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# Matching Behavioral Evolution to Brain Morphology

## Key Words

Bats  
Behavioral distance matrix  
Marsupials  
Path-length distance matrix  
Phylogenetic tree  
Primates  
Statistical test  
Triple-permutation test

## Abstract

A method is presented to test the relationship between a phylogenetic tree derived from brain morphology, and different hypotheses describing the evolution of a behavioral trait. This is a question of interest for evolutionary psychologists and behavioral biologists. The paper first discusses how hypotheses for behavioral evolution should be coded for such a comparison, then a triple-permutation test, originally proposed to compare independently obtained evolutionary trees, is used for the statistical assessment of each hypothesis. Non-parametric correlation coefficients computed between brain components and appropriately coded behavioral states can then be used to suggest what brain components are responsible for the development of the various states of the behavioral trait of interest. The procedure is illustrated with three different applications relating brain evolution to habitat selection in marsupials, locomotory specialization in primates, and trophic adaptation in bats.

## Introduction

The joint evolution of brain and behavior is a topic of great interest to evolutionary psychologists and behavioral biologists [Pirlot, 1987]. Few papers have tackled it, however, [Masterton et al., 1976a, b], because of the methodological difficulties such a comparison implies. Our interest in this problem stems from a work on marsupials that was initiated in 1981 by P.L. and our late colleague Paul Pirlot at Université de Montréal. It aimed at resolving such questions as: does brain morphology contain information as to the evolutionary history of the marsupials? If so, can we establish relationships between brain evolution on the one hand, and the evolution of ecological and ethological characters on the other?

Comparing behavioral data and evolutionary data is not an easy task. Our work remained incomplete, because we

lacked a statistically valid method for comparing empirical evolutionary trees, or for assessing the relationship of a tree to an evolutionary hypothesis. This problem was recently resolved, as reported in a series of papers [Lapointe and Legendre, 1990, 1991, 1992a, b] and in a thesis about the evolution of chiropteran brains [Lapointe, 1992].

The present paper extends the statistical method and allows comparison of an evolutionary tree to hypotheses of behavioral evolution. We will show, in particular, (1) How an hypothesis of behavioral evolution can be coded for such a comparison; (2) How the 'best' or 'most likely' hypothesis of behavioral evolution can be picked; and (3) How an hypothesis of behavioral evolution can suggest what brain components are responsible for the development of the various states of behavior.

The method will be illustrated using three different examples relating brain evolution to (i) habitat selection in

marsupials, (ii) locomotory specialization in primates, and (iii) trophic adaptation in bats. These include cases where all possible behavioral models can be evaluated, as well as situations where we want to assess the best hypotheses given some evolutionary constraints.

## Materials and Methods

We will now briefly present the biological data used in this paper, and describe in detail the new statistical method.

### Brain Data

Twenty-eight species of marsupials [Pirlot, 1981], twenty-six simian primates [Stephan et al., 1988], and twenty-eight microchiropteran bats [Baron et al., 1994] were considered in this study. For all species of marsupials the volume of each of 11 brain components (in cm<sup>3</sup>) was determined: olfactory bulbs (OB), isocortex (IX), olfactory cortex (OX), transitional cortex (TX), septum (SE), striatum (ST), hippocampus (HI), diencephalon (DI), cerebellum (CE), mesencephalon (ME), and medulla oblongata (MO). (This differs from the nomenclature of brain components followed by the aforementioned authors; they call OB 'bulbus olfactorius', IX 'neocortex', OX 'paleocortex', and TX 'schizocortex'.) In addition to these components, the amygdala (AM) was also considered for primates and bats, for a total of 12 components. For a full account of the laboratory methodology, including delineation of the components, measurement techniques, and raw volumes, the reader is referred to Pirlot [1981], Stephan et al. [1988] and Baron et al. [1994]. In addition to these three data sets, data on the brain morphology (11 components) of two edentates [Pirlot, 1980; Pirlot and Kamiya, 1983] and two rodents [Ouedraogo, 1974] were used as outgroups in the phylogenetic analyses, to determine the position of the 'root' of each brain tree.

To standardize the data for size, the brain component volumes were divided by the total brain volume and/or transformed into size indices [Stephan, 1967], depending on what information was available. These standardization methods are discussed by Stephan et al. [1988] and by Lapointe and Legendre [1994].

### Phylogenetic Tree Reconstruction

Euclidean distances were computed among species based on the standardized brain component volumes, and the distance-based phylogenetic tree reconstruction method of De Soete [1983] was applied to each matrix to obtain an additive tree (i.e., cladogram). The root of a tree is located at the point where all marsupials, primates, or bats separate from the members of the outgroup on the tree; the outgroup data will not be used any further in the present analysis.

### Selecting the Best Model of Behavioral Evolution

We will now discuss how to code behavioral evolution hypotheses into behavioral distance matrices and how to select the best model of evolutionary behavior. Three situations will be considered: examination of all possible models of behavioral evolution when there are but a few possible models; a stepwise approach when there are more; and, finally, a constrained approach when considering a behavioral trait possessing many states.

*Case 1: Habitat Selection in Marsupials.* The behavioral trait considered in this test case has three states [Lapointe and Legendre, 1994]: forest dwellers (20 species in our study), ubiquitous (4 species)

and prairie dwellers (4 species). Because this variable has but a few states, enumerating and evaluating all different possible habitat selection models was feasible. We found 22 different models for the evolution of this behavioral characteristic.

The first difficulty was to transcribe these hypotheses, in a non-equivocal way, into a form that could be compared to the brain cladogram. We proceeded in two steps to determine the distance matrix among behavioral states: (a) an unspecified ancestral state, called 'root', was added to the behavior hypothesis tree, and (b) a path-length distance matrix was written, giving the number of steps (number of arrows) separating behavioral states. Adding a 'root' state was important to make the behavioral trees comparable to the cladogram. Figure 1 illustrates these steps for five of our 22 hypotheses. Following that, (c) a behavioral distance hypothesis matrix among species was constructed. Distances among animals were read from the behavior state distance matrix constructed in step (b), according to the pairs of states exhibited by the different species. An example is given in figure 2.

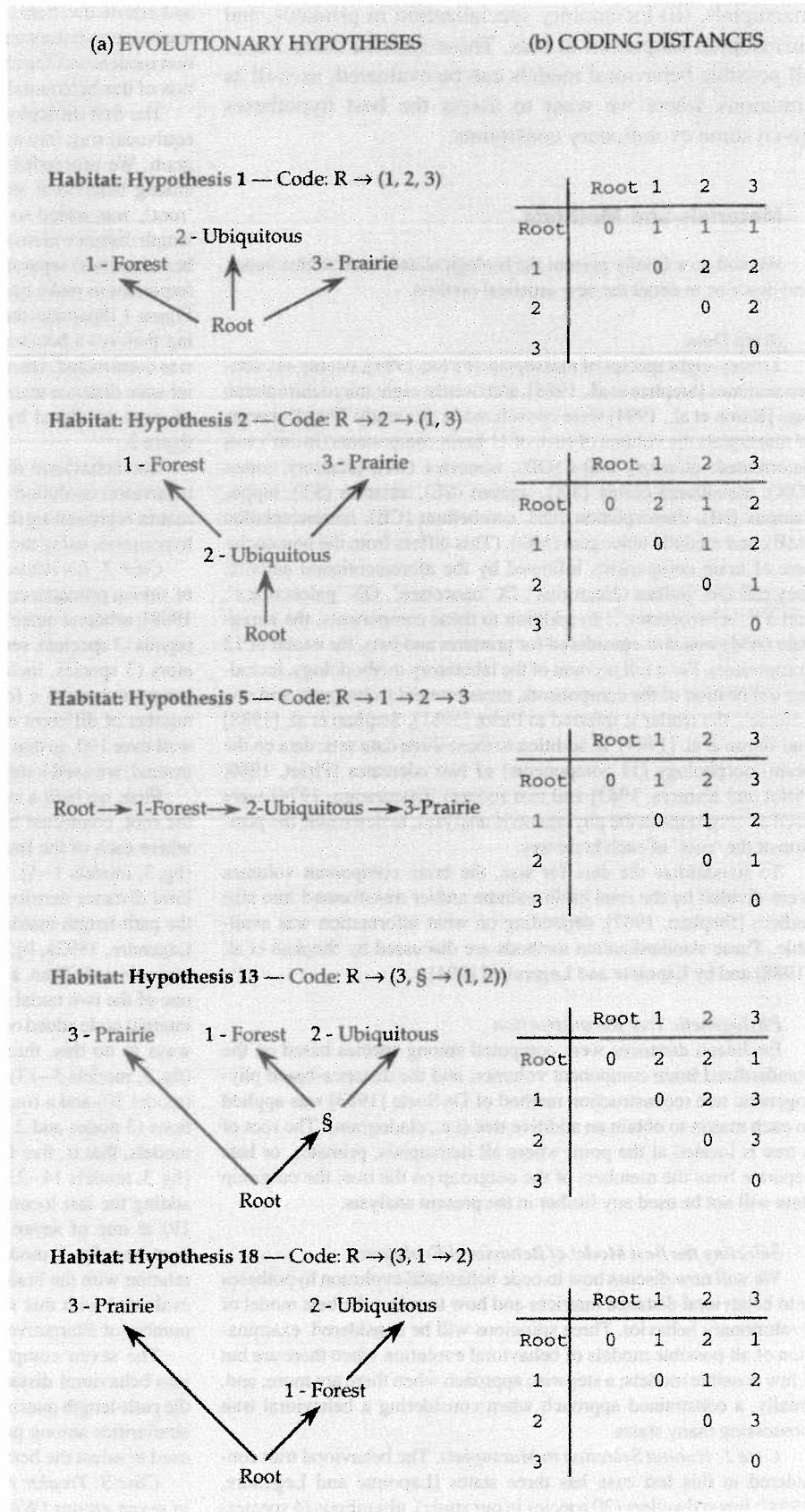
The behavioral distance matrices corresponding to the different behavioral evolution models were then compared to the path-length matrix representing the brain cladogram of marsupials to assess the 22 hypotheses, using the triple-permutation test described below.

*Case 2: Locomotory Specialization in Primates.* The locomotion of simian primates can be classified in four categories [Stephan et al., 1988]: arboreal quadrupeds (14 species in our study), terrestrial quadrupeds (3 species), semi-brachiators (6 species), and modified brachiators (3 species, including the true brachiator *Hylobates lar*, which progress through a forest by swinging from branch to branch). The number of different models of behavioral evolution for four states is well over 100, so that one could not evaluate all possible scenarios. So instead, we used a stepwise approach to find the best model.

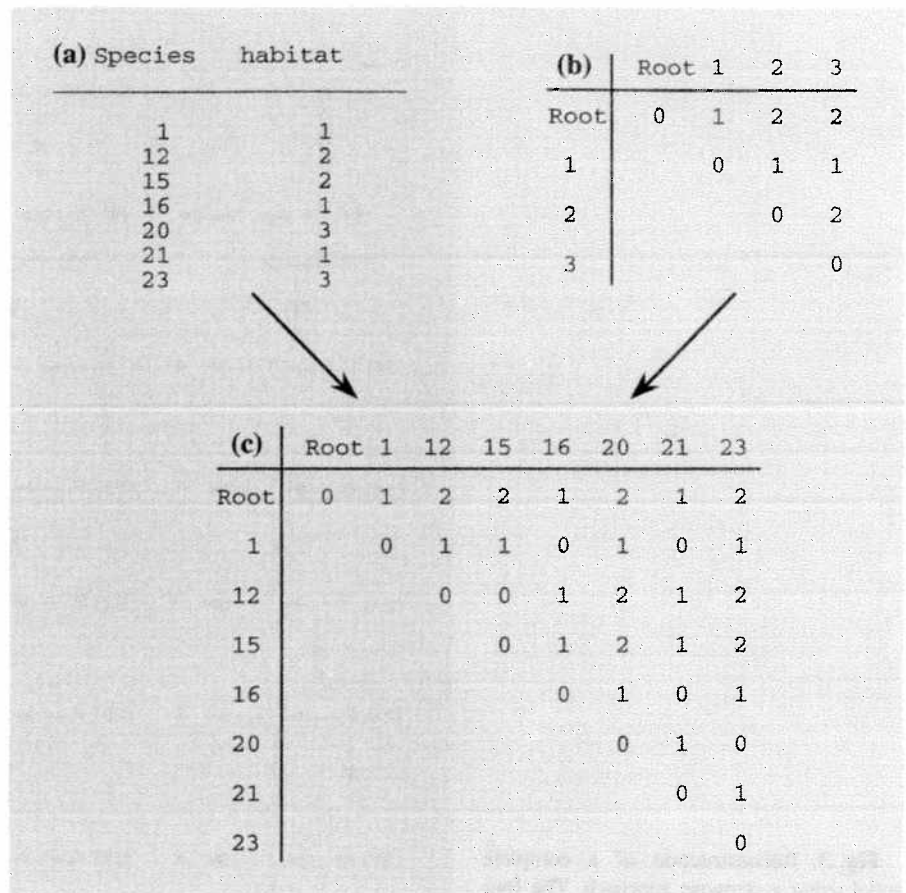
First, we built a simple model with only two nodes: one state and the root, connected by a single branch. There are four such models, where each of the four possible types of locomotion is added in turn (fig. 3, models 1–4). The four models were transformed into behavioral distance matrices among species (as in fig. 2) and correlated to the path-length matrix corresponding to the brain tree [Lapointe and Legendre, 1992a, b]; the one with the highest correlation (model 1) was selected. Then, a third element was included, connected to either one of the two nodal states of the previous model, or to an additional internal node added on the branch of the behavioral tree; there are nine ways to do this, three for each of the remaining locomotion types (fig. 3, models 5–13). The best model with three states was selected (model 10) and a fourth state was added at one of five different positions (3 nodes and 2 additional branches); it sums up to 10 different models, that is, five for each of the two remaining locomotion types (fig. 3, models 14–23). Finally, the complete model was obtained by adding the last locomotion type to the best four-state model (model 19) at one of seven different positions (4 nodes and 3 additional branches: fig. 3, models 24–30), and the model with the highest correlation with the brain tree was selected. The total number of models evaluated with this stepwise approach was 30, greatly reducing the number of alternatives one would have had to consider otherwise.

The seven complete behavioral evolution models, transformed into behavioral distance matrices among species, were compared to the path-length matrix corresponding to the cladogram depicting brain similarities among primates; the triple-permutation test (below) was used to select the best final model.

*Case 3: Trophic Adaptation in Bats.* Diets of bats are categorized in seven groups [Wilson, 1973]: aerial insectivores (8 species in our



**Fig. 1.** Coding hypotheses of behavioral evolution into path-length matrices. To illustrate the procedure, five hypotheses for the evolution of habitat selection are coded into path-length matrices. (a) An unspecified ancestral state 'root' is added to each behavior hypothesis tree. (b) Path-length distances are written in a distance matrix; path lengths are the number of steps (arrows) separating behavioral states. In some cases, as in hypothesis 13, an unspecified intermediate state, represented by §, is included. In table 1, the various hypotheses are coded in linear form, as shown in the figure.



**Fig. 2.** Using the behavioral state data matrix (a), distances among animals are read from a behavioral state distance matrix hypothesis computed as in figure 1 (b) according to the pairs of states exhibited by the animals, to code the behavioral distance matrix among species (c). The following habitat selection hypothesis is used as an example in the present figure:  $R \rightarrow 1 \rightarrow (2, 3)$ .

study), insectivorous gleaners (6 species), frugivores (5 species), nectarivores-pollinivores (3 species), carnivores (3 species), piscivores (1 species), and sanguinivores (2 species). With so many states, the complete enumeration of models is impossible, and so is the stepwise evaluation. We must rely on outside information to set constraints on the search for the best behavioral model. As possible constraints, one may (i) identify pairs of states that should always or should never be connected together, (ii) assign a particular state to the root representing the ancestral condition, (iii) assign a particular state to a terminal node, (iv) order some states in a given sequence, and/or (v) set topological constraints on the behavioral state tree.

Independent studies not involving brain characters can usually provide information on behavioral evolution. In the case of bats, morphological and ecological information was used to define some constraints in building a model of trophic adaptation. One possible set of constraints, from Gillette [1977], is as follows: (1) The primitive condition of microchiropteran bats is insectivory; (2) All diets are derived from insectivory; and, (3) Nectarivores and frugivores are ecologically related.

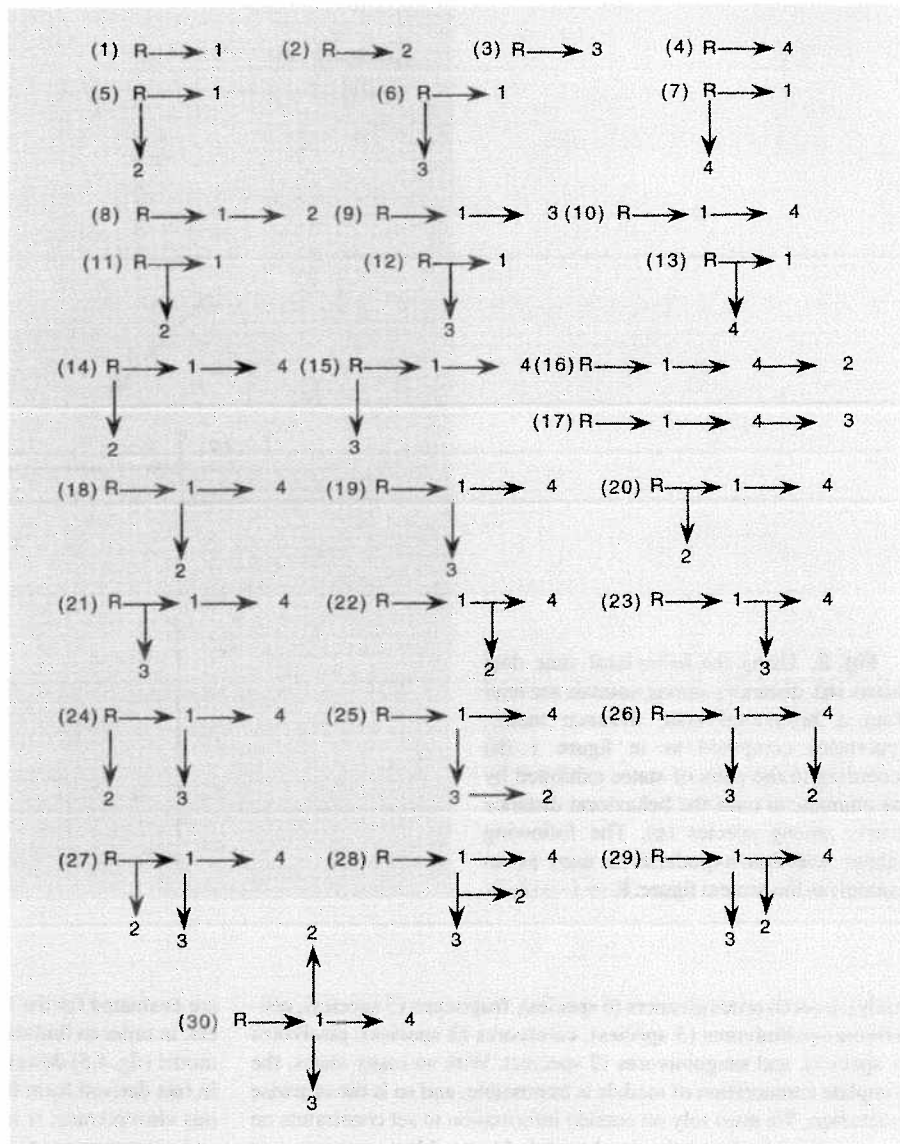
A first model can be derived from this set of constraints (fig. 4.1). This model has only six states since Gillette [1977] did not distinguish aerial insectivores from gleaners. The second model (fig. 4.2) resolves this ambiguity by separating the two insectivore states; the aerial insectivores are represented as more primitive, based on independent morphological studies [Slaughter, 1970]. Then, alternative scenarios

are evaluated for the linkage of frugivores and nectarivores to gleaners, in order to find the best sequence (fig. 4.3 and 4.4). Finally, a last model (fig. 4.5) describes the hypothesis that sanguinivorous bats are in fact derived from frugivores/nectarivores and not from insectivorous chiropterans; it implies that sanguinivores switched from juice and nectar lapping to blood-lapping.

As in the two previous cases, the behavioral evolution models were transformed into behavioral distance matrices among species (see fig. 2). Here again, the triple-permutation test was used to select the behavioral evolution matrix most closely related to the cladogram for bat brains.

#### Further Statistical Analyses

Each brain tree was compared to the behavioral models to assess the best hypothesis. To do so, we used the triple-permutation test of Lapointe and Legendre [1992a]. This method assesses the null hypothesis that two additive trees are not more similar to one another than random trees would be; or, in the present study, that the brain morphology tree is not more similar to a given behavioral evolution model than by chance alone. Statistical evaluation proceeds by generating randomized additive trees (i.e., trees with a randomized topology, randomized taxa positions, and randomized branch lengths) that are correlated in turn with the behavioral distance matrix, whose construction is described in the next section (under Case 1). If most of the correlation values obtained through randomization are of about the



**Fig. 3.** Reconstruction of a complete model using a stepwise approach. The four states of primate locomotory behavior are: (1) arboreal quadrupeds; (2) terrestrial quadrupeds; (3) semi-brachiators; (4) modified brachiators. Models 1 to 4 are obtained at step 1. Model 1 is selected, and models 5 to 13 are derived from it at step 2. Models 14 to 23 are built from model 10 at step 3. The complete models (24 to 30), selected at step 4, are based on the previously selected model 19.

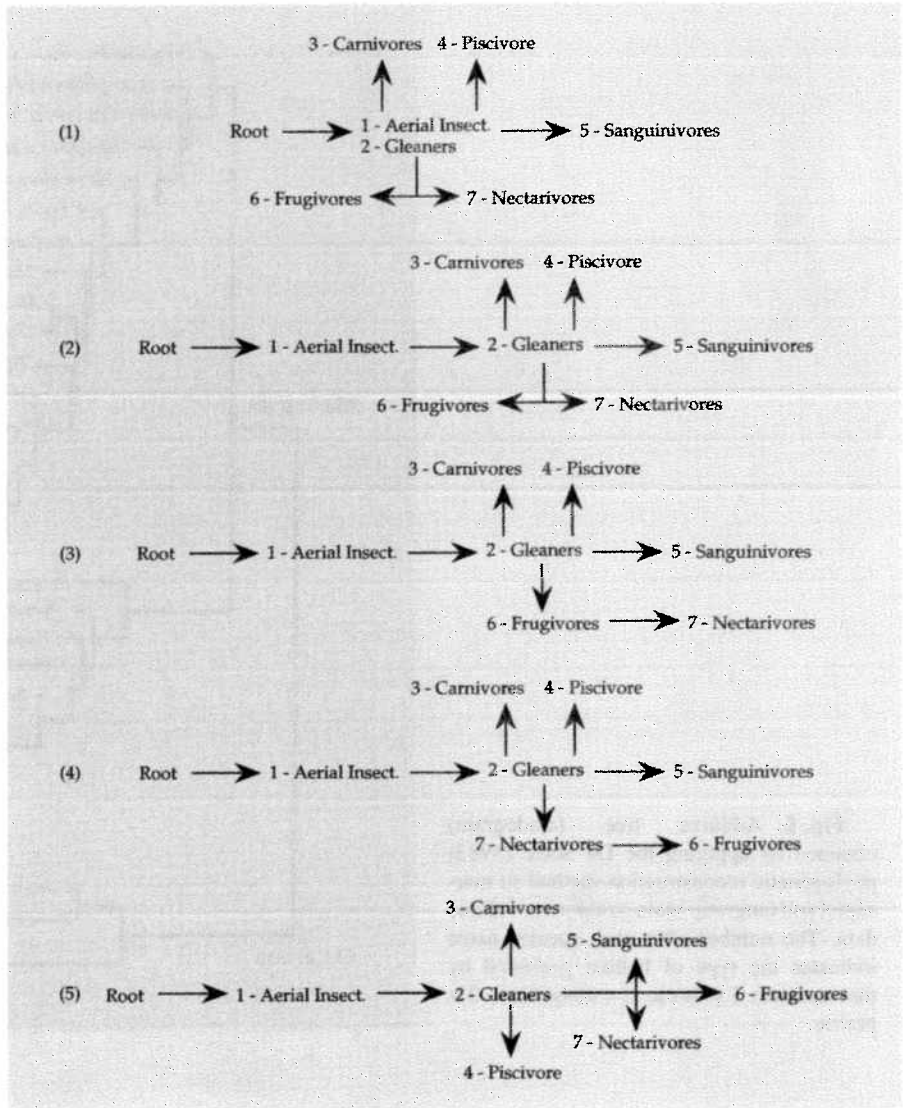
same size as the actual correlation between the brain morphology tree and the behavioral distances, the null hypothesis is accepted; this correlation value could have occurred by chance alone. On the other hand, if the actual value of the correlation is higher than most (say, 95%, for an  $\alpha=0.05$  significance level) random correlation values, the null hypothesis is rejected, implying that the brain morphology tree and behavioral distances are significantly correlated. Models displaying significant correlations with the path-length distances of the brain morphology cladogram are retained for interpretation.

The last problem is to determine which of the brain components are more likely to be responsible for the development of each behavioral state. A new data matrix was assembled, containing all standardized brain component volumes, plus all states of the behavioral characteristic of interest (e.g., habitat selection, locomotory specialization, or trophic adaptation); each state of the behavioral evolution model is

represented by a single variable, coded '1' when present and '0' when absent. The correlation matrix of these new sets of variables contains all the information needed to answer the question; Kendall's non-parametric correlations were used since the three behavioral variables are not continuous but binary-coded.

## Results and Discussion

For selecting the best model of behavioral evolution, our results indicate that a constrained approach, relying on sound evolutionary models, has more heuristic value than exhaustive examination of all possible hypotheses.



**Fig. 4.** Five constrained models of trophic radiation in bats. The seven states are: (1) aerial insectivores; (2) gleaners; (3) carnivores; (4) piscivores; (5) sanguinivores; (6) frugivores; (7) nectarivores.

#### Case 1

The evolutionary tree based on standardized brain component volumes of the 28 species of marsupials is represented in figure 5. Comparisons of this tree to the behavioral distance matrices, derived from the 22 habitat selection evolutionary hypotheses, are reported in table 1. The test clearly rejects several of our hypotheses; it also recognizes 10 of them as being well-supported by the data. What these 10 hypotheses have in common is the following: the two closest behavioral states on the hypothesis tree are always 1 (forest) and 2 (ubiquitous), state 3 (prairie) being more distant from state 1 or state 2, or from both.

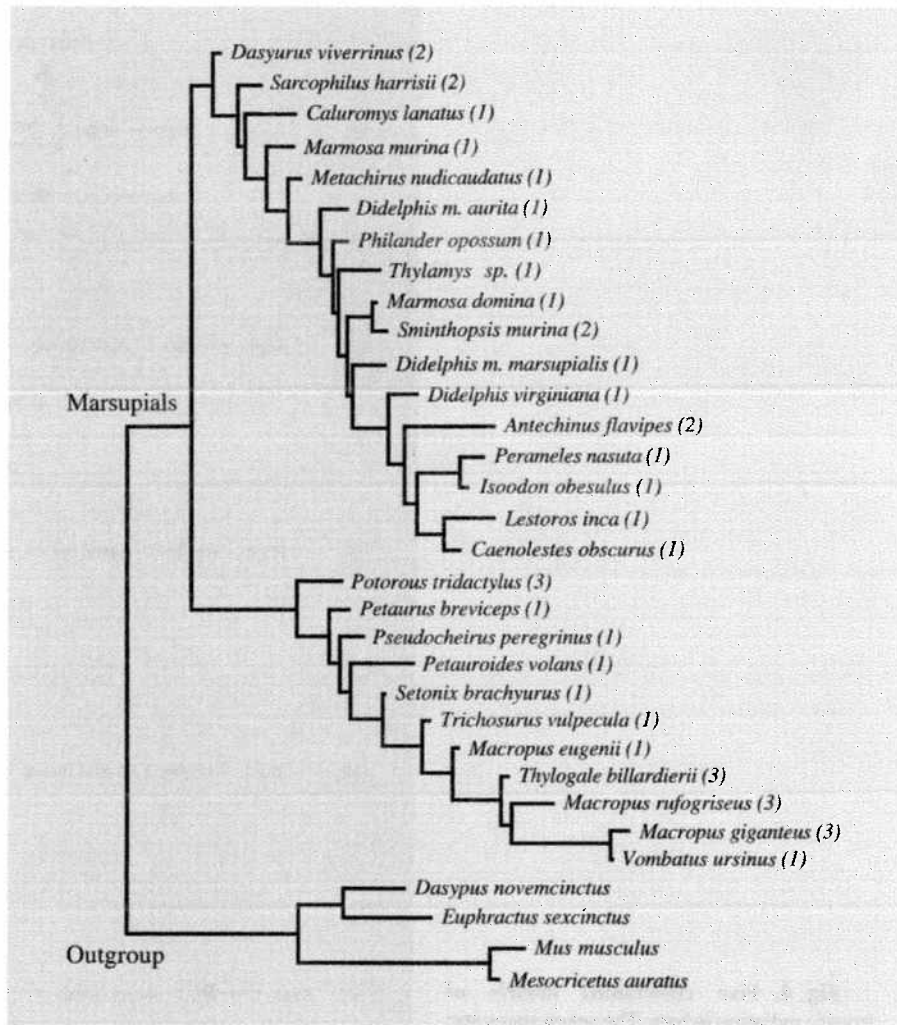
Neurologically speaking, prairie marsupials are indeed very different from other species. The correlation coeffi-

cients reported in table 2 show that prairie species have a larger isocortex and smaller olfactory bulbs, olfactory cortex, transitional cortex, diencephalon, and cerebellum. On the other hand, forest dwellers are characterized by larger transitional cortex than most other marsupials, whereas ubiquitous species display no specially developed brain component. Therefore, prairie dwelling was accompanied by a series of brain modifications that pulled these animals farther away from the other marsupials, while the forest-dwelling and ubiquitous marsupials have remained closer to one another.

This simple case illustrates the problem of choosing among equally likely hypotheses of behavioral evolution in the absence of external constraints. The highest correlations



**Fig. 5.** Additive tree (cladogram) obtained by applying the De Soete (1983) phylogenetic reconstruction method to marsupial and outgroup brain component volume data. The number after each species name indicates the type of habitat preferred by that species: 1 = forest; 2 = ubiquitous, 3 = prairie.



were obtained for two models where the prairie species are considered to represent the primitive condition; this is not supported by other studies on marsupial radiation. However, the models that assume that forest-dwellers or ubiquitous species are primitive also proved to be statistically significant. Even though it is feasible to enumerate all possible models involving few behavioral states, not all hypotheses may be equally likely. The search for the best scenario can be greatly improved by limiting the evaluation to sound evolutionary models, as in case 3.

#### Case 2

The brain similarities based on size indices among the 26 species of primates considered in this study are depicted in the cladogram shown in figure 6. This cladogram was statistically compared to different hypotheses of primate

locomotory behavior (fig. 3) using the stepwise approach; results are summarized in table 3. The best model (it is the only significant one) of locomotory specialization in primates is that of an adaptive radiation from the arboreal quadruped state (model 30, fig. 3).

This model can be interpreted in conjunction with the correlations presented in table 4. The ancestral condition represented by arboreal quadrupeds is characterized by large olfactory bulb, olfactory cortex, transitional cortex, septum, diencephalon, mesencephalon, and amygdala. The path leading to terrestrial quadrupeds is correlated with significant reduction of the transitional cortex and amygdala; the semi-brachiators have evolved reduced olfactory bulbs, olfactory cortex, mesencephalon, and medulla oblongata, while the modified brachiators acquired smaller olfactory cortex and mesencephalon, as well as a larger cerebellum.

**Table 1.** Results from the triple-permutation tests of significance between the evolutionary tree based on brain morphology and the 22 hypotheses about the evolution of habitat selection; the hypotheses are coded as in figure 2. The models are considered fixed, so only the brain morphology tree was permuted for each test of significance (999 permutations)

Brain morphology tree tested against...		Correlation coefficient	Probability of H <sub>0</sub>	p≤0.01
Model 1	R→(1, 2, 3)	0.115	0.462	
Model 2	R→2→(1, 3)	0.251	0.003	*
Model 3	R→1→(2, 3)	0.233	0.001	*
Model 4	R→3→(1, 2)	0.071	0.175	
Model 5	R→1→2→3	0.223	0.002	*
Model 6	R→1→3→2	0.042	0.292	
Model 7	R→2→1→3	0.268	0.001	*
Model 8	R→2→3→1	0.114	0.069	
Model 9	R→3→1→2	0.276	0.001	*
Model 10	R→3→2→1	0.273	0.001	*
Model 11	R→(1, §→(2, 3))	0.083	0.130	
Model 12	R→(2, §→(1, 3))	0.094	0.111	
Model 13	R→(3, §→(1, 2))	0.210	0.003	*
Model 14	R→(1, 2→3)	0.126	0.038	
Model 15	R→(1, 3→2)	0.004	0.479	
Model 16	R→(2, 1→3)	0.097	0.113	
Model 17	R→(2, 3→1)	0.007	0.466	
Model 18	R→(3, 1→2)	0.261	0.001	*
Model 19	R→(3, 2→1)	0.260	0.001	*
Model 20	R→§→(1, §→(2, 3))	0.091	0.099	
Model 21	R→§→(2, §→(1, 3))	0.111	0.068	
Model 22	R→§→(3, §→(1, 2))	0.225	0.002	*

State codes for habitat selection in marsupials: 1 = forest; 2 = ubiquitous; 3 = prairie. § represents an additional, unspecified state.

**Table 2.** Nonparametric correlation coefficients (Kendall's tau) between the binary-coded behavioral states of the habitat selection model (rows) and the 11 standardized brain component variables of 28 marsupial species (columns). Abbreviations for the brain components as in the text

Brain component variables ⇒		1	2	3	4	5	6	7	8	9	10	11
		OB	IX	OX	TX	SE	ST	HI	DI	ME	CE	MO
1	Forest	0.14	-0.20	0.18	0.28 <sup>a</sup>	-0.01	-0.03	0.17	0.16	0.10	0.14	0.10
2	Ubiquitous	0.16	-0.18	0.16	0.06	-0.09	0.15	-0.15	0.25	-0.06	0.23	0.05
3	Prairie	-0.34 <sup>a</sup>	0.44 <sup>c</sup>	-0.39 <sup>b</sup>	-0.42 <sup>b</sup>	0.11	-0.11	-0.07	-0.45 <sup>c</sup>	-0.06	-0.41 <sup>b</sup>	-0.18

<sup>a</sup>0.01 < p ≤ 0.05; <sup>b</sup>0.001 < p ≤ 0.01; <sup>c</sup>p ≤ 0.001.

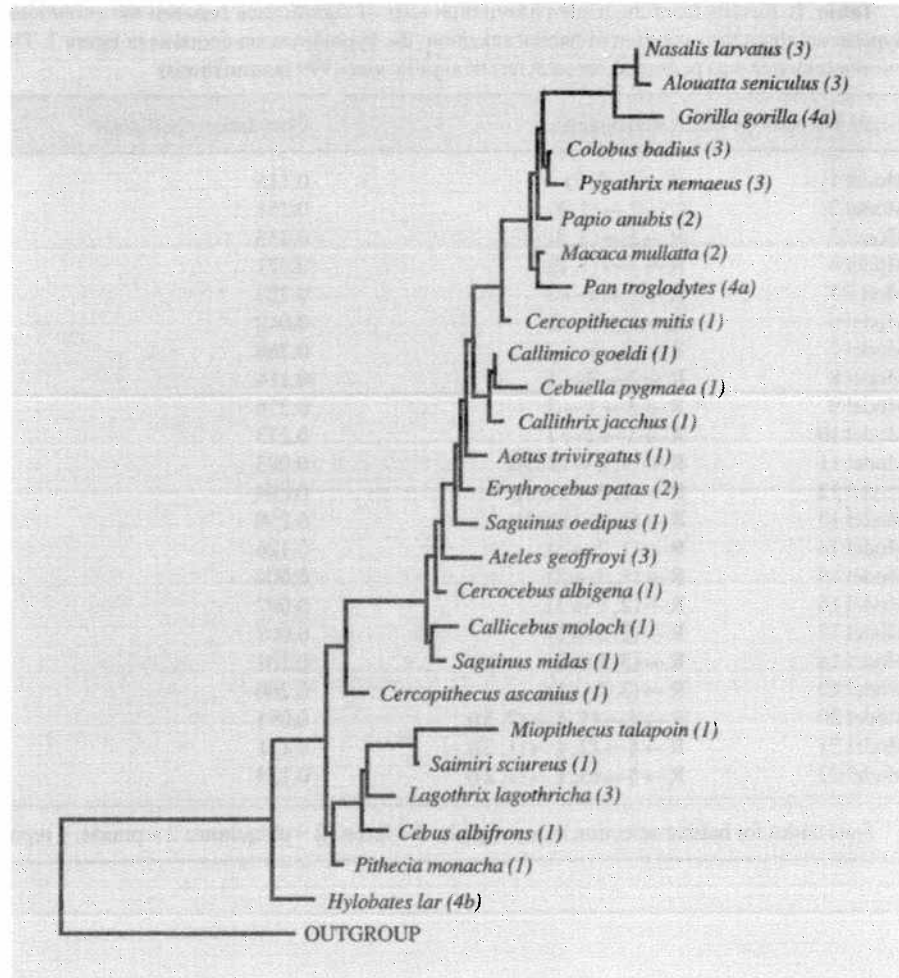
This stepwise approach is very useful when one lacks external constraints and the number of states prevents an exhaustive evaluation of all possible models. In this particular situation, only one scenario was found significant, but this may not always be the case. It is also desirable here to include external information to limit the search for the best behavioral model.

### Case 3

Figure 7 shows the brain cladogram representing possible evolutionary relationships among 28 species of microchiropterans, based on brain similarities computed from size indices. Table 5 presents the results of the comparison tests between the brain tree and some hypotheses of bat trophic radiation, selected by applying a set of evolutionary



**Fig. 6.** Additive tree (cladogram) obtained by applying the De Soete (1983) phylogenetic reconstruction method to primate and outgroup brain component volume data. The number after each species name indicates the type of locomotion preferred by that species: 1 = arboreal quadrupeds; 2 = terrestrial quadrupeds; 3 = semibrachiators; 4 = modified brachiators.



constraints from previous studies. The probabilities alone do not allow one to choose among the possible significant scenarios of bat diet evolution since models 2 to 5 are all statistically related to the brain tree. However, the hypothesis with the highest correlation (model 5) depicts a trophic radiation of bats from aerial insectivores to gleaners, which further evolved into carnivores, piscivores, and a nectarivore-frugivore ancestor leading to the blood-lappers. Therefore, this behavioral model not only confirms statistically the set of constraints, it also supports the hypothesis that sanguinivorous bats are derived from the nectarivore-frugivore state.

The correlation values in table 6 describe the parallel evolution of brain and behavior along the evolutionary lines leading to the different feeding strategies. The primitive aerial insectivores are characterized by small components, and particularly a reduced isocortex, olfactory cortex, septum, striatum, hippocampus, diencephalon, mesencepha-

lon, and cerebellum. The gleaner insectivores, however, have significantly smaller olfactory bulbs and olfactory cortex, but a larger mesencephalon, than do their aerial feeder counterparts. This gleaner state is the one leading to carnivorous and piscivorous bats, which exhibit enlarged diencephalon, mesencephalon, and medulla oblongata components, or a larger amygdala, respectively. The parallel evolution of frugivory and nectarivory is generally associated with an increase in most brain component sizes. In frugivorous bats, this is represented by enlarged olfactory bulbs, isocortex, transitional cortex, septum, striatum, hippocampus, and cerebellum, whereas nectarivorous species show only a larger septum and diencephalon as well as a significantly smaller mesencephalon. Finally, the brain in sanguinivores is definitely closer to that in nectarivores-frugivores than it is to that in insectivores. Blood feeders are characterized by a large olfactory bulb, isocortex, hippocampus, and diencephalon, the first three features held in

**Table 3.** Results of the stepwise procedure; the hypotheses are coded as in figure 3. At each step the model with the highest correlation (\*) is selected. Only the complete models (24 to 30) were tested for significance (999 permutations)

Brain tree tested against...	Correlation	Brain tree tested against...	Correlation
STEP 1 (adding state 1, 2, 3, or 4)			
Model 1 R → 1	0.609*	Model 2 R → 2	0.442
Model 3 R → 3	0.588	Model 4 R → 4	0.423
STEP 2 (adding state 2, 3, or 4)			
Model 5 R → (1, 2)	0.076	Model 6 R → (1, 3)	0.198
Model 7 R → (1, 4)	0.355	Model 8 R → 1 → 2	0.319
Model 9 R → 1 → 3	0.436	Model 10 R → 1 → 4	0.523*
Model 11 R → § → (1, 2)	0.260	Model 12 R → § → (1, 3)	0.353
Model 13 R → § → (1, 4)	0.492		
STEP 3 (adding state 2 or 3)			
Model 14 R → (2, 1 → 4)	0.112	Model 15 R → (3, 1 → 4)	0.180
Model 16 R → 1 → 4 → 2	0.208	Model 17 R → 1 → 4 → 3	0.304
Model 18 R → 1 → (2, 4)	0.328	Model 19 R → 1 → (3, 4)	0.375*
Model 20 R → § → (2, 1 → 4)	0.265	Model 21 R → § → (3, 1 → 4)	0.313
Model 22 R → 1 → § → (2, 4)	0.265	Model 23 R → 1 → § → (3, 4)	0.326
STEP 4 (adding state 2)			
Model 24 R → (2, 1 → (3, 4))	0.063	p = 0.305	
Model 25 R → 1 → (4, 3 → 2)	0.190	p = 0.051	
Model 26 R → 1 → (3, 4 → 2)	0.119	p = 0.158	
Model 27 R → § → (2, 1 → (3, 4))	0.202	p = 0.058	
Model 28 R → 1 → (4, § → (2, 3))	0.172	p = 0.053	
Model 29 R → 1 → (3, § → (2, 4))	0.157	p = 0.101	
Model 30 R → 1 → (2, 3, 4)	0.231*	p = 0.029*	

State codes for locomotory behavior in primates: 1 = arboreal quadrupeds; 2 = terrestrial quadrupeds; 3 = semi-brachiators; 4 = modified brachiators. § represents an additional, unspecified state.

**Table 4.** Nonparametric correlation coefficients (Kendall's tau) between the binary-coded behavioral states of the locomotion adaptation model (rows) and the 12 standardized brain component variables of 26 primates (columns). Abbreviations for the brain components as in the text

Brain component variables ⇒	1	2	3	4	5	6	7	8	9	10	11	12
	OB	IX	OX	TX	SE	ST	HI	DI	ME	CE	MO	AM
1 Arboreal quadrupeds	0.57 <sup>c</sup>	0.04	0.52 <sup>c</sup>	0.36 <sup>b</sup>	0.43 <sup>b</sup>	0.15	0.07	0.31 <sup>a</sup>	0.60 <sup>c</sup>	-0.17	0.51 <sup>c</sup>	0.51 <sup>c</sup>
2 Terrestrial quadrupeds	-0.06	0.10	-0.18	-0.30 <sup>a</sup>	-0.21	-0.05	-0.06	-0.15	-0.21	-0.06	-0.01	-0.30 <sup>a</sup>
3 Semi-brachiators	-0.44 <sup>b</sup>	-0.21	-0.27 <sup>a</sup>	-0.13	-0.25	-0.12	0.05	-0.21	-0.33 <sup>a</sup>	-0.02	-0.38 <sup>b</sup>	-0.23
4 Modified brachiators	-0.25	0.11	-0.27 <sup>a</sup>	-0.09	-0.13	-0.03	-0.11	-0.06	-0.29 <sup>a</sup>	0.35 <sup>a</sup>	-0.21	-0.19

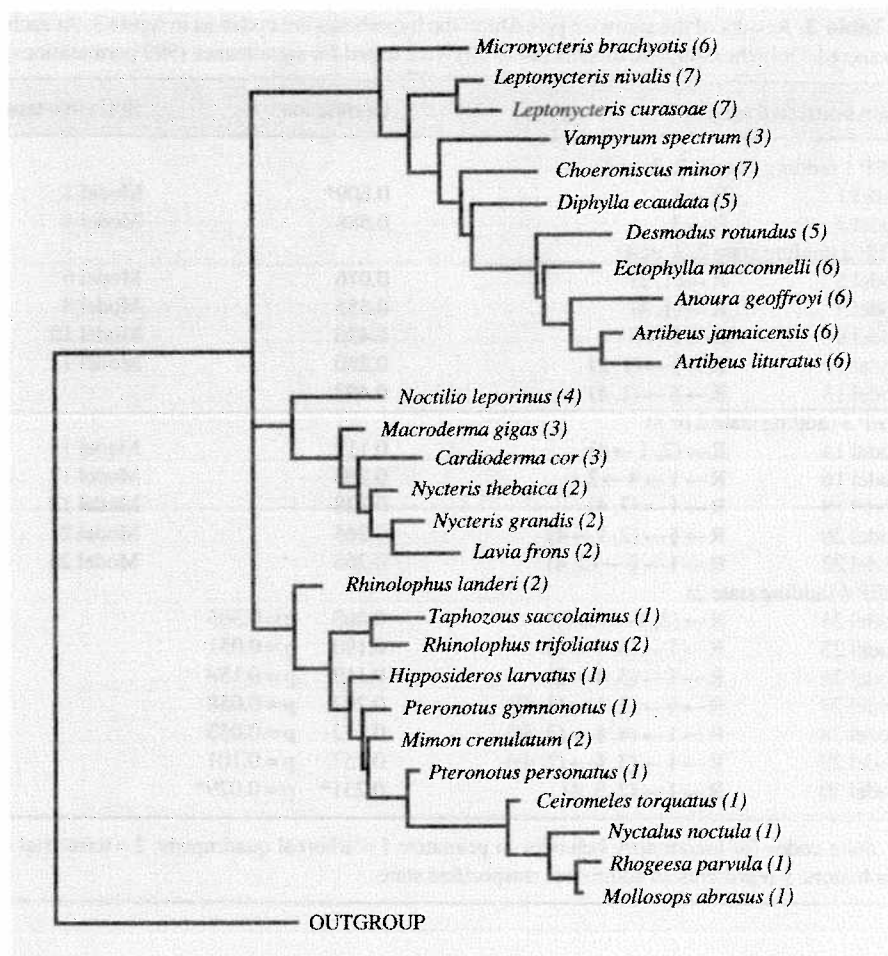
<sup>a</sup>0.01 < p ≤ 0.05; <sup>b</sup>0.001 < p ≤ 0.01; <sup>c</sup>p ≤ 0.001.

common with frugivorous bats, the last one shared by carnivorous species.

This example of adaptive radiation into many states could not have been analyzed exhaustively. The constrained approach, using external information, is very promising and provided interesting results. However, one should bear in mind that in any particular case, more than one model may

be significant. It is even more likely to happen when many states, represented by few taxa, are studied. The statistical power of the method could be improved by selecting equal numbers of taxa for each state, and a large sample size, when possible. We believe, nevertheless, that rare behavioral states (e.g., piscivore) should be used in statistical analyses, despite the low power of such tests.

**Fig. 7.** Additive tree (cladogram) obtained by applying the De Soete (1983) phylogenetic reconstruction method to bat and outgroup brain component volume data. The number after each species name indicates the type of diet preferred by that species: 1 = aerial insectivores; 2 = gleaners; 3 = carnivores; 4 = piscivore; 5 = sanguinivores; 6 = frugivores; 7 = nectarivores.



**Table 5.** Results from the triple-permutation tests of significance between the evolutionary tree based on brain morphology and 5 hypotheses about the evolution of dietary strategies in bats (fig. 4). The models are considered fixed, so only the brain morphology tree was permuted for each test of significance (999 permutations)

Brain morphology tree tested against...	Correlation	Probability of $H_0$	$p \leq 0.01$
Model 1 R → 1+2 → (3, 4, 5, § → (6, 7))	0.237	0.092	
Model 2 R → 1 → 2 → (3, 4, 5, § → (6, 7))	0.508	0.001	*
Model 3 R → 1 → 2 → (3, 4, 5, 6 → 7)	0.502	0.001	*
Model 4 R → 1 → 2 → (3, 4, 5, 7 → 6)	0.489	0.003	*
Model 5 R → 1 → 2 → (3, 4, § → (5, 6, 7))	0.599	0.001	*

State codes for trophic radiation in bats: 1 = aerial insectivores; 2 = gleaners; 3 = carnivores; 4 = piscivore; 5 = sanguinivores; 6 = frugivores; 7 = nectarivores.

## Conclusions

The triple-permutation procedure of Lapointe and Legendre [1992a] has been used in this paper to test the relation between a phylogenetic tree derived from brain mor-

phology, and different hypotheses describing the evolution of behavioral traits. In order to illustrate the behavior of the triple-permutation test in this type of situation, we tested (i) all possible evolutionary hypotheses of habitat selection in marsupials, (ii) seven complete models of locomotory spe-

**Table 6.** Nonparametric correlation coefficients (Kendall's tau) between the binary-coded behavioral states of the trophic adaptation model (rows) and the 12 standardized brain component variables of 28 bat species (columns). Abbreviations for the brain components as in the text

Brain component variables ⇒		1	2	3	4	5	6	7	8	9	10	11	12
		OB	IX	OX	TX	SE	ST	HI	DI	ME	CE	MO	AM
1	Aerial insectivores	-0.22	-0.62 <sup>c</sup>	-0.37 <sup>b</sup>	-0.63 <sup>c</sup>	-0.54 <sup>c</sup>	-0.59 <sup>c</sup>	-0.59 <sup>c</sup>	-0.65 <sup>c</sup>	-0.42 <sup>b</sup>	-0.60 <sup>b</sup>	-0.50 <sup>b</sup>	-0.19
2	Gleaners	-0.44 <sup>b</sup>	-0.05	-0.34 <sup>a</sup>	-0.12	-0.22	-0.05	-0.08	-0.03	0.43 <sup>b</sup>	0.04	0.22	0.08
3	Carnivores	-0.08	0.14	-0.01	0.11	0.02	0.02	0.04	0.28 <sup>a</sup>	0.32 <sup>a</sup>	0.08	0.36 <sup>b</sup>	-0.04
4	Piscivore	-0.03	0.15	0.03	-0.01	0.05	0.15	-0.07	0.03	0.13	-0.09	0.15	0.27 <sup>a</sup>
5	Sanguinivores	0.30 <sup>a</sup>	0.34 <sup>a</sup>	0.26	0.17	0.20	0.36 <sup>b</sup>	0.14	0.34 <sup>a</sup>	0.06	0.16	-0.10	0.11
6	Frugivores	0.48 <sup>c</sup>	0.32 <sup>a</sup>	0.48 <sup>c</sup>	0.43 <sup>b</sup>	0.45 <sup>c</sup>	0.36 <sup>b</sup>	0.40 <sup>b</sup>	0.24	-0.08	0.35 <sup>b</sup>	0.05	0.08
7	Nectarivores	0.16	0.07	0.16	0.30 <sup>a</sup>	0.32 <sup>a</sup>	0.08	0.35 <sup>b</sup>	0.11	-0.29 <sup>a</sup>	0.23	0.01	-0.16

<sup>a</sup>0.01 < p ≤ 0.05; <sup>b</sup>0.001 < p ≤ 0.01; <sup>c</sup>p ≤ 0.001.

cialization in primates, arrived at through a stepwise approach, and (iii) some evolutionary scenarios of trophic radiation in bats, based on a constrained search for the best hypothesis. We limited our inquiry to acyclic and unweighed models; the use of reticulated trees with branches of different lengths would have increased the possible number of models tremendously. This illustration also shows that when analyzing behavioral traits possessing more states, the number of distinguishable models quickly be-

comes intractable. Therefore, actual studies should be restricted, as much as possible, to testing hypotheses that rest on biological ground only.

### Acknowledgments

This work has been supported by NSERC grant No. OGP0007738 to P. Legendre.

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