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TESTING HYPOTHESES ABOUT ECOLOGICAL SPECIALIZATION USING PHYLOGENETIC TREES

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Abstract.—It is often assumed that ecological specialization represents an evolutionary ‘‘dead-end’’ that limits further evolution. Maximum-likelihood (ML) analyses on phylogenies for 15 groups of phytophagous insects revealed that high transition rates both to and from specialization occurred, but that the mean ratio of rates was significantly biased toward a higher rate to specialization. Here we explore the consequences of the fact that transition rates inferred by ML are affected not only by the distribution, but also by the frequency, of character states. Higher rates to the more common state were inferred in the analyses of Nosil (2002), in similar studies published since 2002, and in a small set of simulations. Thus, the ratio of the rate toward versus away from specialization was strongly, positively correlated with the proportion of specialist species at the tips of the phylogeny and whether transitions away from specialization occur remains unclear. Here we reexamine these data using methods that do not rely on directly comparing transition rates. Maximum-likelihood analyses show that models with no transitions in one direction (e.g., irreversible evolution as predicted by the ‘‘specialist as dead end’’ framework) are usually strongly rejected, independent of the proportion of specialists at the tips. Ancestral state reconstruction revealed two instances where generalists were unambiguously derived from specialists. Transition rates would need to biased 100-fold and 5000-fold toward specialization to reconstruct a history where these shifts from specialization toward generalization do not occur. The general conclusions of Nosil (2002) appear to hold; transitions in either direction likely occur such that specialization does not always limit further evolution. Most generally, inferences regarding character evolution can be strengthened by comparing models of character change and examining ancestor states, rather than only comparing parameter values.

Key words.—Ancestral state reconstruction, ecological specialization, maximum likelihood, phytophagous insects, transition rates, sensitivity analysis.

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Many species exhibit specialized patterns of resource use, utilizing only a subset of all suitable and available resource types (Futuyma and Moreno 1988; for reviews, see Jaenike 1990). Ecological specialization is often considered to be a derived state such that it represents an ‘‘evolutionary dead-end’’ which strongly limits further evolution. However, generalist species that use a wide array of resources occur and empirical evidence that specialization restricts further evolution is equivocal (Simpson 1953; Holloway and Hebert 1979; Moran 1988; Janz and Nylin 1998; Kelley and Ferrell 1998). This led Nosil (2002) to examine patterns of specialization and generalization in 15 groups of phytophagous insects using previously published phylogenies and host range data.

For each group, Markov-models of trait evolution and maximum-likelihood (ML) approaches (Pagel 1994, 1999) were used to test whether the transition rate from generalization to specialization (the ‘‘forward’’ rate) differed from the transition rate from specialization to generalization (the ‘‘backward’’ or ‘‘reverse’’ rate) (Nosil 2002). Averaged across groups, these analyses showed that the mean ratio of forward to backward rate was significantly greater than one, indicating that transition rates toward specialization tended to be slightly higher (mean ratio = 1.47). However, higher transition rates both toward and away from specialization, as well as equal rates, were inferred (depending on the insect group examined). In fact, its was rarely possible to statistically reject a model with equal forward and backward rates (equal rates rejected in only two to five of the 15 trees, depending on the method; for details, see Nosil 2002). It was concluded that there may be a weak tendency for directional evolution toward increased specialization but that that specialization does not always or even often represent an evolutionary ‘‘dead-end’’ that strongly limits further evolution.

Transition rates in the Markov model for discrete states are affected by both the evolutionary transition rate and the stationary frequency of character states (Lewis 2001; Takebayashi and Morrell 2001; Huelsenbeck et al. 2003). Pagel’s (1994) invocation of the two-state Markov model assumes the stationary frequency is 0.5 (i.e., equal) for the two states (M. Pagel, pers. comm.), but empirical data might sometimes suggest otherwise. As a result, transition rates estimated using Pagel’s (1994) method will depend on both (1) the distribution of character states and (2) the frequency of states at the tips such that higher transition rates to the more common state might often be inferred. The effects of this assumption of equal stationary frequencies on inferences regarding character evolution are unclear, leading us to reevaluate the results of Nosil (2002).

We show that ML methods for discrete characters do infer higher transition rates toward the more common state. Our results suggest that the ratio of transition rates reported in Nosil (2002) is a poor metric of the directional tendency of host-range evolution in phytophagous insects. Thus, inferences regarding host-range evolution stemming from phylogenetic analysis require further scrutiny and here we reevaluate the data from Nosil (2002) using methods that do not rely on directly comparing transition rates. We do not intend to review the phylogenetic analysis of ancestral states or evolutionary rates (see instead Cunningham et al. 1998;

Stireman 2005). Rather we focus on the specialist/generalist scenario in phytophagous insects because the issues raised above indicate that how often transitions away from specialization actually occur remains unclear.

However, the results of this study are general. The association between transition rate and state frequency that we report could affect all studies using ML estimates of transition rates, and thus extends beyond the specialist/generalist scenario. Moreover, the analyses presented below represent at least one alternative approach to examining the direction and rate of evolution on phylogenies (i.e., one alternative to comparing parameter estimates). Our results suggest that comparing models of character change and examining ancestor states is a useful complement to comparing estimated parameter values. Moreover, the robustness of different evolutionary scenarios should be evaluated (e.g., as by the sensitivity analysis described below).

MATERIALS AND METHODS

Empirical Trends

We first explore the relationship between the ratio of the forward transition rate toward specialization to the backward rate away from specialization and the percent specialists at the tips of the tree for the datasets from Nosil (2002). We examine this association where transition rates were inferred using uniform branch lengths, using Grafen branch lengths (Grafen 1989), and with the root fixed as the more common state (using the average of the forward and backward transition rates in the two cases where specialists and generalists were equally common).

Additionally, we used a Web of Science search to identify studies which have been published since 2002 that examine the evolution of ecological specialization in insects using phylogenetic trees (searched for—insect and phylo* and special*). We assess how these new studies (Stireman 2005; Yotoko et al. 2005) fit with empirical trends detected using the Nosil (2002) dataset.

State Frequencies and Transition Rates—Simulations

We tested whether any empirical associations noted above were anomalous by performing a small set of simulations. Using the core modules of Mesquite (Maddison and Maddison 2004), we simulated 10 two-state discrete characters on each of 15 20-taxon trees (the mean size from the Nosil 2002 dataset) drawn haphazardly from a Yule distribution. Mesquite uses a Markov model as described in Lewis (2001): we constrained the forward and backward transitions rates to be equal and also scaled the rate such that between three and four transitions are expected over each tree. One hundred thirty-two of the 150 simulations produced variation, and 128 of these produced unequal proportions, of the two states at the tips. We then used the Discrete module in MSBayes (Pagel et al. 2004) to estimate q01 and q10 for each tree, without constraining them to be equal. We note that our small test is conservative, in that the true rates were equal and the rate of change relatively low. State designations are arbitrary, such that the root state (0 in these simulations) has no bearing on the results. The set of trees examined illustrates our main point and a larger set of simulations is beyond the scope of our study. However, a technical simulation study that considers the sensitivity of our observed correlation to overall rate, actual rate asymmetry, tree size, and aspects of tree shape may be warranted.

Tests for Irreversible Evolution

Given the associations reported above, our next task was to reevaluate the likelihood of transitions toward specialization and transitions toward generalization. To evaluate whether and to what extent the two ecologies represent dead ends, Nosil (2002) tested whether the forward transition rate from generalization to specialization was different from the backward rate from specialization to generalization. This was done by comparing models where both these rates were free to vary (i.e., estimated from the data) to models where the rates were constrained to be equal, and done in the knowledge that forward and backward rates may not be well estimated (Mooers and Schluter 1999).

Whether it is possible to reject the hypothesis that one of the transition rates is equal to zero was not examined in the original study. This is the most explicit test for irreversible evolution and we implement it here. Evolution can be modeled as irreversible in two ways—either generalist to specialist transitions never occur (‘‘no G to S’’) or specialist to generalist transitions never occur (‘‘no S to G’’). The second scenario is the extreme form of the ‘‘specialists as dead end’’ scenario. We compared the ML fit for the irreversibility model (in which one of the transition rates was fixed to zero) to the ML fit of the model where both the rates were free to vary (the two scenarios must assume that the ancestral state is S for the ‘‘no G to S’’ scenario and that it is G for the ‘‘no S to G’’ scenario and the roots were fixed as such). The two models differ by one free parameter and the resulting value of $-2\log LR$ approximates a chi-square distribution with one degree of freedom (Pagel 1994). Because one parameter value was fixed to a boundary condition (zero), the *P*-values reported are likely very conservative (Ota et al. 2000). Importantly, we also tested whether the magnitude of $-2\log LR$ (a measure of how much worse a model of irreversible evolution fits the data) was correlated with the percent specialists at the tips of the tree. Although these analyses are extreme, they are the most direct test of the ‘‘dead-end’’ hypotheses, and allow us to examine the evolution of ecological specialization without comparing transitions rates to one another.

Ancestor State Reconstruction and Sensitivity Analysis

We performed two additional tests for whether shifts toward generalization ever occur by focusing on ancestral state reconstructions, rather than transition rates per se (Schluter et al. 1997). First, we reconstructed the root state using the ‘‘local’’ approach to ancestor reconstruction, allowing forward and backward rates to take their ML values (Schluter 1995). The local approach is required to compare support for alternative hypotheses at a particular node (Pagel 1999; in this case for a specialist versus for a generalist ancestor at the root) . Strong support for a specialist versus a generalist at the root indicates that a transition from specialization must

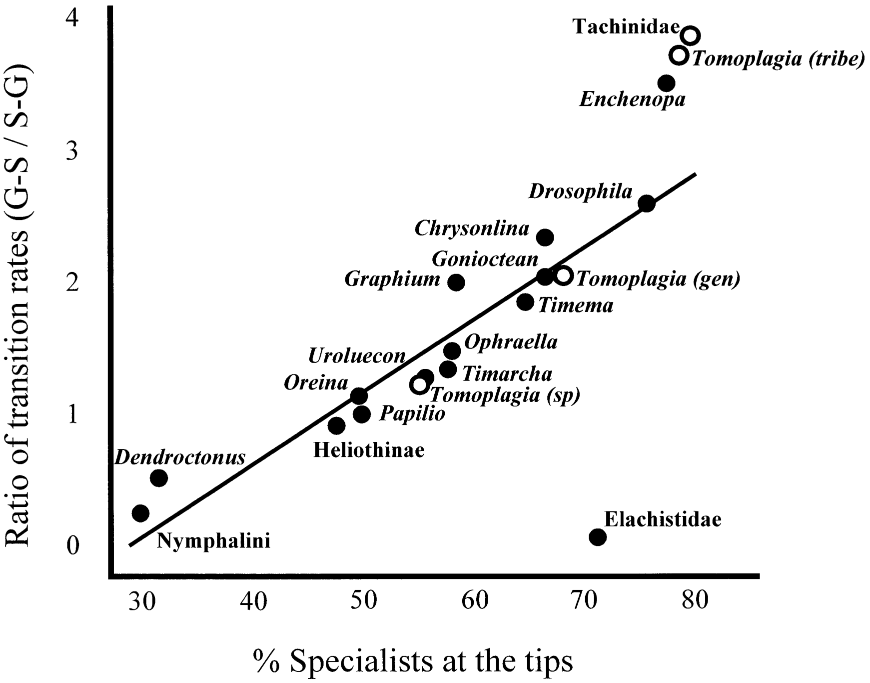

FIG. 1. For the 15 groups of phytophagous insects examined in Nosil (2002), the ratio of the transition rate toward specialization to the transition rate away from specialization is positively correlated with the proportion of specialist species at the tips of the phylogenetic tree ($r = 0.71$, $P < 0.01$, closed circles). Ratios were estimated using unconstrained maximum likelihood (ML). This indicates that ML estimates of transition rates can tend to be higher toward the more common state. Also depicted using open circles is the ratio of transition rates inferred using unconstrained ML by Stireman (2005) and the ratio for three taxonomic levels of host specialization estimated by Yotoko et al. (2005; sp, gen, and tribe refer to specialization at the level of host species, host genus and host tribe, respectively).

have occurred somewhere in a tree with generalists at the tips, even if we cannot locate precisely where.

We then reconstructed ancestor states and looked for subsequent nodes that had significant support, but for opposite character states. Here we are interested not in comparing support for different states at an individual node per se, but rather in determining the most likely state at each node across the tree. The ‘‘global’’ procedure, which considers the relative support for states at all internal nodes simultaneously, is most appropriate for this type of scenario, in which we want to find the maximum-likelihood description of the species data when no hypothesis is made about the value of a particular internal node in the tree (Schluter 1995; Pagel 1999; p. 617). Thus, we reconstructed ancestor states across nodes in each tree using global estimation and the unconstrained ML model (i.e., both transition rates free to vary). We then identified adjacent nodes where there was a shift from one ancestral state to the alternate state (i.e., subsequent nodes both have significant support, defined as >2 log-likelihood units, but for opposite character states).

We conducted a sensitivity analysis to determine how biased the forward transition rates (from generalist to specialists) would need to be to lose certainty in the direction of the transitions inferred using global estimation (i.e., to be uncertain whether nodes leading to the shift toward generalization were actually specialists). The first analysis examined what asymmetry in rates was required to lead to ambiguous reconstructions (i.e., where the support for the two states are roughly equal) at those nodes rather than significant support for reconstruction as a specialist. The second analysis examined what asymmetry in rates was required for generalists (rather than specialists) to be unambiguously reconstructed (defined as support for one state being >2 log likelihood units) at all nodes preceding the shift (i.e., and thus there could be no shift from specialist to generalist). Importantly, the transition rates were allowed to take their maximum-likelihood values under the constraint that they maintain the imposed degrees of rate asymmetry.

All analyses presented here use uniform branch lengths and resolve polytomies (present in two trees) using infinitesimally small branch lengths (for details, see Nosil 2002). Analyses using Grafen branch lengths and resolving polytomies arbitrarily in all possible combinations (Heliothinae, two resolutions; *Gonioctean*, nine resolutions) yielded similar results for all but two tests (highlighted below).

RESULTS

Empirical Trends

In the analyses of Nosil (2002), the ratio of forward transition rate toward specialization to the backward transition rate away from specialization was strongly, positively correlated with the proportion of specialist species at the tips of the tree (Fig. 1; $r = 0.71$, $P < 0.01$, $n = 15$ groups of insects, bivariate correlation). This correlation occurred when the ratios were estimated using uniform branch lengths ($r = 0.71$, $P < 0.01$), Grafen branch lengths ($r = 0.88$, $P < 0.001$), and even when the root of the tree was fixed as the more common state ($r = 0.68$, $P < 0.01$). The association was weakest in the latter case but only slightly so, indicating that fixing the

TABLE 1. Results of likelihood-ratio tests for whether a model where transitions in one direction never occur (i.e., transition rate in one direction, 0; transition estimate in the opposite direction free to vary) is significantly different from a model where transition rates in both directions are free to vary. Tests were applied constraining transitions to generalization to be zero (S to G = 0, root fixed as G) and constraining transitions to specialization to be zero (G to S = 0, root fixed as S). In most cases, a model constraining one transition rate to be zero provides a poor fit to the data (H_0 is rejected). Also shown is the number of specialist (S) and generalist (G) species in each group, as well as the ratio of the transition rate towards specialization to the rate from specialization inferred in Nosil (2002; uniform branch lengths, root free to vary).

			H_0: S to G = 0 H_1: S, G free		H_0: G to S = 0 H_1: S, G free	
Taxon	G, S	Ratio (G to S/ S to G)	−2 log LR	*P*-value	−2 log LR	*P*-value
1. *Timema* walking sticks	6, 11	1.83	6.39	0.011	8.81	0.003
2. Nymphalini butterflies	21, 9	0.29	7.60	0.006	0.75	0.386
3. *Uroleucon* aphids	4, 5	1.25	7.50	0.006	5.62	0.018
4. *Oreina* leaf beetles	6, 6	1.08	38.84	<0.0001	2.06	0.151
5. *Papilio* butterflies	12, 12	1.00	6.02	0.014	3.18	0.075
6. *Ophraella* leaf beetles	5, 7	1.44	4.65	0.031	7.28	0.007
7. Elachistidae	2, 5	0.08	7.92	0.005	0.02	0.885
8. *Chrysolina* leaf beetles	10, 20	2.33	4.27	0.039	4.34	0.037
9. *Timarcha* leaf beetles	14, 19	1.31	19.30	<0.0001	8.39	0.004
10. *Dendroctonus* bark beetles	13, 6	0.46	8.76	0.003	8.34	0.004
11. *Gonioctean* leaf beetles	5, 10	1.99	8.50	0.004	2.47	0.116
12. *Graphium* butterflies	5, 7	1.96	0.75	0.386	5.60	0.018
13. *Enchenopa* treehoppers	2, 7	3.50	5.90	0.015	2.63	0.105
14. *Drosophila* flies	9, 28	2.58	7.65	0.006	2.35	0.125
15. Heliothinae butterflies	11, 10	0.90	0.53	0.467	6.53	0.011

root may have reduced but did not alleviate the effect of state frequencies on the estimation of transition rates. Thus, ML estimates of transition rates on these trees did indeed infer higher rates to the more common state.

Two studies since 2002 have also examined the evolution of specialization in insects using phylogenetic trees (and both these studies implement Pagel's [1994] method). First, an article by Stireman (2005) explicitly highlighted some pitfalls of phylogenetic analyses of host range evolution. In his analysis of the evolution of host range in tachinid flies, Stireman (2005) found contrasting results when examining shifts in ancestral state versus transition rates. Shifts in ancestral state were reconstructed as specialist to generalist, yet transition rates were biased toward specialization and most species were specialists. Moreover, he notes that a study of host range evolution using parsimony (Kelly and Farrell 1998) yielded the opposite conclusion to that gained by re-analysis of the same data using ML by Nosil (2002). Second, an article by Yotoko et al. (2005) examined the trend toward specialization in *Tomoplagia* flies. Their article examined specialization at three different levels of host taxonomy (specialization at the level of host species, host genus and host tribe). As the proportion of specialist species increased at each taxonomic level of host specialization, so did the ratio of the rate toward versus away from specialization. Thus, the data from Stireman (2005) and the three data points from Yotoko et al. (2005) are all highly congruent with the trend from the 15 groups examined by Nosil (2002; see Fig.1).

State Frequencies and Transition Rates—Simulations

Our small set of simulations suggests that the empirical datasets are not anomalous. In the overwhelming majority of cases in our simulations, even though the generating forward and backward rates were equal, the estimated rate toward the majority state at the tips was higher than the estimated rate away from the majority state (104 of the 128 trees, $P < 0.001$ on a binomial test). As with the empirical data, the magnitude of the difference in the rates to and away from a state is also strongly correlated with the proportion of the tips in that state: the rank correlation between the difference in rates to and away from a state and the proportion of tips in that state is positive and strong ($\rho = 0.85$, $P < 0.001$). Setting all branch lengths equal (mimicking our empirical datasets) before estimating the forward and backward rates on the Yule trees produced virtually indistinguishable patterns (105/128 trees, $\rho = 0.87$).

Tests for Irreversible Evolution

Given the associations reported above, the ratio of transition rates appears to be a poor metric of the rate at which shifts toward and away from generalization occur. Most generally, we are interested in whether transitions in either direction ever occur. Thus we tested whether we could reject a model where transitions in one direction never occur. These analyses revealed that transition rates toward specialization and transition rates away from specialization are both very unlikely to be zero in most cases. A model where transition toward specialization was set to zero (which assumes a specialist root) was significantly worse than a model with both rates free to vary for nine of 15 trees (Table 1). More importantly from the point of view of the 'specialist as dead end' scenario, a model with the transition rate away from specialization was set to zero (which assumes a generalist root) was significantly worse than a model with both rates free to vary for 13 of 15 trees (Table 1). This second trend is unlikely to arise by chance ($P < 0.01$, binomial test). Although these analyses examine the extreme scenario of completely irreversible evolution, they do show that transitions both toward and away from specialization do likely occur. A less restrictive test that allows for a low but nonzero

TABLE 2. Likelihood scores for three different models for the state of the root (root free and estimated from the data, root fixed as a specialist (S), and root fixed as a generalist (G).

Taxon	Root free	Root = S	Root = G
1. *Timema* walking sticks	−10.35	−11.04	−10.87
2. Nymphalini butterflies	−14.26	−15.15	−14.59
3. *Uroleucon* aphids	−5.49	−6.19	−6.19
4. *Oreina* leaf beetles	−7.59	−8.32	−8.04
5. *Papilio* butterflies	−15.27	−15.8	−16.06
6. *Ophraella* leaf beetles	−7.45	−8.15	−7.94
7. Elachistidae	−3.17	−3.17	−4.19
8. *Chrysolina* leaf beetles	−17.12	−18.14	−17.45
9. *Timarcha* leaf beetles	−21.72	−21.90	−22.50
10. *Dendroctonus* bark beetles	−11.16	−11.85	−11.84
11. *Gonioctean* leaf beetles	−8.86	−9.51	−9.55
12. *Graphium* butterflies	−4.08	−4.71	−4.77
13. *Enchenopa* treehoppers	−6.94	−7.63	−7.55
14. *Drosophila* flies	−18.28	−18.35	−19.60
15. Heliothinae butterflies	−13.41	−13.89	−14.32

transition rate (set to 0.000001 for all trees) returned very similar results: 10/15 datasets reject such low transition rates to generalization and 14/15 datasets reject such low transition rates to specialization.

Importantly, and unlike the difference in ML parameter values themselves, the magnitude of the difference in likelihood between models (both rates free versus one rate fixed to zero) was not correlated with the percent of specialists at the tips of the tree (no transitions toward specialization, $r = -0.20$, $P = 0.47$; no transitions away from specialization, $r = -0.04$, $P = 0.90$). The same holds true when low but nonzero rates are tolerated (all $P > 0.20$).

Ancestor State Reconstruction and Sensitivity Analysis

We next compared relative support for alternate states at the root of the tree. There was little evidence that one state could be supported over the other. Weak support for a specialist over a generalist at the root was detected in two cases (Elachistidae, *Drosophila*; both with three- to fourfold relative support, Table 2). The greatest support for a generalist over a specialist at the root was with *Chrysolina* leaf beetles, which showed only twofold relative support (Table 2).

Our final set of analyses focused on inferred shifts in ancestral state (i.e., from G to S or from S to G). Unambiguous shifts from one ancestral state to the alternate state between adjacent nodes were rare; only two such shifts were observed on trees with equal branch lengths and both were from specialist to generalist (in the Elachistidae and in the Nymphalini). Thus, for both trees in Figure 2, the nodes preceding node 1 are reconstructed as specialists such that a transition from specialist to generalist occurred somewhere along branch leading to node 1. To be uncertain whether a shift in this direction (i.e., S to G) actually occurred requires ambiguous reconstructions at the nodes leading to node 1 (i.e., all nodes between the root and the shift, along the lineage considered). To generate ambiguous reconstructions at these nodes requires transition rates toward specialization to be 500-fold and 10-fold greater than transition rates toward generalization (for Elachistidae and Nymphalini, respectively). The fit of the resulting rates is 57 and 23 times less likely (for Elachistidae and Nymphalini, respectively) than the corresponding fits with the unconstrained ML rates. To be certain that a shift from specialist to generalist did not occur requires unambiguous reconstructions of generalists at all the nodes leading to node 1. Such a scenario requires transition rates toward specialization to be 5000-fold (128 times less likely) and 100-fold (43 times less likely) greater than transition rates toward generalization for Elachistidae and Nymphalini, respectively.

Under Grafen branch lengths, the only unambiguous S to G reconstruction occurred in the Nymphalini (this tree also furnished the sole unambiguous G to S reconstruction, which was not analyzed further). For this tree, the rate toward specialization must be 50-fold the rate away from specialization to cause ambiguity, and 300-fold to reconstruct all generalist interior nodes.

DISCUSSION

We report a positive association between the ratio of the forward to backward transition rate between character states estimated using unconstrained ML and the percent of a character state at the tips of the phylogenetic tree (Takebayashi and Morrell 2001). Because the stationary frequencies in Discrete (Pagel 1994) are assumed to be equal a priori, when the data suggest otherwise, relative transition rates may be higher toward the majority state. Our observations highlight a qualitative difference between parameter estimation for abundant DNA data and sparse ecological or morphological data on the tips of trees. With a single observation per tip for ecological data, the observed proportion of tips in a particular state will be a poor estimate of the stationary frequency, for instance, we assumed equal forward and backward rates for our simulated data, but on average 75% of the tips were in one of the two states. Indeed, this is why Discrete (Pagel 1994) does not use this information and instead, assumes equal stationary frequencies (M. Pagel, pers. comm.). DNA data, on the other hand, supply a large number of observations per tip, and the observed proportion of states (A,C,G,T) may provide a reasonable estimate of the stationary frequencies (see also Lewis 2001).

We do not advocate comparison of parameters that are poorly fit (Schluter et al. 1997; Mooers and Schluter 1999). Both here and in Nosil (2002), few datasets supported two-rate models, meaning that the transition rates under unconstrained ML will be poorly estimated. The current study focused on reevaluating the data from Nosil (2002) in light of the association detected using methods that do rely on directly comparing transition rates. The general conclusions of Nosil (2002) appear to hold; transitions both toward and away from specialization likely occur in phytophagous insects.

Three lines of evidence support this claim. First, models with transitions in one direction fixed to zero (i.e., irreversible evolution) were usually strongly rejected for both no transitions toward specialization and for no transitions away from specialization. We note that the level of support for models allowing both types of transition over models with irreversible evolution was independent of the proportion of specialists at the tips. Indeed, the irreversible model was often rejected both when transition rates were fixed to zero in the

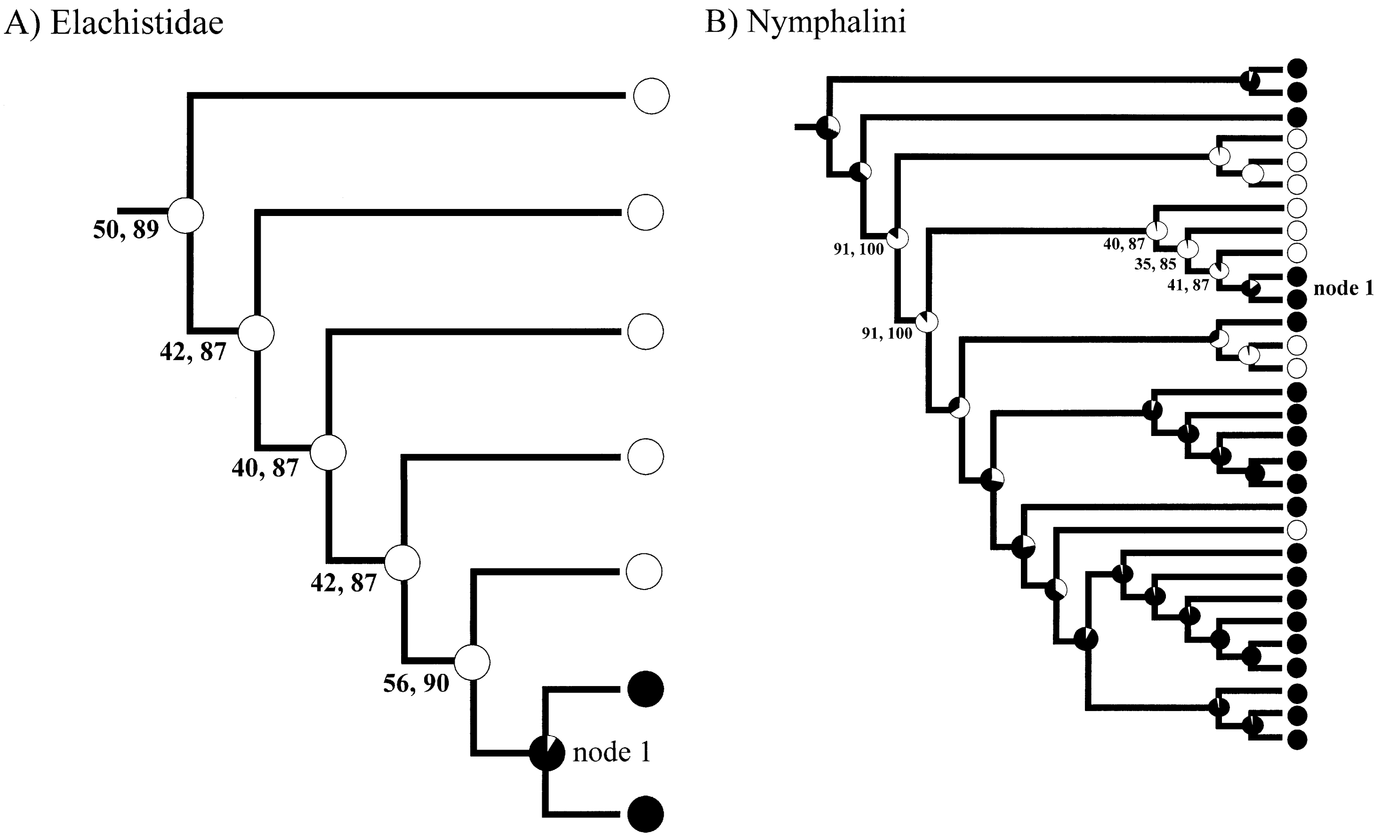

FIG. 2. Phylogenetic trees for the two groups of insects where an unambiguous shift in ancestor state was detected between adjacent nodes using global ancestor reconstruction under unconstrained maximum likelihood (ML) (i.e., both nodes had significant support, but for opposite states). Likelihood pies at the nodes depict the relative marginal probability of specialization in white. In both cases, a shift from specialist to generalist was detected somewhere along the branch leading to node 1. To lose certainty in the direction of this shift (i.e., from specialist toward generalist) requires that the nodes preceding node 1 be ambiguously reconstructed or be reconstructed as generalists. The numbers beside the likelihood pies are the marginal probability of generalism when the asymmetry in transition rates is varied to accomplish these two scenarios. (A) Elachistidae. Free rates: G to S = 0.001, S to G = 0.114; ambiguous reconstruction: G to S = 500*(S to G), where G to S = 0.550; unambiguous reconstruction as G, G to S = 5000* (S to G), where G to S = 0.550). (B) Nymphalini. Free rates: G to S = 0.075, S to G = 0.242; ambiguous reconstruction: G to S = 10*(S to G), where G to S = 0.120; unambiguous reconstruction as G, G to S = 100*(S to G), where G to S = 0.130).

direction supporting, and in the direction opposing, the bias (i.e., both away from and toward the most common state at the tips). As stated previously, these tests for irreversible evolution represent the extreme scenario of totally unidirectional evolution. We note that similar results were found when nonzero but very low rates in one direction were allowed. Bayesian methods offer promise for exploring a wider range of scenarios (e.g., Pagel et al. 2004), but are quite sensitive to the prior probabilities used.

Second, the direction of shifts in ancestral state could rarely be unambiguously determined such that unambiguous shifts from generalist to specialist did not predominate. For example, our trees did not contain enough information to make strong inferences about the root state (see also Mooers 2004). In two of the three cases where support for a transition along a branch was strong (and in all cases using uniform branch lengths), they represented a shift from specialist to generalist near the tips of the tree, contrary to the ''specialist as dead end'' scenario.

Third, transition rates would need to be strongly biased to reconstruct a history without the two shifts toward generalization. We do not imply that the studies examined are representative with respect to the natural diversity of insects. Thus, rather than comment on the relative frequency of different transitions, particularly given the reported association between rate and state frequencies (see below), we simply propose that the present data support the notion that shifts in either direction can occur.

More generally, determining the relative probability of transitions between character states using current phylogenetic methods is difficult (see also Mooers and Schluter 1999). Fixing the root of the tree does not alleviate the association we report. Thus, even a representative sample of insect groups might yield erroneous conclusions regarding host range evolution using unconstrained transition rate estimates alone. We direct readers to Stireman (2005) for a more thorough consideration of potential problems associated with phylogenetic inferences regarding host range evolution.

Another observation is that despite the tendency for rates to reflect state frequencies, the tree and distribution of character states among the tips of the phylogeny also exert an influence. Consider the Elachistidae example, which does not conform to the association between state frequencies and transition rates shown in Figure 1. This tree is comprised of mainly specialists and thus the reported association would predict higher transition rates toward specialization. However, on this tree, higher transition rates are inferred toward generalization, a model with no transitions toward generalization is strongly rejected, a model with no transitions toward specialization is retained, and extremely biased transition rates are required to reconstruct a history without a shift in ancestral state from specialist to generalist (at least when branch lengths are assumed uniform). Importantly, all the generalists in this group form a monophyletic group (Fig. 1); this is consistent with a low rate of transition (Schluter et al. 1997) and suggests that the reported association might be more prevalent when character states are scattered among the tips of the tree and estimated rates are higher. Finally, not only are the generalists in the Elachistidae tree monophyletic, but they are highly nested in the tree, further suggesting that where characters state transitions occur (i.e., which is affected by the distribution of states) also affects parameter estimation.

A phylogenetic study of pollination of *Dalechampia* vines further supports this suggestion (Armbruster and Baldwin 1998). The phylogeny was comprised of primarily specialists (18 of 24 species) yet transition rates estimated by us using unconstrained ML are biased away from specialization (0.0296 away from specialization versus 0.00053 toward specialization). The generalists form a highly nested monophyletic group and the node ancestral to their most recent common ancestor is reconstructed with 100 percent certainty as a specialist. Thus, unconstrained ML analysis applied to this dataset yields conclusions that are congruent with the parsimony analyses used by the authors; a shift from specialized to generalized pollination occurred.

We also highlight how one can test the robustness of a particular evolutionary scenario. A few other studies have employed such sensitivity analyses (they are not yet standard procedure). For example, Oakley and Cunningham (2002) found that 10:1 bias was required to lose confidence in the most likely scenario that compound eyes had evolved multiple times in arthropods. A 30:1 bias was required before a single origin hypothesis was statistically favored (Fig. 2; Oakley and Cunningham 2002). Shi et al. (2005) conducted a similar analysis examining the evolution of vivipary in mangroves. A 12:1 bias was required to lose confidence in the most likely scenario of multiple origins of this trait, and 18:1 bias was required to statistically favor a single origin (Fig. 2; Shi et al. 2005). The biases in transition rates required to lose confidence in the most likely scenario in these other studies were somewhat lower than the biases required in our study (from 10:1 to 5000:1; our Fig. 2). Whether biases of this magnitude are biologically likely or not will likely require additional studies of the ecology of the group in question. Factors that might be considered are levels of genetic variation and the complexity of the relevant traits (e.g., Futuyma et al. 1995 for an example with genetic variation in host-plant use). Thus, models that can incorporate different types of ecological and genetic data, and multiple characters, might help increase the accuracy of inferences made using phylogenetic trees.

In conclusion, our goals were to explicitly report the effect of state frequencies on the estimation of transition rates using unconstrained ML for single characters on phylogenetic trees, and to reevaluate the direction of host range evolution in phytophagous insects in light of this effect. Clearly, further empirical, theoretical and simulation studies are required to better understand the accuracy of inferences made using ML analyses on phylogenetic trees in general, and on the relative rates of change in the breadth of arthropod host range in particular. The simulations reported in the current study are presented only to illustrate the point and studies examining the effects of variation in multiple factors, including tree shape, evolutionary rates, and state frequencies, would be particularly useful.

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