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TESTING HYPOTHESES ABOUT EVOLUTIONARY CHANGE ON SINGLE BRANCHES OF A PHYLOGENY USING EVOLUTIONARY CONTRASTS

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Abstract.—Interspecific comparisons of phenotypes are used extensively to test hypotheses about the evolutionary forces shaping phenotypic variation, but comparative data analysis is complicated by correlations due to the common ancestry of species. The method of evolutionary contrasts removes such correlations by estimating the amount of character change between pairs of closely related species that has occurred since their most recent common ancestors. The original method allows character change to be estimated only along pairs of branches on a phylogeny, but many hypotheses address change along single branches. In this article the method of evolutionary contrasts is extended to allow character change along a set of single branches on a phylogeny to be estimated, expected variances are presented, and it is shown that these extensions also result in a set of contrasts that are not correlated because of common ancestry. These extensions will allow hypotheses to be tested concerning character change associated with host or habitat shifts, changes in breeding system (e.g., monogamy vs. polygyny, monoecy vs. dioecy), changes in life history (e.g., semelparity vs. iteroparity), and changes in quantitative characters in many other situations in which one is interested in character change along single branches.

Interspecific studies of character variation are valuable tools for testing hypotheses about the evolutionary forces shaping phenotypic variation. Comparative analyses are used to generate hypotheses for experimental studies, to complement the results of experimental studies, and to test hypotheses when experimental studies are infeasible (Harvey and Pagel 1991; Gittleman and Luh 1992). Comparative studies search for consistent phenotypic similarities and differences among species that would correspond to the operation of mechanisms hypothesized to generate interspecific pattern (e.g., shared selection pressures, constraints to adaptive evolution). However, phenotypic similarities and differences among species inherently result from phylogenetic relationships (reviewed in Harvey and Pagel 1991). For example, a pair of sibling species will usually be phenotypically more similar to one another than to a distant relative, because the sibling species have had less time to diverge from one another than they have from the distant relative. Therefore, phenotypic patterns due to the phylogenetic relationships among species must be partitioned from the data or removed altogether before testing hypotheses about mechanisms generating interspecific patterns.

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Many statistical techniques have been developed to analyze comparative data (see reviews in Brooks and McLennan 1991; Harvey and Pagel 1991; Harvey and Purvis 1991; Gittleman and Luh 1992). One technique is the method of evolutionary contrasts (Felsenstein 1973, 1985, 1988). To apply the method of evolutionary contrasts, a phylogeny for the species of interest and estimates of branch lengths on the phylogeny must be available. Quantitative characters are assumed to evolve according to a model of Brownian motion along the branches of the phylogeny. This assumption allows evolutionary change on the phylogeny to be partitioned into a set of independent components (i.e., the set of evolutionary contrasts). Each evolutionary contrast estimates the amount of change in a quantitative character that has led to the difference in character values between two species since their most recent common ancestor. Calculating a full set of evolutionary contrasts transforms a comparative data set with potentially correlated values into a set of independent contrasts from which correlations due to common ancestry have been removed (Felsenstein 1973, 1985, 1988). Simulation studies indicate that evolutionary contrasts produce acceptable error rates for hypothesis testing (Martins and Garland 1991; Gittleman and Luh 1992), and models of character change other than Brownian motion can also be used to generate evolutionary contrasts (Martins and Garland 1991).

Evolutionary contrasts have typically been applied to test whether the evolution of two or more characters is correlated. For example, Sessions and Larson (1987) found correlated changes in genome size and rates of development and regeneration in plethodontid salamanders. Losos (1990*a*, 1990*b*) used evolutionary contrasts to study correlated evolution in morphological structures and performance variables in Caribbean anoles. However, evolutionary contrasts are beginning to be employed to test other types of hypotheses about evolutionary change in quantitative characters (see, e.g., Garland 1992; Garland et al. 1993; McPeek 1995).

One limitation of the current method is that evolution along single branches on a phylogeny cannot be estimated; the amount of character change must be estimated along pairs of branches. However, many interesting questions concerning character change involve evolution along single branches: character change associated with host or habitat shifts; changes in breeding system, such as shifts between monogamy and polygyny or monoecy and dioecy; changes in life history, such as shifts between semelparity and iteroparity; changes in diet, such as between monophagy and polyphagy or herbivory and carnivory; and others. In this article I expand the method of evolutionary contrasts to allow character change along single branches of a phylogeny to be included in the transformation of comparative data. This expansion allows the testing of specific hypotheses about evolutionary rates on a set of single branches across the phylogeny.

THE METHOD OF EVOLUTIONARY CONTRASTS

The method of evolutionary contrasts has been expounded in a number of publications (e.g., Felsenstein 1985, 1988; Martins and Garland 1991; Garland et

al. 1992). In this section I present a brief description of Felsenstein's (1985, 1988) methods to lay the groundwork for the extensions that are presented (in the following I use Felsenstein's [1973, 1985] notation; see Felsenstein 1973, 1985, 1988 for a complete description of the method).

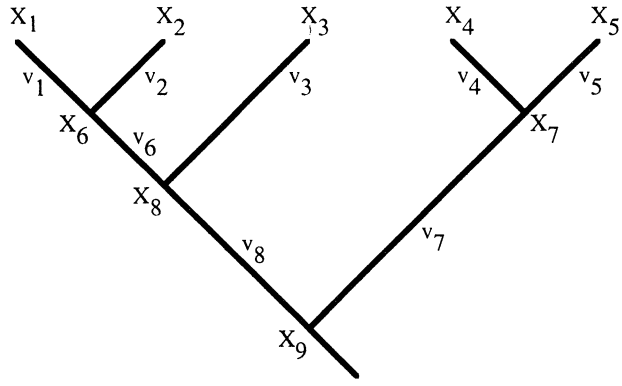
To construct evolutionary contrasts three pieces of information are needed: character values for the species of interest, a phylogeny for the species, and estimates of branch lengths on the phylogeny. The phylogeny and branch lengths are derived from separate analyses and should be constructed from data sets that are independent of the quantitative characters to be examined with evolutionary contrasts. Any method of phylogeny reconstruction (e.g., maximum parsimony, phenetic clustering, and neighbor joining) and of branch length estimation can be used. The method of evolutionary contrasts assumes that the "true" phylogeny and branch lengths are used, which means that evolutionary contrasts are only as good as the phylogeny and branch length estimates used to construct them (Felsenstein 1985, 1988). Analyses should be redone when better estimates become available.

To calculate evolutionary contrasts, character values for extant species are first assigned to the tips of external branches for each species. An evolutionary contrast is constructed by taking the difference between the character values for two species at the tips of two external branches that emanate from the same node. Consider the phylogeny for five species given in figure 1. The first contrast to be constructed would be the difference between the character values for species 1 and 2 (i.e., $X_1 - X_2$). A value is then calculated for the node immediately below the two tip species according to the formula

$$X_k = \frac{v_j}{v_i + v_j} X_i + \frac{v_i}{v_i + v_j} X_j, \tag{1}$$

where i and j are the tip species and k is the ancestral species represented by node k, and the v's are branch lengths. The branches leading to species i and j are then pruned from the tree (i.e., branches $k \rightarrow i$ and $k \rightarrow j$, where $k \rightarrow i$ is read as the branch from k to i). Finally, the branch length leading to the ancestral species is lengthened from v_k to $v_k + [(v_i v_j)/(v_i + v_j)]$. At this point, one contrast has been calculated, and the number of species on the tree has been reduced by one. This series of steps is repeated until only one species is left on the tree. When this is done, $N - 1$ contrasts will be extracted from the original data of N species. The full set of contrasts for the phylogeny in figure 1 is presented as an example. Transforming the character values for the original species into evolutionary contrasts is appealing, because although the original values may be correlated as a result of their shared phylogenetic histories, the covariances among the contrasts are zero (Felsenstein 1973).

An evolutionary contrast infers the amount of character change in the lines leading to adjacent species since they shared a common ancestor. To see this, expand the contrast $X_1 - X_2$ from figure 1 into its component parts associated with each branch emanating from node 6:



Contrast	Variance
$X_1 - X_2$	$v_1 + v_2$
$X_4 - X_5$	$v_4 + v_5$
$X_3 - X_6$	$v_6 + \frac{v_1 v_2}{v_1 + v_2} + v_3 = v_6' + v_3$ where $v_6' = v_6 + \frac{v_1 v_2}{v_1 + v_2}$
$X_7 - X_8$	$v_7 + \frac{v_4 v_5}{v_4 + v_5} + v_8 + \frac{v_3 v_6'}{v_3 + v_6'} = v_7' + v_8'$ where $v_7' = v_7 + \frac{v_4 v_5}{v_4 + v_5}$ and $v_8' = v_8 + \frac{v_3 v_6'}{v_3 + v_6'}$

FIG. 1. A hypothetical phylogeny of five extant species (X_1 through X_5) and the evolutionary contrasts that are derived from this phylogeny according to Felsenstein's (1985) original method. The values X_6 through X_9 are the reconstructed character values associated with the internal nodes of the phylogeny. The values v_1 through v_8 are the branch lengths of the phylogeny. The evolutionary contrasts are presented below the phylogeny, and the variance associated with each contrast is given.

$$
\begin{aligned}
X_1 - X_2 &= \frac{v_1 + v_2}{v_1 + v_2}(X_1 - X_2) \\
&= \frac{v_1}{v_1 + v_2}(X_1 - X_2) + \frac{v_2}{v_1 + v_2}(X_1 - X_2) \\
&= \frac{v_1 + v_2 - v_2}{v_1 + v_2}X_1 - \frac{v_1}{v_1 + v_2}X_2 + \frac{v_2}{v_1 + v_2}X_1 - \frac{v_2 + v_1 - v_1}{v_1 + v_2}X_2 \\
&= X_1 - \left(\frac{v_2}{v_1 + v_2}X_1 + \frac{v_1}{v_1 + v_2}X_2\right) + \left(\frac{v_2}{v_1 + v_2}X_1 + \frac{v_1}{v_1 + v_2}X_2\right) - X_2 \\
&= (X_1 - X_6) + (X_6 - X_2). \qquad (2)
\end{aligned}
$$

If character evolution follows a model of Brownian motion, the variance of a contrast increases with increasing time since the common ancestor. Contrasts must therefore be standardized to a common variance for comparison (Felsenstein 1985). (Other models of character evolution can be accommodated within the method [e.g., punctuational change at speciation events; Martins and Garland 1991], but for present purposes I discuss only the derivation assuming Brownian motion evolution.) The variance of a contrast has components of branch lengths leading to the two species and variance components associated with estimating the values for the two species if they are themselves ancestors of other species in the phylogeny (fig. 1). The variance of the contrast $X_1 - X_2$ in figure 1 has only two components, v_1 and v_2; their sum is the total branch length along which evolution associated with that contrast has occurred. This variance has no components associated with estimating character values, because the values for both species are measured directly.

This is not true for the variance of contrast $X_3 - X_6$. The variance of this contrast has the branch lengths v_3 and v_6 as terms, plus an extra term, $v_1v_2/(v_1 + v_2)$. This extra term is a variance component associated with estimating the character value X_6 (Felsenstein 1985). Likewise, the variance of contrast $X_7 - X_8$ has terms that are the branch lengths v_7 and v_8 and terms associated with estimating X_7 (i.e., $v_4v_5/[v_4 + v_5]$) and X_8 ($v_3v_6'/[v_3 + v_6']$) (fig. 1). Felsenstein (1985) simplifies the practical calculation of a variance by lengthening the branch immediately below an estimated node by the term associated with estimating a value for that node and using the sum of the modified branch lengths as the variance estimate. The discussion presented here only points out the sources of these terms. Also, note that these variance components compound down the tree (i.e., v_6' is used instead of v_6 in the variance of $X_7 - X_8$). Contrasts are standardized for comparison to a common scale by dividing each by the square root of its associated variance (i.e., its standard deviation; Felsenstein 1985). When standardized in this way, evolutionary contrasts estimate the rate of character change along each pair of branches.

AN ALTERNATIVE MODEL FOR CONSTRUCTING EVOLUTIONARY CONTRASTS

Felsenstein's (1973, 1985) method for constructing contrasts assumes a model of Brownian motion for character change in which the expected rates of change

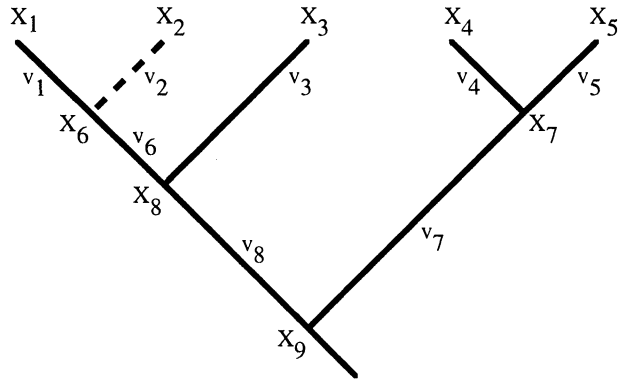
on all branches are identical. However, the evolution of characters in many taxa may not follow this homogeneous model of Brownian motion. For example, rates of character change may be greatly accelerated during habitat shifts because of adaptation to new environments; within each habitat character evolution may follow a Brownian motion model, but when shifts between habitats occur, large and directed changes in the character may occur as a result of adapting to the new habitat. When characters are reconstructed on a phylogeny, a homogeneous Brownian motion model may describe character change along branches for which species remain in the same habitat, but much higher rates of change are expected along branches on which habitat shifts are hypothesized to have occurred. A natural question to ask would be whether character change along branches on which habitat shifts are hypothesized to have occurred is greater than along other branches of the tree. The null hypothesis for this question is that the same rate of change occurred along all branches. To evaluate this null hypothesis against the alternative that rates have been higher along branches on which habitat shifts are hypothesized to have occurred, rates along the set of single branches with habitat shifts must be compared to rates estimated along branches on which habitat shifts are hypothesized not to have occurred. However, character change must always be estimated along pairs of branches in the original method. Modifications of the original method described in this section permit character change along a number of single branches to be estimated along with change along other sets of branches.

A model that isolates change on particular branches can be constructed in the following way: (1) Prune the single branches of interest from the tree. (2) Reconstruct ancestral character values on the tree according to the original methods, and construct contrasts accordingly. (3) Graft the pruned branches back onto the tree. (4) Calculate ancestral values for the nodes of the pruned branches that were not estimated in step 2 by linear interpolation. (5) Construct contrasts for change along these single branches.

To illustrate the alterations to the method, first consider the phylogeny in figure 2. This is the same phylogeny as used above (fig. 1), except that now we are interested in isolating character change along branch $6 \rightarrow 2$. For example, if species 2 is found in one habitat type and the remaining species are found in another habitat type, character reconstruction of habitat affinity using maximum parsimony (Maddison and Maddison 1992) would suggest that a habitat shift occurred along branch $6 \rightarrow 2$ (note that this hypothesis is derived from an independent analysis). The first step is to prune branch $6 \rightarrow 2$ from the phylogeny. This leaves four species, and branch $8 \rightarrow 1$ is $v_1 + v_6$ units long. Three contrasts ($X_1 - X_3$, $X_4 - X_5$, and $X_7 - X_8$) are then constructed according to the usual methods described in the previous section (fig. 2). Branch $6 \rightarrow 2$ is then grafted back onto the tree, and the value for X_6 is estimated by linear interpolation from X_8:

$$X_6 = X_8 + \frac{v_6}{v_1 + v_6}(X_1 - X_8).$$

Substituting for X_8 and $X_1 - X_8$ from analogous forms of equations (1) and (2) above gives



Contrast	Variance
$X_2 - X_6$	$v_2 + \frac{v_1(v_3 + v_6)}{v_1 + v_3 + v_6}$
$X_1 - X_3$	$v_1 + v_3 + v_6$
$X_4 - X_5$	$v_4 + v_5$
$X_7 - X_8$	$v_7 + \frac{v_4 v_5}{v_4 + v_5} + v_8 + \frac{v_3(v_1 + v_6)}{v_1 + v_3 + v_6} = v_7' + v_8'$ where $v_7' = v_7 + \frac{v_4 v_5}{v_4 + v_5}$ and $v_8' = v_8 + \frac{v_3(v_1 + v_6)}{v_1 + v_3 + v_6}$

FIG. 2. The same phylogeny as presented in fig. 1, except that we are now interested in reconstructing character change along the single branch 6 → 2 (i.e., the dashed branch). The alternative set of contrasts that includes the contrast estimating change along branch 6 → 2 is given below the phylogeny with their associated variances. Note that the reconstructed ancestral values for X_6, X_8, and X_9 are different from those presented in fig. 1.

$$X_6 = \left[\frac{v_3}{v_1 + v_3 + v_6} X_1 + \frac{v_1 + v_6}{v_1 + v_3 + v_6} X_3\right] + \frac{v_6}{v_1 + v_6}\left[(X_1 - X_3)\frac{v_1 + v_6}{v_1 + v_3 + v_6}\right],$$

which yields

$$X_6 = \frac{v_3 + v_6}{v_1 + v_3 + v_6} X_1 + \frac{v_1}{v_1 + v_3 + v_6} X_3. \quad (3)$$

This shows that interpolating the value for the node is analogous to calculating

the value by the original method using the values for species X_1 and X_3 and the distances from node 6 to each species (cf. eq. [1]). The contrast $X_2 - X_6$ is then calculated. As with the original method, $N - 1$ contrasts are constructed from N original species. This extension merely allows alternative partitionings of evolutionary change on the phylogeny.

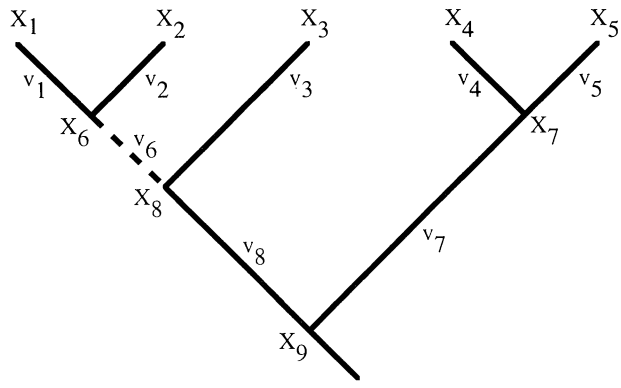
The variances associated with some of the contrasts have also changed, because different combinations of species and nodes are used to estimate ancestral values (fig. 2). The term associated with estimating X_8 in the variance of contrast $X_7 - X_8$ is now $v_3(v_1 + v_6)/(v_1 + v_3 + v_6)$ instead of $v_3 v_6'/(v_3 + v_6')$ (cf. figs. 1 and 2). This is because X_8 is now estimated from species 1 and 3 instead of from species 3 and 6. In other words, v_8 is lengthened to $v_8 + [v_3(v_1 + v_6)/(v_1 + v_3 + v_6)]$ when X_8 is estimated. Also, the variance of the new contrast containing only one branch is given by the branch length plus the variance component associated with estimating X_6 (i.e., $v_1[v_3 + v_6]/[v_1 + v_3 + v_6]$) (fig. 2).

The most appealing property of the original method is that potential correlations among values due to common ancestry are removed from the data. The alternative partitionings of evolutionary change described in this section also result in a set of independent contrasts. To illustrate this consider the covariance between contrasts $X_2 - X_6$ and $X_1 - X_3$ in figure 2, that is, $\mathrm{Cov}((X_2 - X_6),(X_1 - X_3))$. Assuming the null hypothesis of evolution by Brownian motion along all branches and defining $a = (v_3 + v_6)/(v_1 + v_3 + v_6)$, $b = v_1/(v_1 + v_3 + v_6)$, $c = (v_1 + v_6)/(v_1 + v_3 + v_6)$, and σ^2 as the variance in character displacement per unit of branch length (Felsenstein 1973),

$$\begin{aligned}
\mathrm{Cov}((X_2 - X_6),(X_1 - X_3)) &= \mathrm{Cov}((X_2 - aX_1 - bX_3),(X_1 - X_3)) \\
&= \mathrm{Cov}(X_1, X_2) - \mathrm{Cov}(X_2, X_3) - a\,\mathrm{Var}(X_1) \\
&\quad + a\,\mathrm{Cov}(X_1, X_3) - b\,\mathrm{Cov}(X_1, X_3) + b\,\mathrm{Var}(X_3) \\
&= \sigma^2[(v_6 + v_8 + v_9) - (v_8 + v_9) - a(v_1 + v_6 + v_8 + v_9) \\
&\qquad + a(v_8 + v_9) - b(v_8 + v_9) + b(v_3 + v_8 + v_9)] \\
&= \sigma^2[v_6 - a(v_1 + v_6) + bv_3] \\
&= \sigma^2[(1 - a)v_6 - av_1 + bv_3] \\
&= \sigma^2\left[\frac{v_1 v_6}{v_1 + v_3 + v_6} - \frac{v_1(v_3 + v_6)}{v_1 + v_3 + v_6} + \frac{v_1 v_3}{v_1 + v_3 + v_6}\right] \\
&= 0.
\end{aligned} \tag{4}$$

Similar calculations show that $\mathrm{Cov}((X_2 - X_6), (X_4 - X_5)) = \mathrm{Cov}((X_2 - X_6), (X_7 - X_8)) = 0$ (fig. 2).

Similar methods can be applied to internal branches of phylogenies. Figure 3 shows the same phylogeny as used above (i.e., figs. 1 and 2), except that now we are interested in isolating character change along branch $8 \rightarrow 6$. For example, if species 1 and 2 are found in one habitat type and species 3, 4, and 5 are found in another habitat type, character reconstruction of habitat affinity using maximum parsimony (Maddison and Maddison 1992) would suggest that a habitat



Contrast	Variance
$X_1 - X_2$	$v_1 + v_2$
$X_4 - X_5$	$v_4 + v_5$
$X_3 - X_7$	$v_3 + v_7 + \frac{v_4 v_5}{v_4 + v_5} + v_8 = v_3 + v_7' + v_8$ where $v_7' = v_7 + \frac{v_4 v_5}{v_4 + v_5}$
$X_6 - X_8$	$v_6 + \frac{v_1 v_2}{v_1 + v_2} + \frac{v_3(v_7' + v_8)}{v_3 + v_7' + v_8} = v_6' + \frac{v_3(v_7' + v_8)}{v_3 + v_7' + v_8}$ where $v_6' = v_6 + \frac{v_1 v_2}{v_1 + v_2}$

FIG. 3. The same phylogeny as presented in fig. 1, except that we are now interested in reconstructing character change along the single branch 8 → 6 (i.e., the dashed branch). The alternative set of contrasts that includes the contrast estimating change along branch 8 → 6 is given below the phylogeny with their associated variances. Note that the reconstructed ancestral values for X_8 and X_9 are different from those presented in fig. 1.

shift occurred along branch 8 → 6. The first step is to prune branch 8 → 6 from the phylogeny. This leaves one tree with species 1 and 2 and a second tree with species 3, 4, and 5. Three contrasts ($X_1 - X_2$, $X_4 - X_5$, and $X_3 - X_7$) are then constructed according to the usual methods described in the previous section. Branch 8 → 6 is then grafted back to form one tree, and the value for X_8 is estimated from species 3 and 7 as

$$X_8 = \frac{v_7' + v_8}{v_3 + v_7' + v_8} X_3 + \frac{v_3}{v_3 + v_7' + v_8} X_7$$

(which is analogous to eq. [3]). The contrast $X_6 - X_8$ is then calculated. Note that the variance for $X_6 - X_8$ has terms associated with estimating X_6 (i.e., $v_1 v_2 / [v_1 + v_2]$) and X_8 (i.e., $v_3[v_7' + v_8]/[v_3 + v_7' + v_8]$). Calculations similar to those presented in equation (4) demonstrate that the covariances between contrast $X_6 - X_8$ and the other contrasts are all zero.

TESTING HYPOTHESES ABOUT SINGLE CHARACTERS

Contrasts constructed by this method can now be used to test specific hypotheses about the rate of character change along branches of a specific type as compared with the rest of the branches on a phylogenetic tree. I illustrate this method using a data set of the sizes and shapes of the caudal lamellae of larval damselflies (McPeek 1995).

Damselfly species in the genus *Enallagma* (Odonata: Coenagrionidae) segregate among the ponds and lakes of eastern North America on the basis of whether fish are present; one group of species is found as larvae only in fishless ponds and lakes, whereas the remaining species are found as larvae only in ponds and lakes containing fish (Johnson and Crowley 1980; McPeek 1989, 1990*a*). A primary reason that species segregate between the two lake types is because they are differentially susceptible to the predators found in each (Pierce et al. 1985; Blois-Heulin et al. 1990; McPeek 1990*a*). Species in lakes with fish are able to coexist with fish predators but not with the large, active dragonfly predators that are found in fishless lakes; these species move infrequently and slowly, apparently attempting to minimize being detected by fish (Pierce et al. 1985; McPeek 1990*b*). In contrast, species in fishless lakes can coexist with the dragonflies found in fishless lakes but not with fish; these species are active and swim away from attacking predators (Pierce et al. 1985; McPeek 1990*b*).

A study of morphological structures used in locomotion indicated that larvae of fishless-lake species have greatly enlarged caudal lamellae, the structures at the end of the abdomen that act like the caudal fin of a fish to generate thrust for swimming (McPeek 1995). A cladistic analysis also suggested that at least two habitat shifts have occurred in which *Enallagma* species have invaded fishless lakes (fig. 4; see McPeek 1995 for a full description of characters used and the cladistic analysis). The modified method of evolutionary contrasts presented above can be used to test whether the rate of change in the size and shape of caudal lamellae has been greater along branches on which habitat shifts are postulated to have occurred.

The cladogram for a subset of *Enallagma* species given in figure 4 is derived from a cladistic analysis of 41 larval and adult morphological characters using an exhaustive search in PAUP, version 3.1.1 (Swofford 1993; a list of characters and character states for these species and a full description of the analysis is given in McPeek 1995). The number of all possible character state changes in this cladistic data set along each branch was determined with MacClade (Maddison and Maddison 1992) and is used here to estimate branch lengths of the cladogram (Garland et al. 1992).

Figure 5 presents the average values measured in extant species for the lateral surface area and an index of circularity of the median caudal lamella (McPeek

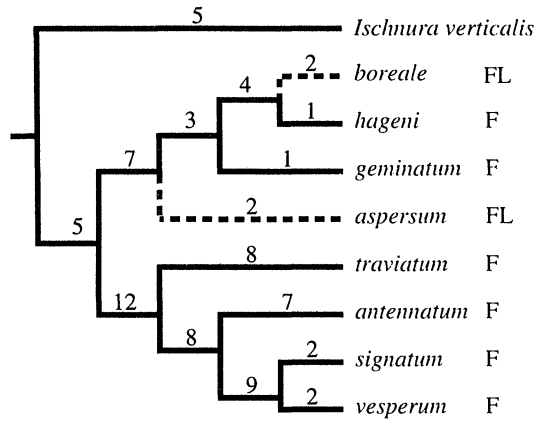


FIG. 4. Phylogeny of eight *Enallagma* damselfly species (Odonata: Coenagrionidae) (from McPeek 1995). This phylogeny was reconstructed from a set of 41 larval and adult morphological characters by maximum parsimony (McPeek 1995). *Enallagma boreale* and *Enallagma aspersum* are associated with fishless lakes (*FL*), and the other *Enallagma* species are associated with fish lakes (*F*). Most parsimonious reconstruction of habitat affinity suggests that habitat shifts from fish to fishless lakes occurred along the dashed branches leading to the two fishless-lake species. The number given over each branch is the total number of character state changes in the data set of 41 characters that occur along that branch (McPeek 1995). These numbers are used as estimates of branch lengths in constructing contrasts in fig. 5.

1995). Evolutionary contrasts are constructed for the cladogram given in figure 4, assuming that separate habitat shifts occurred along the external branches leading to *Enallagma boreale* and to *Enallagma aspersum*. To construct contrasts, these two branches are first pruned from the phylogeny. Contrasts are then constructed among the six fish-lake species. Finally, the branches leading to fishless-lake species are grafted back onto the tree, values for the ancestral nodes are estimated, and contrasts for character change along these two branches are constructed. Contrast values presented in figure 5 have been standardized by their associated standard deviations. Contrasts constructed in this way allow the null hypothesis of homogeneous rates of character change along all types of branches to be tested against the alternative hypothesis of high rates of change along branches on which habitat shifts occurred and lower rates of change along branches on which habitat shifts did not occur.

Parametric tests (e.g., *t*-tests [Sokal and Rohlf 1981]) or nonparametric tests (e.g., Mann-Whitney-Wilcoxon or robust rank-order tests [Siegel and Castellan 1988]) can be employed to evaluate these hypotheses by testing for a difference in the distributions of the two sets of contrasts. For illustration, I employ *t*-tests. For lamella area, the average value for the two contrasts associated with branches on which habitat shifts occur is 2.61 mm^2 per cladistic character state change and the average value for the five contrasts of branches on which habitat shifts do not occur is 0.18 mm^2 per cladistic character state change, which are significantly different in a one-tailed *t*-test (fig. 5*A*, $t_5 = 2.47$, $P < .05$). In contrast, the circularity of the median lamella does not differ between contrasts associated

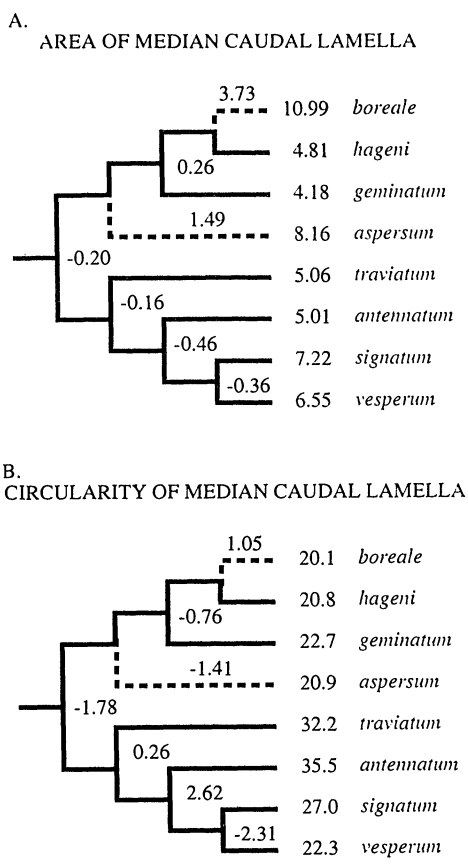


FIG. 5. Reconstruction of the area of the median caudal lamella (*A*) and an index of circularity of the median lamella (*B*) for the eight *Enallagma* species. The circularity index is the surface area of the lamella (the variable given in *A*) divided by the square of the perimeter of the lamella. The numbers given at the tips of the external branches are the means for each species derived from a morphometrics analysis (see app. 2 in McPeek 1995). The numbers given on the phylogeny are the seven contrasts calculated from these data (see text). Contrast values have been standardized by their associated standard deviations. Two contrasts estimate character change along the single dashed lines on which habitat shifts are hypothesized to have occurred. The other five contrasts estimate character change along pairs and triplets of branches for character change within fish lakes.

with and not associated with habitat shifts (fig. 5*B*, $t_5 = 0.14$, $P > .90$). These results support the conclusions of the previous study (McPeek 1995).

If a taxonomically and ecologically more diverse group were under study, more elaborate hypotheses could be tested. For example, imagine a taxon that has greatly diversified into lakes both with and without fish. Multiple habitat shifts have occurred in both directions between fish and fishless lakes in this imaginary taxon, and many speciation events have occurred within both lake types to produce a large number of extant species. On the phylogeny of this imaginary taxon one could potentially identify four types of branches: (1) branches with habitat

shifts from fish to fishless lakes, (2) branches with habitat shifts from fishless to fish lakes, (3) branches associated with only fish lakes, (4) branches associated with only fishless lakes. Using the above methods, contrasts corresponding to these four branch types could be constructed and ANOVA could be applied to test for differences in rates of character change among the four contrast types (see Garland 1992).

DISCUSSION

The techniques described in this article extend Felsenstein's (1973, 1985) method of evolutionary contrasts to allow alternative partitionings of evolutionary character change along a phylogeny. By isolating change along single branches, hypotheses about the magnitudes of rates of change in single characters along particular types of branches can be constructed and tested.

These extensions should be useful for testing hypotheses in a wide variety of studies. For example, phylogenies are increasingly being used to infer when host and habitat shifts have occurred (see, e.g., Futuyma and McCafferty 1990; Brooks and McLennan 1991; Farrell et al. 1992; Brown et al. 1994; McPeek 1995), and the methods presented here open the possibility for testing hypotheses about character change associated with these shifts. Host or habitat shifts usually involve taxa moving between disparate ecological milieus, which often results in radical changes in selection pressures. If host or habitat shifts compel the evolution of new adaptations to use the new environment, change in characters involved in the adaptation should occur rapidly following the shift (Mayr 1942, 1954; Barton and Charlesworth 1984; Carson and Templeton 1984; Barton 1988), and character reconstruction should identify large changes along branches on which shifts are hypothesized to have occurred (see, e.g., McPeek 1995).

The utility of this method is not, however, limited to testing hypotheses about host or habitat shifts; this method can be applied to test hypotheses in any situation in which the researcher is interested in determining the correspondence between change in discrete and quantitative characters. For example, various animal mating systems are hypothesized to impose selection for different degrees of sexual dimorphism and the relative amounts of parental care provided by each parent (Trivers 1972; Thornhill and Alcock 1983). These relationships could be tested by the analysis outlined here in taxa that display variation in mating systems (e.g., monogamy vs. polygyny) across lineages by comparing the amount of change estimated along single branches on which shifts in mating systems are inferred to the amount of character change estimated on other branches (cf. Cheverud et al. 1985). Similar analyses could be applied to study quantitative character change associated with changes in plant mating systems (Donoghue 1989) or plant-pollinator interactions (Armbruster 1988, 1991, 1992; Chase and Hills 1992; McDade 1992). Quantitative character change associated with changes in life history (e.g., changes in longevity and lifetime fecundity associated with shifts between iteroparity and semelparity) could also be fruitfully investigated by this method. Application of this method to these and other problems would allow tests of whether rates of quantitative character change specifically associ-

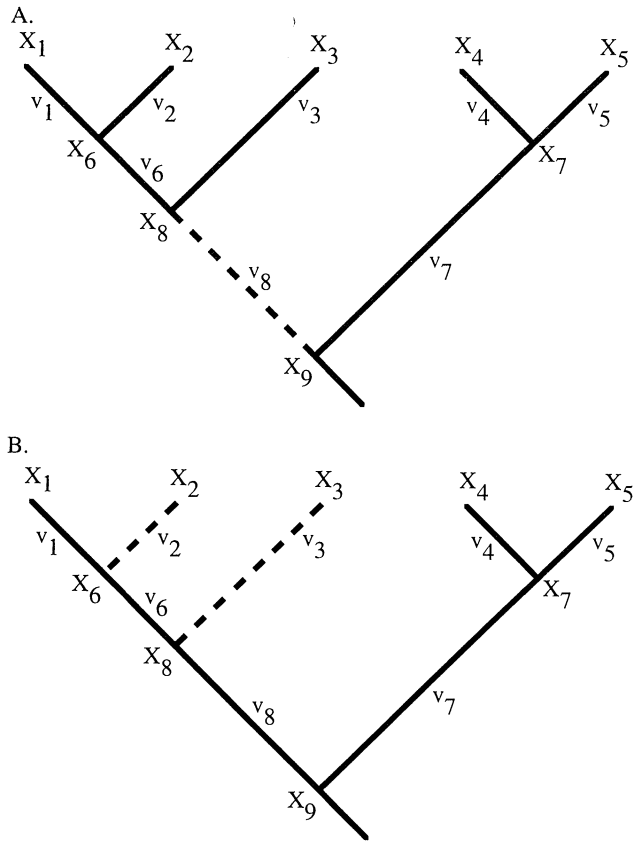


FIG. 6. Two situations in which character change along single branches cannot be reconstructed satisfactorily. In *A*, estimating change along a basal branch of the phylogeny is required, and in *B*, estimating change along two branches that emanate consecutively is required. In each case, contrasts can be constructed, but some contrasts in the resulting set are correlated (see text).

ated with shifts among states of the discrete character are different from rates of character change when states of the discrete character do not change.

This method cannot satisfactorily reconstruct character evolution in two situations when change along single branches is required. The first is when a branch of interest is one of the two basal branches of the tree (e.g., fig. 6*A*). In figure 6*A* the ancestor X_9 cannot be estimated by interpolation. Character X_9 can be estimated by extrapolation from the value of X_7 and the length of v_7. However, in this case $\mathrm{Cov}((X_8 - X_9), (X_4 - X_5)) = v_7$. In other words, when ancestral values must be calculated by extrapolation as in figure 6*A*, the full set of resulting contrasts are not all independent. This situation may be circumvented by including additional taxa in the analysis that would branch before the basal node of the tree (e.g., out-groups).

The other situation in which this method does not produce satisfactory results

is when two single branches are too close to each other on the tree. For example, in figure 6*B* both X_6 and X_8 can be estimated by interpolation from X_1 and X_7, but $\text{Cov}((X_2 - X_6), (X_3 - X_8)) \neq 0$. Here again the contrasts can be estimated, but they are not independent. A general rule of thumb is that all contrasts are independent if only one single branch of interest emanates from each set of branches forming a contrast when the single branches are pruned from the tree. These may be more difficult situations to rectify, especially with species-level phylogenies; an intervening lineage that has not been included in the analysis must be found between the two branches of interest.

Traditional hypothesis-testing statistics (e.g., *t*-tests, ANOVA, Mann-Whitney-Wilcoxon tests) can be used to test for significant differences in the magnitudes of contrasts for single characters. Most previous studies have used correlations between evolutionary contrast values for two or more characters to test hypotheses of adaptation and constraint (see, e.g., Sessions and Larson 1987; Losos 1990*a*, 1990*b*). Traditional hypothesis-testing statistics applied to single characters are appropriate when contrasts can be categorized and each category contains more than one branch (as with the categories of habitat shift and absence of habitat shift in fig. 5). For example, Garland (1992) used evolutionary contrasts in Mann-Whitney *U*-tests to examine whether evolutionary rates differed between the mammal clade Carnivora and the clade of ungulates. His analysis showed that rates of change for body mass, sprint speed, and home range have not differed between the two clades, but that rates of change in the metatarsal/femur and metacarpal/humerus ratios have been significantly higher within the ungulate clade (Garland 1992). The methods elaborated in this article provide greater flexibility in designing a specific set of evolutionary contrasts to test particular hypotheses about character change along various types of branches on a phylogeny.

Comparative analyses may also serve different functions at different stages in a research program. For example, if the differences in selective agents between habitats are not known, reconstructions of characters that should be affected by different selection pressures (e.g., reconstructions of two characters, A and B, where character A is thought to be affected by selection pressure 1, and character B is thought to be affected by selection pressure 2) would allow the discrimination of possible selective agents involved in adaptation during and following habitat shifts. Large change in a character along branches on which host or habitat shifts are inferred would suggest the importance of changes in selection pressures that influence the evolution of that character. To confirm the operation of a particular selective agent in the system, ecological studies of extant species would then be needed to test whether a causal link exists between the character and the hypothesized selective agent in determining fitness. However, the comparative study when done first would allow relatively easy discrimination among multiple selective agents by reconstructing characters that should be influenced by different selection pressures.

Alternatively, if differences in potential selective agents have been identified and the characters that influence fitness in extant species are known, such character reconstructions would test whether these selective agents have caused adap-

tive changes in the phenotype. In the case of the damselflies, previous studies identified that interactions with fish in lakes with fish and with large dragonflies in fishless lakes strongly contributed to generating the segregation of *Enallagma* species between these two lake types (Pierce et al. 1985; Blois-Heulin et al. 1990; McPeek 1990*a*). Also, species in the two lake types differ in behaviors that influence susceptibility to these two predator types (Pierce et al. 1985; McPeek 1990*b*). Reconstructions of morphologies that are important in performing these behaviors indicate that large and directed changes in these characters are associated with habitat shifts into fishless lakes (fig. 5; McPeek 1995). Taken together these studies indicate that dragonfly predation has been a potent selective agent shaping the phenotypes of *Enallagma* species in fishless lakes (McPeek 1995).

Phylogenetic character reconstruction is becoming an increasingly important tool in the analysis of adaptation and constraint in phenotypic evolution (Coddington 1988; Baum and Larson 1991; Brooks and McLennan 1991; Harvey and Pagel 1991). Comparative analyses test for a correspondence between the evolution of a character of interest and changes in selection regime (e.g., habitat or host shifts), in other characters (e.g., life history, diet, mating system), or in the level of taxonomic organization. Although such tests are an integral component of evaluating the mechanisms influencing character evolution, they do not constitute definitive evidence for causal links between hypothesized mechanisms and character change (Leroi et al. 1994); other studies are needed in conjunction with comparative studies. Ecological studies are needed to identify putative selective agents, and functional studies are needed to establish that the phenotype of interest influences fitness via a mechanism acting through the selective agent. Developmental, quantitative genetic, and population-genetics studies are also needed to evaluate the malleability of the phenotype to the forces of evolution. The study of adaptation must be an integrative endeavor that melds studies of ecology, development, genetics, and phylogenetics.

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