 taxa can be calculated from equation 2 of

Heard (1992) as 0.144, and for PDA trees with 100 taxa, I can be extrapolated from Figure 2 of Rogers (1994) as 0.27. We therefore chose trees with shapes that spanned this range for our study to concentrate most of our efforts in the range of shapes where real trees would be expected to fall. To show how the CCT behaves on trees with extreme shapes, we also constructed a 100-taxon tree with nearly perfect balance (symmetrical, as in the part of the tree in Fig. 1 containing taxa A–H) and one that was completely imbalanced (asymmetrical). Perfectly balanced trees can only be constructed with 2^n taxa, where n is an integer.

Once the trees were generated, we evolved 60 random characters onto each tree, using the Evolve Characters option (a Markov model) with each of the following two models of evolution: (1) gains only (e.g., evolution of copulation in water mites; Proctor, 1991): transition probabilities of 0.1 for gains ($0 \longrightarrow 1$ transitions), 0.0 for losses ($1 \longrightarrow 0$ transitions), 0.9 for $0 \longrightarrow 0$ transitions, and 1.0 for $1 \longrightarrow 1$ transitions; and (2) gains and losses (e.g., evolution of dioecy in gymnosperms; Donoghue, 1989): transition probabilities of 0.1 for gains, 0.05 for losses, 0.9 for $0 \longrightarrow 0$ transitions, and 0.95 for $1 \longrightarrow 1$ transitions (Maddison and Maddison, 1992). Character changes were reconstructed onto each tree by using MacClade. The DELTRAN option was used for resolving ambiguous reconstructions of character evolution so as to increase the number of gains reconstructed. Remaining equivocal branches were resolved by assuming that the character state at the tree's root was white (lacked both of the traits of interest) and by allowing only gains (model 1). Dyads of characters were then established by pairing adjacent random characters in the data set. The CCT was conducted for each of the 720 dyads (12 trees, 30 pairs of characters, and 2 models of evolution), using MacClade. Each character contributed to only one test except for the characters on the most-balanced tree, which were used again for the 50- and 25-taxon subset trees (described below). We treated the first random character in each dyad as the independent variable and the second character as the dependent variable. Because of the size of the trees and the number of taxa, the Simulation option was used with 1000 simulations and the Actual changes option was selected.

For each pair of characters, we determined two probabilities:

1. *The probability that an association would be detected between changes in the dependent and independent characters, when the characters evolve independently.* This analysis allows us to assess type I error (the probability that an association will be claimed by the test when none exists). We counted the number of observed gains and losses in the dependent character that occurred on black branches (those reconstructed to have the derived state) of the independent character. In the example shown in Figure 1, three of the four gains in the dependent character are on black branches. We then used MacClade to calculate the probability of getting this many or more gains and as many or fewer losses on black branches, given the total number of gains and losses observed for the dependent character (on all of the independent branches). Since all characters were generated randomly and independently, there should be no significant association—that is, any significant associations observed are due to chance (type I error).
2. *The probability that an association would be detected if all of the changes in the dependent character had occurred on black branches of the independent character.* This analysis allows us to assess type II error (the probability that an association will not be found when one exists). We simulated the extreme situation in which gains in the dependent character occurred only in the presence of the derived state of the independent character and then asked whether the CCT would detect such an association. To do so, we counted the total number of gains and losses of the dependent variable on all independent branches. In Figure 1, for example, there are four gains overall (three on black branches and one other). We then used MacClade to determine the probability

that as many or more gains (e.g., 4 in Fig. 1) and as many or fewer losses would occur by chance on black branches of the independent character. This allowed us to simulate a correlation between the traits without changing the rate of evolution or the methods of reconstructing character evolution. The CCT needs to know the actual pattern of black and white branches only for the independent characters, not for the dependent characters. Other ways of approaching this problem are considered in the *Discussion*.

We next assessed the effect of including white branches on both of these probabilities. The proportion of white branches for each tree and each dyad of characters was calculated by using a C program (available from P.D.L.). Using the MacClade Node List output format for representing the tree and character reconstruction, and the C program, we counted gains and losses as well as white branches. We used nonparametric Spearman rank correlations (corrected for ties when necessary) to examine the relationship between the probabilities calculated by the CCT and the proportion of white branches. The significance level for these correlations were adjusted with sequential Bonferroni because the same test was repeated for each tree (Rice, 1989). We also used nominal logistic regression (Hosmer and Lemeshow, 1989; Trexler and Travis, 1993; SAS, 1994) to test whether an increase in the proportion of white branches (pwb) affected the likelihood that the CCT was significant (for $\alpha = 0.05$ or $\alpha = 0.01$). To do this, we asked whether *pwb* contributed significantly to a model that included the model of evolution, *pwb*, and a tree shape statistic considered later (I), thus comparing the relative importance these three factors. Interactions were included only when they contributed significantly to the models. In our logistic regression analyses, a positive coefficient indicated an increase in the proportion of significant CCT probabilities as the continuous variable increased. When the effect of the model of evolution was considered, a positive coefficient indicated a higher proportion of significant CCT probabilities for the gains-only model than for the model with gains and losses.

To examine the effect of tree shape on Maddison's CCT, we used the trees generated as described above and calculated for each tree a tree balance statistic called the index of imbalance (I, developed by Colless [1982] and corrected by Heard [1992] and Rogers [1994]), calculated as

$$I = \frac{2}{(n-1)(n-2)} \sum_{j=1}^{n-1} |r_j - s_j|$$

where n is the total number of terminal taxa, and r and s are the number of terminal taxa to the right and left, respectively, of a given internal node. I is a good indicator of tree shape because it ranges from 0 for perfectly balanced (symmetrical) trees to 1 for completely imbalanced (asymmetrical) trees. We used this statistic because it has been the focus of much recent research on tree shape (Heard, 1992; 1996; Rogers, 1994; Mooers, 1995; Mooers and Heard, 1997) and because the expected value of the statistic for random trees with n terminals can be calculated (Heard, 1992).

We then examined the effect of tree imbalance on the CCT probabilities. Each tree provided a single value of I. As in the preceding analysis, we determined both (1) the probability that an association would be detected between the evolution of dependent and independent characters when they are independent (to assess the effect of tree shape on type I error) and (2) the probability that an association would be detected if all of the changes in the dependent character had occurred on black branches of the independent character (to assess the effect of tree shape on type II error). To analyze the effect of tree shape, we used two approaches. Linear regression (for multiple y values at each x; Sokal and Rohlf, 1981:477) was used to examine how the CCT probability was influenced by tree imbalance (I). The CCT probabilities had highly skewed distributions. Only the logit transformation was strong enough to normalize these distributions. However, because there are 0's and 1's in our data, for which the logit is undefined, we performed regressions on

the untransformed values to avoid a nonrandom loss of data. We consider the regression with and without the extreme trees (most balanced and imbalanced) to test whether the effect of tree shape on the CCT is different between the range where real trees are more likely to be and across the whole range of tree shapes. We also included I in the nominal logistic regression analysis (see above methods) to test whether an increase in I affected the likelihood that the CCT was significant (for $\alpha = 0.05$ or $\alpha = 0.01$).

To look at the effect of taxon sampling (which varies with both the proportion of white branches and the number of taxa) while holding tree shape more or less constant, we followed the analysis of 100-taxon trees with an analysis on two subsets of a nearly perfectly balanced 100-taxon tree. We did this by calculating the CCT for a 50- and a 25-taxon clade within the larger tree, that is, using the same pairs of characters as in the analyses of the 100-taxon tree but with the additional proviso that there be at least one change (gain or loss) in the dependent character within the selected clade. (If there are no changes, the CCT is fixed at either 0 or 1.) We estimated the same two CCT probabilities (1 and 2 described above). Logistic regression was used to examine the effect on the CCT probability of taxon number and the proportion of white branches together for the two models of evolution. We used subsets of only the most balanced 100-taxon tree for several reasons. First, taking subsets of the most-balanced tree ($I = 0.01$) does not result in trees with drastically different tree balance (0.02 for the 50-taxon subtree and 0.04 for the 25-taxon subtree), which allows us to ignore the effects of tree shape in this part of the analysis. With trees of intermediate balance, any subtree is likely to have a very different shape and I value. It is also difficult to take subsets of a fixed size without changing the structure of the resulting tree and breaking up any clades in the larger tree. Subsets of the least-balanced tree were not used because when traits were gained but not lost, the subset trees were often entirely black.

The CCT relies on reconstructed ancestral states for the characters of interest to judge the numbers of gains and losses on a tree. The implementation of the CCT in MacClade also relies on a null model of random distribution of changes onto the tree to estimate the probability that dependent changes are concentrated on black branches of the independent character (see Maddison [1990] for details). Problems may arise with the test if parsimony reconstruction of ancestral states results in a very different distribution of changes onto the tree than that produced by the null model of random evolution. Fortunately, the effects of this sort of discrepancy on the CCT can be evaluated by using the Reconstruction option in MacClade (for an explanation of the differences between Actual and Reconstructed, see Maddison and Maddison, 1992:310–312). To ensure that the above simulations were not influenced by this sort of reconstruction error, we repeated the above simulations for 30 character pairs (15 with gains only and 15 with gains and losses) on three of the trees, using the MAXSTATE Reconstruction option. Both CCT probabilities (1) and (2) obtained in this way were then compared with those obtained using the Actual changes option, through use of the Wilcoxon signed rank test (with sequential Bonferroni adjustment where appropriate). If there is no effect of using reconstructions, we expect no significant difference between the probabilities obtained when using the Actual and Reconstructed simulations. Because simulations with the Reconstruction option take considerably longer to run, we used the Actual Changes option in the original simulations, and here we repeated the analysis with only 3 of the 12 trees (a, b, and c) for both models of evolution.

Results

Effect of Allowing Gains Only or Both Gains and Losses

When both gains and losses were allowed, more gains were reconstructed onto the trees (using the independent character as an example, for all 12 trees, mean ± SE = 8.56 ± 0.21) than when only gains were allowed

(5.88 ± 0.20). There were 4.51 ± 0.15 losses reconstructed onto the 12 trees. The average number of black branches for the independent character was 106.49 ± 1.90 for the model with losses and gains, and 141.80 ± 2.26 for the model with gains only. Obtaining significant CCT values was more likely when losses were allowed than when they were not (Figs. 2 and 3, and Table 1). A detailed evaluation of these effects is provided in the following sections.

Effect of White Branches

1. Characters evolve independently.—(a) Only gains are allowed.— Considering all 360 pairs of randomly evolved characters (i.e., uncorrelated), only a single significant association was detected at $\alpha = 0.05$ and none was detected at $\alpha = 0.01$. This indicates a realized type I error of 0.0028 (1/360), which is smaller than we would expect: the upper 95% confidence limit based on the Binomial distribution ($n = 360$) is 0.015, which does not include 0.05. If type I errors occurred at a rate of 1 in 20 tests, we would have expected 18 significant tests for $\alpha = 0.05$ and 4 for $\alpha = 0.01$. CCT is therefore conservative with respect to type I error when only gains are allowed.

The proportion of white branches had a small but significant effect on the CCT probability. There was a negative correlation between the proportion of white branches and the test probability when analyses for all 12 trees were pooled ($r_s = -0.34$, $n = 360$, $P < 0.0001$; Fig. 2a). However, when correlations were examined for each tree separately, none were significant (with sequential Bonferroni correction for 13 tests and a tablewide $\alpha = 0.05$), and in two cases the sign was reversed (range: $r_s = -0.52$, $P = 0.005$ to $r_s = +0.31$, $P = 0.09$; $n = 30$ in each case).

TABLE 1. Logistic regression analysis of CCT probability significance ($\alpha = 0.05$ or 0.01) as a function of the model of evolution (gains only vs. gains and losses), the proportion of white branches (*pwb*) and the tree balance statistic *I*. The likelihood ratio χ^2 (LR χ^2) indicates the relative contribution of a given effect to the overall best model that includes the effects shown in that section of the table. Interaction terms were included only if they contributed significantly to the models. Sections 1 and 2 represent the two different probabilities discussed in the text.

Variable	Coefficient	SE	LR χ^{2a}	P^b
1. Characters evolve independently.				
$\alpha = 0.05$				
Model of evolution	–1.880	0.518	36.167	< 0.0001
pwb	–5.507	2.057	8.791	0.003
I	–0.458	0.934	0.259	0.610
Intercept	–3.301	0.644		
2. All changes in the dependent character occur on black branches.				
$\alpha = 0.05$				
Model of evolution	0.227	0.387	0.36	0.551
pwb	17.526	1.619	214.57	< 0.0001
I	–7.358	1.790	30.52	< 0.0001
Model × *I*	–6.253	1.790	17.89	< 0.0001
Intercept	–0.824	0.446		
$\alpha = 0.01$				
Model of evolution	–0.030	0.293	1.023	0.312
pwb	15.251	1.400	208.596	< 0.0001
I	–6.051	1.405	27.601	< 0.0001
Model × *I*	–4.31	1.406	11.149	0.0008
Intercept	–1.813	0.391		

[a] All with $df = 1$.
[b] Probability of getting a greater χ^2 by chance.

(*b*) *Both gains and losses are allowed.*—Twenty-five of the 360 character pairs produced significant associations at $\alpha = 0.05$ and 4 of 360 at $\alpha = 0.01$, resulting in realized type I error of 0.07 and 0.01, respectively. The likelihood of making type I errors is not

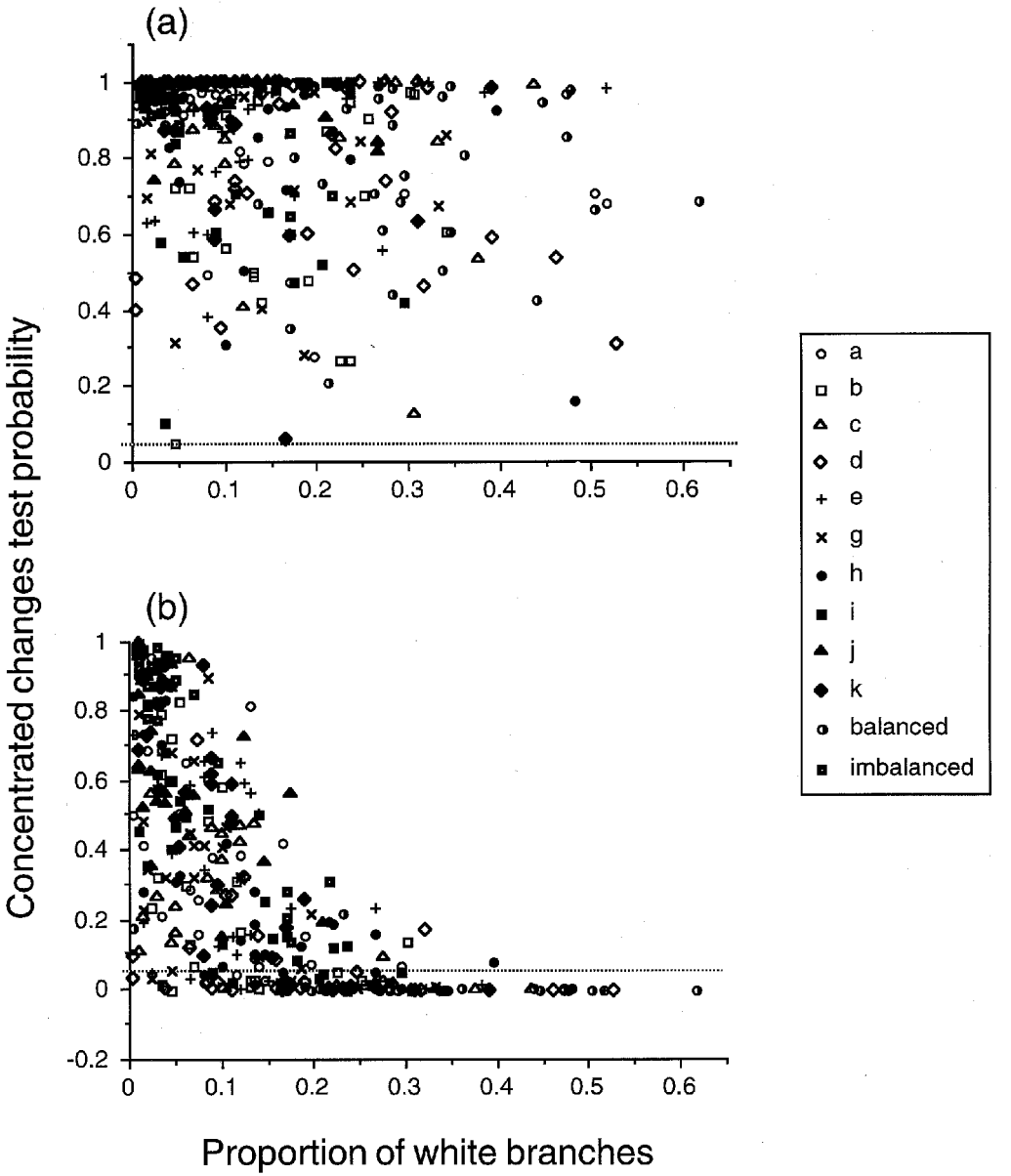

FIGURE 2. The relationship between CCT probabilities and the proportion of branches with neither trait of interest (white branches) when only gains are allowed to evolve is shown for 30 pairs of characters on 12 trees (*a–k*, *balanced*, and *imbalanced*) with 100 taxa. Probabilities are for the cases where (**a**) characters are simulated to evolve independently (probability 1) or (**b**) all gains in the dependent character occur on black independent branches (probability 2). Dotted lines show CCT = 0.05.

significantly different from what we would expect for $\alpha = 0.05$—the lower 95% confidence limit is 0.045 (for 25 significant out of 360), which includes 0.05—and was exactly what we would expect for $\alpha = 0.01$. Neither the overall

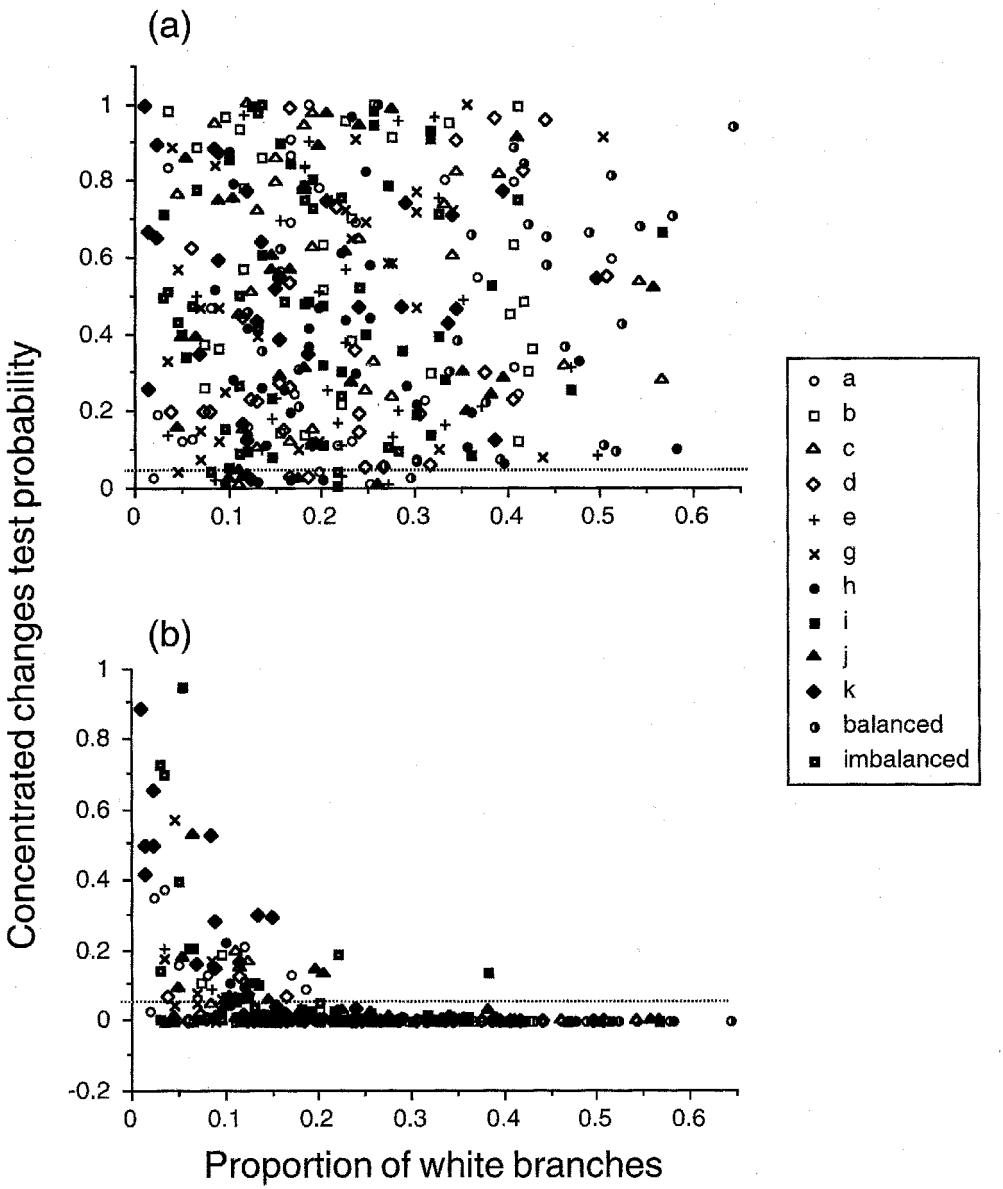

FIGURE 3. The relationship between CCT probabilities and the proportion of branches with neither trait of interest (white branches) when gains and losses are allowed to evolve is shown for 30 pairs of characters on 12 trees (*a–k*, *balanced*, and *imbalanced*) with 100 taxa. Probabilities are for the cases where (**a**) characters are simulated to evolve independently (probability 1) or (**b**) all gains in the dependent character occur on black independent branches (probability 2). Dotted lines show CCT = 0.05.

nor any of the separate correlations were significant at $\alpha = 0.05$ (overall: $r_s = 0.06$, $P = 0.27$; range: $r_s = -0.19$, $P = 0.31$ to $r_s = +0.36$, $P = 0.05$; $n = 30$ in each case).

There was a significant negative contribution of the proportion of white branches to the significance of the CCT probability (at $\alpha = 0.05$), based on the three-variable logistic regression model (Table 1). This is entirely explained by the decrease in the proportion of significant CCT probabilities with the increased pwb seen in the data for the model where both gains and losses were allowed (see points above vs. below dotted line in Fig. 3a). When only gains are allowed, *pwb* does not influence the significance of the CCT, there being only one significant probability under this model. This difference between the models of evolution explains the significant effect of the model of evolution on the analysis (Table 1, part 1). The logistic regression analysis could not be estimated for $\alpha = 0.01$ because there were too few significant CCT probabilities at this α level.

2. All changes in the dependent character occur on black branches.—(a) Only gains are allowed.—When all changes in the dependent character were presumed to have occurred on "black" branches of the independent character, significant associations were detected for 113 of 360 character pairs for $\alpha = 0.05$ and for 73 of 360 for $\alpha = 0.01$ (Fig. 2b). Moreover, there was a highly significant negative correlation between the proportion of white branches and the CCT probability ($r_s = -0.79$, $n = 360$, $P < 0.0001$; data for all 12 trees pooled). When each tree was examined separately, all of the correlations were significant (with Bonferroni-corrected α values for 13 tests) and all were negative (range: $r_s = -0.83$, $P < 0.0001$ to $r_s = -0.47$, $P = 0.01$; $n = 30$ in each case).

(b) Both gains and losses are allowed.—When both gains and losses were allowed, 291 of 360 character pairs gave a CCT probability < 0.05, and 247 of 360 were < 0.01. The proportion of white branches and the CCT probability were highly correlated ($r_s = -0.65$, $n = 360$, $P < 0.0001$; data for all 12 trees pooled; Fig. 3b) with 9 of the 12 trees also reaching significance (after Bonferroni correction for 13 tests; range: $r_s = -0.84$, $P < 0.0001$ to $r_s = -0.35$, $P = 0.06$; $n = 30$ in each case). The ability to detect a significant association between the dependent and independent character was greatly reduced once the proportion of white branches was $< 20\%$, but this loss of power was less marked than when only gains were allowed (compare Figs. 2b and 3b).

There was a highly significant positive contribution of *pwb* to the significance (for both $\alpha = 0.05$ and $\alpha = 0.01$) of this CCT probability, based on the logistic regression model (shown in Table 1, part 2). As the proportion of white branches increases, so does the fraction of CCT values that are judged significant. The model of evolution did not contribute significantly on its own. (The model $\times I$ interaction will be discussed in the next section.)

Effect of Tree Shape

1. Characters evolve independently.—(a) Only gains are allowed.—There was a significant positive relationship between the CCT probability and the tree imbalance statistic (I), and this relationship was stronger when the extreme trees (most balanced and imbalanced) were excluded from the analysis (Table 2, part 1 and Fig. 4a). By excluding the extreme trees, we placed more emphasis on the range of tree shapes in which real trees are expected to be, and the effect of tree shape on this CCT value was stronger in this range. As the trees became more balanced (lower I), the CCT probability decreased slightly. However, even when the tree was nearly fully balanced, only a single type I error was made (at the 0.05 significance level).

(b) Both gains and losses are allowed.—The slope of the regression of the CCT value on I was not significantly different from 0 (with and without the extreme trees; Table 2, part 1 and Fig. 5a).

The contribution of I to the logistic regression that included the model of evolution, the proportion of white branches, and I was not significant (Table 1, part 1). The CCT, therefore, appears not to be affected by tree topology, and this variable does not seem to increase the likelihood of making type I errors.

TABLE 2. Coefficients for regression of two CCT probabilities on tree shape statistic *I*. Values are shown separately for each model of evolution, and for regressions with and without the two extreme trees. Sections 1 and 2 represent regressions for the two different probabilities discussed in the text.

	Including extreme tree shapes				Excluding extreme tree shapes			
	Slope	Intercept	R^2	P^a	Slope	Intercept	R^2	P^a
1. Characters evolve independently.								
Model of evolution								
Gains only	0.17	0.81	0.04	0.046	1.35	0.56	0.06	0.003
Gains and losses	0.05	0.45	0.001	0.551	0.36	0.39	0.0002	0.593
2. All changes in the dependent character occur on black branches.								
Model of evolution								
Gains only	0.74	0.16	0.25	0.001	3.78	–0.48	0.19	0.00005
Gains and losses	0.11	0.02	0.03	0.072	0.80	–0.13	0.06	0.033

[a] Represents the significance level of the regression slope.

2. *All changes in the dependent character occur on black branches.—(a) Only gains are allowed.*—Tree imbalance had a significant effect on the CCT probability in cases where the dependent character changed only on black branches of the independent character (Fig. 4b). There was again a positive relationship between tree imbalance and this CCT probability, both with and without the extreme trees, and again the relationship was stronger when the extreme trees were excluded (Table 2, part 2). Moreover, the ability to detect a significant association between characters was strongly influenced by tree shape. The two trees that were most balanced (lowest *I*; tree *most balanced*, $I = 0.011$; tree *d*, $I = 0.147$) exhibited the greatest proportion of significant associations (27 of 30, and 21 of 30, respectively, for $\alpha = 0.05$; and 24 of 30, and 14 of 30, respectively, for $\alpha = 0.01$). In contrast, 10% or fewer of the associations were significant for the two least-balanced trees (for both $\alpha = 0.05$ and $\alpha = 0.01$; tree *least balanced*, $I = 1$, 0 of 30 associations significant; tree *k*, $I = 0.268$, 3 of 30 associations significant).

(b) Both gains and losses are allowed.—There was a positive relationship between tree imbalance and the CCT probability, both with and without the extreme trees. This relationship was not significant when the extreme trees were included but was significant when they were excluded (Fig. 5b and Table 2, part 2). The two trees that were most balanced exhibited the greatest proportion of significant associations (tree *most balanced*, 29 of 30, and tree *d*, 27 of 30 for $\alpha = 0.05$; and 29 of 30, and 25 of 30, respectively, for $\alpha = 0.01$). In contrast, many fewer of the associations were significant for the two least-balanced trees (tree *least balanced*, 18 of 30 associations significant, and tree *k*, 17 of 30 associations significant for $\alpha = 0.05$; and 2 of 30, and 1 of 30, respectively, for $\alpha = 0.01$).

The results in parts (*a*) and (*b*) of this section were reflected in the logistic regression results for both $\alpha = 0.05$ and $\alpha = 0.01$. There was a significant negative contribution of *I* to the significance of this CCT probability, based on the logistic regression model (Table 1, part 2). Tree topology clearly has an effect on the power of the CCT—the more balanced the tree, the more likely that a significant association will be detected. This effect is significantly stronger when only gains are considered than when losses are also included (seen as significant negative interactions, Table 1, part 2; see also Figs. 4b and 5b and Table 2, part 2). However, the logistic regression results indicate that the proportion of white branches has a greater effect on the CCT probability than either tree topology, the model of evolution, or the model × *I* interaction (greater magnitude of likelihood ratio [LR] χ^2 in Table 1).

Number of Taxa versus Proportion of White Branches

There was only one significant CCT value (at $\alpha = 0.05$ for gains and losses model and none in all other cases) for probability

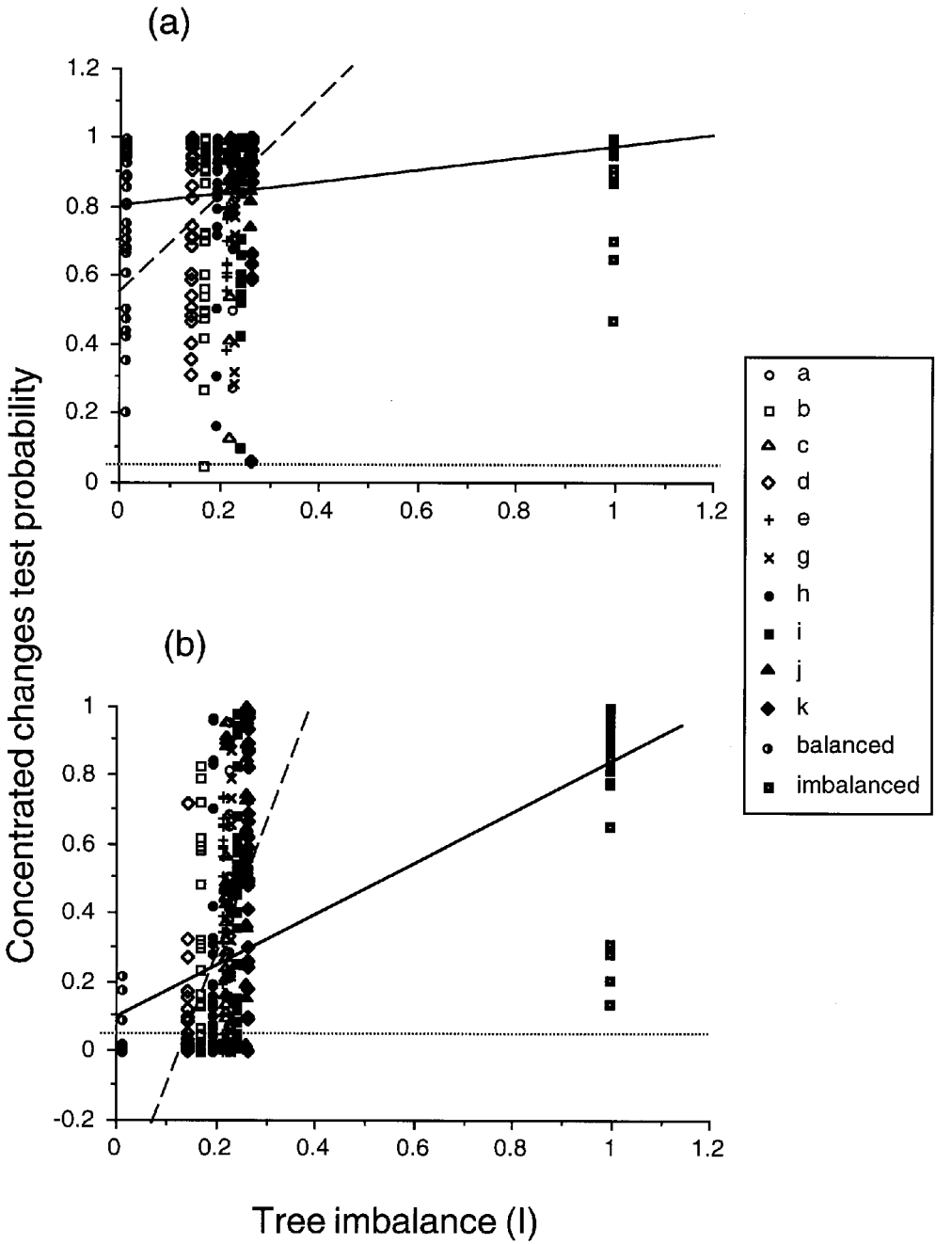

FIGURE 4. The relationship between CCT probabilities and the tree shape statistic *I* when only gains are allowed to evolve is shown for 30 pairs of characters on 12 trees with 100 taxa. Probabilities are for the cases where (**a**) characters are simulated to evolve independently (probability 1) or (**b**) all gains in the dependent character occur on black independent branches (probability 2). Dotted lines show CCT = 0.05, solid lines represent least-squares regression line for all tree shapes, and dashed lines represent the regression line when two extreme tree shapes are excluded.

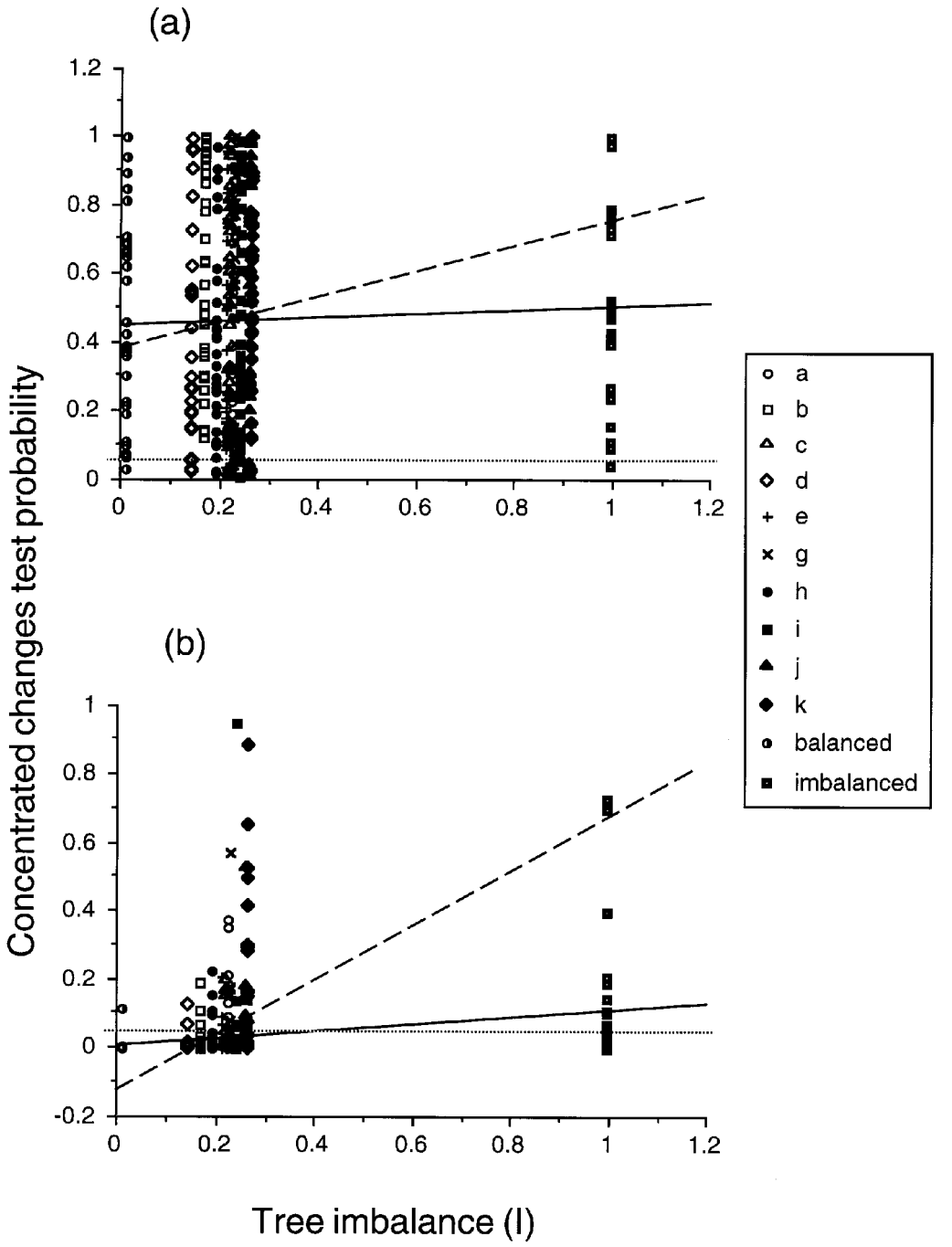

FIGURE 5. The relationship between CCT probabilities and the tree shape statistic *I* when both gains and losses are allowed to evolve is shown for 30 pairs of characters on 12 trees with 100 taxa. Probabilities are for the cases where (**a**) characters are simulated to evolve independently (probability 1) or (**b**) all changes in the dependent character occur on black independent branches (probability 2). Dotted lines show CCT = 0.05, solid lines represent least-squares regression line for all tree shapes, and dashed lines represent the regression line when two extreme tree shapes are excluded.

(1) (i.e., characters evolve independently), which prevented us from analyzing the effects of taxon number on type I error. Logistic regression analysis of CCT probability (2) (i.e., all changes in dependent character occur on black branches) for the 50- and 25-taxon subsets from the 100-taxon balanced tree is shown in Table 3. At $\alpha = 0.05$, *pwb* and the interaction between this variable and the number of taxa both contribute significantly to the regression. Model of evolution and number of taxa by themselves do not contribute significantly. Therefore, once the effect of the interaction is taken into account, increasing the proportion of white branches decreases the fraction of CCT values that are significant (< 0.05). The variable *pwb* is relatively more important than the number of taxa (LR χ^2 for *pwb* > LR χ^2 for number of taxa), indicating that the taxon sampling effects on the CCT are driven primarily by the proportion of white branches. However, this interaction contributes the most strongly to the regression. The significant interaction term indicates that as the number of taxa decrease (especially from 50 to 25), so does the range of *pwb* over which the test has power. Specifically, the point at which there is no power to detect an association between characters increases as the number of taxa decrease (e.g., vertical lines in Fig. 6) until there is no range of *pwb* for which the CCT reliably indicates a significant association. When only gains are allowed, the point at which the test loses power and makes type II errors is around 0.25 *pwb* for 100 taxa, 0.43 *pwb* for 50 taxa, and 0.88 *pwb* for 25 taxa (Fig. 6a, b, c). Results when both gains and losses are allowed follow the same pattern (0.13, 0.28, and 0.88 for 100, 50, and 25 taxa, respectively). This seems to be a straightforward result of the decrease in the number of branches on which character changes could have occurred, which accompanies a decrease in the number of taxa. At the 0.01 significance level, the interaction between *pwb* and number of taxa is the only statistically significant contributor to the regression (Table 3).

Actual versus Reconstructed Changes

Finally, to test whether using reconstructed character states had an effect on the CCT probabilities, we compared values obtained by using the Actual Changes option and those from the MAXSTATE reconstruction option in MacClade for trees a, b, and c. These trees fall within the range

TABLE 3. Logistic regression analysis of CCT significance ($\alpha = 0.05$ or 0.01) as a function of the model of evolution (gains only vs. gains and losses), the proportion of white branches (*pwb*), and the number of taxa. Regressions were performed only when all changes in the dependent character occur on black branches (probability 2). LRχ^2 indicates the relative contribution of a given effect to the overall best model that includes the effects shown in that section of the table.

	Coefficient			
Variable	Mean	SE	LR χ^{2a}	P^b
$\alpha = 0.05$				
Model of evolution	–0.261	0.238	1.232	0.267
pwb	–6.463	3.196	4.706	0.030
Number of taxa	–0.034	0.023	2.335	0.126
pwb × no. of taxa	0.382	0.111	21.156	< 0.0001
Intercept	–0.672	0.902		
$\alpha = 0.01$				
Model of evolution	–0.298	0.213	2.019	0.155
pwb	–2.331	2.257	1.072	0.301
Number of taxa	–0.011	0.017	0.396	0.529
pwb × no. of taxa	0.211	0.063	14.357	0.0002
Intercept	–1.714	0.854		

[a] All with $df = 1$.

[b] Probability of getting a greater χ^2 by chance.

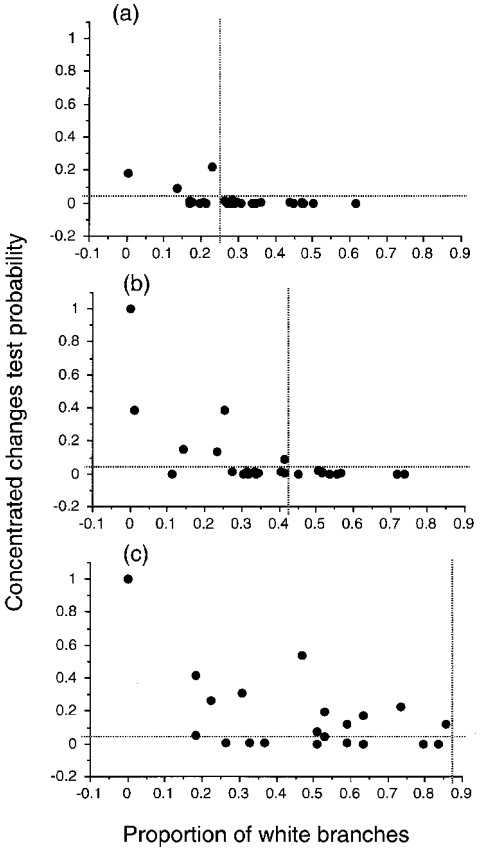

FIGURE 6. The relationship between CCT probability 2 (all changes are presumed to occur on black branches) and the proportion of branches with neither trait of interest (white branches) when only gains are allowed to evolve is shown for trees with (**a**) 100, (**b**) 50, and (**c**) 25 taxa. The horizontal line indicates CCT = 0.05; the vertical line represents the point (in terms of the proportion of white branches) below which the CCT begins to make type II errors (power breakdown point).

of tree shapes into which we expect real trees to be most likely to fit. The differences in the values obtained by these two methods were very small (|median differences| between 0 and 0.007). Wilcoxon signed-rank tests comparing the MAXSTATE-reconstructed CCT values and the "Actual" values for each of the two kinds of probabilities (1 and 2) and both models of evolution (gains only, and losses and gains) for trees *a*, *b*, and *c* found one marginally significant difference among 12 tests (Wilcoxon $z = 2.67$, $P = 0.008$, with Bonferroni correction). For tree c, when only gains were allowed, the Actual changes option caused an underestimate of probability (1). However, the median difference was very small (–0.006). Only a single CCT value for probability (1) was significant when only gains were allowed, and it was not on this tree but tree b. Consequently, the difference in results between the MAXSTATE reconstruction methods and the Actual changes method appears to be small, indicating that our results are not an artifact of having used reconstructed character states.

DISCUSSION

One of the limitations in evaluating fully the power of the CCT is that there are so many variables that can potentially influence the outcome of the test. Many of these variables are interrelated, and assessing the effect of any single variable while holding all others constant becomes difficult. We could have considered additional variables in our simulations, such as different transition probabilities or the resolving option ACCTRAN rather than DELTRAN; however, each added variable increases the number of simulations dramatically and such an exhaustive analysis is difficult to present coherently. We chose instead to focus on four variables that might affect the conclusions drawn with the CCT—the proportion of white branches, tree shape, two basic models of evolution, and, in a simple way, the number of taxa. A summary of the results for each of these variables is presented in Table 4 and each variable is discussed in turn.

Effect of White Branches

For the large realistically shaped trees and the extreme trees used in this study, character pairs with a larger proportion of white branches were not more likely to have significant CCT values when there was no association between the characters (probability 1, Table 4). Increasing the proportion of white branches decreased the likelihood of type I error when gains and losses were allowed but had no effect when only gains

TABLE 4. Summary of the effects of white branches, tree shape, number of taxa, and model of evolution (gains only, or gains and losses) on the outcome of the concentrated changes test.

	Probability 1: Characters evolve independently	Probability 2: All changes in dependent character occur on black branches
Effect of white branches		
a. Gains only	Likelihood of type I error is lower than expected (CCT is conservative) Negative correlation of proportion of white branches with probability 1	Likelihood of type II error increases when proportion of white branches is < 20% Strong, negative correlation of proportion of white branches with probability 2
b. Gains and losses	Type I error as expected (CCT is accurate) No effect of proportion of white branches on probability 1	Likelihood of type II error increases when proportion of white branches is < 20% Strong, negative correlation of proportion of white branches with probabilty 2
Effect of tree shape		
a. Gains only	Likelihood of type I error is low Positive correlation of tree imbalance (I) with probability 1	Likelihood of type II error increases as tree imbalance (I) increases Strong, positive correlation of tree imbalance (I) with probability 2
b. Gains and losses	Likelihood of type I error is low No correlation of tree imbalance (I) with probability 1	Likelihood of type II error increases as tree imbalance (I) increases Weak, positive correlation of tree imbalance (I) with probability 2
Effect of number of taxa		
Gains only and gains and losses	Too few significant CCT values to evaluate	Likelihood of type II error increases as number of taxa decreases, but depends on proportion of white branches Significant interaction with proportion of white branches on probability 2

were allowed. The test therefore seems to behave reasonably with regard to type I error. When there was no association between the characters of interest, the CCT reliably reflected this fact, and adding white branches did not increase the chances of obtaining a significant association when none existed.

In striking contrast, the power of the CCT to detect associations between characters (type II error) was strongly influenced by white branches. Type II errors were made quite commonly when there was a low proportion of white branches (for both models of evolution). We interpret this to mean that the test can be insensitive when there are too few white branches. In our data this occurred when there were fewer than 20% white branches. This result is not entirely unexpected. As more of the branches of a given tree are reconstructed to contain the derived state of the independent character (i.e., are black), the probability of a change in the dependent character occuring on a black branch by chance necessarily increases. The CCT should, and does, reflect this fact. Moreover, we would expect this to be true for

trees of any shape because it is a function of how early the characters of interest evolved. What has been less appreciated, however, is that the power of the test is dramatically reduced when one or both of the traits of interest has evolved early (and hence branches for one or both traits are predominantly black). This power loss is mitigated when both characters can be lost after they have been gained, because losses increase the proportion of white branches again (compare Figs. 2b and 3b).

Effect of Tree Topology

Though less influential than the proportion of white branches, the tree balance statistic *I* is significantly positively related to the CCT value (Figs. 4 and 5 and Table 2)—the more balanced the tree, the more likely that a significant association will be detected. This effect is exaggerated when only gains are reconstructed. Maddison (1990) pointed out that imbalanced trees have high variance in the amount of time represented by each branch. Accordingly, we might anticipate that the CCT value would be lower in imbalanced trees because of the greater likelihood of two characters evolving on the longer branches by chance rather than because of a causal connection (Maddison, 1990).

The CCT could be modified to allow different probabilities of change on different branches by changing the null model from one of random distribution of changes to one where the probability of change on a branch depends on its length (Sanderson, 1991). Pagel (1994a) developed a maximum likelihood approach that gives a measure of the correlated evolution of two traits while taking branch lengths into account. However, our results suggest that the effect of tree shape on the CCT is not solely a result of the influence of branch length. Character changes were not more likely in imbalanced parts of the trees, because changes occurred with equal probability on each branch regardless of length. Therefore, branch length variance alone cannot explain the higher CCT values in more imbalanced trees.

Werdelin and Tullberg (1995) noted that the range of possible CCT values for imbalanced trees was reduced when only gains are allowed. Our data indicate that this may be even more the case when both losses and gains are reconstructed (compare Figs. 4b and 5b). We suggest this pattern results from the different consequences of gains and losses in balanced versus imbalanced parts of trees. There is the potential for higher variance in the number of black branches when changes occur in imbalanced clades. For example, in the imbalanced clade N–U in Figure 1, a gain on the branch that forms the root of clade O–U, one branch away from the root of clade N–U, would result in all branches leading to O–U being black (13 branches). A gain on the other branch from the root of N–U would result in 1 black branch. On the other hand, in an equal-sized balanced clade A–H, an equally basal gain on the root branch of either clade E–H or clade A–D would result in all branches of either clade being black (seven branches). Gains one step away from the root of each clade would result in very different ranges in the amount of black branches. The consequence for the CCT is that the maximum CCT value possible on imbalanced trees is higher than for balanced trees: if most of a clade is black, the numerator and denominator of the CCT approach each other.

Whatever the cause, tree shape influenced the CCT probability for our 12 constructed trees that span the range of tree shapes (in agreement with the results of Werdelin and Tullberg, 1995). Given the effects of tree shape on the CCT, we recommend that users of this test calculate the tree balance statistic *I* for their trees. CCT results for trees with high *I* values should be viewed as more conservative than those based on trees with low *I* values.

Effect of Number of Taxa

The magnitude of the effect of white branches on the CCT was related to the number of taxa (or number of branches) in the tree. Because there were so few significant CCT values detected when characters evolved randomly (probability 1, Table 4), we could not assess the effect of the number of taxa on type I error. However, our analysis indicates that the likelihood of type II errors

increases as the number of taxa decreases, although this also depended on the proportion of white branches on the tree (Fig. 6). For example, in a tree of 100 taxa, type II errors are more likely when the proportion of white branches is < 0.30, whereas in a tree of 25 taxa, type II errors become frequent when the proportion of white branches is < 0.90 (Fig. 6)! This effect occurs because a reduction in the number of taxa reduces the number of places where changes can occur on the tree, leading to higher CCT values and the increased possibility of type II error. Our results (Fig. 6) suggest that researchers should be cautious in applying the CCT to trees with < 50 taxa or with characters reconstructed to have < 30–40% white branches whenever the gains per branch ratio is ≤0.06.

Effect of Model of Evolution: Gains Only versus Gains and Losses

When only gains are allowed, the CCT is perhaps too conservative with regard to type I error. Our results demonstrate a realized type I error that is roughly 18 times less than the generally accepted 5% error rate (0.0028 as opposed to 0.05). Interestingly, Proctor (1991) found that Ridley's (1983) method, which asks whether gains and losses in one trait depend on the state of another trait, was also "extremely conservative when the trait is never lost." Ridley's method appears to treat gains and losses as equally likely; when losses do not occur, the test is conservative. The CCT should not have this weakness: It allows one to ask whether, given a certain number of gains and losses on the whole tree, the number of changes seen on the black branches is more than you would expect by chance. One way to ameliorate this effect would be to adjust the critical value for statistical significance of the CCT (e.g., to $P < 0.10$) when only gains are considered. However, it is difficult to know where to objectively set the critical value for the test. For our data, 1 in 20 results would be deemed significant only if we used a critical value of 0.40, a rather extreme adjustment.

In contrast, the CCT behaves more as we would expect when both losses and gains are allowed (Table 4). It is not clear why the CCT behaves so differently under the two models of evolution. One possibility is that, although the probability of gain was the same for the two models in our simulations, there may have been a smaller number of realized gains when only gains were permitted simply because losses create the opportunity for more gains. Alternatively, a higher proportion of black branches for the independent character might be expected when only gains are allowed. Either of these results (fewer gains or more black branches) would lead to higher values of the CCT probability and hence more conservative test results. Our data support both of these possibilities. The average number of gains of the independent trait is less for the model with gains only (5.88) than for the model including losses too (8.56). In addition, black branches are, on average, 1.33 times more numerous when only gains are allowed than when losses are also allowed. However, the relative numbers of gains and black branches are also affected by other factors, such as our reconstructing the changes onto the trees rather than using the actual changes and our use of DELTRAN resolving options.

Under either model of evolution, two gains, one on each branch descendent from a node, would be reconstructed by the CCT as one gain by parsimony. When losses are allowed, a gain, a loss, and second gain, one after the other, could also be reconstructed as one gain. The DELTRAN resolving option also tends to increase gains and decrease losses when equivocal reconstructions are encountered. Though we do not think that using reconstructions significantly affected our estimates of the CCT probabilities, it still may have affected the relative number of gains and losses. More work clearly is needed to explain the difference in the CCT responses under the two models of evolution.

Recommendations for Future Studies: How Do We Define the Sampling Universe?

Our results clearly demonstrate that inclusion of taxa lacking the derived state of both characters can influence the power of the

CCT, as Maddison suspected. The question then becomes: What determines which, and how many, taxa lacking the traits of interest can or should be added? Several suggestions have been made about how the scope of a study of correlated evolution should be defined (Coddington, 1992; 1994; Pagel, 1994b). Coddington (1994) proposed that as wide an array of organisms as possible should be included in the analysis to maximize the generality of the conclusions. Pagel (1994b), in contrast, argued that tests of correlated evolution involve hypotheses about the selective forces that produce the correlation of interest and, accordingly, only clades that contain taxa with the independent trait of interest should be considered.

Our results also indicate that spurious correlations are not likely to arise simply because a large proportion of a tree is white with respect to two characters of interest, at least for the simulated data presented here. Thus we believe the risk of expanding the sampling universe should be minimal. In contrast, if the independent trait is widespread and has evolved only a few times, limiting the study in the way Pagel suggests may mean that there will be very few white branches. This will make it impossible to use the CCT to detect an association. Sillén-Tullberg's (1993) CST will suffer a similar loss of power, although it may still be possible to use a test based on sister taxon comparisons, such as that of Read and Nee (1995). Consequently, the value of adding clades containing white branches to reduce type II error would seem, based on our data, to offset any small effect (if any) of increasing type I error rates.

We are aware of at least one study in which the effect of a small proportion of white branches may have influenced the outcome of a test for correlated evolution. Hunter (1995) used Maddison's CCT and several other methods to test hypotheses about the evolution of the loss of functional wings in forest Lepidoptera. Because of concern that white branches were "problematic" for the CCT, large clades (97 species) in the Geometridae were omitted from the study (Hunter, 1995:278). The proportion of white branches was not calculated for the phylogenies used in the study, but the lack of significance of the CCT for all hypotheses tested may have been a result of the omission of the white branch–containing taxa. Sillén-Tullberg (1993) also cautions researchers using phylogenies from the literature to be sure that the tree has not been resolved in such a way as to limit white branches.

Assumptions of Our Analyses

We have chosen what some may consider a peculiar way of simulating perfect co- evolution. We have asked what the CCT probability would be if all dependent character changes (those occurring on both black and white branches) had occurred only on black branches. Ideally one would want to make the probability of change in one character depend on the state of another character so that coevolution could be simulated directly. This is currently not possible in MacClade. As an alternative to the method we used, one could consider character pairs in which all changes of the dependent character occur on black branches (6% of our character pairs meet this criterion). Unfortunately, this method can have the undesirable effect of restricting the study to trees that are predominately black (for our data, the changes in independent characters do not all fall on black branches unless the tree is > 77% black). We can think of no simple way to change transition probabilities to deal with this problem. However, our method should not create an unreasonably high number of dependent gains because we use the same transition probabilities for both dependent and independent characters. For example, in no case were there more changes in the dependent character than there were black branches.

We also used reconstructed character states in our analysis of the CCT, which may have affected our results. However, when we compared the results of the MAXSTATE reconstruction methods and the Actual Changes method for a sample of trees, the differences were small and, in 11 of 12 comparisons, not significantly different. Consequently, the patterns we describe do not appear to be an artifact of having used reconstructed character states.

Finally, we assumed certain values for transition probabilities of gains and losses, and we used the DELTRAN resolving option throughout all of our analyses. Our primary objective in doing so was to generate sufficient numbers of gains of the dependent character and a range of trees with various degrees of white branches to be able to fully explore the power of the CCT. Different transitions probabilities or different resolving options may yield different patterns, and we encourage researchers to continue to evaluate these alternatives. In the interim, we believe our analyses provide new insight into the strengths and limitations of the CCT and will help guide others in the use of this test for future comparative studies.

Conclusions

We conclude that the proportion of white branches and the tree shape do influence the results of the CCT, and do so to different extents, depending on whether or not losses are reconstructed onto trees in addition to gains. When the evolution of two correlated characters is reconstructed onto a cladogram, the proportion of the cladogram branches that have neither trait of interest has an important effect on the CCT—not to increase the likelihood that the test will be significant, but to constrain its usefulness. Contrary to our initial expectations, the susceptibility of the test to type I errors is not increased by including a large proportion of white branches. In contrast, if there are too few white branches or too few taxa, the test is not likely to detect even very strong correlations (i.e., is likely to make type II errors frequently). Tree shape can also affect the CCT probability, although for our data set tree shape did so to a smaller degree than the proportion of white branches. Type II errors are more likely in imbalanced trees, and the rate at which their likelihood increases is greater when only gains are considered. Finally, the results of the test are affected by the model of evolution. In particular, the CCT is very conservative with regard to type I error when only gains are reconstructed onto a tree.

Our results have consequences for studies designed to test hypotheses of correlated evolution. It is important not to so limit the scope of the study that clades with neither trait of interest are unnecessarily excluded. If concerns exist about the effect of white branches on a particular tree, simulations such as the ones undertaken in this study can be used to estimate the power of the CCT for a given set of characters and a particular tree shape. We also recommend that users of the CCT report the tree imbalance statistic I for their trees, to allow others to assess the extent to which tree shape may influence the results and conclusions.

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