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Specialization and generalization in the diversification of phytophagous insects: tests of the musical chairs and oscillation hypotheses

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Evolutionary biologists have often assumed that ecological generalism comes at the expense of less intense exploitation of specific resources and that this trade-off will promote the evolution of ecologically specialized daughter species. Using a phylogenetic comparative approach with butterflies as a model system, we test hypotheses that incorporate changes in niche breadth and location into explanations of the taxonomic diversification of insect herbivores. Specifically, we compare the oscillation hypothesis, where speciation is driven by host-plant generalists giving rise to specialist daughter species, to the musical chairs hypothesis, where speciation is driven by host-plant switching, without changes in niche breadth. Contrary to the predictions of the oscillation hypothesis, we recover a negative relationship between host-plant breadth and diversification rate and find that changes in host breadth are seldom coupled to speciation events. By contrast, we present evidence for a positive relationship between rates of host switching and butterfly diversification, consonant with the musical chairs hypothesis. These results suggest that the costs of trophic generalism in plant-feeding insects may have been overvalued and that transitions from generalists to ecological specialists may not be an important driver of speciation in general.

1. Introduction

About half of all eukaryotic species are plant-feeding insects [1]. The evolutionary processes that have driven the diversification of herbivorous insects are poorly understood, but host-plant interactions are thought to be a critical factor. The classic escape and radiate model of Ehrlich & Raven [2] intertwines the diversification of plants with that of the insects that eat them. This hypothesis assumes that plant diversity is limited by insect herbivore pressure and that herbivore diversity is limited by the nutrition and anti-herbivore defences of potential host plants. Under the escape and radiate model, plant or herbivore lineages that evolve ways to break free of these limitations diversify rapidly owing to their expanded ecological opportunities.

Ehrlich and Raven did not specify a mechanism by which exposure to ecological opportunity would promote taxonomic diversification and, in point of fact, that mechanism remains uncertain [3]. Ecological opportunity is expected to result in ecological release, whereby selection becomes more diversifying and increased phenotypic variability is favoured. Yet theoretically, this release can lead either to many ecologically specialized species or to few ecologically generalized species, without rapid speciation [3,4].

Nevertheless, the escape and radiate model successfully predicts some patterns in biodiversity. In particular, the assumption that host-plant associations are instrumental in shaping the diversity of insect herbivores has been supported by a number of empirical studies. For example, Janz *et al.* [5] found a positive relationship between the species diversity of butterflies and the species diversity of their host-plant taxa, and Fordyce [6] reconstructed brief increases in butterfly diversification rates following major host shifts.

One possible mechanism for turning ecological release into rapid species diversification centres on the idea that ‘the jack of all trades is the master of none’ [7]. Diversifying selection regimes will tend to result in taxonomic diversification when there are non-trivial costs for broad niches, e.g. when increased trophic breadth strongly reduces the efficiency of resource use [8]. If we assume that there are fitness trade-offs associated with ecological generalism and that specialists do a better job in their niche than can a generalist, then generalism may be an ephemeral state [9,10]. In the context of plant-feeding insect diversification, this notion has been incorporated in the oscillation hypothesis [5,10]. Under this model, insect herbivore lineages undergo alternating phases of host expansion and contraction. Speciation is thought to be driven by the evolution of specialized populations from a more generalist ancestor, with episodic host-breadth expansions, replenishing the ‘fuel’ consumed by speciation via specialization.

Generalist species are not, however, the only source of new species, as specialists may switch specialties and expand into new adaptive zones without major changes in niche breadth [11]. We refer to this idea as the musical chairs hypothesis, which centres attention on host switching rather than on specialization. Taxonomic diversification of plant-feeding insects may thus be driven by the sheer diversity of niches associated with plants [12], allowing for multiple rounds of host switching. In this case, we expect lineages with more labile host associations to diversify more rapidly than those that switch hosts less often. Note the term ‘musical chairs’ has been used before in the ecological literature to describe the impacts of interspecific competition on niche size and geographical distribution [13]. We do not intend to imply any connection between that and the hypothesis tested here.

In this study, we use a comparative phylogenetic approach to test the impact of host breadth and lability on the diversification of butterflies (Papilionoidea). The oscillation and musical chairs hypotheses make different specific predictions about the effects of host breadth on diversification rates (table 1). The oscillation hypothesis predicts a positive relationship between host breadth and diversification rate, as it is the generalist ancestors that give rise to new specialist species. Because speciation is driven by host specialization, this hypothesis further predicts that speciation events should be associated with a trait shift from polyphagy to monophagy. That is, we would expect host specialization to involve cladogenetic shifts (associated with speciation events) rather than anagenetic shifts (proportional to time). While not a strict prediction of the oscillation hypothesis, we also estimate the relative rates of transition to and from polyphagy, as this would determine how often lineages are likely to be in the generalist state promoting speciation. The musical chairs hypothesis predicts that diversification rates hinge strongly on aspects of butterfly biology that result in faster rates of host switching. Here, we do not attempt to identify specific traits that affect rates of host switching, but examples of such traits include trophic mode (e.g. external folivore, leaf miner), defensive strategy (chemical sequestration, crypsis) and susceptibility to natural enemies [14]. In general, the more complex and intense the ecological interactions between herbivorous insects and their host plants, the less likely it may be for a specialist to switch and survive on a new host. The predictions of the oscillation and musical chairs hypotheses are assessed using likelihood-based trait-dependent diversification models [15–18], applied to

Table 1. Predictions of oscillation and musical chairs hypotheses on the rate and mode of diversification in herbivorous insects. M, monophagous; P, polyphagous; H \uparrow , large host breadth; H \downarrow , small host breadth; r , diversification rate (speciation rate minus extinction rate); p_{Xc} , proportion of speciation events associated with a shift away from state X; L \uparrow , high lability host associations; L \downarrow , low lability host associations.

prediction	oscillation	musical chairs
$r_{H\uparrow} > r_{H\downarrow}$	+	–
$p_{Pe} > p_{Mc}$	+	0
$r_{L\uparrow} > r_{L\downarrow}$	0	+

phylogenies of both the superfamily Papilionoidea and the tribe Heliconiini. Our results shed light on the likely mechanisms by which ecological opportunity promotes taxonomic diversification and on the impact of generalists and specialists on speciation.

2. Methods

(a) Phylogeny estimation

We explored the effect of host-plant breadth on butterfly diversification at two scales: at a relatively coarse scale across the breadth of Papilionoidea diversity, and at a finer resolution in the more densely sampled Heliconiini. DNA sequences from 12 loci (argK, CAD, EF1 α , gapDH, tektin, wingless, rps2, rps5, cytb, nd1, nd5 and 16S) and 2573 species across the Papilionoidea were downloaded as unaligned FASTA files via PhyLoTA rel. 1.5 [19]. Note that PhyLoTA employs a clustering algorithm to minimize the inclusion of paralogous genes, which can mislead phylogenetic inference. Sequences from each locus were aligned with MAFFT [20]. The 16S alignment was filtered through Gblocks to remove hypervariable regions [21], setting the allowed gap positions to half, the minimum length of a block to 5, and the maximum number of contiguous non-conserved positions to 12. MESQUITE v. 2.73 [22] was used to delimit codon positions, delete introns and create a supermatrix of concatenated loci. The maximum-likelihood (ML) phylogeny was estimated with RAxML v. 7.0.4 [23]. The supermatrix was partitioned by genome (mitochondrial or nuclear) and codon position, and parameters of a general time reversible (GTR) nucleotide substitution model with CAT approximation of among-site substitution rate variation were estimated independently for each partition. The ML tree search was then repeated across 100 non-parametric bootstrap (BS) pseudo-replicates of the sequence data, followed by more thorough optimization of the original dataset under GTR + G, using every fifth BS tree as a starting tree. Tips lacking host-plant data (see below) were pruned from the ML tree, leaving 1039 out of the 15 603 known Papilionoidea species (Catalogue of Life Dynamic Checklist) within the tree (6.7%). To obtain ultrametric trees, we then used BEAST v. 1.7.5 [24], which estimated phylogenetic divergence times, fixing the topology on the pruned ML tree. We fixed the topology because, with so many taxa, BEAST was unable to converge on a stationary distribution within a reasonable time frame (convergence still had not occurred after several runs of 50 million steps). The BEAST analysis assumed a global HKY + G nucleotide substitution model, a birth–death model of phylogenetic branching, and an uncorrelated lognormal-relaxed clock model of among-lineage substitution rate variation [25]. An exponential prior with an offset of 33.7 Ma was applied to the age of the stem node of

Papilionidae [26], and a normal prior with a mean of 95 Ma and a standard deviation of 1 Ma was applied to the root node of Papilionoidea [27].

In addition, we performed a finer scale analysis among lineages of Heliconiini, where we had more complete sampling. We used BEAST to simultaneously estimate phylogenetic relationships and divergence times among Heliconiini species, based on an alignment of 4164 sites sampled from 63 of the 113 described species (56%). The model used in the BEAST analysis was the same as that used for the Papilionoidea data, except that the topology was free to vary and the site model was partitioned by codon position and genome. We ran the analysis for 10 million steps and sampled trees once every 10 000 steps, resulting in a set of high-posterior probability trees that captured the extent of tree uncertainty. No fossil Heliconiini are known. Therefore, we standardized the Heliconiini branch lengths such that the root-to-tip distance was 13.7 myr (the age of Heliconiini inferred from the analysis of Papilionoidea), so that rate estimates would be consistent across analyses of the two datasets. As a consequence, the absolute branch lengths and diversification rates will be sensitive to this assumed root age, but their relative values (e.g. comparing speciation rates between traits) would not be. For the comparative analyses, we removed terminal branches from the Heliconiini trees that lacked trait information; the resulting trees included 33 species (29% of the known diversity).

For the Papilionoidea, tests were performed either on 10 trees selected randomly from the set of high posterior probability trees or, when calculation time was prohibitive, on the maximum clade credibility tree—the tree with the maximum product of the posterior clade probabilities. For the Heliconiini, tests were performed on a set of 100 trees randomly chosen from those sampled from the stationary distribution, because the dataset was smaller, and the model fitting calculations were faster.

(b) Host-plant data

Host-plant data were collated from the web interface of the HOSTS database of the world's lepidopteran host plants [28] using a PYTHON script. For the Papilionoidea dataset, we measured host breadth as the number of host-plant families. Because Heliconiini are thought to feed only on Passifloraceae, we measured host breadth for this analysis using phylogenetic diversity (PD: [29]). Using the same methods described above, we estimated the Passifloraceae phylogeny from DNA sequences sampled from 249 species and eight genes (rps4, its1, 5.8S, its2, 26S, trnL-trnF intergenic spacer, trnL-trnT intergenic spacer, trnL) totalling 3589 aligned sites. Relationships among *Passiflora* species were largely congruent with published estimates [30]. PD host-breadth scores were calculated for each Heliconiini species by pruning all non-host species from the Passifloraceae tree, and summing the lengths of the remaining branches, including the stem branch length.

The phylogenetic and host-plant datasets had 1039 species in common. For these species, we were able to retrieve 6987 distinct host-plant species records from the HOSTS database (available from Dryad archive).

(c) Diversification tests

Trait-dependent diversification tests were conducted in the R software environment (R Development Team 2013), using the package diversitree [31]. In these analyses, host breadth was treated as either a binary or a quantitative character. For our main analyses, the data were treated both ways, allowing us to assess sensitivity to the coding of the trait. Some analyses are currently only possible with discrete characters [17], and we thus limited our analyses to the binary coding of host breadth where mentioned. The main parameters of the models are summarized in

figure 1; for the sake of clarity, the specific tests performed are described in the Results section.

For the Papilionoidea dataset with host breadth treated as a binary character, butterfly species feeding on host plants in more than one plant family were considered polyphagous, whereas those with hosts restricted to one plant family were considered monophagous. This definition of monophagy is somewhat broad and arbitrary, but it has been used extensively in the literature [6,32,33], including in a previous study that supported the oscillation hypothesis [5]. Nevertheless, it is important to keep in mind this family-based definition of monophagy when interpreting our results. In a second analysis with the Papilionoidea data, we treated host-plant breadth as a quantitative trait, measured as the number of host-plant families fed upon by each butterfly species. For Heliconiini butterflies, we used PD as a quantitative measure of host breadth. Host breadths of species of Heliconiini were approximately log-normally distributed. To discretize this trait, species with PD scores less than or equal to 0.01 (48%) were treated as specialists ('monophagous'), and those with PD scores > 0.01 were treated as generalists ('polyphagous').

Trait-dependent diversification analyses require that we account for taxonomic sampling across traits. Trait-specific phylogenetic sampling frequencies were approximated by calculating the proportion of polyphagous to monophagous species in the HOSTS database, and then using this ratio to estimate the total number of described polyphagous and monophagous species. With this approximation, the Papilionoidea analyses include 8.3% of the described polyphagous species and 5.8% of the described monophagous species, which were used as the sampling fractions in diversitree. A global sampling frequency of 6.7% was used in analyses that modelled host-plant breadth as a quantitative trait, because trait-specific sampling frequencies have not yet been implemented for quantitative traits in diversitree. The Heliconiini analyses include 29% of the described species diversity. We did not specify the sampling frequency for monophagy and polyphagy separately, because we lacked the information necessary to estimate PD for the unsampled Heliconiini species.

3. Results

(a) Effect of host-plant breadth on diversification rates is negative

Under the oscillation hypothesis, we expect a positive relationship between insect herbivore diversification rates and host-plant breadth. We tested this prediction in an ML framework. First, using Papilionoidea with host breadth as a binary character, we fit the full binary-state speciation and extinction (BiSSE) model [15] (see figure 1 for all parameter definitions). We then used a likelihood ratio test to compare the fit of the full model with a model in which speciation rates of monophagous and polyphagous species were constrained to be equal. We repeated these tests over 10 BEAST trees to account for phylogenetic uncertainty. The full BiSSE model estimated more than threefold higher speciation rates in monophagous lineages (mean $\lambda_M = 2.28$ speciation events per Ma) than in polyphagous lineages (mean $\lambda_P = 0.688$) and was preferred over a model in which speciation rates were constrained to be equal across states (median p -value = 1.17×10^{-5} ; $p < 0.05$ for 10/10 BEAST trees). Extinction rates were estimated to be considerably lower in polyphagous lineages (mean $\mu_M = 2.11$; $\mu_P = 0.633$; median p -value = 3.22×10^{-5} ; $p < 0.05$ for 10/10 BEAST trees), but net diversification rates of monophagous lineages remained significantly higher.

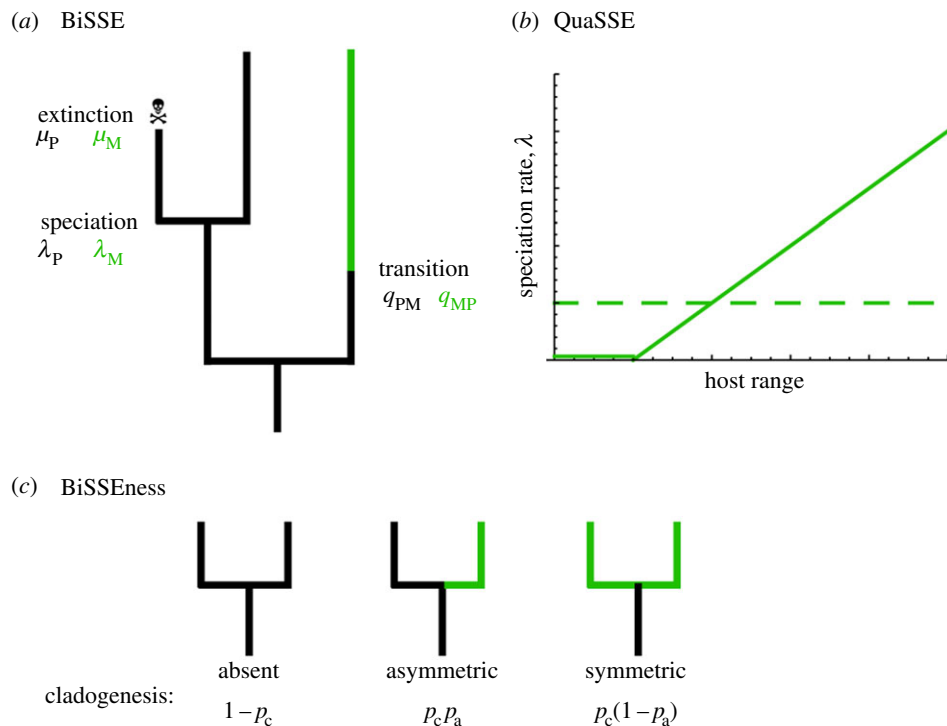


Figure 1. Models relating the state of a single trait to speciation and extinction. (a) The binary-state speciation and extinction model (BiSSE) allows the speciation rate (λ) and extinction rate (μ) to depend on the state of the trait (e.g. M, monophagy; P, polyphagy), simultaneously modelling transitions between the trait states (at rates q_{MP} : monophagy to polyphagy and q_{PM} : polyphagy to monophagy). (b) The quantitative state speciation and extinction model (QuaSSE) handles continuous-state traits (e.g. host breadth). To reduce the number of parameters, we assume that the extinction rate (μ) is constant and fit only linear models relating the speciation rate (λ) to the trait state. The key parameter here is the slope (λ_{slope}), showing either a positive relationship (solid) or no relationship (dashed) to the trait state. Note that QuaSSE automatically sets the speciation rate to zero if ever it becomes negative (see FitzJohn 2010 for fitting other functional relationships). (c) BiSSEness extends BiSSE by allowing cladogenetic shifts in the trait to coincide with speciation events, as would be expected if trait changes cause speciation (or vice versa). The illustration assumes that the species was monophagous, generating two monophagous daughter species with probability $(1 - p_{cM})$. The probability of cladogenesis, p_{cM} , is then further subdivided into cases where one (p_{aM}) or both ($1 - p_{aM}$) daughter species switch state. Because the likelihood of the data is generally only weakly affected by the asymmetry parameter, we set $p_{aM} = p_{aP} = 0.5$, which is ambivalent about the nature of cladogenetic events. (Online version in colour.)

Second, treating host-plant breadth in Papilionoidea as a quantitative trait and using the maximum clade credibility BEAST tree, we compared the fit of two quantitative state speciation and extinction (QuaSSE) models [16]: (i) a model in which speciation rates change as a linear function of host breadth, and (ii) a constant rate model in which speciation rates are independent of host breadth (figure 1). The slope of the linear QuaSSE model relating speciation rate to host breadth was negative (figure 2, $\lambda_{\text{slope}} = -0.091$), and the linear model was a much better fit than a model in which diversification rate was independent of number of hosts ($p\text{-value} = 2.9 \times 10^{-8}$ on the maximum clade credibility tree).

Finally, we performed the same BiSSE and QuaSSE analyses using PD measures of host breadth in Heliconiini butterflies, repeating the tests over 100 BEAST trees. Results of the analysis of Heliconiini diversification using PD measures of host breadths confirm results from analyses of the Papilionoidea dataset (figure 2). Using the binary coding, net diversification was higher in monophagous lineages, although support for host breadth associated differences in diversification rates was not significant (median $p\text{-value} = 0.27$; $p < 0.05$ for 4/100 BEAST trees). Analysing PD as a continuous character, QuaSSE fit a linear model again with a negative slope (mean $\lambda_{\text{slope}} = -0.084$) for the relationship between host breadth and diversification rate, which was a marginally better fit than a constant rates model (median $p\text{-value} = 0.077$; $p < 0.05$ for 18/100 BEAST trees).

(b) Cladogenetic change in host-plant breadth

If butterfly speciation is driven by host-plant specialization, as expected under the oscillation hypothesis, we should see changes in host breadth coupled in time with speciation events (i.e. we should reject the hypothesis that there is no cladogenetic change, $p_{cM} = p_{cP} = 0$), and speciation should more often involve decreases in host breadth than increases ($p_{cM} < p_{cP}$). To test this, we estimated the parameters of a binary-state speciation and extinction-node enhanced state shift (BiSSEness) model [18] applied to the Papilionoidea and Heliconiini phylogenies using the binary host breadth data. This method estimates the proportion of speciation rates associated with a switch in trait state, p_c (figure 1), but the method is currently not available for quantitative traits. BiSSEness also estimates the probability, p_a (figure 1), that cladogenetic shifts involve only one daughter species versus both daughter species. We fixed p_a at 0.5 to allow for both possibilities without requiring the estimation of additional parameters, as recommended by Magnuson-Ford & Otto [18]. We then compared the fit of the full BiSSEness model with the fit of (1) a model in which the proportion of cladogenetic change in host breadth was constrained to be equal in polyphagous and monophagous lineages and (2) a model in which all changes in host breadth were anagenetic.

The results provide no support for the oscillation hypothesis. For the Papilionoidea dataset, the estimated proportion of speciation events coupled with a shift in host breadth for polyphagous lineages ($p_{cP} = 5.1 \times 10^{-7}$) was estimated to be lower than the

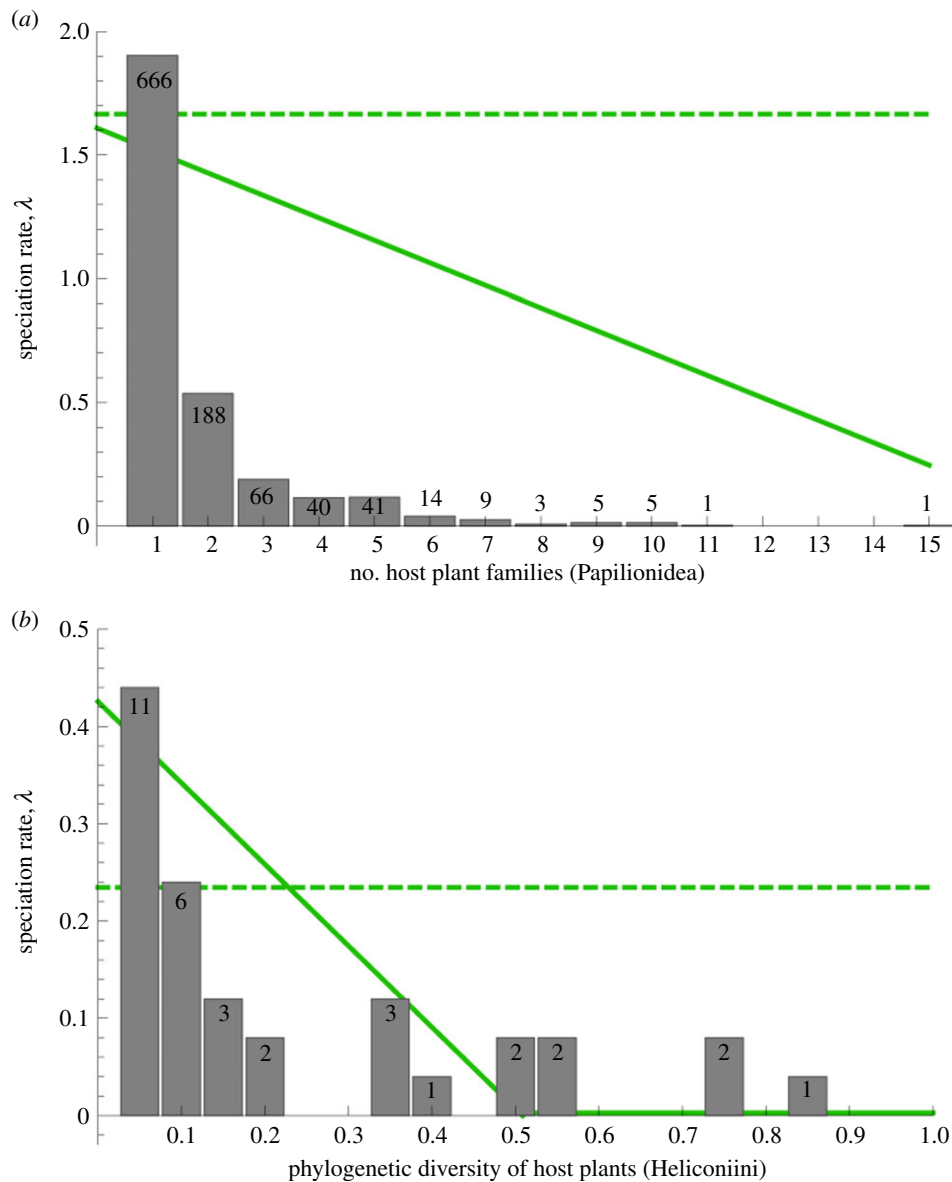


Figure 2. Inferred speciation rate as a function of host breadth. (a) The number of host-plant families used by each of the 1039 Papilionoidea species included is shown in a histogram, alongside the ML estimate of the speciation rate for the QuaSSE model allowing speciation rate to depend on host breadth (solid line with $\lambda = 1.61 - 0.091 \times (\text{host breadth})$), which fit significantly better (p -value = 2.9×10^{-8}) than a model with a constant speciation rate (dashed line, $\lambda = 1.67$). (b) Phylogenetic diversity of host-plant families for the 33 Heliconiini species, with the ML estimate of the linear speciation rate model (solid line, averaged over 100 trees, $\lambda = 0.43 - 0.84 \times (\text{PD})$), which fit marginally better (median p -value = 0.077) than a model with a constant speciation rate (dashed line, averaged over 100 trees, $\lambda = 0.23$). (Online version in colour.)

proportion for monophagous lineages ($p_{\text{CM}} = 2.5 \times 10^{-2}$; electronic supplementary material, table S1). The full model allowing cladogenetic change in both monophagous (p_{CM}) and polyphagous (p_{CP}) lineages did not fit significantly better than a constrained model where these rates were equal ($p_{\text{CM}} = p_{\text{CP}}$) or a model in which only anagenetic change was permitted ($p_{\text{CM}} = p_{\text{CP}} = 0$). Likewise, for the Heliconiini dataset, the full BiSSEness model was no better than a model in which host breadth only evolves anagenetically. These results suggest that most changes in host breadth have not been associated with speciation. We were thus unable to detect a signature of cladogenesis driven by generalist butterflies evolving more specialized diets, or specialist butterflies evolving more generalized diets.

(c) Host-plant breadth and dispersiveness

Host-plant breadth and dispersiveness might be correlated, so that any effects on species diversification ascribed to polyphagy/monophagy may truly be caused by a correlation with

dispersal ability. We tested this possibility using dispersiveness data available for species of Heliconiini; Brown [34] classified Heliconiini species as either philopatric, dispersive or migratory. Theoretically, migratory species are exposed to many of the same risks as randomly dispersing species, but they maintain their associations with natal habitats. The analyses were performed with migratory species coded as philopatric and then repeated with migratory species coded as dispersive. Treating both host breadth ($\text{PD} \leq$ or > 0.01) and dispersiveness in Heliconiini as binary traits, we used multi-trait multi-state speciation and extinction (MuSSE) model comparisons [17] to test whether [1] host breadth and dispersiveness independently affect speciation rates, and [2] a combination of host breadth and dispersiveness is associated with elevated speciation rates (figure 3). To reduce the number of parameters, extinction rates were assumed to be constant ($\mu_{\text{PD}} = \mu_{\text{PN}} = \mu_{\text{MD}} = \mu_{\text{MN}}$; figure 3). Analyses were repeated over 100 BEAST trees. We performed this analysis only on the Heliconiini dataset with host breadth treated as a binary character, because multi-trait

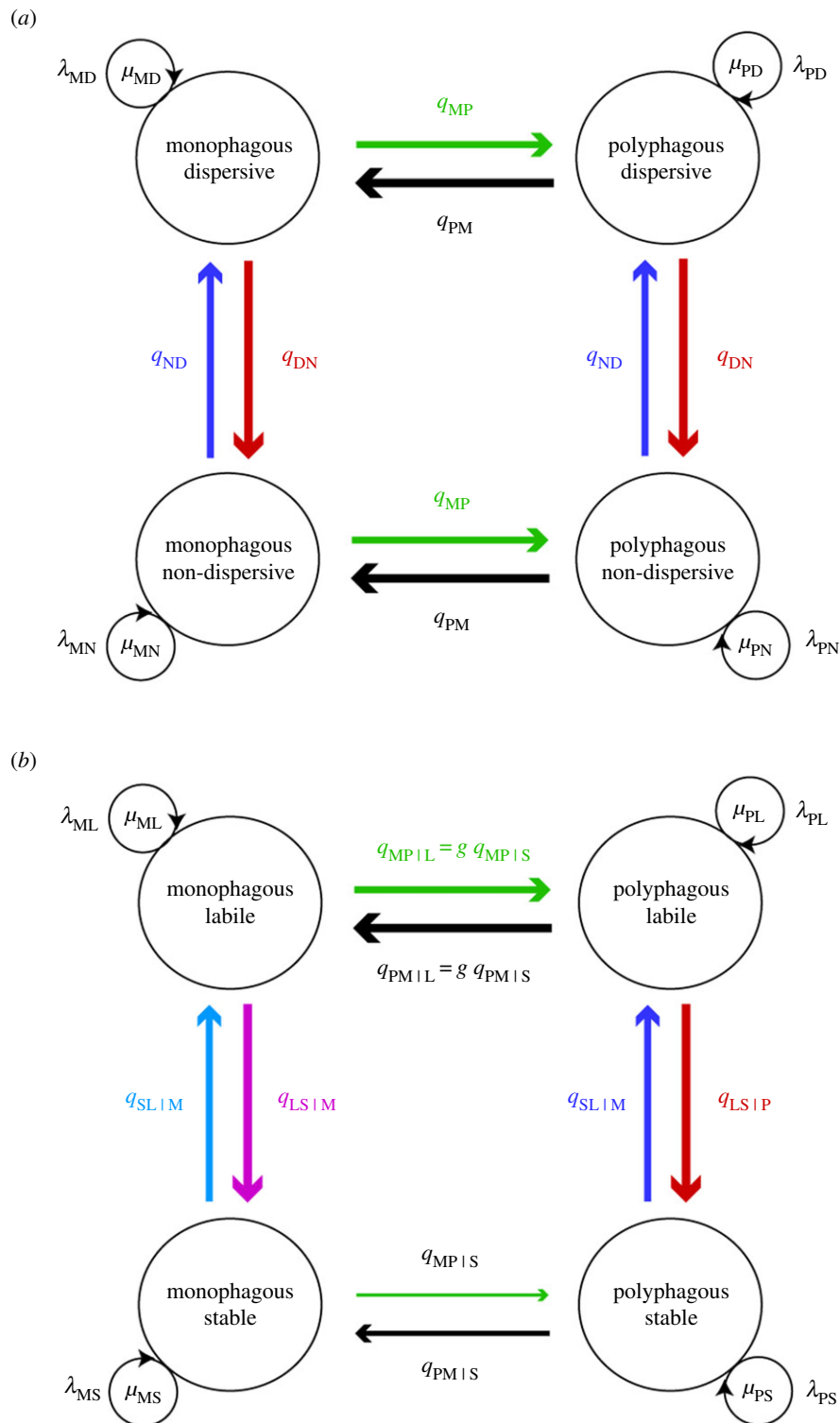


Figure 3. Models relating the state of two traits to speciation and extinction. The multiple state speciation and extinction model (MuSSE) was used to explore two hypotheses. (a) Diversification effects may be driven by alternate characters than the focal trait of interest. Here, we explored two binary-state traits, monophagy (M) versus polyphagy (P) and dispersive (D) versus less dispersive (N), to assess the relative impact of the two traits on speciation (λ) and extinction (μ). The transition rates between the two states (q_{ij}) for each trait were assumed to be independent of the state of the other trait. (b) Diversification effects may be driven by the propensity to change more than the state itself. A hidden trait was incorporated via MuSSE to alter the rate of transitions between monophagy and polyphagy. These rates occurred g times faster when the hidden trait was in the 'labile' state than when it was in the 'stable' state. The model was fit allowing transitions in the lability trait to depend on the host-breadth trait, which fit better than assuming independent transition rates (i.e. constraining $q_{SL|M} \sim q_{SL|P}$ and $q_{LS|M} \sim q_{LS|P}$).

analyses are not currently available for quantitative characters, and because there is insufficient information on dispersiveness across the Papilionoidea dataset.

Regardless of whether migratory species were classified as dispersive or non-dispersive, the negative relationship between

host-plant breadth and butterfly diversification rates remained, even after dispersiveness was added as a character, although the result was not significant in the Heliconiinae (median p -value = 0.19; $p < 0.05$ for 1/100 BEAST trees). Furthermore, the model in which dispersiveness did not influence speciation

rates ($\lambda_{PD} = \lambda_{PN}$ and $\lambda_{MD} = \lambda_{MN}$) was no worse than the full model (median p -value = 0.61; $p < 0.05$ for 0/100 BEAST trees), and there was no evidence for an interaction between host breadth and dispersiveness on speciation rates (median p -value = 0.46; $p < 0.05$ for 0/100 BEAST trees).

(d) Effect of host-plant lability on diversification

Under the musical chairs model, the primary driver of speciation in phytophagous insects is the lability of host associations, i.e. the rate at which host associations evolve (whether or not these changes affect host breadth). We tested this prediction using two approaches. In the first approach, we allowed a second (hidden) trait to alter the rate at which host breadth shifts. Again, we classified host breadth (H) as a binary trait, but the transition rates between low and high host breadth were allowed to depend on the state of a second trait (L , lability). For example when a lineage has high host lability ($L = 1$), then the transition rates for host breadth ($H1 \leftrightarrow H0$) were allowed to be a factor g times higher than when $L = 0$ using the constrain function in diversitree (figure 3). Note, although our definition of lability includes switching within and between host breadth classes (specialist or generalist), our approach detects changes only between classes.

We then used MuSSE [17] to test whether the lability in host breadth affects speciation by comparing the fit of a model in which speciation rates are constrained to be equal for high and low lability lineages to a model in which these parameters are free to vary. Although it would be valuable to repeat this analysis while coding host breadth as a quantitative trait, current methods do not (yet) allow a simultaneous analysis involving quantitative traits with more than one trait.

In the multi-trait MuSSE analysis with Papilionoidea, the hidden lability trait had a strong impact on diversification rates, compared with a model where only host breadth affected diversification (p -value $\ll 0.001$), with diversification being highest in monophagous lineages with high host lability (electronic supplementary material, table S1). Constraining polyphagous and monophagous taxa to the same diversification rate led to a significant decrease in the fit of the model (electronic supplementary material, table S1: p -value $\ll 0.001$). In the analysis of Heliconiini, there was no significant effect of the lability trait, suggesting that transitions between monophagy and polyphagy are not critical to speciation over this limited taxonomic scale. Although this method does not directly measure lability among extant species, it is consistent with the musical chairs model in suggesting that it is the switching rate, especially among monophagous species, that drives diversification.

In a second approach, we measured the correlation between rates of host switching and rates of diversification across butterfly clades of similar age. To quantify rates of host switching, we focused our attention only on monophagous species and quantified the rate at which these switched host-plant families. Specifically, we removed all polyphagous species from the maximum-clade-credibility ultrametric Papilionoidea tree, and then coded the remaining host-plant associations as a multi-state trait with 166 states (one for each host-plant family in the dataset). Next, a set of subtrees were assembled by cutting branches at 25 Ma from the tips, and discarding any tree with fewer than 10 tips. The rate of host switching was then estimated under an equal rates model of discrete trait evolution, using the fitDiscrete function of the R package geiger [35]. To

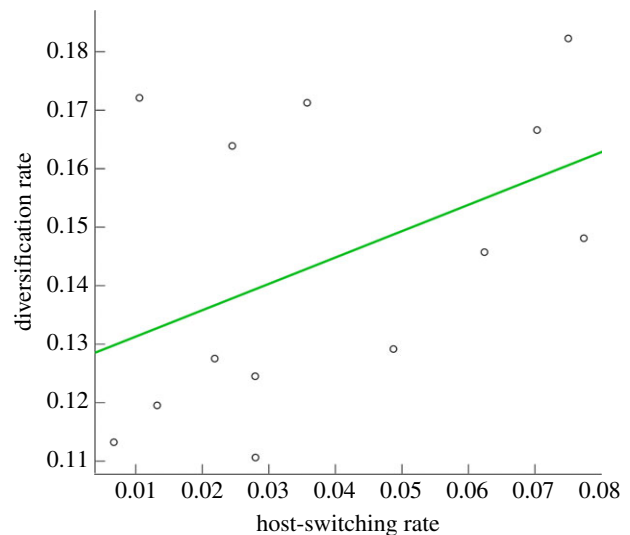


Figure 4. Relationship between rate of host switching and diversification in equal clades. Host associations were coded as a multi-state character for each monophagous (restricted to one host family) species included in the Papilionoidea phylogeny. For each 25 Ma old clade, we estimated the rate of an equal rates model of character change for the monophagous lineages (the character being the host-plant family), and the net diversification rate given all of the taxa in the clade (monophagous and polyphagous). Estimates of diversification rates in each clade were state-independent. (Online version in colour.)

obtain the diversification rate, we returned the polyphagous species to each subtree (but keeping the same branch-cut position), and estimated the net diversification rate using the geiger function rate.estimate, which implements the method of Magallon & Sanderson [36]. Finally, the relationship between rates of host switching and rates of diversification was computed using Pearson's r correlations in R. The significance of the correlation was assessed with a randomization test, i.e. a null distribution of Pearson's r -values was constructed by creating 100 datasets of the same size as the observed data, composed of random pairings of diversification rate and host switching rate.

We recovered a positive relationship between rates of host switching and diversification rate across 25 Ma old butterfly clades, although the result was only marginally significant (figure 4; Pearson $r = 0.457$, randomization two-tailed p -value = 0.09). This correlation remained positive when cut-off times were set instead to 20 or 30 Ma, but the sample sizes were then lower and the correlations less significant (Pearson $r = 0.341$ and 0.515 , p -values = 0.34 and 0.20, respectively).

4. Discussion

Here, we provide evidence that host-plant associations are an important factor affecting the diversification of butterflies. We tested two hypotheses for how these associations might affect diversity: diversification is driven by generalists giving rise to specialist daughter species (the oscillation hypothesis), or diversification is driven by switches in host-plant use (the musical chairs hypothesis). We present multiple lines of evidence suggesting that the musical chairs hypothesis better explains diversification rate differences among butterflies.

Evolutionary biologists frequently envision adaptive radiation as species diversifying across peaks in an adaptive landscape, with intermediate phenotypes suffering lower fitness [3,37]. In the context of herbivorous insects, it is

commonly assumed that ‘a jack of all trades is a master of none’. That is, a specialist species that feeds on one host plant is expected to outcompete a generalist that feeds on many, including the specialist’s host [38]. Under the oscillation hypothesis, following a host breadth expansion event, these fitness trade-offs are expected to favour the evolution of specialists, a prediction that has at least some empirical support [39,40]. The oscillation hypothesis also predicts that this trend towards specialization will drive speciation among generalist ancestral lineages. However, despite numerous attempts to measure fitness trade-offs among host-plant specialists and generalists, none has been identified; indeed, generalist species often have higher fitness than specialists across multiple hosts [41,42].

Contrary to expectations under the oscillation hypothesis, we recovered a strong negative relationship between host-plant breadth and diversification rate, both in a broad-scale analyses of Papilionoidea and a more narrowly focused analysis of Heliconiini, regardless of whether host breadth was treated as a binary or quantitative character. In addition, we found little evidence that changes in host breadth in either direction accompany speciation events (cladogenetic shifts). Thus, using comparative analyses of diversification rates, our study finds no evidence that butterfly speciation is driven by ecological specialization. In fact, generalist species exhibited significantly lower rates of speciation. Interestingly, generalist species also exhibited significantly lower extinction rates, which is consistent with the notion that generalism is a bet-hedging strategy that reduces extinction risk in a variable environment. Nevertheless, the net diversification rate (speciation minus extinction rate) remained lower for generalist species. The negative correlation between niche width and speciation rate could be explained by specialists and generalists responding differently to disruptive selection: specialists switch hosts and speciate, whereas generalists broaden their niche width without speciating. Furthermore, disruptive selection is expected to be weaker in populations that feed on multiple resources [8], which would also reduce the likelihood of taxonomic diversification in generalists. Alternatively, the negative relationship between niche width and taxonomic diversification may be the result of traits that covary with niche width. For example, generalist species may disperse more successfully among patches, restricting opportunities for divergence. Effectively, both higher dispersal and the lack of specialization of generalist species may flatten the adaptive landscape, explaining the negative association between host breadth and speciation rate.

By contrast, multiple lines of evidence supported the musical chairs hypothesis. First, as described above, specialist species exhibited higher diversification rates, as expected under the hypothesis that switching hosts drives speciation. Second, we modelled lability as a hidden binary character (high/low) underlying among-lineage variation in the rate at which host-associations evolve. This binary trait was then used along with the binary classification of each species as monophagous versus polyphagous to assess correlations with diversification. The multi-trait MuSSE analysis of the Papilionoidea data indicated that high lability among monophagous lineages is particularly strongly associated with diversification, suggesting that host switching, rather than host specialization, is a driver of speciation in this group. A more quantitative model would allow rates of host-association evolution to change continuously over the

branches of the phylogeny and estimate rates of host switching in addition to changes in number of hosts, but current methods do not yet allow such tests. Third, analysing subclades of equal age, we detected a positive relationship between rates of host switching and species diversity, although the relationship was only marginally significant.

Host-plant switching is thought to have driven speciation, in a manner consistent with the musical chairs hypothesis, across a broad array of insects [37,43], including some of the most famous examples of incipient species. For example, *Rhagoletis* flies have switched from hawthorne hosts to apple trees in association with agriculture [44,45], and distinct ecotypes of *Timema cristinae* walking sticks are characterized by their host associations (either with *Ceanothus* or *Adenostoma*) [46]. Niche switching is also thought to drive taxonomic diversification among species involved in interspecific interactions other than those between plants and herbivores. For example, Whittall & Hodges [47] found that changes to nectar spur length among different *Aquilegia* plant species were associated with shifts in specialist pollinators. Host switching is also thought to drive diversification among the estimated 20 000 species of *Gyrodactylus* fish ectoparasites [48].

At first glance, our results appear to contradict the conclusions of Janz *et al.* [5], who found that resource diversity was correlated with species richness in the butterfly family Nymphalidae and suggested ‘recurring oscillations between host expansions—the incorporation of new plants into the repertoire—and specialization, as an important driving force behind the diversification of plant-feeding insects’. The analysis by Janz *et al.* was based, however, on data that summarized host-plant breadth and species number for each genus. Thus, their result is also consistent with the musical chairs hypothesis, in that clades that include more labile specialists, switching host plants more often in a manner that generates new species, would lead to genera that both use a broader array of hosts and are more speciose.

The oscillation hypothesis and the musical chairs hypothesis can be viewed as ‘bottom-up’ models of herbivorous insect diversification, in which ecological interactions with host plants are of primary importance. Alternatively, in ‘top-down’ models, interactions with natural enemies are assumed to be the most important drivers of taxonomic diversification [14]. Models in both classes (bottom-up, top-down) emphasize interspecific interactions. They also assume that host-plant switching will be coupled with speciation, either through trophic divergence or escape from natural enemies. However, in this study, we estimated very low levels of cladogenetic change in host-plant breadth among Papilionoidea lineages. This result could be an artefact of sparse taxonomic sampling across the Papilionoidea, which limits our ability to detect cladogenesis. However, little cladogenetic change in host-plant breadth was also estimated for Heliconiini, for which taxonomic sampling was more comprehensive. Finally, it is possible that cladogenetic shifts from generalist to specialist insects occur primarily within host families of plants, which would require a more refined, species-level analysis of host plant use. Alternatively, our inference of a low level of cladogenetic change may be because speciation events are not actually associated with switches in host-plant breadth (i.e. shifts between polyphagy and monophagy) but are instead associated with which host plants are being used. Given the large number of host plants used across the large-scale phylogenies considered here,

we were unable to test whether specializing or switching among host plants—analysed at the level of plant species—was more associated with cladogenesis. Future analyses, focusing on smaller scale phylogenies (e.g. within genera) with fewer host plants, would be a valuable complement to the broad-scale analyses presented here.

This study supports theoretical models that generalists are expected to diversify at lower rates because of their broad niches, whereas specialists with narrow niches are more likely to speciate [49]. This suggests that a more nuanced interpretation of the escape and radiate hypothesis for insect–plant interactions is needed, with specialist insects radiating into underused niches (niche switching), but generalist insects expanding into them (niche broadening). Whether the patterns found here with butterflies will be seen in other clades that exhibit variation in niche breadth is a fascinating open question. One grey area is how to define the relevant niche with respect to diversification, as generalists along some axes (e.g. resource use) may be specialists along others (e.g. in host–pathogen interactions). While theoretical models have shown that speciation is more likely when phenotypes are multi-dimensional [50], this raises a challenge for empiricists who must identify the phenotypic axes exhibiting the strongest diversifying selection in order to detect relationships between niche breadth and speciation.

Comparative analyses allow us to examine transitions across vast expanses of the evolutionary tree of life, but several caveats must be kept in mind in interpreting these results. First and foremost, the associations revealed here between host switches and diversification are exactly that, associations. Correlation does not prove causation, and the correlations that we observe between monophagy and speciation and between host-breadth lability and diversification are just that, correlations. In particular, other traits that we have not considered may be causative. We allowed for the possibility that dispersiveness might have been the true driver of diversification, but this did not eliminate the negative relationship between polyphagy and speciation. But dispersiveness is only one of a large number of traits that could truly be responsible for variation in diversification rates. In addition, we found that high lability in host breadth was associated with speciation rates, but we caution that this result was based on inferred values of lability, whose influence may reflect other hidden characters. Future analyses would benefit from gathering and using more direct data to estimate lability, such as variation within species. Another potential complication is that we lack trait and genetic data for many species of butterflies. While previous work has shown that sampling only a proportion of species does not bias estimates of speciation rates [31], the method that we have employed (the ‘skeleton tree’ method) assumes that species are sampled randomly, and we have little

understanding at present of how the sampling strategies of biologists might affect diversification inferences. We also caution that while estimates of speciation rates were little affected by sampling in the simulations performed to date, whether evolutionary change tends to occur with speciation (cladogenesis) or with time (anagenesis) should be much harder to assess when sampling is poor, because many of the internal phylogenetic nodes, which might record cladogenetic change, are missing. Finally, we caution that our analyses are limited to the data at hand, with potential errors in species designations, trait states and phylogenies, as well as missing data and discrepant phylogenetic signal coming from different genes. That said, we accounted for uncertainty in the phylogenies and for species that were not sampled, and we obtained consistent results using either Papilionoidea butterflies or the Heliconiini subclade and treating host breadth as either a binary or quantitative trait.

5. Conclusion

The general notion that herbivorous insect diversity is intricately connected with the diversity of their host plants is non-controversial, but the details of the connection remain unclear. The preponderance of evidence presented in this study is inconsistent with the oscillation hypothesis. Generalist species did not tend to speciate at higher rates, and cladogenetic shifts in host breadth were inferred to be rare. The phylogenetic distribution of host-plant associations in extant butterflies better supports the musical chairs hypothesis, in which host switching, not host specialization is the key. To better understand the ecology of taxonomic diversification, we need to reformulate the concept of ecological opportunity and give more explicit consideration to how specific opportunities can affect the potential for niche widening versus niche switching.

Data accessibility. The data are currently available from <http://apes.skullisland.info/bag/Hardy-Otto-Data.zip>, and are available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.bv689> (i) the maximum-clade-credibility tree, and a sample of 10 high posterior probability BEAST trees estimated from the Papilionoidea data; (ii) a sample of 100 high posterior probability BEAST trees estimated from the Heliconiini data; (iii) the Passifloraceae phylogeny used to compute PD measurements of host-plant breadth in Heliconiini, and (iv) host-plant dataset and the R code used to perform the analyses. The estimated ML parameter values obtained from each of the analyses are given in the electronic supplementary material, table S1.

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