# Notes and Comments

## On the Analysis of Evolutionary Change along Single Branches in a Phylogeny

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ABSTRACT: Comparative biologists are sometimes interested in estimating the evolutionary rate along single branches in a phylogeny. I evaluate two methods by which the evolutionary rate along single branches can be compared with the evolutionary rate throughout the rest of the tree. The first is McPeek's contrasts method, and the second is a likelihood method presented independently in two recently published studies. Although the latter method was developed primarily for the comparison of rates among clades, the approach is equally suited for the analysis of evolutionary rate along single or isolated branches. I find that Type I error is acceptable in both methods but that power and parameter estimation are relatively poor in McPeek's method as it is typically applied.

*Keywords:* independent contrasts, comparative method, evolutionary rate, quantitative characters, phylogenetics.

In recent years it has become widely recognized that in studies of comparative biology, observations from species related by a phylogenetic tree are nonindependent (Felsenstein 1985; Harvey and Pagel 1991). This nonindependence requires that evolutionary analyses be conducted in the context of the phylogenetic relationships among the species in the study (Cheverud et al. 1985; Felsenstein 1985; Larson and Losos 1996). Numerous statistical methods have been developed in order to analyze species data in the context of their shared and separate histories as represented by a phylogeny (Ridley 1983; Felsenstein 1985; Grafen 1989; Harvey and Pagel 1991; Hansen 1997).

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The most commonly used method for continuously distributed characters is the phylogenetically independent contrasts method of Felsenstein (1985). In this approach, differences are calculated between the character values (known or inferred) at sister nodes, including tip nodes. Under the common assumption that covariance among taxa is accumulated in proportion to shared history, the differences between sister nodes are independent because they consist of observations unfettered by the common history that creates statistical dependence among the observations at the tips of the tree. These differences, the phylogenetically independent contrasts, can thus be substituted for the species data in many standard statistical analyses.

In some cases, researchers are also interested in the amount or direction of change along particular branches in the phylogeny, as opposed to solely differences between sister nodes. McPeek (1995) developed an innovative approach, based on Felsenstein's (1985) contrasts method, to analyze the rate of evolutionary change along a single branch or set of isolated branches in the phylogeny. In McPeek's (1995) method, the researcher is first required to identify branches of interest on the phylogenetic tree. Generally, these are branches for which we have some prior information to hypothesize that exceptional evolution might have occurred. Such branches could include internodes or terminal branches on which a transition in habitat, ecology, morphology, or genetics is thought to have taken place. For example, if a population bottleneck is hypothesized for a particular branch, one might suspect that the rate of evolution by drift was elevated along that branch. Alternatively, if an ecological transition between environments or parasite hosts is hypothesized to have transpired along a particular branch in the phylogeny, one might expect that rapid adaptive evolution had accompanied that transition (e.g., McPeek 1995; Stoks and McPeek 2006).

Once exceptional branches have been hypothesized, two types of contrasts are calculated. The first type are standard sister-node contrasts calculated for a phylogeny from which the branches of interest have been pruned. The

second type are parent-daughter–node contrasts calculated along the pruned branches. These two types of contrasts are then compared (McPeek 1995).

Various authors have used the method of McPeek (1995) or similar approaches (e.g., Klingenberg and Ekau 1996; McPeek and Brown 2000; Crespi and Teo 2002; Revell et al. 2007b; Wiens et al. 2007). In most studies using McPeek's method, the absolute values of both types of contrasts are used (e.g., Klingenberg and Ekau 1996; McPeek and Brown 2000; Crespi and Teo 2002; Stoks and McPeek 2006). This is because independent contrasts calculated between sister nodes have arbitrary signs (Garland et al. 1992). When the absolute values are compared, McPeek's method is a test for a change in the evolutionary rate along particular branches of the tree compared with that in the other branches in the phylogeny (McPeek 1995, 1999).

Using the approach of phylogenetic generalized least squares (PGLS; Grafen 1989), O'Meara et al. (2006) and Thomas et al. (2006) independently developed highly similar likelihood tests for single or multiple shifts in the evolutionary rate in the phylogeny. Developed and implemented primarily to compare rates among clades (especially by O'Meara et al. 2006), in theory the likelihood approach should be equally applicable to hypotheses concerning the evolutionary rate along a single or multiple isolated branches in a phylogeny. Although these are exactly the types of hypotheses that McPeek's (1995) method was designed to address, the properties of the likelihood method for the analysis of evolutionary rates have never been compared with those of McPeek's.

In fact, the statistical properties of McPeek's (1995) method have not been evaluated. Although the properties of the likelihood method have been examined in other studies (O'Meara et al. 2006; Revell and Harmon 2008), these studies did not focus on changes in the evolutionary rate along single or isolated branches. In this study, I first describe the implementation of each approach to the analysis of evolutionary change along single branches in a phylogenetic tree. I then compare the statistical properties of the two methods in terms of error rate, parameter estimation, and power.

## McPeek's Method

In McPeek's (1995) method for testing hypotheses about evolution along single branches, we first identify internal or terminal branches hypothesized to have been subject to exceptional evolutionary conditions. We prune those branches from the phylogeny. If the pruned branch is nonterminal (i.e., if it is an internal branch), then pruning will result in two trees. This is because removing any internal branch in a phylogenetic tree will bisect the tree

into two subtrees. When the tree being pruned is a rooted phylogeny, as in this study, one of the resulting trees will be composed of the branches below the pruned internode, while the other will be composed of the branches above the pruned internode (McPeek 1995). We then calculate phylogenetically independent contrasts across all internal nodes in the pruned tree or trees. If the daughter nodes are also tip nodes, then the contrasts are simply calculated as the difference between tip states. If one or both daughters are internal nodes themselves, then node states are inferred by taking the weighted average of the descendants, in which the weights are inversely proportional to the branch lengths leading to the descendant (where the branches have been adjusted in length following Felsenstein 1985).

To then calculate contrasts for all pruned branches, we first calculate the state for the end closer to the root of each pruned branch. The end farther from the root of each pruned branch either is a tip, whose state is known, or has a state inferred by taking the weighted average of the descendants, as above. The state at the end closer to the root is estimated as a weighted average of the endpoints of the contrast that it interrupts, in which the weights are calculated from the branch lengths to each endpoint (McPeek 1995). Contrasts are then calculated along each pruned branch as the difference between the known or inferred states at either end of the branch.

As in the typical implementation of independent contrasts, all differences are then standardized by their expected variances. For contrasts between tips, the expected variance is proportional to the sum of the branch lengths (Felsenstein 1985). For all other contrasts, the expected variance is adjusted to accommodate extra variance associated with the estimation of ancestral states at internal nodes (Felsenstein 1985; McPeek 1995). Standardization is performed by dividing each contrast by the square root of its expected variance (Felsenstein 1985).

The rate test is then performed by comparing the mean absolute values of the contrasts using a *t*-test or a nonparametric equivalent (McPeek 1995, 1999). This is a test for a change in the evolutionary rate because the absolute values of the contrasts should scale, on average, as a function of the rate of evolution (Garland 1992). More details, including a worked example, are provided in McPeek (1995, 1999).

## A Likelihood Method

A likelihood method for the comparison of evolutionary rates was provided independently by O'Meara et al. (2006; the "noncensored" approach) and Thomas et al. (2006). I describe and provide a worked example of this method in an appendix in the online edition of the *American Nat-*

*uralist* and provide a simplified and condensed description of the method here.

In the likelihood approach, we first identify all exceptional branches (numbering $m^*$) hypothesized to exhibit a rate of evolution different from that in the rest of the tree. The lengths of these branches are added into the $n \times n$ (for $n$ taxa) matrix $\mathbf{C}^*$, in which the branch length $v_{(i,j,k)}$ (for the branch preceding a node with descendant taxa $i$, $j$, and $k$) is added to the matrix elements $C^*(i, j)$, $C^*(i, k)$, and $C^*(j, k)$, as well as to their transposes (e.g., Revell and Harmon 2008). All remaining ("unexceptional") branch lengths are added to the elements of the matrix $\mathbf{C}_0$ according to the same method. From this procedure, the matrix $\mathbf{C}_0 + \mathbf{C}^*$ is proportional to the expected covariance matrix for the values at the tips, given a single rate of evolution, whereas the matrix $\mathbf{C}_0 + h\mathbf{C}^*$ is proportional to the expected covariance matrix at the tips, given that the rates along exceptional branches are scaled by the constant, $h$, to be estimated using likelihood.

We then calculate two sets of evolutionary rates, using the equation

$$\hat{\sigma}_0^2 = \frac{(\mathbf{x} - \hat{a}\mathbf{1})'(\mathbf{C}_0 + h\mathbf{C}^*)^{-1}(\mathbf{x} - \hat{a}\mathbf{1})}{n}, \quad (1)$$

based on O'Meara et al. (2006) and Thomas et al. (2006). Here, $\mathbf{x}$ is an $n \times 1$ vector of trait values at the tips, $\hat{a}$ is the phylogenetic mean for the trait (Rohlf 2001; Revell and Harmon 2008; also see the appendix), and $\mathbf{1}$ is an $n \times 1$ column vector of 1.0s. The value of $h$ is either set to 1.0, corresponding to a hypothesis of rate homogeneity, or determined by maximizing the likelihood equation

$$L(\hat{\sigma}_0^2, \hat{a}, h | \mathbf{x}, \mathbf{C}_0, \mathbf{C}^*) =$$
$$\frac{\exp\{-(\mathbf{x} - \hat{a}\mathbf{1})^{\mathrm{T}}[\hat{\sigma}_0^2(\mathbf{C}_0 + h\mathbf{C}^*)]^{-1}(\mathbf{x} - \hat{a}\mathbf{1})/2\}}{\sqrt{(2\pi)^n \cdot |\hat{\sigma}_0^2(\mathbf{C}_0 + h\mathbf{C}^*)|}},$$
$$(2)$$

in which $\hat{\sigma}_0^2$ is determined by equation (1) and $\hat{a}$ is a function of $\mathbf{C}_0$, $\mathbf{C}^*$, and $h$ (see first section of the appendix).

From this procedure, parameter estimates and likelihoods for two hypotheses can be calculated. In the case of hypothesis A, corresponding to a single homogeneous rate, or $h = 1.0$, $\hat{\sigma}_0^2$ in equation (1) provides the maximum likelihood estimate (MLE) of the evolutionary rate, under the constraint of a single rate throughout the tree. Equation (2) provides the likelihood of that rate, given the data and tree. In hypothesis B, corresponding to heterogeneous rates, or $h \neq 1.0$, $\hat{\sigma}_0^2$ in equation (1) and $\hat{\sigma}_*^2 = h \cdot \hat{\sigma}_0^2$ provide the MLEs of the evolutionary rates on "unexceptional" and "exceptional" branches, respectively. Equation

(2), using the MLE of $h$, provides the likelihood of the rates $\hat{\sigma}_0^2$ and $\hat{\sigma}_*^2$, given the data and phylogeny. The likelihoods of hypotheses A and B can be compared by computing the log-likelihood ratio test statistic $-2 \cdot \log[L(A)/L(B)]$ and comparing it with a $\chi^2$ distribution with 1 df (for the single additional parameter, $h$, estimated in the numerator model). More details, a more thorough conceptual justification, and a worked example of this method can be found in the appendix.

One small difference exists between the likelihood methods employed by O'Meara et al. (2006) and Thomas et al. (2006). In the former, one estimates a single phylogenetic mean for all branches, whereas in the latter, a design matrix is specified so that different phylogenetic means can be estimated for different parts of the tree (the different rate regimes). Under heterogeneous-rate Brownian motion, it is not clear why a different phylogenetic mean should be specified for different branches that are themselves united only by rate regime, particularly when the branches are polyphyletic. Therefore, I focus my attention on the approach of O'Meara et al. (2006), although it should be kept in mind that the method of Thomas et al. (2006) is highly similar.

## Modeling the Evolutionary Process

An important assumption of both methods is that the evolutionary process under which the data at the tips of the tree arose can be modeled as a Brownian motion process (Edwards and Cavalli-Sforza 1964; Felsenstein 1985, 1988; Harvey and Pagel 1991). Under Brownian motion, the expected variance among lineages is proportional to the time (usually assumed to itself be related to the phylogenetic branch length) separating them (Felsenstein 1985, 1988).

Brownian motion is a standard evolutionary model used in numerous methods for the analysis of quantitative trait data in a phylogenetic context (e.g., Felsenstein 1985; Garland et al. 1993; Schluter et al. 1997; Garland and Ives 2000; Rohlf 2001; O'Meara et al. 2006). Brownian motion is a suitable model for drift under broad conditions and for selection under restrictive ones (reviewed in O'Meara et al. 2006; Revell and Harmon 2008). It is an unsuitable model for many circumstances of natural selection (e.g., Butler and King 2004); however, it remains a very useful starting point for the evolutionary analysis of quantitative characters.

## Simulation Tests

To explore the two methods, I used numerical simulations. I first generated 1,000 stochastic, continuous-time, constant-rate, pure-birth phylogenies, each containing $n =$

100 taxa. I then assigned "exceptionality" to $m^* = 20$ random branches of the $2n - 2 = 198$ branches in each phylogeny, under the constraints that no exceptional branches were directly adjoining or adjoining the same internal branch and that the daughter branches from the root node were both unexceptional. These are constraints required by McPeek's (1995) method because single-branch contrasts cannot be evaluated along adjacent branches or branches adjacent to the root.

I then simulated the evolution of a single continuous character on each phylogeny under several different Brownian motion models: homogenous rate ($h = 1.0$) and heterogeneous rate with $h = 2.0, 4.0, 8.0,$ and $16.0$. The quantity $h$ indicates the true relative rate of evolution along isolated, exceptional branches, compared with the rate throughout the rest of the tree.

I next analyzed the simulated data sets using each method. I performed McPeek's (1995) analysis, in which sister-node and parent-daughter–node contrasts are computed separately for unexceptional and exceptional branches, respectively. I compared the two types of contrasts using three statistics. First, I performed a standard $t$-test on the absolute values of the contrasts; second, I performed a Wilcoxon rank-sum $U$-test on the absolute values of the contrasts; and third, I computed the mean square of each type of contrast and compared the mean squares using an $F$-test with df $= 20, 79$. The third analysis is not a standard approach used in prior studies to compare single-branch and sister-node contrasts. However, the mean square of sister-node independent contrasts provides an unbiased estimate of the evolutionary rate (Garland 1992; Garland et al. 1999; Revell 2007; Revell et al. 2007*a*). We might therefore expect that the ratio of the mean square of the contrasts obtained along single branches and the mean square of the contrasts obtained between sister nodes would have an expected value of 1.0 under the null hypothesis of rate homogeneity and be distributed as an $F$ statistic with the degrees of freedom given above.

Next, I performed the likelihood analysis of O'Meara et al. (2006) and Thomas et al. (2006). I determined the MLE for the rate-scaling constant, $h$. I also calculated a likelihood ratio for the rate estimates, given the data and tree. I compared the likelihood ratio to a $\chi^2$ distribution with df $= 1$.

In order to compare McPeek's method and the likelihood method, I estimated the Type I error rates of each method as the fraction of times that a significant result was yielded for each statistical test using each method when the rate of evolution was homogeneous throughout the tree. I also compared the power of the methods as the fraction of times that a significant result was produced for each test using each method when the rate was heterogeneous, for each value of $h$. Finally, I evaluated the pa-

rameter estimates of the methods by comparing the mean squares ratio with the MLE of the rate ratio, $h$. The mean square of each type of contrast should provide an estimate of the evolutionary rate of each, as detailed above. I performed all analyses using software available on request.

In addition to the aforementioned simulations, I also performed simulations with fewer exceptional branches ($m^* = 1, 2, 10$), more exceptional branches ($m^* = 30, 99$), and fewer taxa ($n = 10, 20$). The results from these simulations were generally consistent with the results obtained for 100 taxa and 20 exceptional branches. They are presented in "Number of Exceptional Branches; Number of Taxa" in the appendix. For some of the simulation conditions, particularly when $m^*$ was very small, I also generated the null distribution for hypothesis testing via simulation.

## Results

### Error Rates

When the null hypothesis was true (i.e., in the homogeneous-rate simulations), Type I error was not significantly elevated in the statistical hypothesis tests based on McPeek's method as typically applied ($t$-test: Type I error $= 0.047$, $P$(true Type I error $\leq 0.05$) $= .69$; $U$-test: Type I error $= 0.051$, $P$(true Type I error $\leq 0.05$) $= .54$). However, Type I error was significantly elevated in the uncentered variance ratio (mean squares ratio) test ($F$-test: Type I error $= 0.064$, $P$(true Type I error $\leq 0.05$) $= .03$). The likelihood method also exhibited appropriate Type I error under the null hypothesis (likelihood ratio test: Type I error $= 0.048$, $P$(true Type I error $\leq 0.05$) $= .63$).

### Power

I measured statistical power as the proportion of tests that were significant when the null hypothesis was false. With McPeek's method, I performed several different statistical tests. Power was similar when I compared the means of the absolute values of the two types of contrasts using a $t$-test or a nonparametric $U$-test (fig. 1). By contrast, power was considerably higher when I evaluated the rates using a variance ratio test and compared the variance ratio to an $F$ distribution with df $= 20, 79$ (fig. 1).

With the likelihood method, the likelihood ratio test exhibited substantially greater power to reject the null hypothesis when it was false (i.e., for all cases of rate heterogeneity) than both the $t$- and $U$-tests with McPeek's contrasts. However, the power of the likelihood ratio test on the PGLS rate estimator was comparable to that of the variance ratio test based on McPeek's contrasts (fig. 1).
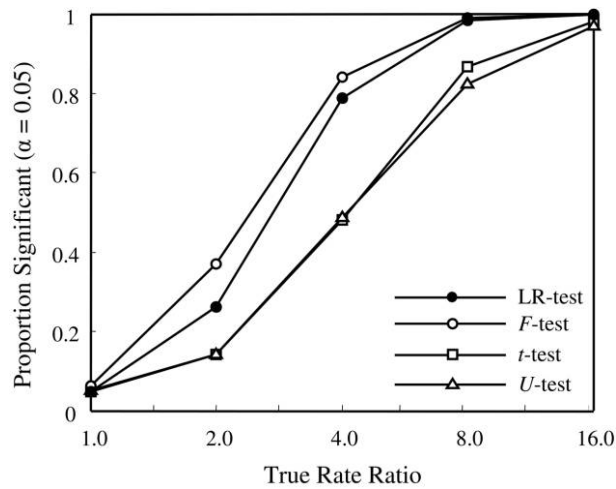
**Figure 1:** Proportion of significant hypothesis tests from 1,000 simulated data sets and trees for various degrees of rate heterogeneity. The proportion significant when the rate heterogeneity rate ratio is 1.0 is equivalent to the Type I error. Open symbols correspond to hypothesis tests based on McPeek's contrasts, and filled symbols correspond to results from the likelihood method. In particular, the *t*-test and *U*-test represent McPeek's method as typically applied; the *F*-test is a modification of McPeek's method developed in this study; and the LR-test is the likelihood method developed by O'Meara et al. (2006) and Thomas et al. (2006) and elaborated in this note.

### Parameter Estimation

When McPeek's method was used, the variance ratio provided a slightly but significantly upwardly biased estimate of the rate ratio, expected to be 1.0 when the rate is constant ($\overline{F} = 1.07$; $t = 5.38$; df = 999; $P < .001$). However, when the rate was heterogeneous throughout the tree, the variance ratio was increasingly downwardly biased relative to the true value of the rate ratio (fig. 2).

When the likelihood method was used, the branch length–scaling constant $h$ provided a relatively unbiased estimate of the relative rate ratio, regardless of the true value of the rate ratio (fig. 2), although for large values of the rate ratio, $h$ was slightly upwardly biased (for a true rate ratio of 16, $\overline{h} = 16.95$, $t = 4.46$, df = 999, $P < .001$; fig. 2).

### Discussion

McPeek's (1995) method for the analysis of evolutionary change along single branches in a phylogeny provides a framework within which to test more specific hypotheses about evolutionary rates than can be tested with Garland's (1992) method using independent contrasts. In particular, McPeek's method allows hypothesis tests about the rate of evolution along particular, prespecified branches in the

phylogenetic tree. This is in contrast to previous methods based on independent contrasts (e.g., Garland 1992), in which the rate is estimated only for sets of sister-node comparisons.

Analyses with McPeek's method have both succeeded (e.g., McPeek 1995, 1999; Klingenberg and Ekau 1996; McPeek et al. 1996; Crespi and Worobey 1998; Harrison and Crespi 1999; McPeek and Brown 2000; Crespi and Teo 2002; Stoks et al. 2003; Stoks and McPeek 2006) and, in some cases, failed (e.g., McPeek 1995, 1999; Crespi and Teo 2002; Stoks et al. 2003) to reveal exceptional evolutionary rates along specified branches in the phylogeny. I found that McPeek's method generally had appropriate Type I error when the true evolutionary rate is homogeneous throughout the tree, regardless of which statistical test was used to compare rates among branches (fig. 1).

This is a very encouraging result. It suggests that the statistically significant changes in evolutionary rate along phylogenetic branches identified in previous studies were obtained under conditions with acceptable Type I error, a property not previously explored for this method.

Unfortunately, I also found that McPeek's method, as typically applied, has low power when the rate of evolution is heterogeneous. Using simulated data in which 20 branches in 100-taxon trees are subject to an elevated rate of evolution, the power of *t*- or *U*-tests to identify exceptional evolutionary rate was low. For example, only 48% of data sets were inferred to have evolved under a het-
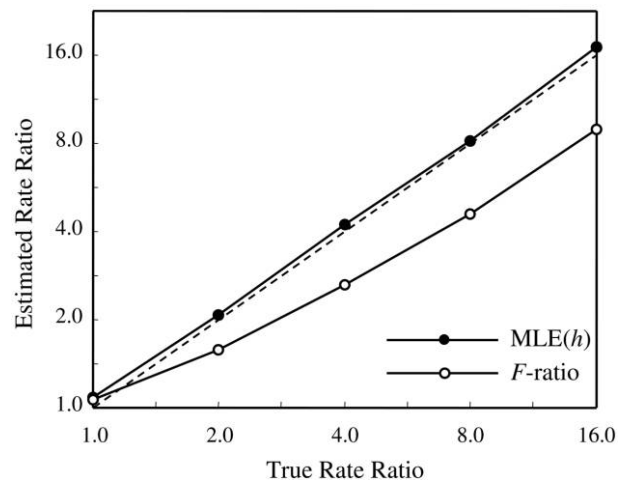


**Figure 2:** Mean estimated rate ratio for homogeneous and heterogeneous rate simulations from 1,000 simulated data sets and trees for various degrees of rate heterogeneity. The dashed line indicates the 1 : 1 line (along which the parameter estimate is equal to its true value). MLE(*h*) is the maximum likelihood estimate of the rate ratio based on the likelihood method, and the *F* ratio is the mean squares ratio of the contrasts based on McPeek (1995).

erogeneous rate when the rate heterogeneity factor was set to 4.0 (i.e., when the rate of evolution was four times as fast along exceptional branches), and only 82%–87% of data sets were inferred to have evolved by a heterogeneous rate when the rate factor was 8.0 (fig. 1).

I also calculated a variance ratio from the mean squares of changes along single branches and sister-node contrasts. This ratio was intended to provide a parameter estimate for the rate heterogeneity factor. However, it is also expected to be distributed as an *F* statistic with degrees of freedom equal to the number of single branches and the number of sister-node contrasts (because the variances are uncentered and so a degree of freedom is not lost for the calculation of the mean in each category). Using the variance ratio for hypothesis testing yielded higher statistical power, but the statistic also had the highest Type I error when the evolutionary rate was homogeneous (fig. 1). Furthermore, I did not find the variance ratio to provide a reasonable estimate for the rate heterogeneity factor under any condition other than the null hypothesis of rate constancy (fig. 2). This is because the mean square of the parent-daughter–node contrasts calculated using McPeek's method consistently underestimated the evolutionary rate along "exceptional" branches (fig. 3). This may result from inappropriate variance correction in the method of McPeek (Felsenstein 2004) or because the computation of contrasts along isolated branches results in contrasts that are nonindependent (Felsenstein 2004; J. Felsenstein, personal communication).

One use of McPeek's contrasts method that I did not explore in this study is a test for the direction of evolution (e.g., Stoks et al. 2003). This is because tests for the direction of evolution rely on the comparison of unsigned (or "nondirectional" sensu Harvey and Purvis 1991) contrasts with signed (or "directional") contrasts. Unsigned sister-node contrasts and signed parent-daughter–node contrasts have neither the same expected value nor the same expected distribution under the null hypothesis of constant-rate Brownian motion evolution (see supplementary app. 5 in Revell et al. 2007*b*), and thus their comparison may be inappropriate (following McPeek 1999). Alternative methods for the analysis of directional change have recently been suggested (Revell et al. 2007*b*; Wiens et al. 2007), although the properties of these methods are not well explored.

O'Meara et al. (2006) and Thomas et al. (2006) provide an alternative likelihood approach by which to address the hypotheses (i.e., pertaining to rate heterogeneity) for which McPeek's (1995) method is intended. This method is based on the PGLS approach of Grafen (1989) and involves direct maximum likelihood estimation of the rate heterogeneity factor.

The likelihood method exhibited low Type I error when
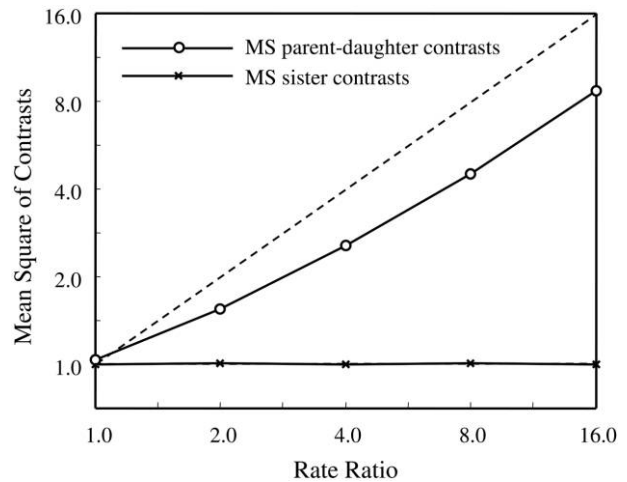


**Figure 3:** Mean square (MS) of the independent contrasts from sister-node contrasts ("unexceptional" rate of evolution) and parent-daughter–node contrasts ("exceptional" evolution) calculated using McPeek's (1995) method. The dashed lines indicate the expected values if the MS were providing us an unbiased estimate of the rate. For the unexceptional-rate contrasts, the dashed line showing the expected rate is largely obscured by the line for the observed rate.

the evolutionary rate was homogeneous (fig. 1). It also exhibited power comparable to the power of the variance ratio from McPeek's contrasts method for all levels of the rate heterogeneity factor (fig. 1). The main advantage of this approach over McPeek's method, however, is in terms of parameter estimation. The likelihood approach provides explicit parameter estimates for the rate heterogeneity factor. This factor is generally estimated more or less without bias for all levels of rate heterogeneity explored by this study (fig. 2), although the parameter estimates are biased under some conditions (see appendix).

In exploring the performance of the McPeek's contrasts method and the likelihood method, I made several assumptions. However, I explored the consequence of these assumptions with some additional simulations.

First, I assumed that the positions of exceptional branches in the phylogeny were known without error. To explore the consequence of misspecifying the exceptional branches in the phylogeny, I conducted some additional simulations in which the hypothesized exceptional branches were "nudged" to adjacent branches with substantial probability. The results, presented in "Consequences of Misspecifying Exceptional Branches" in the appendix, suggest nothing more nefarious than underestimation of the degree of rate heterogeneity and slightly decreased power.

Second, in this study I assumed that the rate of evolution was homogeneous along all $m^*$ exceptional branches in

each phylogeny. Alternatively, rate heterogeneity might exist among exceptional branches as well as between exceptional and unexceptional branches. I conducted some additional simulations to explore the consequences of this assumption and found that, in general, even substantial heterogeneity among exceptional branches was of relatively minor consequence. The results of these analyses are presented in "Heterogeneity in the Evolutionary Rate along Exceptional Branches" in the appendix.

Finally, I assumed that the rate along exceptional branches was invariably higher than the rate along unexceptional branches. There are biological reasons for this assumption. For example, an adaptive hypothesis is frequently postulated for rate heterogeneity, and adaptive evolution can proceed rapidly (reviewed in Hendry and Kinnison 1999). However, an elevated rate is not a necessary assumption of the likelihood method, and, in fact, a decreased rate of evolution on exceptional branches can also be detected by this method (see "Low Rate on Exceptional Branches" in the appendix).

## Summary

McPeek (1995) provided an interesting and innovative approach for the analysis of evolutionary change along single branches in phylogenetic trees. This approach has been used by numerous researchers. In this study, I show that McPeek's approach has some good statistical properties, including appropriate Type I error. In addition, the likelihood approach requires computationally intensive numerical optimization, whereas McPeek's method is an algorithm in which contrasts are calculated using a single pass through the tree. Consequently, computer analysis is much faster for McPeek's method than under the likelihood approach. However, the method of McPeek has low power as typically applied.

An alternate approach to the analysis of evolutionary change along single branches is presented in this note. This likelihood approach is based on the methods of O'Meara et al. (2006) and Thomas et al. (2006). These very similar methods were designed to test for rate heterogeneity among different parts of a phylogenetic tree. I found that the likelihood approach has low Type I error as well as other desirable properties, including relatively high power and appropriate parameter estimation under most circumstances.

Not considered in this note are comparative methods based on explicitly adaptive models for the evolutionary process (e.g., Hansen 1997; Butler and King 2004). In empirical studies, hypotheses of exceptional evolution along particular branches of the phylogeny are often thought to have an adaptive basis (e.g., adaptation to a new environment; McPeek 1995, 1999; Revell et al. 2007*b*).

Other authors have shown that Brownian motion, the model for the likelihood test used in this study, is an appropriate model for some, but not all, situations of adaptive evolution (Hansen and Martins 1996; also discussed in "Modeling the Evolutionary Process"). Although beyond the scope of the present study, explicitly adaptive models for the evolutionary process may represent a viable third alternative to the methods presented here for circumstances in which heterogeneity in the evolutionary rate among branches is hypothesized to have an adaptive basis.

Although McPeek's method for the analysis of evolutionary changes along single branches has appropriate Type I error, I found that the likelihood approach has several preferable properties. In particular, under most circumstances the likelihood method has low Type I error, appropriate parameter estimation, and relatively high power. Thus, future studies in which the researcher is interested in exceptional evolutionary change along single branches should consider the findings of this study in choosing their method for analysis and when interpreting their results.

## Acknowledgments

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