

Does plasticity drive speciation? Host-plant shifts and diversification in nymphaline butterflies (Lepidoptera: Nymphalidae) during the tertiary

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How and why the great diversity of phytophagous insects has evolved is not clear but, if the explanation is the diversity of plants as a resource, colonizations of novel plant taxa can be expected to be associated with higher net speciation rates. In the present study, we make use of recent advances in plant and butterfly systematics to trace the evolution of host-plant utilization in the butterfly subfamily Nymphalinae (tribes Nymphalini, Melitaeini, and the probably paraphyletic ‘Kallimini’). A clear historical pattern emerges, with an ancestral host-plant theme of ‘urticalean rosids’ and two major colonizations of novel distantly-related plant clades. The asterid order Lamiales was colonized by an ancestor of ‘Kallimini’ + Melitaeini and the family Asteraceae in Asterales was later colonized by Melitaeini butterflies. These colonization events appear to have been followed by increases in the rate of net butterfly diversification. Two not mutually exclusive scenarios to explain such patterns have been suggested: (1) adaptive radiation due to release from competition following host-plant shifts or (2) higher rates of net speciation during a relatively long-lasting potentially polyphagous (plastic) state. In support of the ‘plasticity scenario’, phylogenetic traces of a long-lasting stage with some potential to feed on more than one host-plant clade can still be seen, despite the ancient age of the colonizations. When angiosperm communities changed after the K/T boundary due to extinctions and subsequent diversification, herbivore taxa that could occupy several alternative niches may have had the greatest opportunity to diversify in turn. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **94**, 115–130.

ADDITIONAL KEYWORDS: feeding – generalist – niche – phenotypic plasticity – polyphagy – specialization.

INTRODUCTION

Phytophagous insects represent a large proportion of global biodiversity, and insect clades feeding on plants are generally more species-rich than their sister taxa (Mitter, Farrell & Wiegmann, 1988). These patterns strongly suggest that plant-feeding in insects promotes speciation and diversification, but it is not clear how and why. Evolution of the ability to feed on plants may be something of an evolutionary ‘hurdle’ for insects, a key innovation opening up new routes of evolution, as suggested by Mitter *et al.* (1988). The important factor may be the diversity of plants (chemical and otherwise) as a resource compared to,

for example, feeding on other insects, in particular the great diversity of angiosperms (Ehrlich & Raven, 1964; Farrell, 1998). But, if so, exactly how does plant diversity drive the origin of insect diversity? After all, most phytophagous insects are relatively specialized on their host plants (Thompson, 1994), so where is the opportunity for plant diversity to affect insect speciation?

In a now classic study, Ehrlich & Raven (1964) suggested a coevolutionary scenario with adaptive radiations after shifts to competition-free host plants, using butterflies and their larval hosts for illustration. However, with the phylogenetic knowledge available at the time, the data could only be presented in the form of lists of associations between taxa, showing that related butterflies feed on related plants. In the

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present study, we make use of recent advances in plant and butterfly systematics and the dating of lineages to trace the evolution of host-plant utilization in the entire butterfly subfamily Nymphalinae, in an effort to shed more light on how current butterfly diversity evolved and the role of host plants in this process. In particular, we investigate to what degree the patterns observed fit Ehrlich and Raven's 'escape and radiate' scenario and/or a scenario where plasticity in host-plant utilization is what drives diversification. This 'plasticity scenario' or 'oscillation hypothesis' has been explained elsewhere (Janz, Nylin & Wahlberg, 2006; Weingartner, Wahlberg & Nylin, 2006; Janz & Nylin, 2007) but, in brief, the idea is that host-plant shifts occur via a broadening of the potential host-plant range, a transient but relatively long-lasting evolutionary stage with plastic host utilization (i.e. a stage where ovipositing females respond to a broader range of plant cues and larvae can tolerate a broader range of hosts). This, in turn, sets the scene for sympatric speciation via host races or for allopatric speciation via locally adapted, more specialized, subpopulations.

In the generally accepted classification (Harvey, 1991), Nymphalinae consists of three tribes: Nymphalini, Melitaeini, and Kallimini. The first two tribes have been the separate subjects of previous detailed investigations of evolution of host-plant utilization (Janz, Nyblom & Nylin, 2001; Wahlberg, 2001). They have very different host-plant themes (Nymphalini being dominated by Urticaceae and related families as hosts; Melitaeini by Acanthaceae and related families as well as Asteraceae), and the new knowledge of their relationships presents an ideal situation for exploring the phylogenetic origin of butterfly-plant associations of the type first pointed out by Ehrlich & Raven (1964). We proceed to investigate whether there is evidence that the colonization of new, distantly-related plant clades have promoted butterfly diversification and, if so, which ecological processes may have been involved.

MATERIAL AND METHODS

BUTTERFLY PHYLOGENY

The relationships among nymphalid butterflies has until recently been poorly known. This has also been true for Nymphalinae, but a series of phylogenetic studies on the family and subfamily have cleared up many questions (Brower, 2000; Nylin *et al.*, 2001; Wahlberg & Zimmermann, 2000; Wahlberg & Nylin, 2003; Wahlberg, Weingartner & Nylin, 2003; Freitas & Brown, 2004; Wahlberg, Brower & Nylin, 2005). The current subfamily Nymphalinae consists of the tribes Nymphalini, Melitaeini, Kallimini, Victorinini,

Junoniini, and possibly Coeini (for genera included in these taxa, see Table 1). The sister group to Nymphalinae is not yet clear, but the most recent molecular studies suggest that it is one of the clades Cyrestini, Pseudergolini, Biblidinae, or Apaturinae (Wahlberg *et al.*, 2003, 2005).

Within Nymphalinae, relationships have been elucidated by a morphological study (Freitas & Brown, 2004) and a molecular study with data from the mitochondrial gene cytochrome oxidase subunit *I* and the nuclear genes *EF1- α* and *wingless* (Wahlberg *et al.*, 2005). Coeini (which contains the genera *Historis* and *Baeotus* only) appears to be the sister group to the rest of the species. The remaining tribes form a stable well-supported clade, with Nymphalini being sister to the rest of the species. What was once considered a poorly defined tribe Kallimini (Harvey, 1991) has now been shown to comprise several tribes that form a grade to a monophyletic Melitaeini (Wahlberg *et al.*, 2005). Three genera with single species in each (*Kallimoides*, *Rhinopalpa*, and *Vanessula*) have unstable positions. This uncertainty limits the possibilities of unambiguously reconstructing host-plant shifts, and we discuss the implications of different topologies here.

Figures 1, 2, 3, 4 show the relationships between genera supported by the most recent and most complete study (Wahlberg *et al.*, 2005; a maximum parsimony analysis), along with inferences (from taxonomical statements in the literature) for genera not sampled in that study (*Antillea*, *Atlantea*, *Phystis*, *Dagon*, *Ortilia*, and *Tisona*). The alternative topology in Figure 5 is a result of a Bayesian analysis presented in Wahlberg (2006b). Note especially the different positions for *Rhinopalpa* and *Vanessula*.

HOST PLANTS

We have followed the most recent plant classification available, the one suggested by the Angiosperm Plant Phylogeny group (APGII, 2003), based on molecular evidence. The new order Rosales is more inclusive than the traditional one and includes the former Urticales. Still, the families Ulmaceae, Cannabaceae, Moraceae, and Urticaceae (the former Urticales) together evidently form a monophyletic clade with high support, and we refer to this clade in the following as the 'urticalean rosids'. Regarding 'Kallimini' and Melitaeini, the new classification simplifies presentation of the host-plant utilization patterns observed, by bringing together important host families formerly in the orders Lamiales, Plantaginales, and Scrophulariales in the modern-sense Lamiales.

Host-plant records for Nymphalini in the narrow sense are mostly from Janz *et al.* (2001) and records for Melitaeini from Wahlberg (2001); see these papers

Table 1. Host plants and species counts for genera of Nymphalinae and the possible sister taxon Cyrestinae

Higher taxon	Genus	Host orders	References	Number of species	References, alternative speciosity
Cyrestinae	<i>Cyrestis</i>	U, R?	5, 6, 8–11	23	Savela
	<i>Marpesia</i>	U	3, 12, 13	19	Savela
	<i>Chersonesia</i>	U	5	7	Savela
	<i>Pseudergolis</i>	U	6	2	Savela
	<i>Amnosia</i>	U	5	1	Savela
	<i>Stibochiona</i>	U	5	2	Savela
	<i>Dichorragia</i>	S, etc.	5, 11	2	Savela
Coeini	<i>Historis</i>	U	3, 12	2	Savela, Wa2, DV
	<i>Baeotus</i>	Unknown		4	Savela, Wa2 (DV: 2)
Nymphalini	<i>Colobura</i>	U	3	2	Savela, Wa2
	<i>Tigridia</i>	U	3	1	Savela, Wa2, DV
	<i>Smyrna</i>	U	3	2	Savela, Wa2, DV
	<i>Pycina</i>	U	3	1	Savela, Wa2 (DV: 2)
	<i>Araschnia</i>	U	7	8	Savela, Wa2
	<i>Symbrenthia</i>	U	7, 11	15	Wa2 (Savela: 12)
	<i>Mynes</i>	U	7, 11	10	Wa2 (Savela: 9)
	<i>Antanartia</i>	U, A?	7–9	6	Savela, Wa2, Ack
	<i>Hypanartia</i>	U	7	14	Savela, Wa2
	<i>Vanessa</i>	U, A, Ma, etc.	7	16	F (Wa2, Savela: 18)
	<i>Aglais</i>	U	7	5	Wa1, Wa2 (incl. Inachis)
	<i>Nymphalis</i>	U, M, R, etc.	7	6	Wa1, Wa2 (incl. Roddia)
	<i>Polygonia</i>	U, M, Sf, etc.	7	13	We, Wa2
	<i>Kaniska</i>	Monocots	7	1	We, Wa2
Uncertain	<i>Rhinopalpa</i>	U	5	1	Savela, Wa2
	<i>Vanessula</i>	Unknown		1	Savela, Wa2, L1, L2
	<i>Kallimoides</i>	L	9	1	Savela, Wa2, L1, L2
Victorinini	<i>Anartia</i>	L	3, 12, 13	5	Savela, Wa2 (DV: 4)
	<i>Siproeta</i>	L	3	3	Savela, Wa2 (DV:5, Sm: 4–5)
	<i>Metamorpha</i>	Unknown		1	Savela, Wa2
	<i>Napeocles</i>	Unknown		1	Savela, Wa2
	Junoniini	<i>Junonia</i>	L, Ma, So, A?	1, 2, 8–14	32
<i>Salamis</i> s.s.		U	8, 9	3	Wa2, L2 (Ack, L1: 2)
<i>Yoma</i>		L, Ma	6, 10, 11	2	Savela, Wa2 (B: 1)
<i>Protogoniomorpha</i>		L	8, 9	5	L2 (Ack, Wa2: 4)
<i>Precis</i>		L, D	8, 9	18	L1 (L2: 16, Savela: 18–21, Wa2: 14)
<i>Hypolimnas</i>		U, L, C, Ma, etc.	1, 5, 6, 8–11, 14	23	Savela (Wa2: 22)
Kallimini		<i>Kallima</i>	L	2,5	10
	<i>Doleschallia</i>	U, L	1, 2, 5, 10, 11, 14	11	Savela
	<i>Catacroptera</i>	L	8, 9	1	Savela, L1, L2
	<i>Mallika</i>	Unknown		1	L1
Melitaeini	<i>Euphydryas</i>	L, D, G	16	13	Wa2 (Savela: 16)
	<i>Chlosyne</i>	L, A, C	16	30	Wa2 (Savela: 33 incl. Thessalia, Charidryas)
	<i>Poladryas</i>	L	16	2	Wa2, Savela
	<i>Atlantea</i>	L	13	4	Wa2, Savela, Sm
	<i>Texola</i>	L, A	16	3	Wa2 (as Microtia), Savela
	<i>Dymasia</i>	L	16	1	Wa2 (as Microtia) (Savela: 2)
	<i>Microtia</i>	Unknown		1	Wa2 (Microtia s. s.), Savela

Table 1. *Continued*

Higher taxon	Genus	Host orders	References	Number of species	References, alternative speciosity
	<i>Melitaea</i>	L, G, D, A	16	68	Wa2, Savela
	<i>Higginsius</i>	Unknown		2	Wa2, Savela
	<i>Gnathotriche</i>	Unknown		2	Wa2 (Savela: 4)
	<i>Mazia</i>	Unknown		1	Wa2, Savela
	<i>Tegosa</i>	A	16	14	Wa2 (Savela: 15)
	<i>Phyciodes</i>	A, L, So	16	11	Wa2, Savela
	<i>Eresia</i>	L, U	16	37	Wa2 (Savela: 34)
	<i>Janatella</i>	Unknown		3	Wa2, Savela
	<i>Anthanassa</i>	L	16	24	Wa2 (Savela: 22)
	<i>Castilia</i>	L	16	13	Wa2, Savela
	<i>Telenassa</i>	Unknown		8	Wa2 (Savela: 15)
	<i>Antillea</i>	L	4,13	2	Wa2, Savela, Sm
	<i>Phystis</i>	Unknown		1	Wa2, Savela
	<i>Dagon</i>	Unknown		3	Wa2 (Savela: 4)
	<i>Ortilia</i>	L?	15	9	Wa2 (Savela: 10)
	<i>Tisona</i>	Unknown		1	Wa2, Sav

Classification according to Wahlberg *et al.* (2005).

U, 'Urticalean rosids'; R, other rosids; L, Lamiales; A, Asterales; S, Sapindales; M, Malphigiales; Ma, Malvales; Sf, Saxifragales; So, Solanales; D, Dipsacales; C, Caryophyllales; G, Gentianales.

Host plant references: 1, Braby (2000); 2, Corbet & Pendlebury (1992); 3, DeVries (1987); 4, Fernandez (2004); 5, Igarashi & Fukuda (1997); 6, Igarashi & Fukuda (2000); 7, Janz *et al.* (2001); 8, Larsen (1991); 9, Larsen (2005); 10, Parsons (1991); 11, Parsons (1998); 12, Scott (1986); 13, Smith *et al.* (1994); 14, Tennent (2002); 15, Trigo (2000); 16, Wahlberg (2001).

Alternative species numbers, with reference, in parentheses: Ack, Ackery, Smith & Vane-Wright (1995); B, Braby (2000); DV, DeVries (1987); F, Field (1971); L1, Larsen (1991); L2, Larsen (2005); Savela, Savela (2006); Sm, Smith *et al.* (1994); Wa1, Wahlberg & Nylin (2003); Wa2, Wahlberg (2006a); We, Weingartner *et al.* (2006).

for source references. The same sources used for these studies were scrutinized for records from other members of Nymphalinae and Coeini and, to a less intensive degree, also for the other possible sister taxa: Cyrestinae, Biblidinae, and Apaturinae. We also added some sources: Smith, Miller & Miller (1994); Igarashi & Fukuda (1997, 2000); Parsons (1998); Braby (2000); Tennent (2002); and Larsen (2005). As in the previous studies, we were conservative when making a choice whether to include a host-plant record or not, and took care not to include dubious records; that is, isolated reports of nontypical hosts were omitted unless the observations had been carefully documented.

CHARACTER OPTIMIZATION

We reconstructed plant utilization at the level of plant orders. However, we show the results of tracing use of 'urticalean rosids' rather than Rosales as a whole because the other families in Rosales are used only by a couple of species in the subfamily. By the same token, only the results of tracing use of

Lamiales and Asterales are shown in addition to the urticalean rosids (these are the only higher host taxa of general importance; Table 1), but we discuss a few other orders of minor importance. We used the 'Trace character' option in the computer program, MESQUITE (Maddison & Maddison, 2006), using parsimony reconstruction of ancestral states. The host taxa were optimized independently of each other as well as using a character model assigning a separate state to each combination of host orders (including minor orders used by more than one genus). For both character models, we explored the effects of giving gains of plant taxa as hosts (colonizations) the same weight as losses, as well as giving gains a weight twice that of losses (using a step-matrix).

Maximum likelihood reconstruction was considered but, because of the very few evolutionary events involved, the parsimony reconstruction was deemed adequate, as well as more transparent to readers in showing, for example, the influence of alternative topologies and weighting schemes. More sophisticated model-based methods will not alleviate the fact that

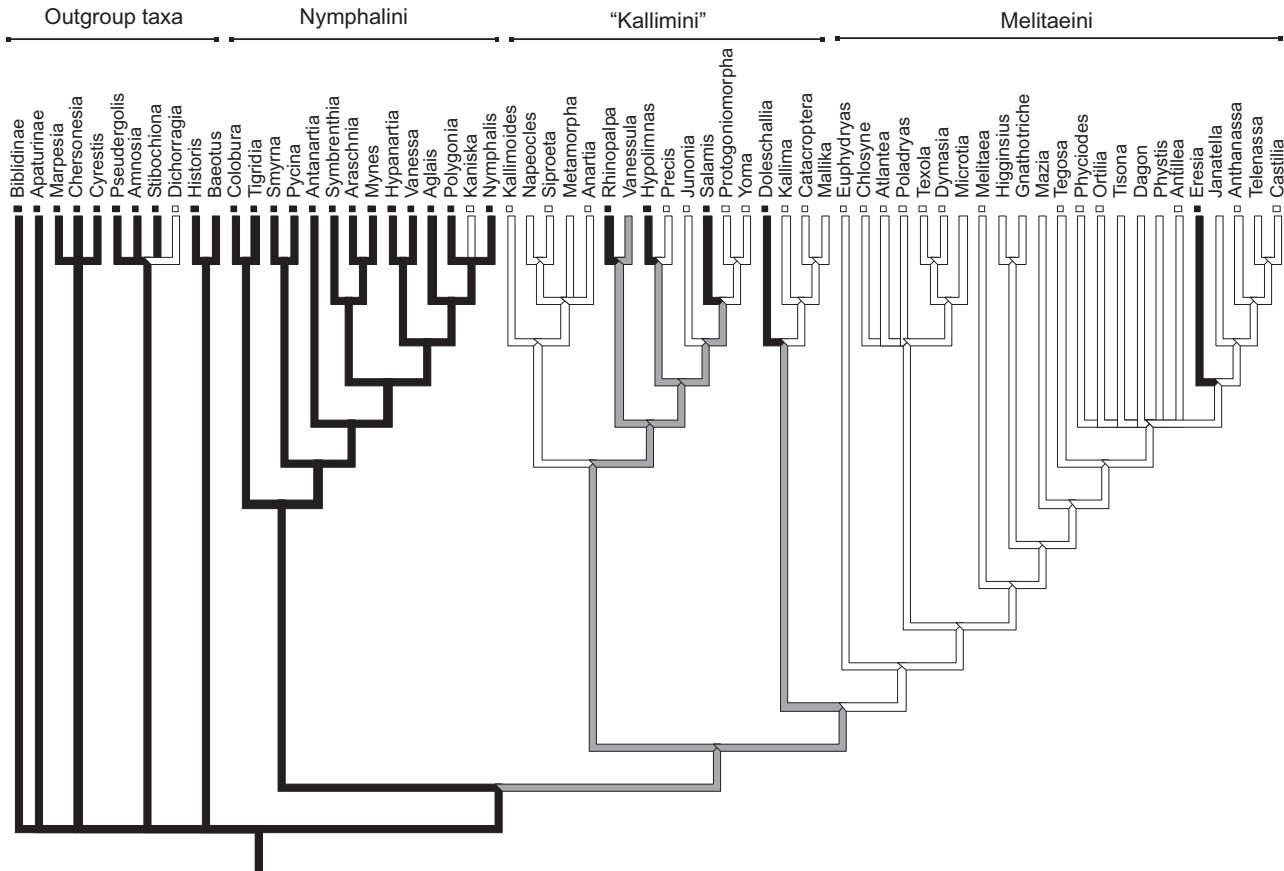


Figure 1. Character optimization for use of plants in the 'urticalean rosids' (former Urticales) as larval hosts, in the butterfly subfamily Nymphalinae and possible sister taxa. Topology from parsimony analysis of DNA data (Wahlberg *et al.*, 2005) and inferred positions for a few additional genera. Branches in black show reconstructed host use when gains and losses of the host taxon were given equal weight, whereas grey branches show additional lineages reconstructed as using urticalean rosid hosts when gains were instead given a weight twice that of losses.

any reconstruction is highly dependent on both topology and the choice of model.

DIVERSIFICATION OF BUTTERFLIES

Two simple techniques were used to study and illustrate diversification rates and patterns of diversification in the butterflies. Again, more sophisticated model-based techniques are available but, because patterns were clear using straightforward and transparent methods, we judged these to be preferable. First, we compared species number in sister clades before and after the major host-plant shifts. The probability of obtaining the observed differences in species number entirely by chance was estimated using the method of Slowinski & Guyer (1993). The probability is $(N-r)/(N-1)$ where N is total number of species in the inclusive clade and r is number of species in the clade (of the two sister clades) that possess the trait to be tested for influence on

speciation rates; in this case, the clade formed after a host-plant shift.

Second, we used the age estimates for clades in Nymphalinae provided by Wahlberg (2006b) to date the host-plant shifts and to study the patterns of butterfly diversification, also relative to ages of plant taxa reported in the literature. Wahlberg's datings are based on Bayesian relaxed clock methods, calibrated using the fossil record for Nymphalinae. We then used the method of Davies *et al.* (2004) to illustrate inferred changes in net speciation rates. Net diversification rates for clades in the tree were first estimated using $\log(N)/t$, where N is the number of species in the clade and t is the time since the clade diverged from its sister clade. Changes in diversification rates can then be calculated by subtracting the estimated rate for a given clade from the rate of its immediate more inclusive clade; thus, an increase in diversification results in a negative number. We selected cut-off points to best bring out and illustrate

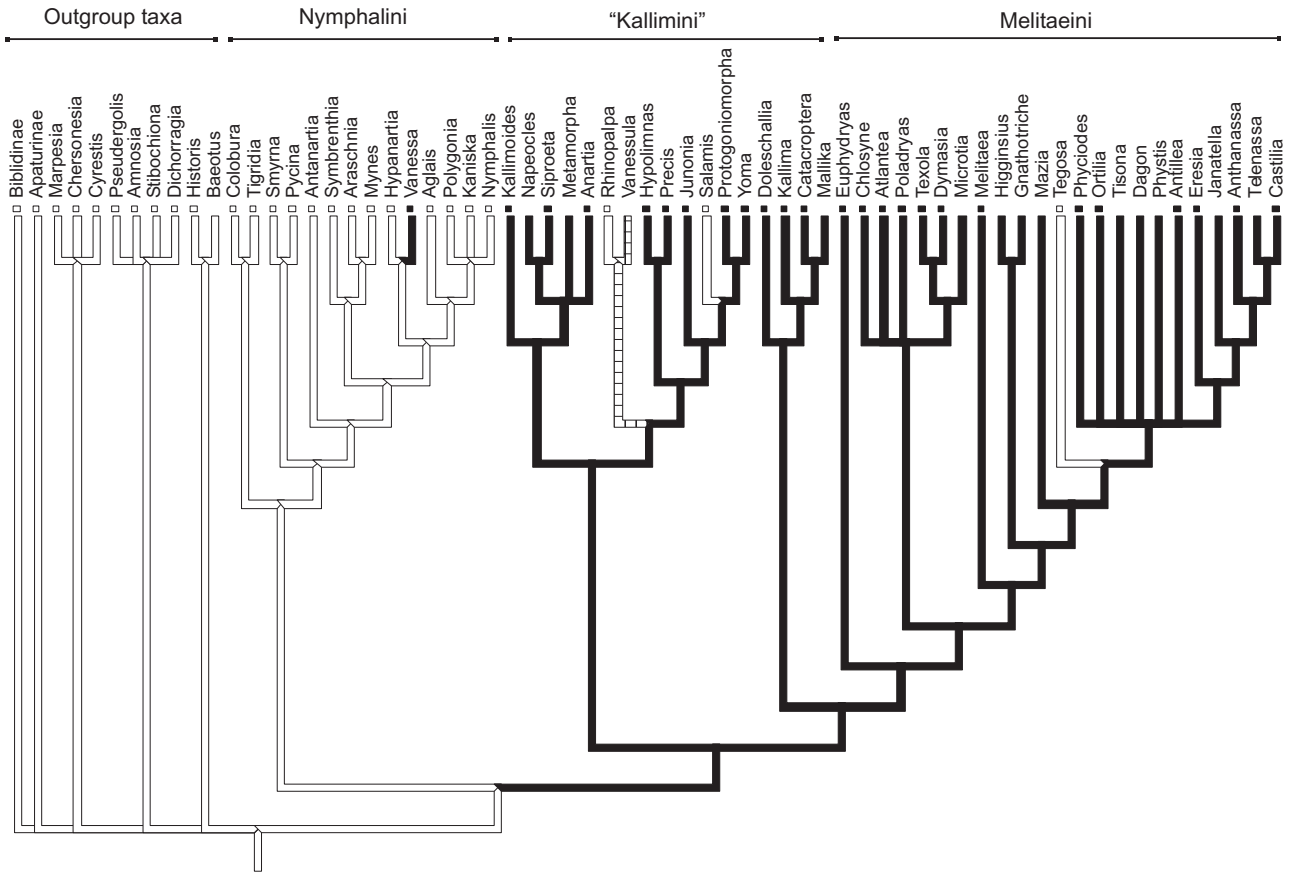


Figure 2. Character optimization for use of plants in the order Lamiales as larval hosts, in the butterfly subfamily Nymphalinae and possible sister taxa. Topology from parsimony analysis of DNA data (Wahlberg *et al.*, 2005) and inferred positions for a few additional genera. Branches in black show reconstructed host use, which was identical when gains and losses of the host taxon were given either equal weight or gains a weight twice that of losses. In branches with hatched patterns, reconstruction of Lamiales use is equivocal due to lack of host records for *Vanessula* butterflies.

the variation present in rates of diversification (see Supplementary material). We did this for both the parsimony topology and the Bayesian topology from Wahlberg (2006b).

RESULTS

HOST PLANTS

Records of host-plant orders for the most probable sister groups to Nymphalinae and for the genera in Nymphalinae are presented in Table 1. Regarding more distantly-related outgroup taxa not included in Table 1, for members of the subfamily Biblidinae, the dominating host-plant order is Malpighiales, but Sapindales is also used and, in a few genera, urticalean rosids. For members of Apaturinae, the main host-plant association is urticalean rosids, primarily Ulmaceae, but Malpighiales is used by *Apatura*.

In brief, the information in Table 1 can be summarized phylogenetically. In the probable sister groups,

larvae of members of both Cyrestinae and Coeini primarily feed on urticalean rosids, but *Dichorragia* has a more divergent pattern of host-plant utilization. Within the tribe Nymphalini, all basal genera feed on urticalean rosids, as do *Antanartia* and *Hypanartia*, for which the phylogenetic positions are still uncertain. With the exception of the polyphagous *Vanessa cardui* (and its close relatives) and the section *Nymphalis* + *Polygonia* (including *Kaniska*), with several polyphagous species, most other species in Nymphalini are also specialists on urticalean rosids.

The tribe Kallimini of Harvey (1991) has now been found to be most likely paraphyletic (Wahlberg *et al.*, 2005). The exact phylogenetic positions of three of the genera, *Rhinopalpa*, *Vanessula*, and *Kallimoides*, are uncertain, but the available evidence suggests that they belong to the more basal clade in the grade 'Kallimini'. The single species of *Rhinopalpa* feeds on urticalean rosids and the single species of *Kallimoides* on a host in Lamiales (Table 1). The proposed tribes

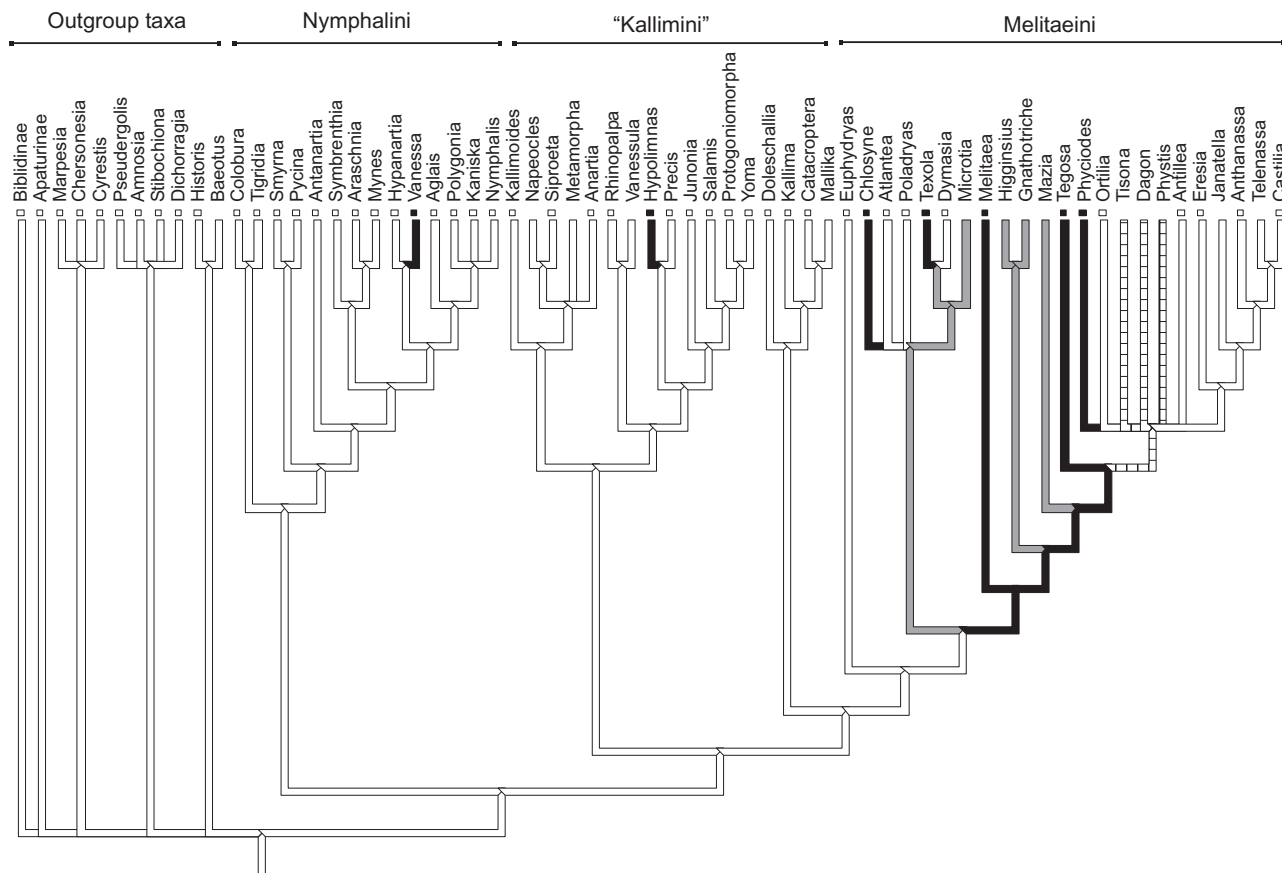


Figure 3. Character optimization for use of plants in Asteraceae (or the order Asterales) as larval hosts, in the butterfly subfamily Nymphalinae and possible sister taxa. Topology from parsimony analysis of DNA data (Wahlberg *et al.*, 2005) and inferred positions for a few additional genera. Branches in black show reconstructed host use when gains and losses of the host taxon were given equal weight, whereas grey branches show additional lineages reconstructed as using Asteraceae hosts when gains were instead given a weight twice that of losses. In branches with hatched patterns, reconstruction of Asteraceae use is equivocal due to lack of host records for several genera of butterflies, in combination with an unresolved phylogeny.

Victorinini and Junoniini also belong to this basal clade. Known hosts of Victorinini are in the Lamiales. In Junoniini, species of *Junonia* also primarily feed on hosts in the Lamiales. *Yoma* and *Protogoniomorpha* feed on Lamiales but the closely-related *Salamis* instead on urticalean rosids. *Precis* feeds on hosts in Lamiales and *Hypolimnas* has both highly polyphagous species and species more or less restricted to either urticalean rosids or Lamiales hosts. The sister clade to Melitaeini may be the new narrow-sense Kallimini (Wahlberg *et al.*, 2005). In this tribe, *Kallima* and *Catacroptera* feed on Lamiales hosts and species in *Doleschallia* feed on Lamiales and urticalean rosids. At least one species, *Doleschallia bisaltide*, apparently may feed on both plant clades (Corbet & Pendlebury, 1992; Igarashi & Fukuda, 1997).

Host-plant records for the most basal clade in the tribe Melitaeini, the genus *Euphydryas* (Figs 1, 2, 3,

4), are from a wide range of families in Lamiales, Gentianales and Dipsacales. Species in the genus *Melitaea* feed on several families in Lamiales as well as on Gentianales, Dipsacales, and Asterales. Most remaining genera feed exclusively on Lamiales and/or Asterales, with the exception of the urticalean rosid Urticaceae reported for one species in *Eresia*.

COLONIZATIONS OF PLANT CLADES

Results from optimizing host taxa independently or together did not differ, and for clarity of illustration, Figures 1, 2, 3 show the independent optimizations.

Reconstruction of ancestral states by parsimony strongly suggests that the ancestor of Nymphalinae fed on host plants in the urticalean rosids clade (Fig. 1). As noted above, another order in the Eurosid I clade, Malpighiales, is an important host group for outgroup taxa. This order may then have been

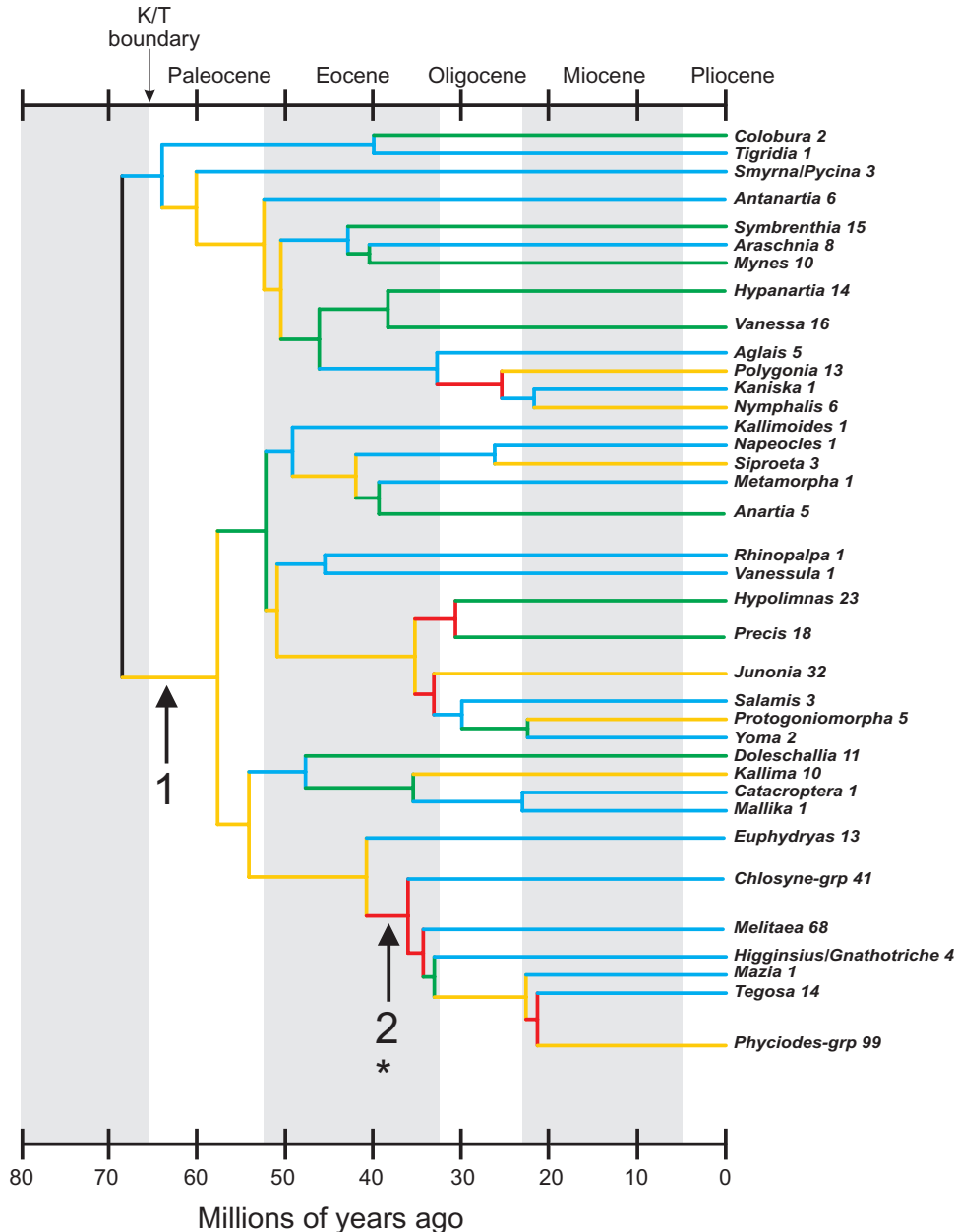


Figure 4. Inferred changes in net speciation rates over the history of the butterfly subfamily Nymphalinae. Topology based on parsimony analysis of DNA data (Wahlberg *et al.*, 2005), dated phylogeny from (Wahlberg, 2006b). Branches with large positive changes relative to the immediate more inclusive clade given in red, smaller positive changes in yellow. Large negative changes are shown in blue, smaller negative changes are shown in green. Arrow 1 shows the inferred colonization of the order Lamiales, arrow 2 shows the inferred colonization of Asterales (Asteraceae). *Clade utilizing the novel host taxon is more diverse than its sister clade at $P = 0.05$. Number of species is given by number after the taxon name.

included in the range of potential hosts for the ancestor of Nymphalinae.

According to the maximum parsimony reconstruction, a major shift in host-plant theme took place at the base of the grade 'Kallimini', when the order Lamiales in the Euasterid I clade was colonized

(Figs 1, 2: black branches). Plants in this order are not known to be used by any species in the possible sister taxa to Nymphalinae, and the same is true for other Euasterid families. Outside of the 'Kallimini' + Melitaeini, the use of Euasterid plants is only known with any certainty from the strongly

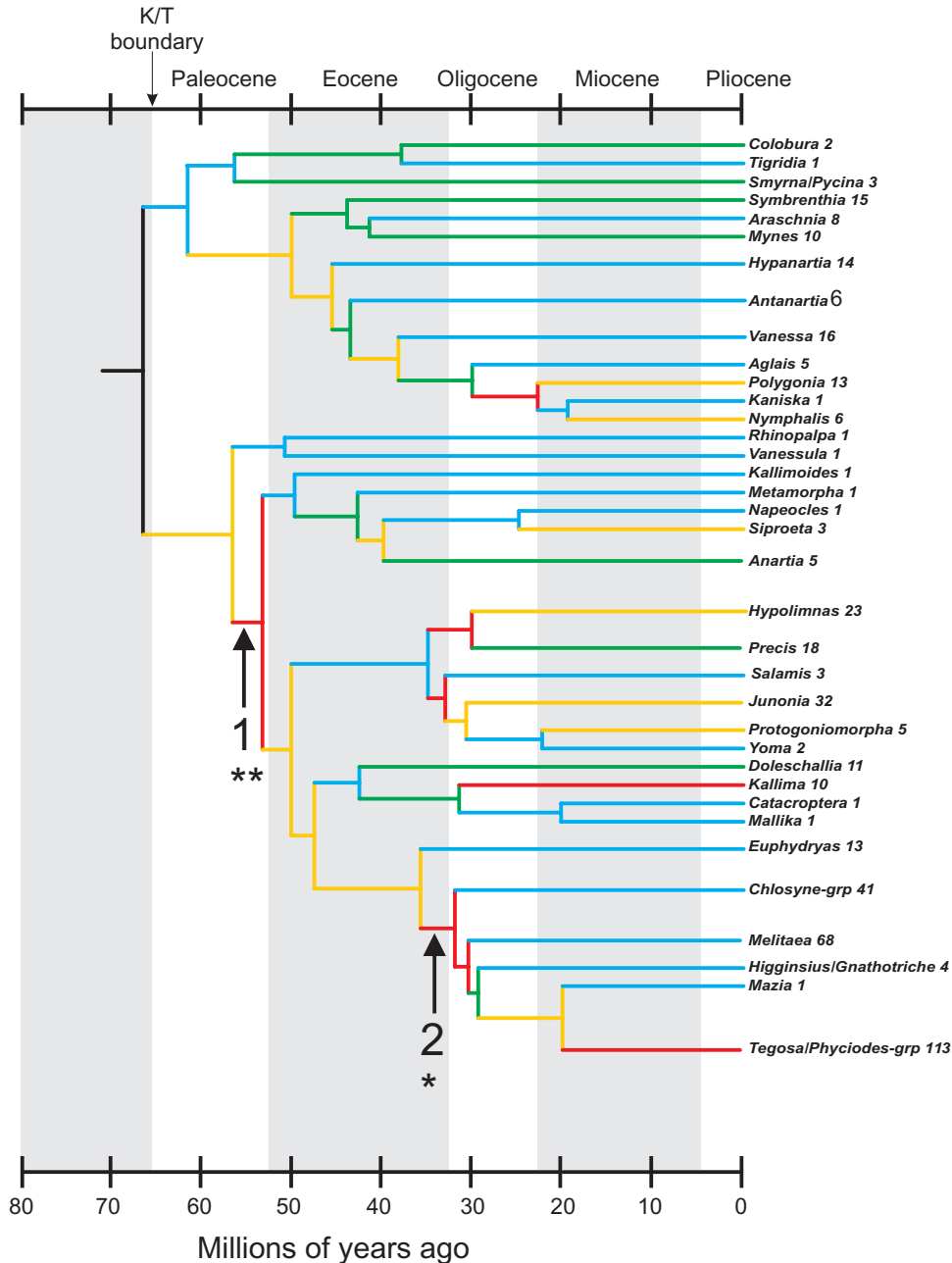


Figure 5. Bayesian topology and dating of the butterfly subfamily Nymphalinae (Wahlberg, 2006b), and inferred changes in net speciation rates. Branches with large positive changes relative to the immediate more inclusive clade given in red, smaller positive changes in yellow, larger negative changes are shown in blue, smaller negative changes are shown in green. Arrow 1 shows the inferred colonization of the order Lamiales, arrow 2 shows the inferred colonization of Asterales (Asteraceae). *Clade utilizing the novel host taxon is more diverse than its sister clade at $P = 0.05$. ** $P < 0.01$. Number of species is given by number after the taxon name.

polyphagous *Vanessa* in Nymphalini. By contrast, almost all genera in ‘Kallimini’ and many Melitaeini utilize Lamiales (Table 1). For this reason, character optimization of use of Lamiales as host gives the same result if gains of plant taxa are weighed twice that of losses (Fig. 2).

However, some basal ‘Kallimini’ use urticalean rosoid families as hosts (Fig. 1, Table 1) and, when gains of plant host taxa were given a weight twice that of losses, the use of urticalean rosoids was reconstructed as never being lost in these taxa (Fig. 1: black + grey branches). This was true also using a

character model where each combination of host orders was given a separate character state (i.e. in this case, the lineages represented by the grey branches were inferred to feed on a combination of urticalean rosids and Lamiales; not shown). Under these models, with costly gains, loss and re-colonization of urticalean rosids is more costly than never losing them in the first place. We suggest that such models reflects evolution of potential host-plant range better than equal-weighting schemes because it is reasonable to assume that to first acquire a novel plant order as a potential host is less evolutionarily likely than subsequently losing it.

Topologies of Nymphalinae based on Bayesian analysis are similar, but differ in the position of *Rhinopalpa* and *Vanessula* (Fig. 5). Since larvae of *Rhinopalpa* use hosts in the urticalean rosids, and the hosts of *Vanessula* are unknown, this topology would suggest that the colonization of Lamiales did not occur until after *Rhinopalpa* + *Vanessula* split off from the remaining 'Kallimini' + Melitaeini. Character optimization of host-plant data performed on the Bayesian topology seen in Figure 5 (with gains given twice the weight of losses) again suggests that the capacity to feed on urticalean rosids was never lost in the lineages leading to *Hypolimnas*, *Salamis*, and *Doleschallia* (not shown).

A second important colonization was that of Asteraceae in Asterales (Euasterid II clade). This family is used by some species in *Vanessa* and by polyphagous species in *Hypolimnas* (and perhaps by some *Antanartia*) but, otherwise, not by any nymphaline butterflies outside of Melitaeini (Table 1). Moreover, it is not used (or only very rarely used) by the basal genus in this tribe, *Euphydryas*. When gains and losses are weighed equally, use of Asterales is reconstructed as having evolved at least four times within Melitaeini (Fig. 3: black branches), but the weighting scheme with more expensive gains suggests that the potential to feed on Asterales more probably only evolved once in this clade (Fig. 3: black + grey branches). If so, this potential may have been later lost again in the section of the tree which includes American genera such as *Eresia*, but this reconstruction could also be the result of a paucity of host data from the neotropics.

BUTTERFLY DIVERSIFICATION

In Table 1, the number of species in the studied genera are listed, based mainly on the continuously updated web resources maintained by Savelle (2006) and (in particular for genera in the Melitaeini) Wahlberg (2006a). Additional references used to modify these compilations, or sources of alternative views, are also listed. The exact number of species given for

these genera sometimes varies between studies, but this does not obscure the main patterns of diversity.

In Figures 4, 5 (based on lineage datings reported in Wahlberg, 2006b), the phylogeny of all sampled species of Nymphalinae (topology and dates based on parsimony and Bayesian analysis, respectively) is shown with branch lengths relative to estimated evolutionary time. Shown in different colours for branches are the inferred changes in net speciation rates (see for detailed data behind these calculations, Supplementary Material).

Nymphalini evidently diverged from the remaining Nymphalinae in the Palaeocene, most likely approximately 60–65 Mya. The host order Lamiales was then colonized at some time after this event before the diversification that produced the different clades observed today in 'Kallimini' + Melitaeini. In the Nymphalini, feeding mainly on the ancestral host clade of urticalean rosids, there are approximately 100 species. In the sister clade 'Kallimini' + Melitaeini, following the shift in major host theme, there are approximately 372 species representing several major lineages with an origin in late Palaeocene or early Eocene. This pattern is consistent with an increase in net diversification rate following the shift, but the difference in species number could also have arisen by chance ($P = 0.21$). However, if the Bayesian topology (Fig. 5) is correct, the appropriate sister-group comparison would be between *Rhinopalpa* + *Vanessula* (two species) and the remaining 'Kallimini' + Melitaeini, with approximately 370 species. This would be a significant deviation from random branching patterns ($P < 0.01$).

In both topologies, a slight increase in net diversification rates is indicated early in the evolution of 'Kallimini' + Melitaeini, relative to Nymphalinae as a whole (Figs 4, 5). In the parsimony topology this coincides with the colonization of Lamiales, but speciation rates accelerate again both at the base of strict-sense Kallimini + Melitaeini and at the base of Melitaeini (Fig. 4) without an inferred change in major host-plant theme. In the Bayesian topology, a steeper increase in rate of speciation coincides precisely with the inferred colonization of Lamiales, after the split with *Rhinopalpa* + *Vanessula* (Fig. 5).

As noted above, a second major colonization was that of the Asteraceae family (and at the same time of the order Asterales) which appears to have occurred in the late Eocene or early Oligocene, 33–40 Mya, after the *Euphydryas* split off from the remaining Melitaeini (Figs 3, 4, 5). There are approximately 13 species in the *Euphydryas*, compared to approximately 240 in the sister clade, a borderline significant deviation from random branching ($P = 0.05$). In both topologies, accelerated rates of diversification are indicated in the Melitaeini after the colonization of

Asteraceae (Figs 4, 5). The temporal pattern of radiation also provides some support for an increased diversification rate following the colonization event. The tribe Melitaeini seems to be surprisingly ancient, perhaps 50 Myr or more, yet all extant genera originated long after the Melitaeini split off from the Kallimini. Three of the four major clades (the *Chlosyne* group, the *Melitaea* group, and the *Phyciodes* group) originated at around the same time; interestingly, this was soon after the colonization of Asteraceae (in the late Eocene, Fig. 4; or early Oligocene, Fig. 5).

One final pattern is worth noting from Figures 4, 5. In the Nymphalini, a decrease in diversification rates is inferred at the base, followed by increases at the base of the section that today contains most of the species. However, in all parts of the clade, subsequent decreases in rates of diversification are inferred in late Eocene, and this declining speciation trend is only reversed at the base of the *Polygonia* + *Kaniska* + *Nymphalis* clade, coinciding with a widening of host-plant use to include not only the ancestral urticalean rosids but also a range of other plant taxa (Janz *et al.*, 2001; Weingartner *et al.*, 2006). The difference in diversity, however, is not significant ($P = 0.21$).

AGE OF PLANT VERSUS BUTTERFLY CLADES

The techniques for phylogenetic age estimates using rates of sequence divergence are constantly improving, but there are still many uncertainties both when it comes to the datings themselves (Wahlberg, 2006b) and the choice of relevant taxonomical levels for associated groups of organisms (Magallon & Sanderson, 2001).

Nevertheless, some patterns of interest can be noted. The age of the probable ancestral host clade for Nymphalinae, the 'urticalean rosids' in Rosales, may be approximately 65–69 Myr from both molecular and fossil evidence (Magallon & Sanderson, 2001; Wikström, Savolainen & Chase, 2001). This is similar to the estimated age of the entire crown-group Nymphalinae (Figs 4, 5; Wahlberg, 2006b), placing the stem-group age (split from the sister group) before the estimated origin of the urticalean rosids. Since the order Malphigiales is most likely older, perhaps 88–91 Myr (Wikström *et al.*, 2001), it could be speculated that this related rosid clade was the major host-plant theme in 'nymphalines' before the origin of the 'urticalean rosid' clade, as it is in the Biblidinae today.

The age of Lamiales has been estimated to 71–77 Myr from molecular evidence (Wikström *et al.*, 2001) but later, and independently, was estimated to be 106 Myr Bremer, Friis & Bremer (2004). The age of

'Kallimini' + Melitaeini may be of a similar magnitude to the former estimate; a likely stem age for this butterfly clade is approximately 68 Myr (Figs 4, 5; Wahlberg, 2006b). The earliest occurrence of Lamiales in the fossil record puts the age of the order at only 37–44 Myr (Magallon & Sanderson, 2001; Wikström *et al.*, 2001). This has been suggested to severely underestimate the true age (Wikström *et al.*, 2001; Bremer *et al.*, 2004), and the dating of its colonization by nymphalid butterflies to already have occurred approximately 68–58 Mya supports this suggestion.

Regarding the Asterales, this order may be as old as 100 Myr or more (Wikström *et al.*, 2001; Bremer *et al.*, 2004; Sanderson *et al.*, 2004) but more relevant in the case of families utilized by the Melitaeini is the age of the family Asteraceae, which has been proposed to be 'at least 38 Myr' based on fossil pollen (Bremer & Gustafsson, 1997). The probable age of the split between *Euphydryas* and the remaining Melitaeini, at approximately 35–40 Mya, suggests an early colonization of Asteraceae. However, the much older Lamiales remains the main host theme for most genera (Table 1). Two other important host orders for Melitaeini, including the basal genus *Euphydryas*, are Gentianales and Dipsacales. Both have their origin approximately 85–90 Mya (Wikström *et al.*, 2001) or even earlier (Bremer *et al.*, 2004) and the inclusion of these orders in the host range clearly represent late colonizations.

DISCUSSION

Advances in plant and butterfly phylogenetics have now made it possible to examine some of the butterfly–plant associations originally observed by Ehrlich & Raven (1964) in more detail, and we present one such case study. The results show two ancient major colonizations of novel distantly-related plant clades, from the even more ancient relationship with urticalean rosids, which together account for the broad patterns of host-plant themes observed today within the subfamily Nymphalinae. This is consistent with both the general pattern reported by Ehrlich & Raven (1964), suggesting that clades of butterflies appear to feed on clades of related plants, and their interpretation that this reflects butterfly colonizations of plant taxa followed by diversification. Notably, the plant taxa involved are known to differ in their chemical characteristics, with in particular a class of secondary plant metabolites called iridoids being of prime importance in Lamiales and for host use in the Melitaeini (Wahlberg, 2001). This importance of plant chemistry as a constraint on host use was another cornerstone of the co-evolutionary scenario suggested by Ehrlich & Raven (1964). Such patterns should not

be taken to mean that plant chemistry, or even plant taxon, is the only factor determining butterfly host use; ecological factors such as natural enemies will typically be strongly involved as well (Bernays & Graham, 1988; Heard *et al.*, 2006). However, it is clear that, for most species in Nymphalinae the higher host-plant taxon sets the outer limits for what is a potential host, with ecological factors being of more importance at the level of utilization of particular plant families, species, or individuals.

Janz *et al.* (2001) concluded that there is strong evidence that the 'urticalean rosids' (formerly 'Urticales', now placed in the order Rosales) is the ancestral host-plant clade for the tribe Nymphalini, and we show in the present study that this association most likely is ancestral also for the entire subfamily Nymphalinae. The extensive use of 'urticalean rosids' as hosts for both the subfamily Nymphalinae and several other higher lineages that are possible sister taxa is consistent with a rapid diversification in this entire section of Nymphalidae (the 'Nymphaline' clade; *sensu* Wahlberg *et al.*, 2003) after early colonization of 'urticalean rosids', already around the time of the K/T boundary. However, lack of phylogenetic resolution in Nymphalidae prevents further analysis of this putative diversification event for the time being. Some potential to feed on hosts in Malphigiales is also likely to be an ancestral trait for the Nymphalinae. Colonization of the order Lamiales, in the Euasterid I clade, occurred near the base of the 'Kallimini' + Melitaeini butterfly clade, suggesting that it could have been a major factor in its diversification. The same is true for the colonization of the family Asteraceae (order Asterales, Euasterid II clade) by members of the tribe Melitaeini after *Euphydryas* had split off, after which all the main lineages of the tribe evolved during a relatively short period of time with elevated diversification rates. In both cases, there are also more species in the butterfly clade that has colonized the novel clade, compared to its sister clade.

Two (not mutually exclusive) scenarios have been suggested for how diversity of host-plant use could drive the diversification of phytophagous insects. It may be that the plant diversity creates opportunities for shifts to novel host plants, representing the opening up of new adaptive zones and promoting adaptive radiation on the new host taxon after ecological release from competition, in 'empty niches'. This 'escape and radiate' scenario was another key feature of Ehrlich & Raven's (1964) theory of coevolution between butterflies and plants. Another suggestion is more connected to theory on plasticity as a key feature in the origin of biodiversity (West-Eberhard, 1989, 2003; Janz *et al.*, 2006; Janz & Nylin, 2007). It may be that colonization of novel host plants

represent not so much a shift into a new adaptive zone with release from competition, but a widening of the evolutionary possibilities: an escape from all the evolutionary constraints of specialization rather than from competition in particular. More types of potential host plants means a larger number of potential niches ('empty' or not) and often larger areas of distribution, especially during a polyphagous stage around the time of the colonization, and hence more potential for speciation following re-specialization. A lower risk of extinction could also contribute to higher net speciation rates in clades with more diverse host use. Butterfly diversity patterns consistent with a process where diversification is favoured by such oscillations in host-plant range have recently been reported from other taxonomical levels (Janz *et al.*, 2001, 2006; Weingartner *et al.*, 2006), and the present study complements these observations.

It is not straightforward to distinguish between the two scenarios using phylogenetic reconstructions (if this is even a meaningful task; see below). The 'escape and radiate' scenario should produce a fast radiation, but only immediately after the colonization event, when the colonizers can benefit from being free of competition. The shift to a novel host could also be expected to be completed relatively rapidly because the adaptive radiation is assumed to be a result of higher fitness on the new host. The higher diversification rate suggested by the 'plasticity scenario' should be less pronounced but instead could be sustained for a longer time, until host-plant specialization has again become the norm in all species and even the potential for polyphagy has disappeared. Under this model, novel host plants will often be suboptimal hosts that can be utilized merely as a side-effect of existing plasticity in host use, suggesting that when complete host-plant shifts do occur, this can take a very long time. The available evidence from Nymphalinae (Figs 4, 5) does suggest an increased rate of diversification which is not only concentrated to the time of the colonization events, but cannot clearly distinguish between the two scenarios. Can the reconstructed patterns of host-plant utilization tell us anything about whether there was a 'phylogenetically instantaneous' shift from a single plant clade to another single plant clade, or whether there was in fact a long-lasting polyphagous or potentially polyphagous stage? There are in fact a number of observations to support the latter interpretation.

Regarding the first major colonization event (Lamiales), the utilization of 'urticalean rosids' or Lamiales as hosts may at first glance appear to be mutually exclusive (Figs 1, 2), supporting a rapid shift from the former to the latter before the 'Kallimini' + Melitaeini clade diversified. However, within the grade 'Kallimini', several patterns in recent species (reflected by

the parsimony reconstruction of ancestral states; see Results) suggest that the shift was not completed rapidly. The single species in the genus *Rhinopalpa* (*R. polynice*) feeds on Urticaceae. According to the parsimony topology, this implies a reversal to the 'urticalean rosids' more than 10 Myr after the colonization of Lamiales, but the phylogenetic position of this taxon is uncertain, and according to the Bayesian topology (Fig. 5) Lamiales may not yet have been colonized at the time of the speciation event which eventually produced the clade *Rhinopalpa* + *Vanessula*. A more clear and even later reversal took place in the ancestor of *Salamis* (in the strict sense), because this genus feeds on Urticaceae, in contrast to the closely-related *Junonia*, *Yoma*, and *Protogoniomorpha* (the latter formerly included in *Salamis*; but see also Vari, 1979; Wahlberg *et al.*, 2005), feeding on Acanthaceae and other Lamiales. These recurrences of 'urticalean rosids' as hosts were most likely facilitated by some retained capacity to feed on the ancestral host, long after the colonization of the novel plant host (Futuyma, Keese & Scheffer, 1993; Janz *et al.*, 2001). Such a capacity is also indicated by extant species in the grade 'Kallimini' that are able to feed on both plant groups, but no other, which is true for some species of *Doleschallia*. Furthermore, in the genus *Hypolimnas*, there are a couple of exceptionally polyphagous species, but most are actually specialists on either Lamiales or 'urticalean rosids', or use both taxa as host plants.

Regarding the second major colonization (Asteraceae), it can easily be seen by comparing Figures 2 and 3, which demonstrate that, in the Melitaeini, there was no 'instantaneous' shift from Lamiales to Asteraceae. The use of Lamiales is still retained in almost all extant genera, often alongside the Asteraceae (and even Urticaceae reappears as a host for *Eresia*). The combined capacity to feed on hosts in Lamiales and Asteraceae is even more notable considering the differences in plant chemistry (Wahlberg, 2001). Host families in Lamiales often contain iridoid glycosides (or secoiridoids in the case of the basal Oleaceae), whereas plants in Asteraceae appear to lack iridoids. Wahlberg suggested that the family Plantaginaceae (where host taxa contain iridoid glycosides) is the most probable ancestral host for Melitaeini, and that this conclusion and the role of iridoids could be tested with a good phylogenetic hypothesis for the entire subfamily Nymphalinae. We can now note that use of Plantaginaceae in outgroup taxa (genera in 'Kallimini') is restricted to *Junonia*, which is certainly not the sister taxon to Melitaeini. Instead the family Acanthaceae, used by *Doleschallia*, *Kallima*, and *Catacroptera* in the probable sister clade (Kallimini in the strict sense), and by most genera of Victorinini and Junoniini as well, is a much

more pervasive theme. This difference between Melitaeini and its closest relatives in the grade 'Kallimini' may, however, reflect biogeography rather than a host shift because both Acanthaceae and 'Kallimini' have more tropical distributions than Plantaginaceae & Melitaeini. Ehrlich & Raven (1964) suggested that the host-plant repertoires of *Junonia* and Melitaeini have converged due to shared chemistry of hosts, but it now appears to be the case that the use of hosts in Lamiales in both butterfly taxa is due to shared ancestry.

The pattern in Melitaeini clearly demonstrates that the potential to use an ancestral host taxon can be evident in a clade many million years after the colonization of a novel host. Since most insect herbivores are specialists, such a potential is probably more often present only as a very imperfect ability to use plants other than those in the present range, but it would still give the opportunity for expansions of host range through oviposition 'mistakes' (Larsson & Ekbom, 1995). If use of the alternative host is favoured by selection, selection can then act further to modify and perfect host finding, metabolism, and so on, in a process of 'genetic accommodation' (West-Eberhard, 2003).

If observations such as these reflect a plastic phase around a host shift where a potential to use both the old and new host is retained, we find it likely that at least initially the potential range typically extends also to other host plants, although phylogenetic traces may not always remain of these secondary hosts. Around the time of a historical shift to a novel host-plant clade, the phytophagous insect cannot have been a strict specialist. At the very least, some poorly developed potential to oviposit, feed and survive on the novel plant must already have been present, and we suggest that at least the old and the new plant group most likely were used side-by-side for a time; host-plant shifts by other means are hard to envisage. Moreover, shifts to distantly-related plant taxa would seem very unlikely in an extreme specialist. Such species can be expected to be very discriminating in their choices of oviposition sites (Janz & Nylin, 1997), as well as typically lacking the capacity to feed on a novel plant following an oviposition mistake.

Indeed, in the tribe Nymphalini, there is evidence that major host-plant shifts have only occurred after a polyphagous stage (Janz *et al.*, 2001). For example, a polyphagous habit evolved in an ancestor of *Nymphalis*, *Polygonia* and *Kaniska* (coinciding with a possible increase in net speciation rates, Figs 4, 5), and this led to colonization of a whole range of plant families and orders, including even a complete shift to monocotyledons in *Kaniska*. For this reason, it is also of interest for the 'plasticity scenario' to note that the same kind of patterns occur in the rest of Nympha-

linae, the 'Kallimini' + Melitaeini clade. Here, the themes of Lamiales and/or Asteraceae host plants are very consistent, alongside some other groups that recur in several genera of Melitaeini (Gentianales, Dipsacales). Most of the few exceptions involve re-colonizations of urticalean rosids, or occur in butterfly genera, such as *Hypolimnias* and *Junonia*, which contain some highly polyphagous species. Furthermore, in the Melitaeini, the most basal branch, the genus *Euphydryas*, has the most diverse total range of host-plant families, suggesting a polyphagous ancestor. The families used by this genus recur later in different branches of the Melitaeini.

The patterns observed in the Nymphalinae are in many respects consistent with the patterns and process envisaged by Ehrlich & Raven (1964). It does appear that butterflies are constrained in their host use by the chemical properties of plants over very long time periods, and that butterfly diversification often follows after colonization of a novel plant clade. Although there are many uncertainties concerning the dating, the three key plant lineages (urticalean rosids, Lamiales, Asteraceae) appear to have been colonized early in their evolution, rather than after most of their diversification had already taken place as would have been suggested by the 'sequential evolution' theory of Jermy (1984). It also is likely that, at some point in time, aspects of the plant chemistry in the taxa involved were modified by selection from herbivores, to function as a defense against them, in which case the whole process could rightly be described as diffuse coevolution in the strict sense of Janzen (1980; albeit more probably between herbivores and plants than specifically between butterflies and plants). Herbivore–plant coevolution may also have played a more specific role in the diversification of both taxa (Farrell, Dussourd & Mitter, 1991; Berenbaum, 2001; Becerra, 2003), but there is still a lack of conclusive evidence that this role extends much beyond contributing to the chemical niche diversity of angiosperms as a resource.

Also, the patterns observed in nymphalid butterflies at various taxonomical levels (present study; Janz *et al.*, 2001, 2006; Weingartner *et al.*, 2006) appear to be more consistent with the 'plasticity scenario' than with at least one aspect of Ehrlich & Raven's (1964) theory: the 'escape and radiate' scenario. The fact that old hosts are often kept in a potential repertoire long after colonizations of novel hosts, and frequently re-colonized at a later date, suggests that the observed insect diversification is not due to release from competition but to greater niche diversity as a consequence of greater plasticity in taxa with wider potential host range. Perhaps, however, the 'plasticity scenario' should not be contrasted too strongly with Ehrlich & Raven's (1964)

views. They also seem to have considered a process reminiscent of this scenario, as indicated by a quote from the discussion in their paper: 'The degree of plasticity of chemoreceptive response and the potential for physiological adjustment to various plant secondary substances in butterfly populations must in large measure determine their potential for evolutionary radiation'. Still, this argument is not referred to elsewhere in the paper and, over the years, these authors have instead been cited as describing a co-evolutionary arms race process with episodes of ecological release (Futuyma, 1983).

CONCLUSION

We suggest a picture of herbivore–plant evolution in the Tertiary that is more in line with the spirit of the quote about plasticity from Ehrlich & Raven (1964) given above. Regarding butterflies, it now appears that several higher clades were present before the K/T event but that many of the present lineages originated during a time of rapid diversification soon after the event (Wahlberg, 2006b). When the angiosperms likewise continued to diverge after the K/T boundary to produce the astounding plant diversity that we can see today (Davies *et al.*, 2004), herbivore taxa that could occupy several alternative niches were the ones that had the greatest opportunity to take advantage of this new and different diversity, and to diversify in turn. A similar reasoning could be applied to parasite–host (Zarlenga *et al.*, 2006) and other species interactions, and the plasticity scenario thus has the potential to function as a broadly applicable principle in historical explanations for the composition of extant biodiversity.

The plant utilization patterns in Nymphalinae presented here, when coupled with the dating of butterfly lineages, highlight the extreme evolutionary conservatism of host-plant use present in some taxa of phytophagous insects. At the same time, they point to the challenge of understanding how and why such constraints can sometimes be broken, to provide the fuel for subsequent re-specialization and diversification.

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SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Table S1. Detailed data for Figures 4 and 5.

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