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Linking the Investigations of Character Evolution and Species Diversification

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abstract: Variation in diversification rates is often studied by investigating traits related to species' ecology and life history. Often, however, it is unknown whether these traits evolve gradually or in punctuated bursts during speciation. Using phylogenetic data and species' present-day trait information, we present a novel approach to assessing the mode of character change while accounting for trait-dependent speciation and extinction. Our model, "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness), estimates both the rate of change occurring along lineages and the probability of change occurring during speciation, as well as independent speciation and extinction rates for each character state. Using simulations, we found that BiSSE-ness is able to distinguish along-lineage and speciational change and accurately estimate the parameters associated with character change and diversification rates. We applied BiSSE-ness to an empirical primate data set and found evidence for along-lineage changes in primate mating systems and social behaviors, whereas shifts in habitat were associated with speciation. In cases where trait changes may be linked to the speciation process itself (e.g., niche-related traits), BiSSE-ness provides a suitable framework with which to simultaneously address questions regarding species diversification and character change.

Keywords: macroevolution, speciation, phylogeny, punctuated equilibrium, primates.

Introduction

The mode of character evolution—whether traits change gradually along a lineage or rapidly at speciation—is a topic that has received considerable attention from evolutionary biologists and paleontologists (see review in Monroe and Bokma 2010). Darwin proposed that evolutionary change accumulates gradually via natural selection, resulting in the phenotypic differences we observe among species (Mayr 1982). However, since the publication of Eldredge and Gould's (1972) theory of punctuated equilibria, there has been increasing support for the concept that for some traits in some species, there are stretches of time where phenotypes remain relatively static that are interspersed with periods of rapid change coinciding with speciation. In cases of ecological speciation (Schluter 2001; Rundle and Nosil 2005), trait evolution may even drive the speciation process. For example, as niche-related traits diverge, reproductive isolation between two populations may occur, leading to ecological speciation.

Many studies have made use of the fossil record to demonstrate the tempo and mode of character change (Eldredge and Gould 1972; Cheetham 1986; Gould and Eldredge 1993; Benton and Pearson 2001), but the development of tools that use phylogenetic and species' present-day trait information has also furthered investigation of this topic (Monroe and Bokma 2010). To illustrate how phylogenies contain information regarding the mode of character change, Avise and Ayala (1975) reasoned that if change occurs gradually, in proportion to the age of a lineage, then we may expect a correlation between genetic differentiation (or, more generally, character differentiation) and the age of a lineage (i.e., branch length on a time-calibrated phylogeny). In this case, recently diverged species would appear relatively similar (Bokma 2002). This type of change occurring along a lineage, termed anagenetic change, has been detected by using phylogenetic methods in a variety of cases, including unison calls in cranes (family Gruidae; Mooers et al. 1999) and body size and shape in salamanders (genus *Plethodon*; Kozak et al. 2006; Adams et al. 2009).

In contrast, if character change tends to occur in punctuated bursts in association with speciation, then we may expect a correlation between character differentiation and the number of speciation events, and thus, recently diverged species may exhibit substantial differentiation (Avise and Ayala 1975; Mooers et al. 1999; Bokma 2002). Evidence for this type of "speciational" or cladogenetic change has been found in the evolution of body size in

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mammals (Mattila and Bokma 2008; Monroe and Bokma 2009) and birds (Bokma 2004), as well as pollen spur length in columbine flowers (genus *Aquilegia*; Whittall and Hodges 2007) and traits associated with the β-niche in rockfish (genus *Sebastes*; Ingram 2011).

Phylogenetic methods have been developed to investigate a wide range of questions regarding character evolution, including to test for correlated evolution between two traits (Pagel 1994; Maddison 2000; Pagel and Meade 2006), to infer ancestral character states (Losos 1999; Bokma 2008), and the focus of this study, to determine the tempo and mode of character evolution (Mooers et al. 1999; Oakley 2003; see also the review in Pagel 1999). Early phylogenetic methods for examining rates of character change (e.g., Pagel 1997; Mooers et al. 1999; Paradis 2005) posed several limitations, such as the assumptions that extinction has not occurred and character change occurs either entirely gradually or with speciation. A recent method allows for estimation of the speciation and extinction rates from the phylogeny as well as the relative rates of anagenetic and cladogenetic change for continuous characters, assuming that the characters do not influence the diversification rates (Bokma 2008). When this method was used to investigate body-size evolution in mammals, Mattila and Bokma (2008) found that a large portion of changes in body size were associated with speciation. Because fossil data suggest that large mammals have greater speciation and extinction rates (Liow et al. 2008; a trend not accounted for in Mattila and Bokma 2008), we might expect this higher rate of lineage turnover coupled with cladogenetic changes in body size to result in higher rates of body-size evolution in larger mammal lineages compared with smaller-bodied lineages. Monroe and Bokma (2009) found some support for this hypothesis by analyzing inferred changes in body size as a function of average body size, but only among older branches along which lineage turnover was plausible. Body-size evolution provides an interesting example of where the process of speciation and the rate of character change are interdependent in two ways: first, speciation may accelerate the rate of body-size evolution via cladogenetic change and, second, body size may affect the rates of speciation and extinction. To estimate these interdependent processes requires methods that allow cladogenetic and anagenetic character change and that explicitly allow the state of a character to influence speciation and extinction rates. We begin by developing such a method for a binary-state character, leaving for future studies the development of analogous methods for continuous characters.

Phylogenies have also been used extensively to address the relationship between species' traits and rates of diversification (Barraclough et al. 1998; Paradis 2005; Jablonski 2008; Purvis 2008). Maddison (2006) indicated the challenge of separating the effects a trait has on speciation and extinction rates from differences in the rates of character change. For example, sister-clade analyses, which compare the size of two clades that are, by definition, of the same age but differ in a trait of interest, have been used to draw an association between a particular state and a clade experiencing rapid or depressed diversification. However, it is possible that it is simply the rates of change between different character states that are asymmetric and the diversification rate is relatively constant (Maddison 2006). Subsequently, Maddison and colleagues (2007) developed the "Binary-State Speciation and Extinction" (BiSSE) model, which estimates the speciation and extinction rates separately for each state of a binary character, as well as the rate of change between states. As originally formulated, the BiSSE model accounts for only the character change that occurs along lineages. Here we modify the BiSSE model to allow for cladogenetic change in addition to anagenetic change, which thus enables us to address the mode of character change while simultaneously accounting for the effect the character may have on rates of speciation and extinction.

This new model, "BiSSE–node enhanced state shift" (BiSSE-ness), may be used to investigate a variety of questions regarding character evolution and diversification. Indeed, we have already used the BiSSE-ness model to examine the association between ploidy level and lineage diversification within angiosperm and seed-free vascular plant groups (Mayrose et al. 2011). Because a shift from diploidy to polyploidy is a well-studied mechanism of plant speciation (Coyne and Orr 2004; Wood et al. 2009), the BiSSE-ness model could account for changes in ploidy at speciation (Mayrose et al. 2011). The study by Mayrose and colleagues (2011) revealed that the diversification rate of polyploid lineages was significantly lower than that of their diploid congeners, and the use of the BiSSE-ness model allowed the authors to attribute this pattern of diversification in part to shifts from diploidy to polyploidy that occurred concurrently with speciation. Similarly, a model closely related to BiSSE-ness ("ClaSSE") was recently developed and has been applied to explore the role of self-compatibility versus incompatibility in the diversification of Solanaceae (Goldberg and Igić 2012). Again, the ability to self has long been thought to shift during the speciation process, thereby necessitating a model of diversification that allows for cladogenetic trait change.

The BiSSE-ness model may also be a useful tool for investigating the mode of change in ecological or niche-related traits and for assessing whether such changes might drive speciation. For example, pollinator shifts may contribute to speciation in columbine flowers (Whittall and Hodges 2007). Conversely, the degree of pollinator specialization may affect the overall rates of extinction and speciation (see references in Jablonski 2008). Other ecological and reproductive traits have also been associated with differential rates of diversification, such as host specificity and sexual dimorphism (see references in Jablonski 2008). Thus, the BiSSE-ness model provides a flexible framework in which questions regarding the mode of trait change and trait-dependent diversification may be tested simultaneously.

To be useful, however, the BiSSE-ness model must be able to distinguish cladogenetic and anagenetic trait change for traits that affect diversification while simultaneously estimating speciation and extinction rates. Here we generate simulated data sets to assess, for the first time, the power and accuracy of BiSSE-ness. We then apply the model to an empirical data set of 233 primate species, using a complete phylogeny. The evolution of primate diversity has long been studied, from a number of different perspectives and by using a variety of approaches, including paleontological and comparative methods. A wealth of information, including molecular phylogenies, ecological data, and life-history information, is available for primates. Nevertheless, many questions regarding primate evolution still remain. For example, the use of molecular data has revealed several significant shifts in diversification rate within primates that have not been explained by the traits examined to date, such as body size (Paradis 2005; Fabre et al. 2009). Several aspects such as the colonization of a new geographic area and shifts in activity period (i.e., from nocturnal to diurnal or vice versa) are thought to be important innovations that have allowed new niches to be occupied, potentially also impacting species diversification (Ankel-Simons and Rasmussen 2008; Fabre et al. 2009). We use BiSSE-ness to test some existing hypotheses concerning primate evolution, thereby illustrating the use of the model with empirical data. We consider five binary traits: activity period (diurnal/nocturnal), habitat type (forest-savanna/forest), mating system (nonmonogamous/monogamous), social behavior (solitary/social), and terrestriality (arboreal/terrestrial). Specifically, we investigate the degree to which changes in these traits occur along lineages or are concentrated at speciation, and we examine whether any of these five traits are associated with differential speciation and/or extinction rates.

BiSSE-ness Model

For a binary character (with states 0 and 1), the BiSSE model computes the combined probability of both a phylogeny and the corresponding character states of each terminal taxon having evolved precisely as observed, with the ancestor of the lineage in question in either state 0 or state 1 (Maddison et al. 2007; see the differential equations [A1] and [A2] in the appendix). Each state is associated with independent parameters for the rates of speciation (λ_0, λ_1) and extinction (μ_0, μ_1) and a change in state (q_{01} for 0 → 1 and q_{10} for 1 → 0). In addition to calculating the probabilities of a lineage evolving as observed from each possible root state (0 or 1), BiSSE also calculates the probability that a lineage becomes extinct before the present, given that it was in state 0 or 1 at time t (differential equations [A3] and [A4] in the appendix). The BiSSE model computes diversification and extinction probabilities by numerically solving these differential equations along each lineage and for each state, starting at the tips (present day) and working backward in time (for more detail, see Maddison et al. 2007).

At each node, the probabilities calculated for the two daughter clades are combined, yielding one probability pertaining to the joint clade. The BiSSE model makes the assumption that no change in state occurs simultaneously with speciation. Therefore, to calculate the probability (D) that a lineage just ancestral to node A at time t_A is in state 0 and evolved as observed, BiSSE multiplies the rate of speciation in state 0, λ_0, by the probability that both daughter lineages (N and M) are in state 0 and evolved as observed:

$$D_{A0}(t_A) = \lambda_0 D_{N0}(t_A) D_{M0}(t_A) \tag{1}$$

(from eq. [4a] in Maddison et al. 2007; similarly, eq. [4b] in Maddison et al. 2007 describes the case for state 1, $D_{A1}(t_A)$). At the root, the diversification probabilities are combined to give an overall likelihood, using the relative probabilities of observing the data, following FitzJohn et al. (2009; $D_{\text{root}} = D_0 \times D_0/(D_0 + D_1) + D_1 \times D_1/(D_0 + D_1)$).

To allow state change to occur simultaneously with a speciation event, we include four parameters to describe change at nodes, in addition to the 6 parameters used in BiSSE. Two of these new parameters are the probabilities that there is a change in character state associated with the speciation process and, thus, one or both of the daughter lineages are in the state opposite that of the ancestral lineage. These parameters, p_{0c} and p_{1c}, correspond to when the ancestral lineage is in state 0 and 1, respectively, and c represents a change in character state. The remaining two parameters, p_{0a} and p_{1a}, refer to the probability that, given a change in character state has occurred during speciation and the ancestral lineage is in states 0 and 1, respectively, this change is asymmetrical. That is, one daughter lineage retains the same state as the ancestral lineage (with a 50% probability of being the left lineage and a 50% probability of being the right lineage on the phylogeny), while the other lineage changes to the opposite state. Alternatively, the probability that both daughter lineages change state is given by $1 - p_{0a}$ when the ancestor was in state 0 and a change associated with speciation occurred.

When both p_c parameters are 0, the p_a parameters become irrelevant and the 10-parameter BiSSE-ness model reduces to the 6-parameter BiSSE model. To account for all possible changes that could occur during the speciation process, we revise equation (1), the equation for node calculations, as follows: Given that ancestor A is alive at time t_A and is in character state 0, the probability of accounting for the phylogeny and extant character states for both of the daughter lineages (N and M) is the sum of

$$D_{A0}(t_A) = \lambda_0 D_{N0}(t_A) D_{M0}(t_A)(1 - p_{0c}), \tag{2a}$$

for when there is no change at speciation, plus

$$+\frac{1}{2}\lambda_0[D_{N1}(t_A)D_{M0}(t_A) + D_{N0}(t_A)D_{M1}(t_A)]p_{0c}p_{0a} \tag{2b}$$

for when one lineage changes, plus

$$+\lambda_0 D_{N1}(t_A)D_{M1}(t_A)p_{0c}(1 - p_{0a}) \tag{2c}$$

for when both lineages change. By interchanging the 1s and 0s, we obtain a corresponding equation, $D_{A1}(t_A)$, to calculate the probability that the lineage was in state 1 just before node A. Even along branches of the tree where no nodes appear, speciation events may have occurred if one daughter lineage became extinct before the present, so we also update the differential equations describing diversification and extinction (eqq. [A1]–[A4] in the appendix).

The BiSSE-ness model may be used in a likelihood or a Bayesian framework, thereby generating the most likely parameter set (maximum likelihood estimates) or a distribution of parameter estimates (Bayesian posterior distribution). We focus on analyses using maximum likelihood (ML), but we also present the results of a Bayesian analysis of the primate data set. Our use of BiSSE-ness within the ML framework allows us to generate and rapidly analyze large numbers of data sets, using likelihood ratio tests. Alternatively, the use of BiSSE-ness with Bayesian methods provides an efficient way of assessing uncertainty in parameter estimates, as well as determining correlations among parameters when analyzing a specific data set (for a more in-depth discussion, see Huelsenbeck et al. 2002). The BiSSE-ness model was written and executed in R (R Development Core Team 2010), using the package diversitree (ver. 0.6-1; FitzJohn et al. 2009). The R code and numerical data used to produce the results presented in this article may be downloaded from Dryad (Dryad data: http://dx.doi.org/10.5061/dryad.2sr417nv). The BiSSE-ness model is now available as a built-in option within the R package diversitree (ver. 0.9-1; http://www.zoology.ubc.ca/prog/diversitree/; courtesy of R. FitzJohn).

Simulations

Methods

We simulated speciation, extinction, and character changes in order to test the accuracy and power of the BiSSE-ness model. To simulate phylogenies, we modified the "tree.bisse" function in the diversitree R package to allow for cladogenetic trait change. With this new function, we generated simulated data sets under a variety of different parameters, which allowed us to assess the performance of BiSSE-ness under a range of scenarios. We then used both the BiSSE and BiSSE-ness models to estimate the most likely parameter set for the simulated data. This allowed us to investigate how accurately and precisely the BiSSE-ness model can recover the true parameter values, as well as to infer the true mode of character change.

We conducted several sets of simulations in order to address two main questions. First, how often must character change occur with speciation for us to detect significant differences between BiSSE and BiSSE-ness? We explored this question by simulating trees under parameters that varied in both their values for the rate of anagenetic character change (q_{01} and q_{10}) and the probability of cladogenetic change (p_{0c} and p_{1c}) and then setting these parameters equal between states ($q_{01} = q_{10}$ and $p_{0c} = p_{1c}$; see set 1 in table 1). It has been suggested that traits that change at a high rate may obscure any signals of change associated with speciation (Cheverud et al. 1985; Mooers et al. 1999); therefore, one advantage to our approach is that we may compare cases with similar proportions of anagenetic and cladogenetic character changes but different numbers of total character changes.

Second, we investigated how accurately and precisely BiSSE-ness estimates the nature of trait changes at speciation. In particular, given that some amount of cladogenetic change occurs, how well can BiSSE-ness differentiate between symmetrical and asymmetrical state changes at a speciation event? To address this, we simulated trees under various p_a values in combination with low, medium, and high levels of cladogenetic change (p_c), and set all remaining parameters to constant values (set 2 in table 1). We also examined how BiSSE and BiSSE-ness compare in their abilities to estimate accurately the rate parameters pertaining to speciation (λ), extinction (μ), and along-lineage character change (q).

For the tree simulations, the initial character state at the root was determined by drawing randomly according to the equilibrium frequencies of states 0 and 1 determined by the model (see eq. [A6] in the appendix). For each parameter set, 10 trees were simulated. Each simulation proceeded until the desired number of species plus 1 was generated; the tree was then pruned back to before the last node, so that the tree contained the correct number

Table 1: Parameter sets under which trees were simulated with the "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness) model

Model parameter	Definition	Set 1	Set 2
λ_0	Speciation rate, state 0	.1	.1
λ_1	Speciation rate, state 1	.2	.2
$\mu_0 = \mu_1$	Extinction rate	.003	.003
$q_{01} = q_{10}$	Rate of character state change along lineages	.005, .01, .05, .1	.01
$p_{0c} = p_{1c}$	Probability of character change at speciation	0, .0001, .0005, .001, .005, .01, .05, .1, .5, 1	.005, .05, .5
$p_{0a} = p_{1a}$	Probability of asymmetrical character change	.5	.1, .3, .5, .7, .9

Note: Set 1 was used to determine the ability of the BiSSE-ness model to distinguish speciational and along-lineage change. Set 2 was used to explore the ability of the BiSSE-ness model to differentiate between asymmetrical versus symmetrical change at speciation. Both sets 1 and 2 were used with 500-species trees; for the 50- and 200-species trees, we used a subset of parameter set 1, where $p_c = 0$, .005, .05, .5, 1 and $q_{01} = q_{10} = 0.01$. For each set, all combinations of parameters were simulated and replicated 10 times.

of taxa and all terminal branch lengths were nonzero. We first considered large data sets involving 500-species trees, simulating 10 trees for each of 55 unique parameter sets (in table 1: set 1, 40 sets; set 2, 15 sets). To examine the effect of using a smaller phylogeny, we also simulated trees with 50 and 200 species, using a subset of the set 1 parameters ($p_c = 0$, 0.005, 0.05, 0.5, 1 and $q_{01} = q_{10} = 0.01$). Again, for each of these 10 unique parameter sets, 10 independent simulations were performed. Thus, in total, we analyzed 650 phylogenetic trees (550 with 500 species, 50 with 200 species, and 50 with 50 species).

Although our parameter sets included trait-dependent diversification ($\lambda_1 > \lambda_0$), the BiSSE-ness model may also be used in cases where diversification is independent of the trait in question. In this case, BiSSE-ness may be considered as the discrete analog to the methods developed by Bokma (2008) for continuous traits. Additionally, the BiSSE-ness model is fully compatible with BiSSE methods that account for incomplete taxon sampling, incomplete trait information, and unresolved clades (see FitzJohn et al. 2009). These methods are important, as they allow the application of BiSSE (and BiSSE-ness) to a much larger number of data sets, including those in which not all species have been incorporated into a phylogeny. Previous simulations with BiSSE have shown that little loss of power occurs as long as the missing data are properly included in the likelihood calculations (FitzJohn et al. 2009); however, we do not test these issues here.

We conducted likelihood searches in a stepwise fashion to facilitate reaching the maximum likelihood point. The starting parameter values for the likelihood searches using BiSSE were determined from a heuristic search that makes use of the state-independent birth-death model (the "starting.point.bisse" function in the diversitree R package). We then used the ML estimates of BiSSE with all p parameters set to 0 as starting parameters for the BiSSE-ness likelihood search. In order to compensate for a potential bias in the BiSSE-ness estimates toward BiSSE parameter estimates due to these starting conditions, we also used a second starting condition with the ML parameter estimates obtained from a BiSSE-ness likelihood search where the values for the rates of character change along lineages (q_{01} and q_{10}) were constrained to 0. The starting values for probabilities of cladogenetic change (p_{0c} and p_{1c}) were set to the values for q_{01} and q_{10} determined by starting.point.bisse, and p_{0a} and p_{1a} were set to 0.5. Because the q parameters are rate estimates based on the tree root-to-tip distance and the p_c parameters are probabilities, the starting values for p_{0c} and p_{1c} are somewhat arbitrary; however, we do not expect this to impact the overall results of our analyses.

We found that the BiSSE-ness ML searches were largely insensitive to the two different starting points (corresponding to the reduced models where all state changes occur along lineages or at speciation) and converged on the same ML and parameter estimates. However, when the data were simulated with high proportions of speciational change, we found that higher ML estimates were sometimes found when we used the starting point that assumed speciational change. All subsequent analyses were completed using output from the BiSSE-ness search that yielded the most likely parameter combination. Likelihood ratio tests were performed between BiSSE and BiSSE-ness by comparing twice the log likelihood with a χ^2 distribution with 4 degrees of freedom (accounting for the two p_c and two p_a parameters).

Results

We first consider the simulated trees that had 500 species each. For these trees, the tree depths (distances from root to tip) were approximately log-normally distributed and averaged 36.44 ± 7.64 (±SD) units of time. The root-to-tip distance of the phylogeny may be multiplied by the rate parameters to estimate the approximate number of events that occurred in a single lineage over time. For example, when λ_1 is estimated to be 0.1 and the tree depth is 30, we expect a single lineage to undergo approximately three speciation events throughout its history.

Among the data sets in which character change occurred both along lineages and at speciation (273/400 simulations in set 1, excluding cases where no speciational change occurred during the simulated history of the clade, which happened frequently when $p_c < 0.001$), we found that BiSSE-ness was able to infer the mode of character change with reasonably high power under the conditions simulated. When at least 10% of the total character change occurred at speciation ($n = 181$), BiSSE-ness provided a significantly better fit than BiSSE 59% of the time ($P < .05$, likelihood ratio test). As proportionately more of the character changes occurred at speciation rather than along lineages (moving to the right in fig. 1), the ML parameter estimates obtained using BiSSE-ness became increasingly more likely than those obtained by BiSSE. Thus, the higher the degree of cladogenetic change, the more power BiSSE-ness has to distinguish between modes of character change. In cases where cladogenetic change had occurred but BiSSE was not rejected in favor of BiSSE-ness (despite the latter being the true model), the proportion of character change that occurred at speciation averaged 17% and the median proportion was just 7.5% ($n = 166$).

Conversely, when there was no character change at speciation ($n = 127$), the BiSSE-ness and BiSSE models returned statistically indistinguishable likelihoods. Typically, we would expect BiSSE-ness to yield significantly higher likelihoods than BiSSE for ~5% of cases, corresponding to our critical P value, 5%. Our observation of significantly fewer Type I errors than expected ($P = .003$, two-tailed binomial test) provides evidence that the use of a likelihood ratio test with 4 degrees of freedom is conservative. This is not unexpected, given that the p_a parameters are conditional on cladogenetic change and contribute weakly to the overall

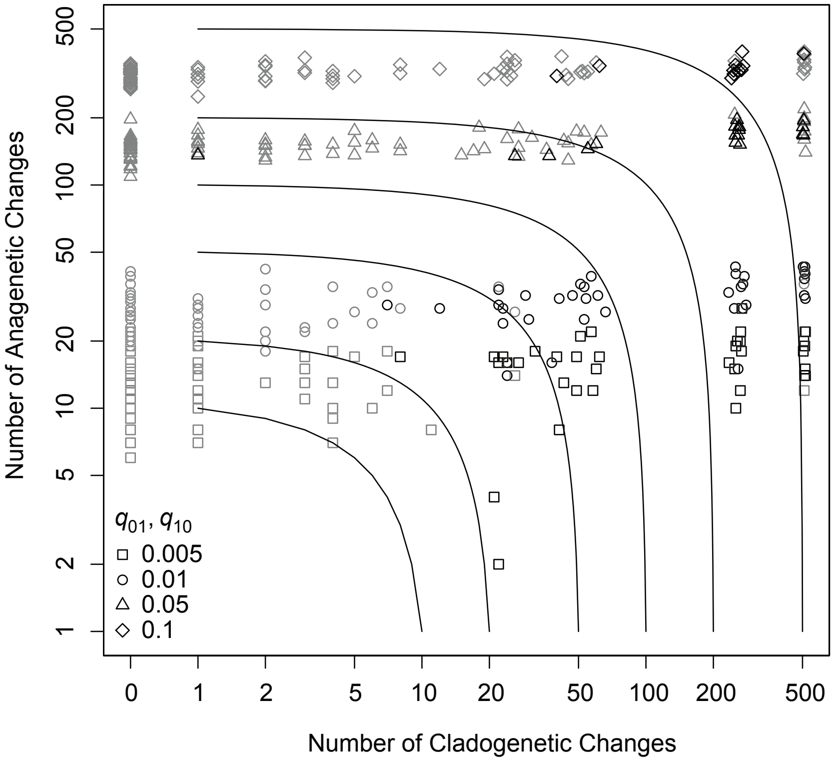

Figure 1: Amount and type of character change for each of the simulated trees in set 1. The *X*- and *Y*-axes give the actual number of events that occurred during the simulation, excluding extinct lineages. Cases in which our model, "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness) significantly outperformed the "Binary-State Speciation and Extinction" model (BiSSE) are indicated in black ($P < .05$, likelihood ratio test, df = 4); cases where BiSSE-ness did not significantly improve the fit are shown in gray. Cases in which cladogenetic change did not occur in the simulated history ($p_c = 0$ or, by chance, when p_c was small) are also included (placed at 0 along the otherwise logged *X*-axis). Curves show a constant total number of character changes.

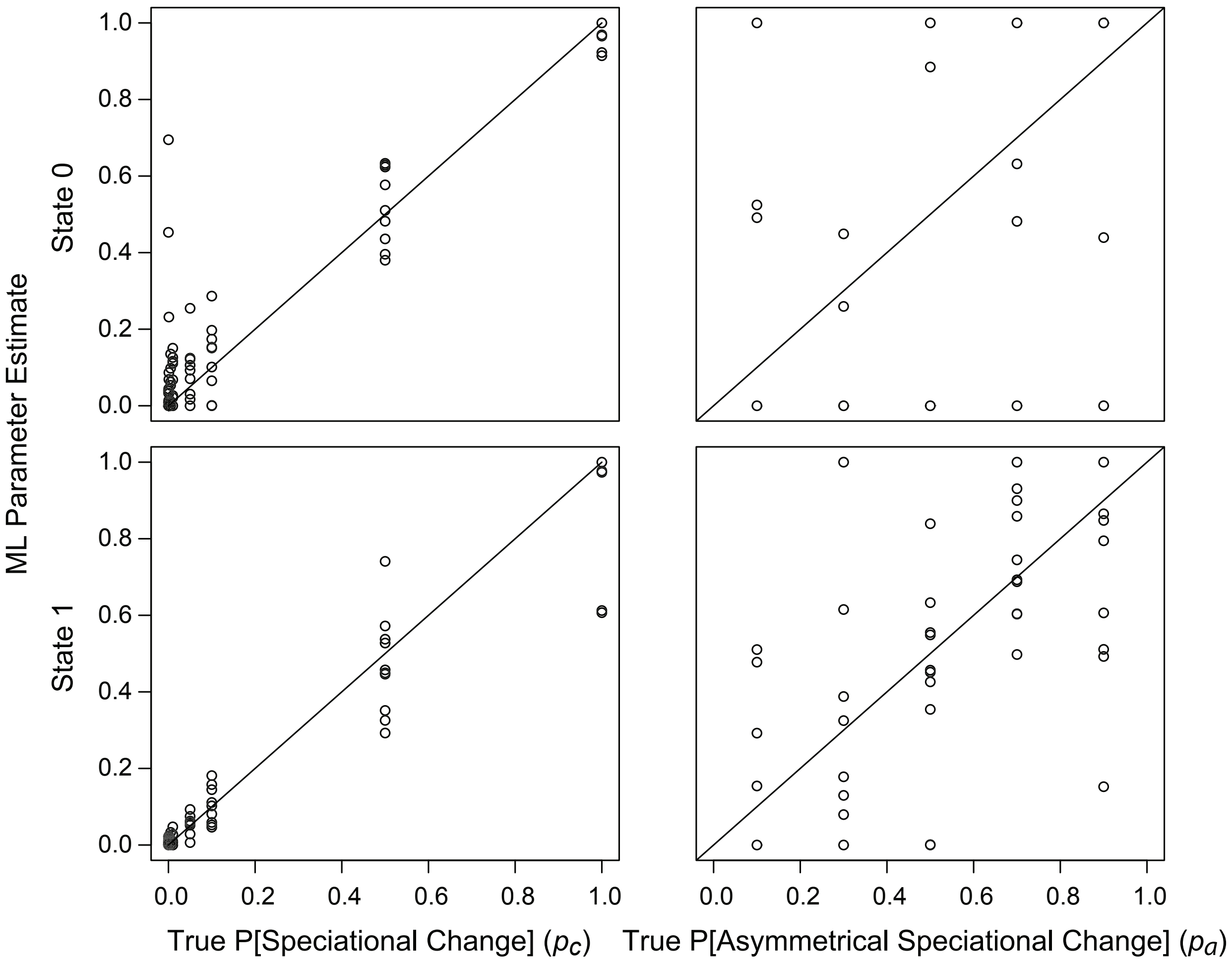

Figure 2: Parameter estimates associated with cladogenetic character change. For each parameter value on the *X*-axis, the 10 points show the inferences from 10 independently simulated trees. Our model, "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness), accurately and precisely estimated the probability of speciational character change for simulated data with varying p_{0c} and p_{1c} values (*left*: $p_{0c} = p_{1c}$, $p_{0a} = p_{1a} = 0.5$, $q_{01} = q_{10} = 0.01$). The BiSSE-ness model also provided accurate estimates, on average, of the degree of asymmetrical character change, p_{0a} and p_{1a} (*right*: $p_{0a} = p_{1a}$, $p_{0c} = p_{1c} = 0.05$, $q_{01} = q_{10} = 0.01$), although with considerably more scatter (less precision) in the individual estimates than with the p_c parameters. Lines indicate the 1 : 1 ratio; that is, if all of the estimates fell on the lines, then BiSSE-ness would have perfectly recovered the actual parameter values under which the data were simulated.

likelihood of the data (see below). When we conducted the likelihood ratio test with 2 degrees of freedom, the test remained conservative (0% Type I errors). When only 1 degree of freedom was used, slightly more than the desired number of Type I errors were observed (8/127 = 6.3%). Thus, to avoid an overly conservative likelihood ratio test (but while remaining conservative), we recommend that BiSSE-ness be compared with BiSSE using 2 degrees of freedom, preferably with the p_a parameters set either to 0.5 (if the user is agnostic about the possibility of transitions in one or both daughter species) or to 0 or 1 (if an assumption that only symmetric or only asymmetric cladogenetic shifts can be biologically justified).

The BiSSE-ness model was able to estimate the probability of speciational character change, p_c, with both high accuracy and high precision (fig. 2). In contrast, the p_a parameters, which describe the probability that only one daughter lineage exhibited character change, were on average estimated reasonably accurately, but with considerably less precision. This is likely due to a lack of statistical power, because these parameters are conditional on the occurrence of speciation change. Because λ_1 is greater than λ_0 in all of our simulations, more speciation events occurred when the ancestral lineage was in state 1. Consequently, the p_a parameters were estimated more precisely for state 1 (fig. 2). As the relative proportion of speciational change increased, both the p_c and p_a parameters were estimated with more precision (data not shown).

Both models were able to estimate the diversification parameters with a high degree of accuracy (fig. 3). As the probability of cladogenetic character change approached 1, each model became less precise in estimating the effect of the trait on speciation and extinction, because the amount of character change approached saturation. However, BiSSE tended to produce more variable estimates than BiSSE-ness. As the amount of cladogenetic change increased, the likelihood surface for the rate of along-lineage character change became increasingly flat using BiSSE-ness, making it difficult to distinguish the true rate of anagenetic change from 0 (see wide horizontal spread in fig. 3, right panels). In contrast, BiSSE tended to overestimate anagenetic change, because the changes that occurred at speciation were mistaken for along-lineage changes. In the extreme case where character change always occurred at speciation ($p_{0c} = p_{1c} = 1$), the character states became essentially randomized on the tree; in this worst-case scenario, BiSSE tended to estimate very high values of anagenetic change.

Similar results were obtained when analyzing phylogenies consisting of 50 and 200 species, although parameter precision and statistical power declined, especially for the 50-species trees (figs. S1–S4, available in a PDF). Again, when cladogenetic changes made up 10% or more of the total character shifts ($n = 34$ for the 50-species trees; $n = 33$ for the 200-species trees), BiSSE-ness performed significantly better than BiSSE 9% of the time with 50 species and 48% of the time with 200 species ($P < .05$; see fig. S1 in the PDF). This result demonstrates that the power to detect cladogenesis increases with clade size. When there was no character change at speciation, BiSSE-ness never returned a significantly higher likelihood than BiSSE (i.e., no Type I errors with 4 degrees of freedom), although our sample sizes were low (only 16 and 15 simulations involved no cladogenetic change with 50 and 200 species, respectively).

Overall, our simulation results indicate that BiSSE-ness can infer the mode of character change with reasonable power given large phylogenetic trees. Interestingly, when the wrong model was used (e.g., cladogenetic change was present but ignored by BiSSE), estimated rates of speciation and extinction were not strongly biased, although the parameters describing trait change were (fig. 3).

Primate Diversification

Methods

Because they are a large, well-studied, monophyletic group, primates provide us with an excellent opportunity to study trait evolution. Primates have a well-established phylogeny consisting of 233 species (Vos and Mooers 2006), and for a high proportion of these species, many aspects of the biology and ecology are well studied. In general, mammal diversity is thought to be associated with ecological divergence (Bininda-Emonds et al. 2007; Fabre et al. 2009). For example, Malagasy primate diversity has been associated with climatic niche divergence (Kamilar et al. 2010). If speciation is the result of a shift into novel niche space (Funk et al. 2006), then we expect that accounting for character change at speciation events may improve the fit of the model to the data. We test this explicitly by examining five traits related to primate species' behavior, reproduction, and ecology and comparing output from the BiSSE and BiSSE-ness models.

The five binary traits represent different axes of primate niche space (data were taken directly from appendix S2 of Redding et al. 2010): activity period (diurnal/nocturnal; $n = 233$), habitat type (forest-savanna/forest; $n = 215$), mating system (nonmonogamous/monogamous; $n = 146$), social behavior (solitary/social; $n = 205$), and terrestriality (arboreal/terrestrial; $n = 197$; fig. 4). There is evidence that shifts in activity period (from nocturnal to diurnal and vice versa) have occurred several times over the course of primate evolution (Ankel-Simons and Rasmussen 2008). These shifts are thought to be associated with filling empty diurnal and nocturnal niches (Ankel-Simons and Rasmussen 2008). Changes in habitat type and terrestriality are also likely to have been the result of species adapting to novel niches (Conroy 1990). Less is known about how changes in mating system and social behavior are related to niche structure in primates, because these features cannot be explicitly analyzed in fossils and there may be considerable variability in both traits even within species (Dixson 1998; Kappeler and van Schaik 2002), but both may be involved in the early steps of speciation as isolating mechanisms.

We used a recently constructed primate supertree (Vos and Mooers 2006) for our analyses. Because this tree contains several polytomies (representing phylogenetic uncertainty) that are not allowed in the BiSSE or BiSSE-ness models, we followed FitzJohn (2010) and used a set of 10,001 fully resolved trees that had been generated from the supertree, using methods developed by Kuhn et al. (2011; fig. 4). In order to account for phylogenetic uncertainty, 20 phylogenies were randomly selected from this set of primate phylogenies for use in the maximum likelihood searches (note that this set reflects only alternative resolutions of polytomies that exist in the primate supertree, and not the full posterior distribution of trees). In our experience with this data set, the level of uncertainty in parameter estimation is much greater than the variation observed among phylogenies, as indicated by the posterior distribution of parameter estimates generated using Bayes-

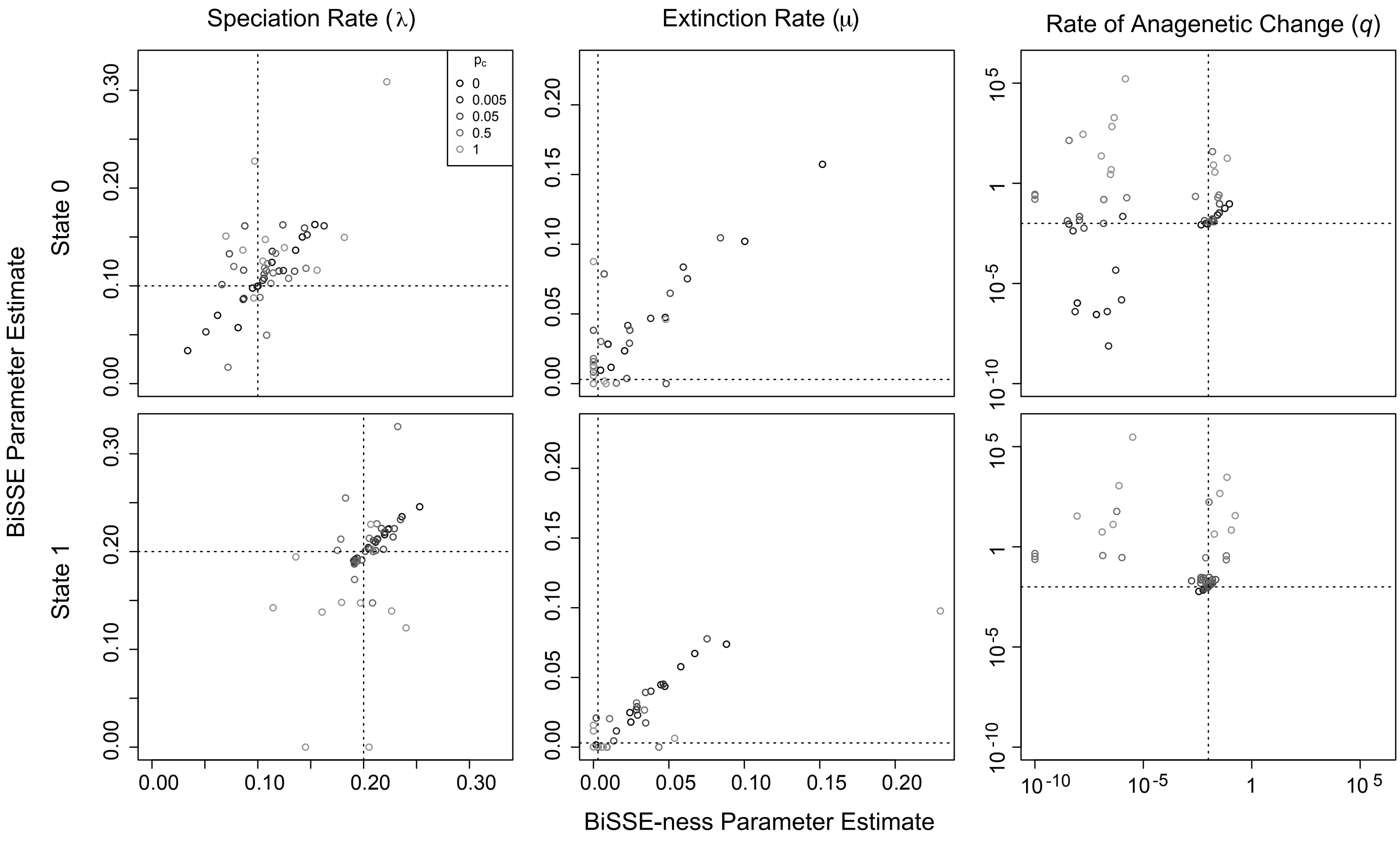

Figure 3: Comparing ML parameter estimates between our model, “Binary-State Speciation and Extinction–node enhanced state shift” (BiSSE-ness), and the “Binary-State Speciation and Extinction” model (BiSSE; a subset of set 1; $q_{01} = q_{10} = 0.01$). Crosshair lines indicate the true parameter values under which the data were simulated. Allowing character change at speciation events ($p_c > 0$) had relatively little effect on the mean estimates of speciation and extinction rates. The precision of the estimated speciation rates decreased, however, as the amount of speciational change increased under both BiSSE-ness (λ_0: SD = 0.037 and 0.048 for $p_c = 0$ and 1, respectively) and BiSSE (λ_0: SD = 0.041 and 0.064 for $p_c = 0$ and 1, respectively; similar patterns for λ_1). Note the use of a logarithmic scale for the rates of anagenetic character change (*top*: 0 → 1; *bottom*: 1 → 0).

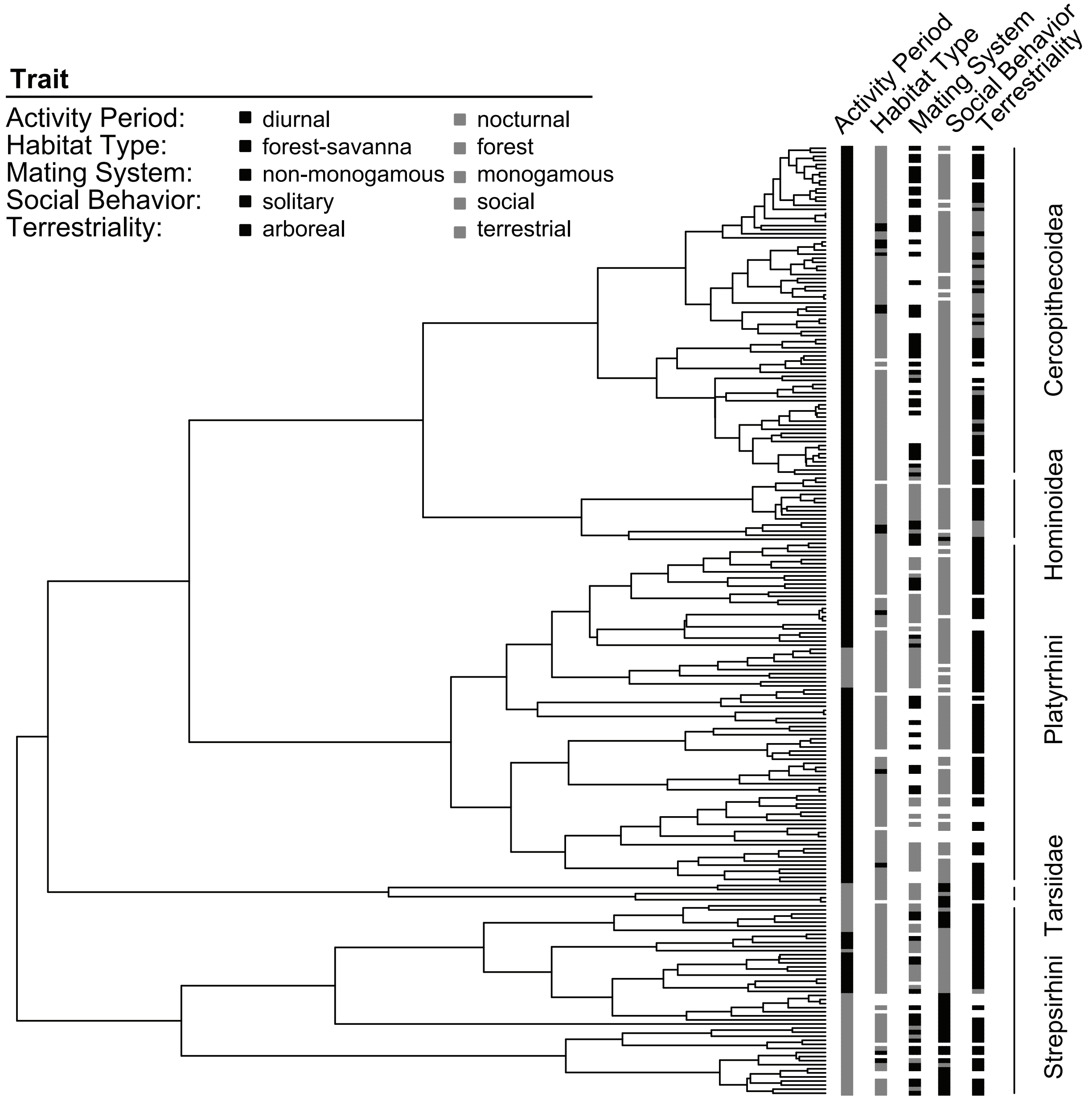

Figure 4: One of the 20 primate phylogenies used in our analyses that were randomly selected from the set of 10,001 resolved trees ($n = 233$). The five character traits used in this study are also shown.

ian Markov chain Monte Carlo (MCMC) searches (see below). Consequently, all results presented are mean values across the 20 trees, and we present average P values over trees, indicated as $\overline{P}$ (individual tree analyses are available upon request). In addition, we found similar significance levels and parameter estimates when using MCMC and ML searches, and so we focus on the ML results for brevity.

To investigate the importance of the mode of character change, we compared two extreme cases: when character change occurs only along lineages (BiSSE) and when it occurs only at speciation (BiSSE-ness with q parameters set equal to 0). Because these are not nested models, we used Akaike Information Criterion (AIC) scores and Akaike weights (Burnham and Anderson 1998), rather than likelihood ratio tests, to compare these models. We found that the degree of asymmetrical versus symmetrical speciational change did not have a large impact on the overall likelihood of the data, because there were negligible differences between the full 10-parameter BiSSE-ness model and the 8-parameter BiSSE-ness model where speciational change is equally as likely to occur symmetrically or asymmetrically ($p_{0a} = p_{1a} = 0.5$; $\overline{P} > .05$ for all traits except terrestriality, where $\overline{P} = .041$, df = 2; likelihood ratio test). In addition, we explored a reduced BiSSE-ness model (with 6 parameters) where character change occurs only at speciation events and could equally affect one or both daughter lineages ($p_{0a} = p_{1a} = 0.5$, with $q_{01} = q_{10} = 0$). We expected that the 6-parameter BiSSE and BiSSE-ness models should be comparable representations of contrasting modes of character change. Overall, we analyzed two models in which character change can occur either along lineages or at speciation and two models in which character change can occur both along lineages and at speciation (the 10-parameter BiSSE-ness model and the 8-parameter BiSSE-ness model, where $p_{0a} = p_{1a} = 0.5$).

We also used BiSSE and BiSSE-ness to investigate whether these traits are associated with differences in speciation and extinction rates. Shifts in diversification rates within primate groups have been well studied and are supported by both molecular and fossil evidence (Fabre et al. 2009), and several traits are thought to have influenced rates of primate diversification. Matthews et al. (2011) recently used BiSSE and found that large-bodied primates are associated with higher rates of speciation. Primate species with small geographic ranges, those with "slow" life-history traits (such as small litter size and late sexual maturity), and island endemics are also thought to exhibit higher current extinction risks than other primates (Purvis et al. 2000). For the traits studied here, nonmonogamous mating systems may facilitate sexual selection, which may in turn increase the rate of speciation because sexual isolation may occur more readily (Coyne and Orr 2004). Diurnal activity has been associated with increased current extinction risk (Purvis et al. 2000), and social behavior is thought to increase extinction risk, as Allee effects may be more severe in social than in solitary species (Courchamp et al. 1999).

As with the simulation analyses, we conducted all BiSSE-ness ML searches with the primate data set, using two opposing sets of starting parameters corresponding to the restricted models that allow character change only along lineages or only at speciation. Again we found that the resulting maximum likelihood point and associated parameter estimates typically converged on the same values (the likelihood values identified by each of the ML searches were always within 0.12% of each other). All subsequent analyses made use of the search that yielded the most likely parameter combination.

Results

Overall, when we compared AIC scores and Akaike weights for the four models of character change, we found that shifts in habitat type are likely to occur during speciation whereas shifts in mating system and social behavior occur primarily along lineages. For habitat type, when we compared models of equal complexity, those with cladogenetic change only (6-parameter BiSSE-ness model) yielded better AIC scores than those with anagenetic change only (BiSSE model), and by a considerable margin (ΔAIC = 6.77; table 2; Akaike weight, 83.0%; fig. 5). As we increased the number of free parameters in the BiSSE-ness model, the AIC scores became progressively worse. The 95% confidence set based on Akaike weights for habitat type included the restricted 6- and 8-parameter BiSSE-ness models.

For mating system and social behavior, we found that BiSSE typically outperformed BiSSE-ness, indicating that changes in these traits predominantly occur along lineages at rates proportional to time (BiSSE) rather than number of speciation events. When comparing BiSSE with the next-best-fitting model, the 8-parameter BiSSE-ness model accounting for anagenetic and cladogenetic change, the change in AIC was 3.93 and 3.79 log-likelihood units for mating system and social behavior, respectively (table 2). Together, these two models make up the 95% confidence set for both mating system and social behavior, with the remaining BiSSE-ness models contributing less than 4% of the Akaike weights for both traits (fig. 5).

For activity period and terrestriality, we did not detect strong evidence for or against cladogenetic change. For activity period, the BiSSE-ness model of cladogenetic change only (6 parameters) produced better AIC scores than did the model of anagenetic change only (Akaike weight, 57.2%), but the difference in AIC was minor (1.09; fig. 5). The 95% confidence set based on Akaike weights included all models except the full 10-parameter BiSSE-ness model. Similarly, we found that the terrestriality data could be well explained by models of both anagenetic and cladogenetic change (the full BiSSE-ness model) and pure anagenetic change (BiSSE; Akaike weights, 46.4% and 25.6%, respectively; fig. 5). Because the 95% confidence set includes all four models for terrestriality and all models except the most parameter-rich BiSSE-ness model for activity period, we remain uncertain as to how changes in these traits have occurred in primates.

To further examine the patterns of cladogenetic change in habitat type and anagenetic change in mating system and social behavior, we performed several likelihood-ratio tests. For habitat type, the model that included cladogenetic change (8-parameter BiSSE-ness model) performed significantly better than the model that included only an-

Table 2: Parameter estimates based on maximum likelihood analyses

Trait, model, no. parameters estimated	ΔLog likelihood	Speciation		Extinction		Anagenetic change		Cladogenetic change			
		λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	p_{0c}	p_{1c}	p_{0a}	p_{1a}
Activity period:[a]											
BiSSE:											
6	−1.11	.20	.12	.077	.072	.0010	.0048	0	0	...	...
BiSSE-ness:											
5	−2.53	.18	.18	.050	.15	0	0	.0056	.015	.5	.5
6[b]	−.57	.20	.12	.085	.075	0	0	.0041	.034	.5	.5
8	−.57	.20	.12	.085	.074	2.4×10^{-7}	4.1×10^{-7}	.0041	.034	.5	.5
10	0	.20	.12	.083	.069	6.2×10^{-8}	7.9×10^{-8}	.0037	.032	4.0×10^{-6}	4.5×10^{-6}
Habitat type:[c]											
BiSSE:											
6	−3.68	.23	.19	.0029	.12	.30	.0095	0	0	...	...
BiSSE-ness:											
5	−.47	.19	.19	.12	.10	0	0	.67	.042	.5	.5
6[b]	−.30	.27	.18	.080	.11	0	0	.72	.040	.5	.5
8	−.22	.20	.19	.0059	.11	.14	4.1×10^{-9}	.54	.041	.5	.5
10	0	.17	.19	.10	.11	.075	1.4×10^{-9}	.53	.039	1.0	.44
Mating system:[d]											
BiSSE:											
5	−3.84	.18	.18	.024	.13	.035	.014	0	0	...	...
6[b]	−.24	.24	.12	.12	.038	.021	.030	0	0	...	...
BiSSE-ness:											
6	−4.46	.25	.14	.16	.062	0	0	.068	.16	.5	.5
8	−.20	.24	.12	.12	.038	.021	.030	.0014	.00051	.5	.5
10	0	.24	.12	.13	.035	.017	.030	.013	8.1×10^{-7}	1.0	.45
Social behavior:[e]											
BiSSE:											
5	−1.33	.17	.17	.14	.027	.0087	7.1×10^{-8}	0	0	...	...
6[b]	−.15	.11	.19	.066	.049	.014	1.3×10^{-7}	0	0	...	...
BiSSE-ness:											
6	−3.87	.13	.20	.087	.081	0	0	.092	.00074	.5	.5
8	−.050	.11	.19	.066	.049	.014	5.5×10^{-8}	.0020	1.1×10^{-7}	.5	.5
10	0	.11	.19	.066	.049	.013	7.4×10^{-6}	.0036	9.6×10^{-8}	.71	.65
Terrestriality:[f]											
BiSSE:											
6	−4.59	.17	.25	.11	2.2×10^{-6}	.0027	.15	0	0	...	...
BiSSE-ness:											
6	−5.11	.16	.33	.097	1.9×10^{-6}	0	0	.013	.47	.5	.5
8	−3.30	.16	.29	.097	9.3×10^{-7}	.0025	.070	3.5×10^{-7}	.27	.5	.5
9	−5.07	.20	.20	.14	2.8×10^{-6}	.0017	.081	.0013	.25	1.0	.95
10[b]	0	.16	.32	.093	6.1×10^{-7}	.0015	4.5×10^{-7}	6.5×10^{-7}	.49	.65	1.0

Note: All estimates are averaged across all 20 trees. The difference in log likelihoods between the full "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness) model and all submodels, including "Binary-State Speciation and Extinction" (BiSSE), is also given. Change in the Akaike Information Criterion value (ΔAIC) can be calculated as twice the difference in log likelihood (Δlog likelihood) minus twice the difference in number of parameters. The models with 5 or 9 parameters restrict the speciation rates to be equal between states based on the model of character change that has the lowest AIC value for each trait.

[a] Diurnal = 0, nocturnal = 1.

[b] Model with the lowest AIC value among the models without constraints on the speciation and extinction rates.

[c] Forest/savanna = 0, forest = 1.

[d] Nonmonogamous = 0, monogamous = 1.

[e] Solitary = 0, social = 1.

[f] Arboreal = 0, terrestrial = 1.

agenetic change (BiSSE; $\overline{P} = .032$; df = 2; see also fig. S5 in the PDF). Similarly, when comparing the BiSSE-ness model that restricts change to occur only at speciation (6 parameters) with the model that allows both cladogenetic and anagenetic change (8-parameter BiSSE-ness), we found that allowing for along-lineage change provides significantly better fits to the mating system and social behavior data sets ($\overline{P} = .021$ and .034, respectively; df = 2). However, when we correct for multiple comparisons (Bonferroni $\alpha = 0.01$ with five traits or using sequential Bonferroni; Holm 1979), these results are just marginally significant. As in our simulation study, we generally ob-

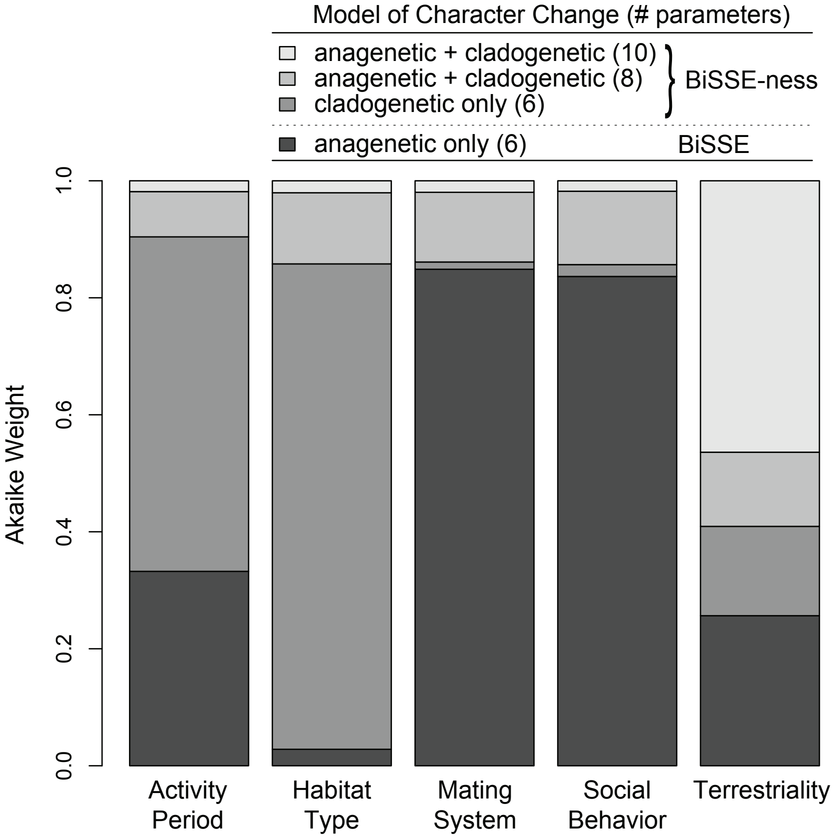

Figure 5: Comparing Akaike weights of the "Binary-State Speciation and Extinction" model (BiSSE) and our model, "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness), averaged over 20 trees, for each of the five traits. To compare models of equal complexity (i.e., equal number of parameters), a 6-parameter BiSSE-ness model was constructed such that $q_{01} = q_{10} = 0$ and $p_{0a} = p_{1a} = 0.5$. In addition, we explored an 8-parameter model that permitted character change both along lineages and at speciation (again holding $p_{0a} = p_{1a} = 0.5$). Finally, we explored the full 10-parameter BiSSE-ness model, which estimates the proportion of speciational change that occurred in one or both lineages (p_{0a}, p_{1a}).

tained similar diversification parameter estimates across all models and traits, especially for the speciation rates (see table 2).

We also examined differential rates of speciation and extinction against reduced models in which the speciation and extinction rate parameters were constrained to be equal between character states. We completed these analyses for each of the four different models of character change discussed above. Overall, using different models of character change did not have a substantial impact on the detection of differential rates of speciation or extinction (data available upon request). Thus, the $\overline{P}$ values and rate estimates below pertain to the best model for each trait, as determined by the AIC score averaged across the set of 20 trees (see parameter estimates in table 2).

In addition, we performed MCMC analyses for 2,000 steps for each of the 20 trees. We then concatenated the final 1,000 steps for each tree (20,000 steps in total) to obtain a posterior probability distribution. The MCMC posterior distributions of rate estimates for all traits under the full 10-parameter BiSSE-ness model are illustrated in figure 6. We used an exponential prior for all rate parameters (with a mean equal to twice the state-independent speciation rate; as discussed in Johnson et al. 2011) and a uniform prior from 0 to 1 for the parameters that describe cladogenetic change. Uncertainty in each parameter estimate is given by the 95% highest posterior density interval of the posterior distribution (see bars along the horizontal axis in fig. 6 and also table S1 in the PDF).

We found that the models that account for differential speciation performed substantially better for three traits: activity period, mating system, and terrestriality. That is, for these traits, the models that included separate speciation rates for each state performed significantly better than the reduced models where a single speciation rate was estimated (table 2). We found that diurnal species tended to have

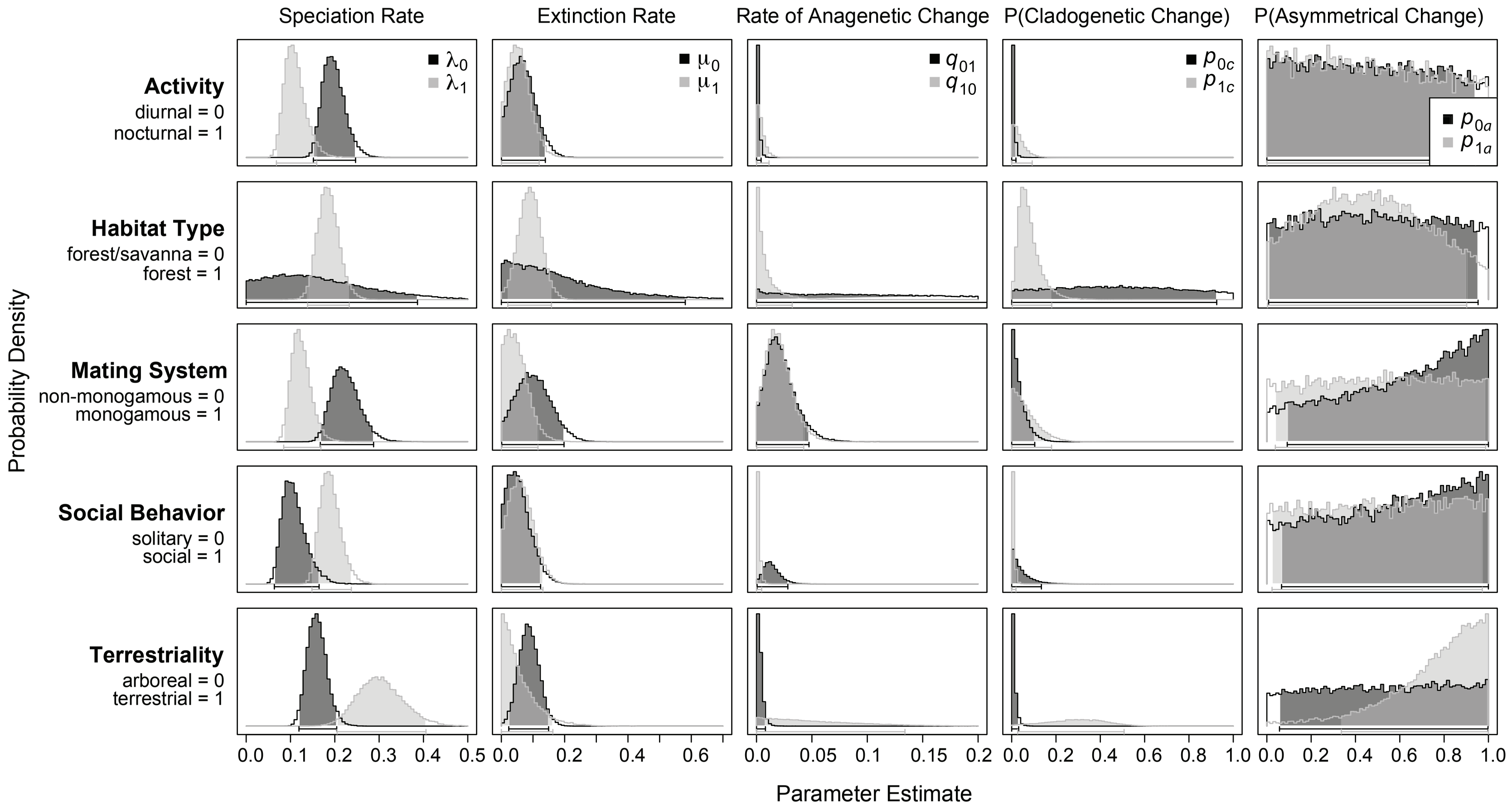

Figure 6: Posterior distributions of the parameter estimates using the full 10-parameter "Binary-State Speciation and Extinction–node enhanced state shift" (BiSSE-ness) model for the five traits and across the 20 trees included in the primate diversification analysis. The bars beneath each plot correspond to the shaded areas, indicating the 95% highest posterior density interval (see table S1, available online in a PDF).

speciation rates that were almost twice as high as those of nocturnal species (mean $\lambda = 0.20$ and 0.12, respectively; table 2), a difference that is marginally significant under the best-fitting model (the BiSSE-ness 6-parameter model with cladogenetic change only; $\overline{P} = .055$). Our results suggest that primate species that are classified as polygynous, polyandrous, or polygynandrous tended to speciate at a rate almost two times higher than that of monogamous species (mean $\lambda = 0.24$ and 0.12, respectively; $\overline{P} = .0073$ under the best-fitting BiSSE model; table 2). Finally, terrestrial species had significantly higher speciation rates than those of arboreal species (mean $\lambda = 0.32$ and 0.16, respectively; $\overline{P} = .0014$ under the best-fitting 10-parameter BiSSE-ness model; table 2). However, the effect of terrestriality on speciation was only marginally significant ($\overline{P} = .085$) under BiSSE, which also had a substantial Akaike weight (25.6%). Although habitat type and social behavior were not significantly associated with differences in speciation rates, λ_0 and λ_1 were estimated consistently across the set of phylogenies and across models (see table 2). For primate mating system and terrestriality, these results remain significant after correction for multiple comparisons (under the best-fitting model; Holm 1979).

We did not observe any association between species' traits and rates of extinction under the BiSSE and BiSSE-ness models. As a consequence, results for diversification rates ($\lambda - \mu$) largely mirror those for speciation rates. In general, extinction rate estimates were very similar across models (see table 2).

Discussion

Distinguishing between Modes of Character Change

Our new model, BiSSE-ness, may be used to estimate the degree of along-lineage and speciational change in a binary trait, using phylogenetic and present-day species information. Our simulations demonstrate that when character change is concentrated at speciation, the BiSSE-ness model performs significantly better than the simpler model, BiSSE, which assumes only anagenetic change. We found that as the proportion of character change occurring at speciation increases, the ability of BiSSE-ness to distinguish between modes of character change increases, except in cases where the total amount of character change is extremely high. At this point, trait changes become saturated and the signal of anagenetic versus cladogenetic change is essentially erased from the phylogenetic data.

With regard to parameter estimation, we found that the precision with which cladogenetic change was estimated depended on the rate of speciation in a particular state. In our simulations, more speciation events occurred for lineages in state 1 (because $\lambda_1 > \lambda_0$), resulting in more precise estimation of the probabilities of cladogenesis in state 1 (p_{1c} and p_{1a}). In contrast, we obtained similar estimates of the diversification parameters (rates of speciation and extinction), regardless of whether the model under which the data were simulated matched the model used for inference (BiSSE or BiSSE-ness). This result is important, as it suggests that speciation and extinction rate estimates are reasonably robust to model mis-specification with respect to the mode of character evolution. That said, as the total amount of character change increases, estimates of the speciation rates become less precise, particularly for BiSSE, because the frequent switching between states obscures any signal of state-dependent speciation.

Because our simulations represent just a small portion of possible parameter combinations, several questions regarding the ability of BiSSE-ness to distinguish between various patterns of diversification remain to be addressed by future studies. For example, how well can the diversification rates be estimated if, say, the rate of character change from state 0 to 1 is much higher than the rate of change from 1 to 0? Also, we have used a relatively low extinction rate in our simulations. We expect that a high rate of extinction may obscure the ability of BiSSE-ness to detect differences between anagenetic and cladogenetic change, because a character change occurring with speciation but followed by extinction of one daughter lineage would appear as an along-lineage change.

Another caveat of phylogenetic inference methods such as BiSSE-ness is that phylogenies that describe a large number of species must be used in order to have sufficient power to detect trait-dependent speciation and extinction rates and to differentiate cladogenetic and anagenetic change. The power to detect cladogenesis was greatest in the 500-species trees and declined when the 200- or 50-species trees were examined. Nevertheless, BiSSE-ness remained able to detect cladogenetic change, especially as the rate of such change increased. We also note that the BiSSE-ness model is fully compatible with the BiSSE methodologies that have developed to accommodate incompletely resolved phylogenies and incomplete taxon sampling and trait information (FitzJohn et al. 2009). Therefore, a significant amount of power may be regained with these methods when there is a large number of species in a clade but only a limited number are included on a phylogeny.

Detecting Patterns of Character Change in Primates

When we applied the BiSSE and BiSSE-ness models to the primate data set, we found that changes in habitat type (forested vs. forest-savanna regions) tended to occur with speciation. Because habitat type represents a major component of the primates' ecological niche, changes in this trait during speciation support the hypothesis of a role for ecological speciation in primates (Conroy 1990). Also consistent with our findings are the results of Curnoe et al. (2006), which provide evidence that primate speciation may be driven by prezygotic isolating mechanisms. Relatively few primate species inhabit savanna regions (14/215). These species are, however, distributed relatively evenly throughout the phylogeny and are often recently diverged from forest-dwelling sister species (see fig. 4). This pattern of recently diverged species occupying forest-savanna habitats is likely the signal that is generating support for speciational change, because the chance of anagenetic change occurring along short branches near the present is relatively small.

We found evidence that changes in primate social behavior (social vs. solitary) and mating system (monogamous vs. nonmonogamous) tend to occur gradually along a lineage rather than concurrently with speciation. Social behavior and mating system are both traits that can exhibit considerable variation among groups within species (Kappeler and van Schaik 2002). Therefore, changes in the degree of sociality and mating system may occur independently of speciation in response to other factors related to changes in ecology (e.g., resource defense) and/or demography (e.g., distribution of females; Kappeler and van Schaik 2002).

For two traits, primate activity period and terrestriality, no single model of character change was strongly preferred over any of the other models considered (anagenetic change only: BiSSE; cladogenetic change only: BiSSE-ness with 6 parameters; mixture of models: BiSSE-ness with either 8 or 10 parameters; see fig. 5). It is possible that these traits did evolve in a manner consistent with one of the models of character change explored, but there is not enough statistical power to obtain a strong signal. Alternatively, it may be that these traits evolved according to a different process than is represented by the gradual and speciational change models presented here. Mooers et al. (1999) and Oakley et al. (2005) outlined several models of character change in addition to the ones used here, each of which differently weights aspects such as phylogenetic structure, genetic change, and patterns of character change. For example, the genetic model presented by Mooers et al. (1999) tests the association of character change with the number of genetic changes (inferred substitutions) that have occurred. Because there is evidence that the evolution of primates is not best represented by a strict molecular clock (Fabre et al. 2009), the genetic model may provide different results from the anagenetic model, which examines character change in proportion to time. Another possibility is that a discrete character may be the manifestation of an underlying continuous trait, where shifts in the discrete character occur when the continuous trait crosses a threshold value (Felsenstein 2012).

Overall, we find support for gradual diversification and punctuated evolution in primates among the traits included here, although there remains considerable model uncertainty. Our analyses using neontological data and the BiSSE-ness model represents just one aspect of the study of patterns of character evolution and, as in all modeling exercises, can only assess the relative likelihoods of the hypotheses proposed to explain past events. The degree to which our models can inform us about historical processes is limited by the size of the data set (i.e., statistical power) as well as any biases inherent in using phylogenies to examine diversification. For example, even though larger phylogenies may generate more precise parameter estimates, our picture of diversification will be skewed if we study only large clades—and not small ones—of a given age (Ricklefs 2007). When only large clades are studied, we obtain higher speciation and/or lower extinction rates than the global average rates would suggest (Ricklefs 2007).

To confirm our results and gain a more complete understanding of primate diversification, we recommend that future analyses include trait information for more species, use the most recent molecular data (e.g., see Perelman et al. 2011), and be conducted over a set of trees that are consistent with the data, to account for phylogenetic uncertainty. Fossils provide an additional source of potential information in phylogenetic analyses (Ricklefs 2007; Jablonski 2008), contributing unique information that cannot be obtained with neontological data, such as minimum estimates of when certain characteristics first evolved (Fleagle 2002). Developing BiSSE-like models to incorporate data from the fossil record is a promising future direction, given that the fossil record is an excellent resource for historical information relating to evolutionary change (Conroy 1990; Fleagle 2002), particularly for primates, which have a well-studied fossil record. Another fruitful avenue worth exploring is to allow diversification rates to vary over time. Because several studies have noted differences in diversification rates among primate clades (Curnoe et al. 2006; Fabre et al. 2009), future BiSSE and BiSSE-ness analyses that account for these shifts might be revealing (e.g., see FitzJohn 2010).

Investigating Trait-Dependent Diversification using BiSSE-ness

BiSSE-ness is distinct from other models that investigate the mode of character change in that it explicitly accounts for trait-dependent speciation and extinction (Mooers et al. 1999; Bokma 2008). Because many of the characters that have been intensively studied with respect to the tempo and mode of character evolution, such as polyploidy in plants (Wood et al. 2009) and body size and range size in mammals (Mattila and Bokma 2008; Carotenuto et al. 2010), are also thought to influence the rates of speciation and extinction (respectively, Mayrose et al. 2011; Liow et al. 2008; Cardillo et al. 2003), we believe that methods that simultaneously account for these effects will be widely applicable. Although in this article we have focused on the contributions our model makes to studies regarding the tempo and mode of character change, BiSSE-ness also provides a more flexible framework for studies investigating how traits are associated with differences in diversification rates. That is, BiSSE-ness may be used in place of BiSSE for studies that primarily investigate how a trait influences diversification rates (e.g., FitzJohn et al. 2009; Wilson et al. 2011), with the advantage that it provides additional insight into the evolution of the trait or, at the very least, simply remains agnostic regarding the mode of character change.

We found evidence for an association between three out of five primate traits and differential rates of primate diversification: activity period, mating system, and terrestriality. Overall, diurnal activity, nonmonogamous mating systems, and terrestriality tended to be associated with increased rates of speciation when compared with nocturnal, monogamous, and arboreal lineages ($\overline{P} = .055$, .0073, and .0014, respectively). The relationship with diurnality is only marginally significant and must be confirmed with more extensive analyses, but it points to the possibility that diurnal species have increased opportunities for diverging into novel niches.

To the extent that nonmonogamous mating systems experience stronger sexual selection, our findings support the hypothesis that increased sexual selection is associated with higher speciation rates. This positive association has also been found in birds (Mitra et al. 1996; Owens et al. 1999, considering plumage dichromatism), lizards (Agamidae; Stuart-Fox and Owens 2003), and insects (Arnqvist et al. 2000). However, this effect was not detected when analyzing other sexually selected traits in birds (social polygamy and body size dimorphism; Owens et al. 1999), nor was it detected in two more recent studies investigating mammals. Both Gage et al. (2002) and Isaac et al. (2005) used different measures of sexual selection, the degree of polyandry (measured using relative testes size) and sexual size dimorphism, respectively, which may contribute to the inconsistency among the results (Isaac et al. 2005).

In contrast to mating system, we are unaware of an obvious explanation for higher speciation rates in terrestrial lineages as compared with arboreal lineages. What is driving this pattern may be the fact the most terrestrial primates belong to the species-rich Cercopithecidae family (Old World monkeys), a recent radiation that is thought to have relatively high speciation rates (Purvis 1995; Paradis 2005; Fabre et al. 2009). This group is also characterized by locomotion morphology that is not highly specialized to arboreal or terrestrial habitats, and at least for guenon monkeys (genus *Cercopithecus*), switches between these habitats may have occurred a number of times (Gebo and Sargis 1994; Fabre et al. 2009).

Although our analyses showed an association between a higher speciation rate and diurnal activity, nonmonogamy, and terrestriality, we note that our methods (and comparative methods in general) cannot distinguish a causal relationship from a correlation. That is, terrestriality, for example, does not necessarily cause an increase in the rate of speciation; it may be that yet another trait that is similarly distributed among the extant species affects the diversification rate directly (e.g., body size; Paradis 2005). Further analyses investigating correlated trait evolution may be useful in teasing apart these relationships (Maddison 2000; Pagel and Meade 2006; see also Nunn and Barton 2001).

We found that inferences about rates of speciation and extinction remained largely consistent across different models of character evolution. Therefore, although previous analyses that used BiSSE methodologies did not account for the mode of character change, our results with both the primate data set and the simulations indicate that inferences about trait-dependent diversification tend to be robust to mis-specifying the mode of character change. This is not true, however, when it comes to estimating the parameters involving trait changes themselves, and we expect that anagenetic character change has been overestimated in cases where substantial cladogenetic change occurs.

Applications of BiSSE-ness

As the number of large, well-resolved phylogenies increases and as global databases of life-history and ecological traits become more comprehensive, our ability to address a vast range of macroevolutionary questions with neontological data will likely improve tremendously. Recent analyses have, for example, taken advantage of growing phylogenetic information in plants to explore the impact of polyploidy (Mayrose et al. 2011) and the impact of selfing (Goldberg and Igić 2012) on diversification rates. In both cases, prior work has suggested that these traits are likely to undergo cladogenetic change. Although Mayrose et al. (2011) used the BiSSE-ness method developed here, Goldberg and Igić independently derived a similar method (ClaSSE) to accommodate both anagenetic and cladogenetic trait changes. ClaSSE is also based on BiSSE methodologies and was used to explore the evolution of self-incompatibility in the nightshade family (Solanaceae). Goldberg and Igić found that a loss of self-incompatibility has often been associated with the speciation process (cladogenetic change). While BiSSE-ness describes changes in a trait that occur during the speciation process (the p_c and

p_a parameters), ClaSSE uses an alternative parameterization that specifies additional speciation rate parameters. That is, in ClaSSE, the total speciation rate in state 0 (λ_0) comprises two parameters corresponding to rates of speciation leading to daughter species that differ (λ_{001}, where one daughter is in state 0 and one is in state 1) and daughter species that both remain in state 0 (λ_{000}). Although the application to Solanaceae assumed that both daughter species did not change state, ClaSSE could also be applied to this case (by introducing two additional parameters, λ_{011} and λ_{100}; E. Goldberg, personal communication). In an ML analysis, BiSSE-ness and ClaSSE would produce identical results because likelihood is not sensitive to alternative parameterizations of the underlying model. However, in a Bayesian analysis, the natural priors for the p_c and p_a parameters of BiSSE-ness differ from the natural priors for the λ_{ijk} parameter of ClaSSE, and the user might prefer one or the other parameterization depending on the question at hand. Regardless of the exact implementation used, our simulations are the first to explore the ability of these methods to distinguish cladogenetic from anagenetic change and to assess the impact of the mode of trait evolution on diversification.

Overall, the BiSSE-ness and ClaSSE family of models provide a flexible framework with which to simultaneously address questions regarding the mode of character evolution and the effect of a trait on species diversification. Importantly, even in cases where processes such as trait-dependent diversification or speciational change are expected to be absent or negligible, these methods may still be used, even though some parameters are expected to be zero or similar to other parameters. Alternatively, the user may restrict the model to test a particular hypothesis (e.g., to test whether anagenetic, cladogenetic, or both types of change have occurred) or in accordance with prior information (e.g., concerning irreversibility of trait change, as in Goldberg et al. 2010). Extending the BiSSE-ness model to accommodate multistate and continuous trait data would be a worthwhile endeavor, increasing the number of traits that could be studied and the number of questions that could be addressed.

APPENDIX

BiSSE-ness Likelihood Equations

The diversification probabilities for states 0 and 1, respectively, change over time according to equations (A1) and (A2):

$$\begin{aligned}\frac{dD_{\mathrm{N0}}}{dt} = {} & -(\lambda_0 + \mu_0 + q_{01})D_{\mathrm{N0}}(t) + q_{01}D_{\mathrm{N1}}(t) + 2\lambda_0 E_0(t)D_{\mathrm{N0}}(t)(1 - p_{0\mathrm{c}}) \\ & + 2\lambda_0\left(\frac{E_0(t)D_{\mathrm{N1}}(t)}{2} + \frac{E_1(t)D_{\mathrm{N0}}(t)}{2}\right)p_{0\mathrm{c}}p_{0\mathrm{a}} \\ & + 2\lambda_0 E_1(t)D_{\mathrm{N1}}(t)p_{0\mathrm{c}}(1 - p_{0\mathrm{a}}),\end{aligned} \tag{A1}$$

$$\begin{aligned}\frac{dD_{\mathrm{N1}}}{dt} = {} & -(\lambda_1 + \mu_1 + q_{10})D_{\mathrm{N1}}(t) + q_{10}D_{\mathrm{N0}}(t) + 2\lambda_1 E_1(t)D_{\mathrm{N1}}(t)(1 - p_{1\mathrm{c}}) \\ & + 2\lambda_1\left(\frac{E_1(t)D_{\mathrm{N0}}(t)}{2} + \frac{E_0(t)D_{\mathrm{N1}}(t)}{2}\right)p_{1\mathrm{c}}p_{1\mathrm{a}} \\ & + 2\lambda_1 E_0(t)D_{\mathrm{N0}}(t)p_{1\mathrm{c}}(1 - p_{1\mathrm{a}}).\end{aligned} \tag{A2}$$

By setting the $(1 - p_{0\mathrm{c}})$ and $(1 - p_{1\mathrm{c}})$ terms equal to 1 (corresponding to the probabilities that cladogenetic character change does not occur), the first line in each equation describes the original BiSSE model (Maddison et al. 2007). The last two lines in equations (A1) and (A2) account for speciational change (BiSSE-ness). In these equations, speciation along an unbroken branch in the tree must lead to extinction of one of the daughter lineages by the present (described by $E_0(t)$ and $E_1(t)$ above), because no node is observed along the branch. Thus, the terms describing speciation followed by extinction are each weighted by 2, because it is possible to account for the data if either the left or the right lineage becomes extinct before the present. The probabilities of extinction in states 0 and 1, respectively, change over time according to

$$\begin{aligned}\frac{dE_0}{dt} = {} & \mu_0 - (\mu_0 + q_{01} + \lambda_0)E_0(t) + q_{01}E_1(t) + \lambda_0 E_0(t)^2(1 - p_{0c}) \\ & + \lambda_0\left(\frac{E_0(t)E_1(t)}{2} + \frac{E_1(t)E_0(t)}{2}\right)p_{0c}p_{0a} \\ & + \lambda_0 E_1(t)^2 p_{0c}(1 - p_{0a}),\end{aligned} \tag{A3}$$

$$\begin{aligned}\frac{dE_1}{dt} = {} & \mu_1 - (\mu_1 + q_{10} + \lambda_1)E_1(t) + q_{10}E_0(t) + \lambda_1 E_1(t)^2(1 - p_{1c}) \\ & + \lambda_1\left(\frac{E_1(t)E_0(t)}{2} + \frac{E_0(t)E_1(t)}{2}\right)p_{1c}p_{1a} \\ & + \lambda_1 E_0(t)^2 p_{1c}(1 - p_{1a}),\end{aligned} \tag{A4}$$

where again, the second and third lines account for cladogenetic trait change (BiSSE-ness). These coupled ordinary differential equations were solved numerically in R with diversitree (ver. 0.6-1; FitzJohn et al. 2009), which was modified to allow speciation-associated trait changes. The diversitree package uses these results to traverse branches, along with equation (1) for BiSSE or equation (2) for BiSSE-ness to traverse nodes, until the root of the tree is reached. For more details, see Maddison et al. (2007).

Following Nee et al. (1994) and Maddison et al. (2007), we condition the overall likelihood of the data on clade survival, meaning that neither of the two daughter lineages of the root node are allowed to become extinct before the present day. This conditioning is accomplished by dividing the D_0 and D_1 values at the root by the probability of clade survival, which equals

$$\sum_{i=0}^{1} \frac{D_i}{D_0 + D_1}\lambda_i[(1 - p_{ic})(1 - E_i)^2 + p_{ic}p_{ia}(1 - E_i)(1 - E_j) + p_{ic}(1 - p_{ia})(1 - E_j)^2], \tag{A5}$$

where E_i and D_i represent their values at the root. In other words, (A5) takes the probability that the root is in state 0 or 1 (first fraction), multiplies this by the probability of speciation at the root node (λ_i), and then multiplies this by the probability that both daughter lineages do not become extinct, while accounting for the possibility of cladogenetic trait shifts.

The equilibrium frequencies of states 0 and 1 can also be calculated using the BiSSE-ness model. This differs slightly from the procedures used in the BiSSE model (see eq. [13] in app. 2 of Maddison et al. 2007), and in this study it is used only to determine the root state of the simulated trees. Specifically, the frequency of lineages in state 0, $x = n_0/(n_0 + n_1)$, is described by the differential equation

$$\begin{aligned}\frac{dx}{dT} = {} & gx(1 - x) - xq_{01} + (1 - x)q_{10} \\ & - x\{p_{0c}\lambda_0[2(1 - p_{0a}) + p_{0a}]\} + (1 - x)\{p_{1c}\lambda_1[2(1 - p_{1a}) + p_{1a}]\},\end{aligned} \tag{A6}$$

where $g = \lambda_0 - \mu_0 - \lambda_1 + \mu_1$ is the difference in diversification rates between states. The second line in equation (A6) includes the terms involving cladogenesis that are added to the original BiSSE model.

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Online Supplementary Material: Linking the Investigations of Character Evolution and Species Diversification

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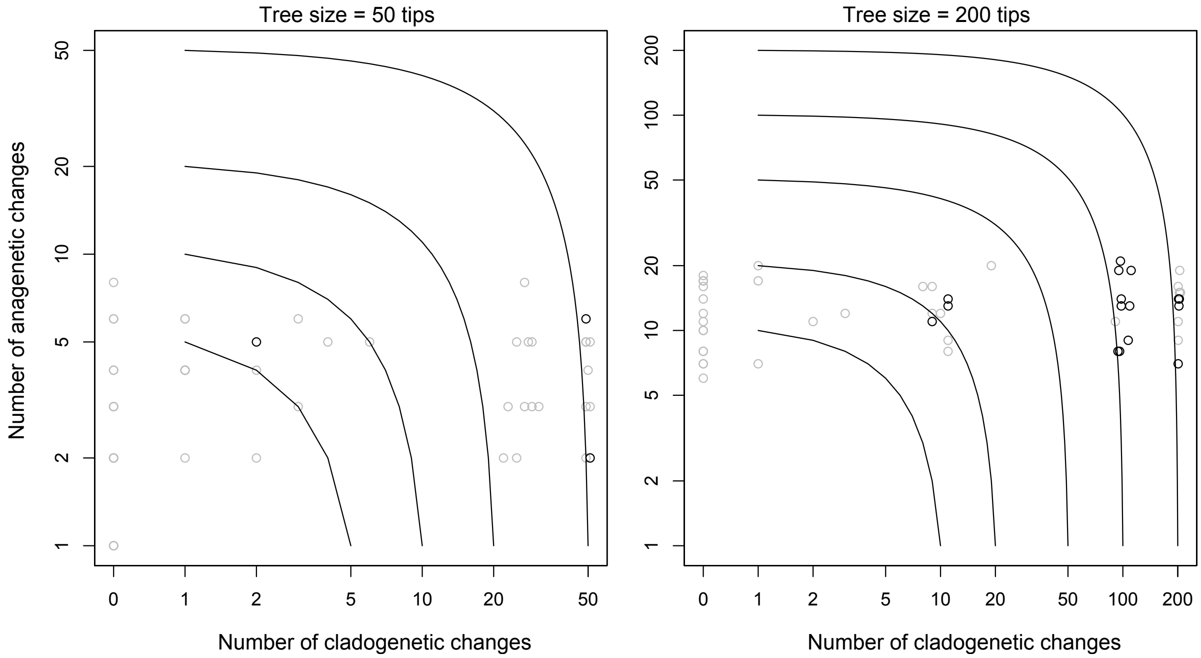

Figure S1: The amount and type of character change for each of the simulated trees with 50 species (left) and 200 species (right). The x- and y- axes give the actual number of events that occurred during the simulation, excluding extinct lineages. Cases where BiSSE-ness significantly outperforms BiSSE are indicated in black ($p < 0.05$, likelihood ratio test, d.f. = 4). Curves show a constant total number of character changes. The parameter set used to generate these trees was a subset of Set 1 (see table 1). The tree depths averaged 23.85 ±6.92 and 32.89 ±7.25 for the 50-species and 200-species trees, respectively.

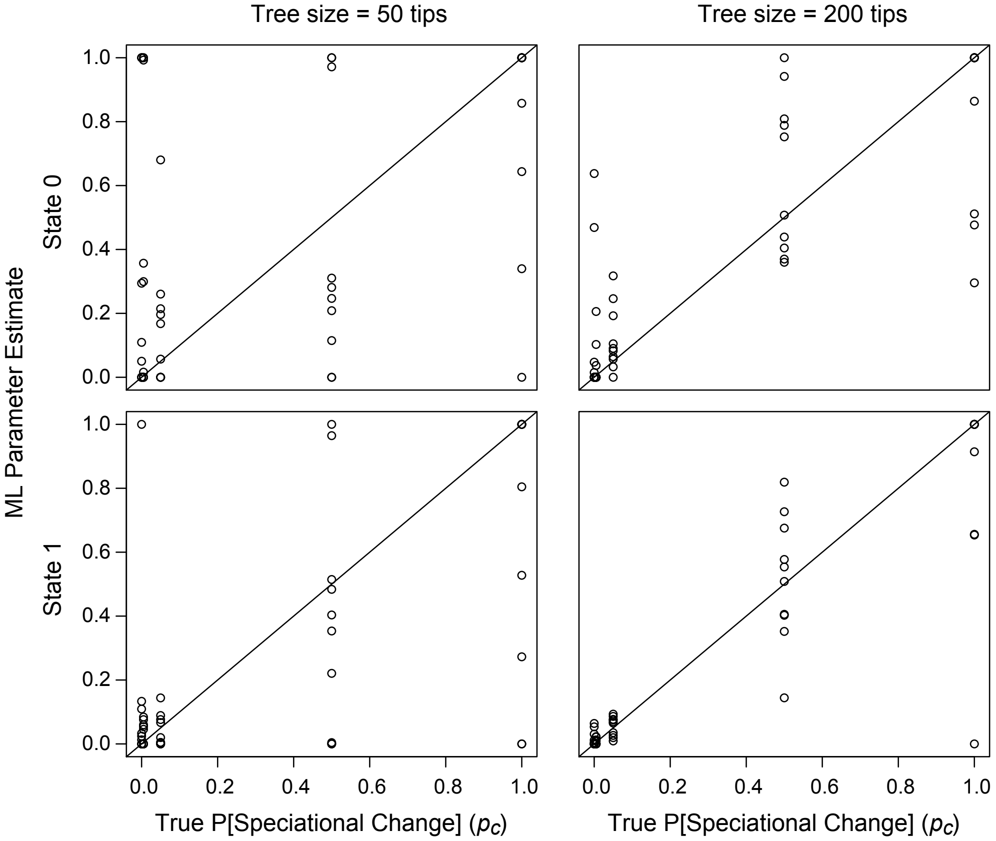

Figure S2: Parameter estimates associated with speciational character change for the simulated trees with 50 species (left panels) and 200 species (right panels). BiSSE-ness accurately estimated the probability of speciational character change for simulated data with varying p_{0c} and p_{1c}values ($p_{0c} = p_{1c}$; $p_{0a} = p_{1a} = 0.5$; $q_{01} = q_{10} = 0.01$). Lines indicate the 1:1 ratio; that is, if all the estimates fell on the lines, then BiSSE-ness would have perfectly recovered the actual parameter values under which the data was simulated.

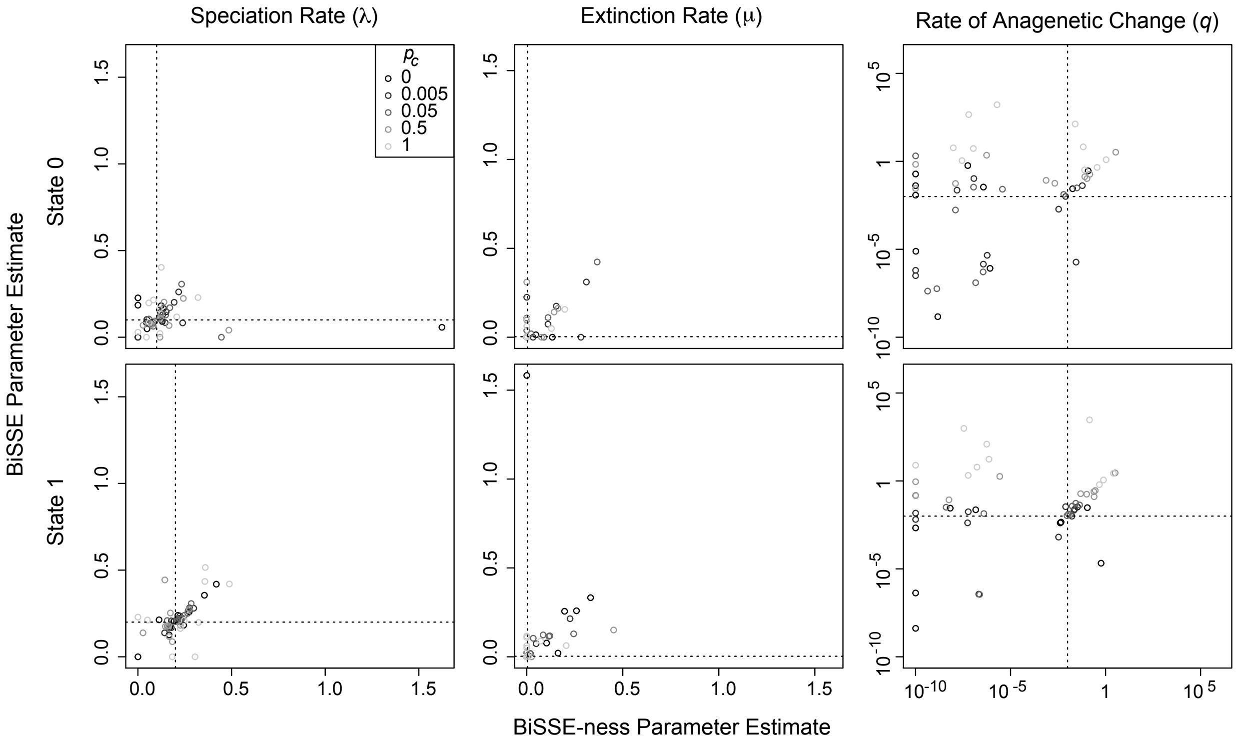

Figure S3: Comparing ML parameter estimates between BiSSE-ness and BiSSE for the simulated trees with 50 species. Crosshair lines indicate the true parameter values under which the data was simulated. Allowing character change at speciation events ($p_c > 0$) had relatively little effect on estimates of speciation and extinction rates. Note the use of a logarithmic scale for the rates of anagenetic character change.

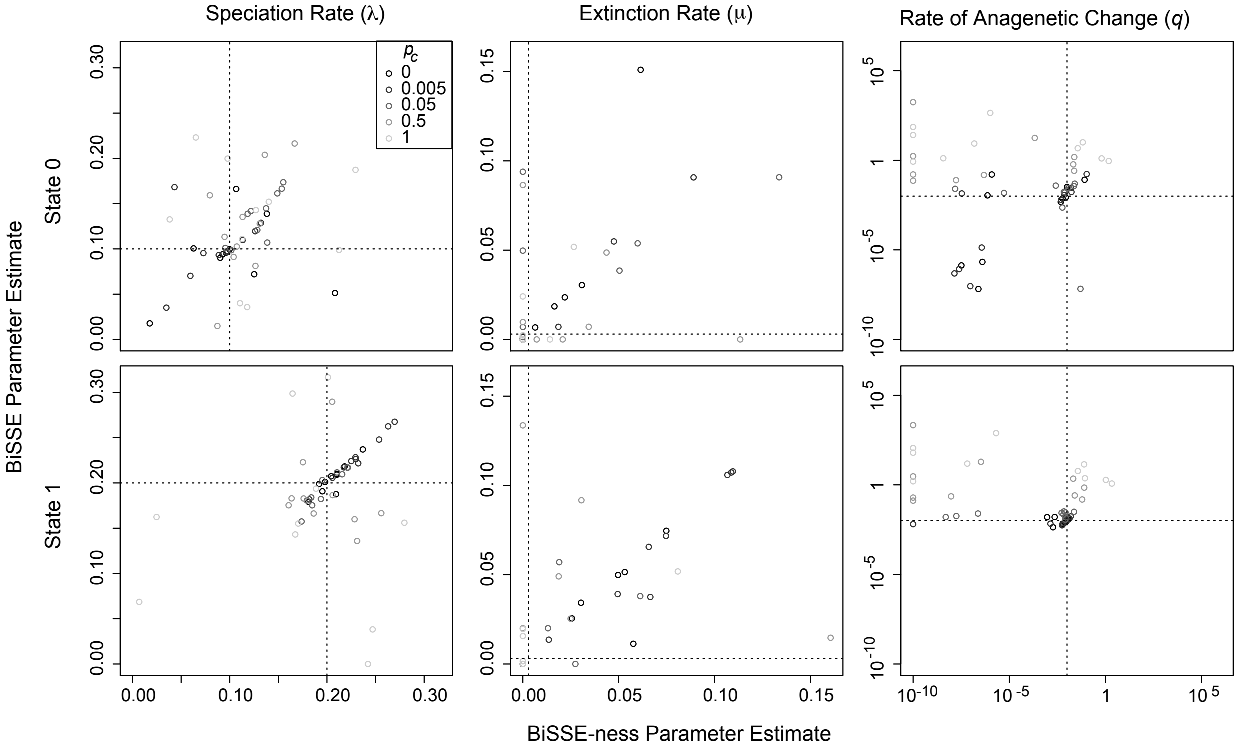

Figure S4: Comparing ML parameter estimates between BiSSE-ness and BiSSE for the simulated trees with 200 species. Crosshair lines indicate the true parameter values under which the data was simulated. Allowing character change at speciation events ($p_c > 0$) had relatively little effect on the mean estimates of speciation and extinction rates. The precision of the estimated speciation rates decreased, however, as the amount of speciational change increased under both BiSSE-ness (λ_0: SD = 0.049 and 0.058 for p_c = 0 and 1, respectively) and BiSSE (λ_0: SD = 0.041 and 0.063 for p_c = 0 and 1, respectively; similar patterns for λ_1). Note the use of a logarithmic scale for the rates of anagenetic character change.

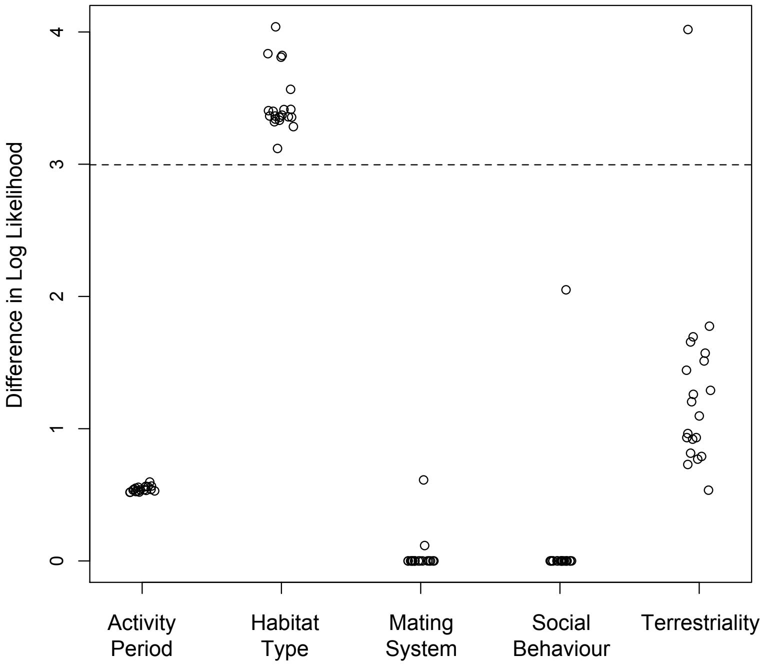

Figure S5: Comparing differences between log likelihoods for the BiSSE model (6 parameters) and the BiSSE-ness model where character change at speciation occurs both symmetrically and asymmetrically in a 50:50 ratio (p_{0a}=p_{1a}=0.5; 8 parameters). Differences in log likelihood values greater than 3.00 correspond to significant support for the more complex BiSSE-ness model (dashed line; p <0.05, likelihood ratio test, d.f. = 2). Each point represents a different phylogenetic tree drawn from the set of trees (n = 20 for each trait). Allowing for cladogenetic change in habitat type results in a significantly better fit than when change is limited to occur only along lineages ($\overline{p}$ = 0.0320).

Table S1: 95% credibility intervals for the posterior distributions of the parameter estimates generated through Bayesian MCMC analyses (see Figure 6 in the main text).

	Activity Period	**Habitat Type**	**Mating System**	**Social Behaviour**	**Terrestriality**
0 =	diurnal	forest/savanna	non-monogamous	solitary	arboreal
1 =	nocturnal	forest	monogamous	social	terrestrial
λ_0	0.15 – 0.25	6.5x10^{-6} – 0.39	0.17 – 0.29	0.064 – 0.16	0.12 – 0.20
λ_1	0.068 – 0.16	0.14 – 0.23	0.085 – 0.17	0.15 – 0.24	0.21 – 0.41
μ_0	0.00024 – 0.14	3.3x10^{-5} – 0.58	0.0012 – 0.20	1.4x10^{-5} – 0.12	0.023 – 0.15
μ_1	0.00019 – 0.12	0.020 – 0.16	2.5x10^{-6} – 0.12	2.1x10^{-5} – 0.13	3.2x10^{-6} – 0.16
q_{01}	1.1x10^{-7} – 0.0041	1.2x10^{-6} – 0.47	3.4x10^{-6} – 0.047	0.00054 – 0.028	2.2x10^{-7} – 0.0081
q_{10}	3.1x10^{-7} – 0.011	4.5x10^{-7} – 0.032	0.00023 – 0.043	6.5x10^{-8} – 0.0046	1x10^{-5} – 0.13
p_{0c}	1.4x10^{-7} – 0.019	3.4x10^{-5} – 0.93	3.4x10^{-6} – 0.10	1.7x10^{-6} – 0.13	8.3x10^{-7} – 0.032
p_{1c}	7.5x10^{-6} – 0.093	0.0032 – 0.18	1.3x10^{-5} – 0.18	7.8x10^{-8} – 0.02	0.027 – 0.51
p_{0a}	4.3x10^{-5} – 0.94	0.0079 – 0.95	0.092 – 1	0.066 – 1	0.057 – 1
p_{1a}	0.00065 – 0.94	0.0031 – 0.90	0.038 – 0.99	0.023 – 0.97	0.34 – 1