

EVOLUTIONARY DYNAMICS OF HOST-PLANT SPECIALIZATION: A CASE STUDY OF THE TRIBE NYMPHALINI

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Abstract.—Two general patterns that have emerged from the intense studies on insect–host plant associations are a predominance of specialists over generalists and a taxonomic conservatism in host-plant use. In most insect–host plant systems, explanations for these patterns must be based on biases in the processes of host colonizations, host shifts, and specialization, rather than cospeciation. In the present paper, we investigate changes in host range in the nymphalid butterfly tribe Nymphalini, using parsimony optimizations of host-plant data on the butterfly phylogeny. In addition, we performed larval establishment tests to search for larval capacity to feed and survive on plants that have been lost from the female egg-laying repertoire. Optimizations suggested an ancestral association with Urticaceae, and most of the tested species showed a capacity to feed on *Urtica dioica* regardless of actual host-plant use. In addition, there was a bias among the successful establishments on nonhosts toward plants that are used as hosts by other species in the Nymphalini. An increased likelihood of colonizing ancestral or related plants could also provide an alternative explanation for the observed pattern that some plant families appear to have been colonized independently several times in the tribe. We also show that there is no directionality in host range evolution toward increased specialization, that is, specialization is not a dead end. Instead, changes in host range show a very dynamic pattern.

Key words.—Colonization, generalist, host-plant range, host shift, polyphagy, specialist, speciation.

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Insect host-plant utilization is one of the most intensely studied species interactions, which is hardly surprising considering the immense impact this interaction has on virtually all terrestrial ecosystems, including man-made agricultural systems. Moreover, the species involved are often relatively accessible because they are often conspicuous and easy to maintain and rear under laboratory conditions. Consequently, there is an unusual availability of comparative data because of the extensive attention this group has received, not only by professionals but also by collectors and amateur naturalists over several centuries. This is particularly true for butterflies, a group that is rapidly establishing itself as an important ecological and evolutionary model system (Nylin and Janz 1999; Boggs et al. 2001).

Although the many studies of insect host-plant use has provided us with a multifaceted and complex picture of this species interaction, a couple of general patterns have emerged that call for explanation: a strong predominance of specialists over generalists and a strong taxonomic conservatism in host-plant utilization. The high proportion of host-plant specialists has been a common observation among researchers in the field (cf. Futuyma 1991; Bernays and Chapman 1994; Thompson 1994). Precise estimates are difficult to obtain, due to sampling issues and inconsistency in the definition of the terms. However, Janzen (1988) estimated that around 50% of the caterpillars of Santa Rosa National Park in Costa Rica use only one or a few related host plants. The pattern is not restricted to tropical faunas; a compilation of host-plant data for 329 North American butterfly species from Scott (1986) reveals that around 90% of these predominantly temperate species only use plants from one family. Moreover, species with seemingly wide host ranges can often be made up of more specialized populations (Fox and Morrow 1981; Thompson 1994), so current estimates should tend to underestimate the number of specialists, if anything. Conservatism in host use, where groups of insects tend to be associated

with the same sets of plants, was one of the major points in Ehrlich and Raven's (1964) classical study on butterflies and it has later been confirmed in many other studies (e.g., Ackery 1991; Mitter and Farrell 1991; Fiedler 1996; Futuyma and Mitter 1996; Janz and Nylin 1998). Although not universally true, these patterns are remarkably consistent among widely separated groups of plant-feeding insects. The development of phylogenetic methods for use in studies of character evolution and association has provided a new set of powerful tools to investigate these and other aspects of the insect–host plant interaction (Wanntorp et al. 1990; Brooks and McLennan 1991; Harvey and Pagel 1991; Mitter et al. 1991). Phylogenetic congruence is rarely found in insect–host plant systems, and consequently, cospeciation of associated lineages is not likely to explain patterns of host-plant association (Benson et al. 1975; Smiley 1978; Miller 1987; Mitter and Farrell 1991; Anderson 1993; Funk et al. 1995; Weintraub et al. 1995; but see Farrell and Mitter 1990). Thus, several authors have suggested a shift in focus from cospeciation to explicitly investigating the processes of host shifts and specialization (Ronquist and Nylin 1990; Funk et al. 1995; Ronquist 1995; Janz and Nylin 1998), and this is the approach we shall take here.

A priori, neither the predominance of specialists over generalists nor the taxonomic conservatism over large time scales is easy to explain. There are many good reasons why a broader range of host plants would be favorable, such as an increased likelihood of encountering a favorable host and an increased realized fecundity. Likewise, the evident bond with particular plant groups over long periods of time is difficult to understand in the face of several reports of relatively rapid host-plant evolution on an ecological time scale (Tabashnik 1983; Singer et al. 1992, 1993; Fraser and Lawton 1994; Radtkey and Singer 1995; Fox et al. 1997). It is counterintuitive that the potentially rapidly evolving processes of plant colonization, specialization, and host shifts, all leading to

changes in host-plant use, could give rise to conservatism in host use. However, a series of studies on the leaf beetle genus *Ophraella* have demonstrated that host shifts are affected by constraints on genetic variation for host use, making shifts to plants that have been used by the lineage in the past more likely than shifts to novel host plants (Futuyma et al. 1993, 1994, 1995; Funk et al. 1995; Keese 1998). This offers a compelling explanation for host-plant conservatism without cospeciation: Even when the number of host shifts in a lineage is relatively large, in retrospect the macroevolutionary pattern of host-plant use can appear conservative, because most shifts will go back and forth between the same plants. This conservatism should be less strict than what would be expected from cospeciation, insect lineages would be expected to be only loosely associated with a collection of plant groups. Incidentally, this is exactly the pattern that emerges from the association between butterflies and their host plants (Ehrlich and Raven 1964; Janz and Nylin 1998).

A related observation is that the range of plants that can successfully support larval growth is often wider than the range of plants actually used by the females for oviposition (Wiklund 1975; Smiley 1978; Roininen and Tahvanainen 1989). In addition, these larval and adult traits appear to have different genetic determination (Thompson 1988; Thompson et al. 1990; Keese 1996; Janz 2001), implying that they are not so tightly linked to each other as one might think. This raises the possibility that plants that females have ceased using as hosts can, for whatever reason, linger in the larval host-plant repertoire for extended periods of time. A prediction from these observations is that there should be a bias toward the ancestral host-plant association, both when an insect species specializes again after a colonization of an additional host and regarding the targets of the colonizations themselves.

In spite of the large-scale taxonomic conservatism in host-plant use, in most plant-feeding insects selection for specialization per se seems to be as important as selection for specialization on any particular group of plant (e.g., Futuyma and Moreno 1988; Menken 1996). This means that there must exist a general driving force toward specialization that is not linked to any particular plant characteristic. This could be achieved by a general trade-off between capacities to feed on more than one plant species (e.g., Futuyma and Wasserman 1981; Carriere and Roitberg 1994; Joshi and Thompson 1995; Fry 1996), better predator avoidance by specialists than generalists (e.g., Bernays 1989), or higher accuracy and/or shorter decision times for specialists when searching for host plants (e.g., Bernays and Wcislo 1994; Janz and Nylin 1997; Bernays 1998; Bernays and Funk 1999). The apparent trend toward increased specialization has led to the much debated view that the process of specialization will result in a loss of genetic variation for the ability to use alternate hosts, eventually taking the specialized species into an evolutionary dead end (e.g., Futuyma and Moreno 1988; Moran 1988; Wiegmann et al. 1993; Thompson 1994; Thompson et al. 1997; Kelley and Farrell 1998). A few studies have tried to test this hypothesis on phytophagous insects, and results are mixed. Moran (1988) found evidence for the hypothesis among aphids, whereas Thompson et al. (1997) pointed out that even for extreme specialists such as *Greya* moths, plant

polyploidization could provide a route out of specialization as a dead end. Kelley and Farrell (1998) noted that although host range evolution among *Dendroctonus* bark beetles have clearly occurred in both directions, specialization appeared to have a more “tippy” distribution in the phylogeny, indicating that it was more often a derived condition. This, however, does not seem to be the case among chrysomelid beetles (Futuyma et al. 1995; Dobler et al. 1996). Obviously, specialization among plant-feeding insects is not completely irreversible. The question, as Kelley and Farrell (1998) puts it, is whether specialists are *more often* phylogenetically derived than generalists. In other words, is there a phylogenetic bias toward increased specialization that can account for the predominance of specialists over generalists?

In this paper, we have used a combination of phylogenetic (character optimizations) and experimental methods (larval rearings) to test hypotheses regarding host-plant specialization and conservatism in the butterfly tribe Nymphalini of the family Nymphalidae. We have compared optimizations of actual host use with optimizations of potential host use, where the dataset is expanded to include plants that are not used for oviposition but that the larvae can feed on.

Apart from a descriptive investigation of the patterns of host use and specialization in the clade, we have tested the following explicit predictions: (1) the frequency and direction of host shifts should be biased toward ancestral host-plant associations; (2) the apparent number of independent colonizations should be lower when optimizing potential than actual host use, because a loss from the actual repertoire that is followed by a later recolonization of the same host will appear as a single colonization event using the dataset on potential hosts; (3) if there is a bias toward increased specialization, polyphagy should be the ancestral state, with a general trend in the clade toward specialization; and (4) likewise, if such a bias exists, gains of plant families should be rare compared to losses, and when gains occur they should typically lead to host shifts (gain of new family, loss of old), not to host range expansions.

METHODS

Study Group and Definitions of Terms

The study was carried out on the butterfly tribe Nymphalini (sensu Harvey 1991). This tribe includes the two large genera *Nymphalis* and *Polygonia*, as well as the *Vanessa* group (including *Cynthia* and *Bassaris*) and several smaller genera such as *Inachis*, *Aglais*, and *Araschnia*. The Nymphalini is represented in all major biogeographic zones of the world, but especially *Nymphalis* and *Polygonia* have their main distribution in the Holarctic (Table 1). The study has taken its point of departure in the unusually wide host range of the Palearctic *Polygonia c-album* (Nylin 1988; Janz et al. 1994). Host-plant ranges in the rest of the tribe vary considerably, from the strictly monophagous, such as *Araschnia levana* and *Aglais urticae*, to the very polyphagous *Cynthia cardui* (Table 1). This makes the tribe well suited for an investigation into the processes behind host range evolution.

Terms like “specialist” and “generalist” are difficult to define because they always depend on context and scale. In a group of extreme specialists, using only one host plant

TABLE 1. Host plant families used by genera in the tribe Nymphalini and the outgroups used in the phylogenetic reconstruction, with number of species and geographic distribution. Sources are abbreviated as follows: A, Ackery 1988; BJB, Bascombe et al. 1999; CP, Corbet and Pendlebury 1992; CW, Common and Waterhouse 1972; DV, DeVries 1987; E, Ebert 1993; F, Field 1971; L, Larsen 1991; N, Nylin et al., 2001; Pa, Parsons 1991; PO, personal observations; Sc, Scott 1986; Sm, Smart 1975.

Genus	No. of species	Distribution	Host-plant families	Sources
<i>Hypanartia</i>	8–9	Neotropical	Urticaceae, Ulmaceae	DV, Sm
<i>Antanartia</i>	6	Africa	Urticaceae	L
<i>Mynes</i>	6–9	Australasian	Urticaceae	CW, Sm
<i>Symbrenthia</i>	6	Southeast Asia	Urticaceae	A, Sm
<i>Araschnia</i>	6?	Palaearctic	Urticaceae	A, Sm
<i>Bassaris</i>	2	Australia, New Zealand	Urticaceae	F
<i>Vanessa</i>	5	cosmopolitan	Urticaceae	F
<i>Cynthia</i>	9	cosmopolitan	Urticaceae, Asteraceae, Malvaceae, Boraginaceae, Fabaceae, Rosaceae, Ulmaceae, Rhamnaceae, Chenopodiaceae, Hydrophyllaceae, Lamiaceae, Verbenaceae, Solanaceae, Convolvulaceae, Plantaginaceae, Brassicaceae, Apiaceae, Polygonaceae, Cucurbitaceae, Rutaceae, Poaceae	F, Sc
<i>Inachis</i>	1	Palaearctic	Urticaceae, Cannabidaceae	A, Sm
<i>Aglais</i>	2–3	Palaearctic	Urticaceae	A, E, Sc, Sm
<i>Nymphalis</i>	5	Holarctic	Ulmaceae, Salicaceae, Rosaceae, Rhamnaceae	A, E, N, Sc, Sm
<i>Roddia</i>	1	Holarctic	Salicaceae, Betulaceae, Ulmaceae	A, Sc, N
<i>Kaniska</i>	1	Southeast Asia	Smilacaceae	CP
<i>Polygonia</i>	~11	Holarctic	Urticaceae, Ulmaceae, Cannabidaceae, Salicaceae, Betulaceae, Grossulariaceae, Ericaceae, Corylaceae	A, N, Sc, PO
Outgroup: <i>Hypolimnas</i>	~20	Old World tropics, Neotropics	Urticaceae, Portulacaceae, Acanthaceae, Convolvulaceae, Malvaceae, Urticaceae, Amaranthaceae	DV, L, Sc, Sm, Pa
<i>Argynnis</i>	2	Palaearctic and Oriental	Violaceae	A, Sm
<i>Precis/Junonia</i>	~30	Africa, Neotropics	Plantaginaceae, Scrophulariaceae, Verbenaceae, Acanthaceae, Lamiaceae, Amaranthaceae	L, Sc, Sm, CP, BJB

species each, an insect feeding on several plant species could arguably be considered a generalist. In groups where specialization is less extreme, as in most butterfly groups, a species that is constrained to one plant family can be considered a specialist (cf., Wahlberg 2001). In essence, degree of specialization is a relative measure, and we have chosen to use the term in such a manner in our manuscript, rather than trying to force it into an arbitrary definition. The terms “monophagy” and “polyphagy” are used in a similar manner and should be interpreted as relative, not definitive states.

Phylogeny and Character Coding

Phylogenetic relationships among the Nymphalini were taken from a companion paper with a total evidence analysis of morphological and molecular (mitochondrial *nd1* and nuclear *wingless*) data (Nylin et al., 2001). The morphological matrix consists mainly of wing pattern characters, together with a few larval, pupal, and adult eye morphology characters. Inclusion of host-plant data into the matrix did not influence the phylogenetic reconstruction (Nylin et al., 2001). This phylogenetic analysis was concentrated on the two large Holarctic genera *Polygonia* and *Nymphalis*, along with the closely related *Kaniska*, *Roddia*, *Aglais*, and *Inachis*. Relationships within and among other Nymphaline groups that

were included in the analysis, such as *Hypanartia*, *Antanartia*, and the *Vanessa-Cynthia* group, are less certain because only samples of these genera were included in the analysis. However, this is not a large problem for the present study, because most of the dynamics of host-plant utilization appear to have occurred within *Nymphalis* and *Polygonia*, with the exception of *Cynthia*. This last genus, however, deserves a treatment of its own when a detailed phylogeny is available for the group. All genera in the tribe (sensu Harvey 1991) have been sampled for the analysis (Table 1), and we have no reason to believe that host-plant associations in nonsampled relatives should differ from the ones sampled. The phylogeny (using *Argynnis*, *Precis*, and *Hypolimnas* as outgroups) is presented in Figure 1. This phylogeny is a strict consensus tree of two equally parsimonious reconstructions, differing only in the reversed positions of *P. interrogationis* and *P. c-aureum*. A completely resolved phylogeny was used for the character optimizations and is shown in Figure 2; this was the tree that was favored by successive weighting (Nylin et al. 2001). Because *P. interrogationis* and *P. c-aureum* have very similar host-plant ranges (Appendix 1), there were no differences between the two equally parsimonious reconstructions for the results that are presented in this paper.

All optimizations were made with MacClade 3.07 (Mad-

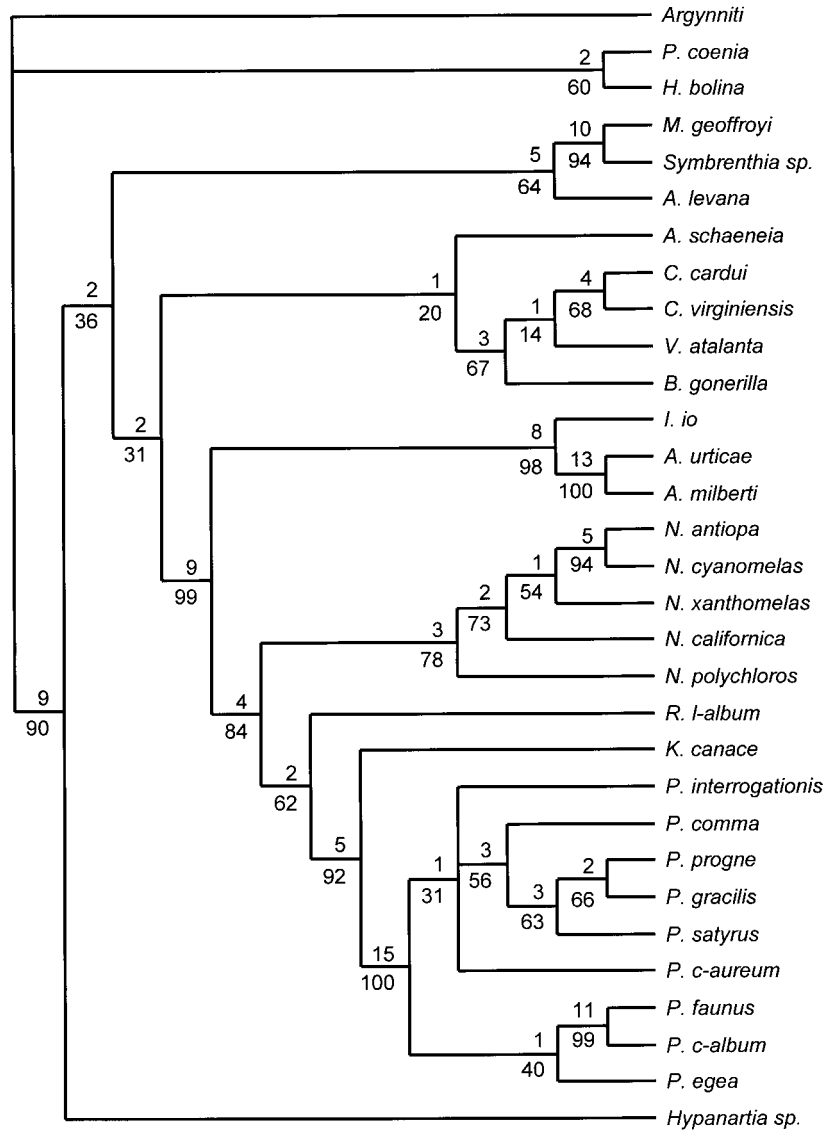


FIG. 1. Phylogeny of Nymphalini, based on a total evidence analysis of morphological and molecular (*nd1*, *wingless*) data (Nylin et al. 2001). The reproduced phylogeny is a strict consensus tree of the two most parsimonious trees (1272 steps) with CI = 0.402 and RI = 0.601. The two equally parsimonious trees differed only in that the positions of *Polygonia interrogationis* and *P. c-aureum* were reversed. Decay indices (top) and bootstrap values (bottom) on branches show level of support. *Nymphalis cyanomelas* is not included in the host-plant analyses due to lack of host plant data.

dison and Maddison 1992). Host-plant data (as all species associations) are not trivial to code and optimize on a phylogenetic tree. There are several reasons for this. First, the characters (host plants) are evolving and bifurcating themselves, and if the aim is not explicitly to explore cospeciation in the lineages, it is necessary to use host groups that are inclusive enough to have kept their identity over the time for diversification of the associated group. Second, there is to date no entirely satisfactory way of coding multiple associations; there are a number of alternative methods, but all have their problems (Maddison and Maddison 1992; Janz and Nylin 1998). Third, host-plant use is a compound character. It is the result of the interaction between two rather different traits: oviposition preference and larval performance, which will make optimizations problematic.

For the purpose of this study, the first problem is not difficult. In most analyses, we have used plant families as host characters, most of which are older even than the butterflies as a whole (Janz and Nylin 1998). The second problem is more difficult. Unfortunately, coding all host-plant associations as one multistate character is not an option in this study because we want to explicitly address dynamics in host-plant range, and this type of coding does not allow for a correct treatment of multiple host associations. Treating it as ambiguity would lead to serious loss of data and misleading optimizations. MacClade does have an option to treat multiple associations as polymorphisms, but it does not allow these polymorphic states to be assigned to internal nodes of the phylogeny. Binary optimizations, in contrast, are easy to code and interpret but may produce inaccurate reconstruc-

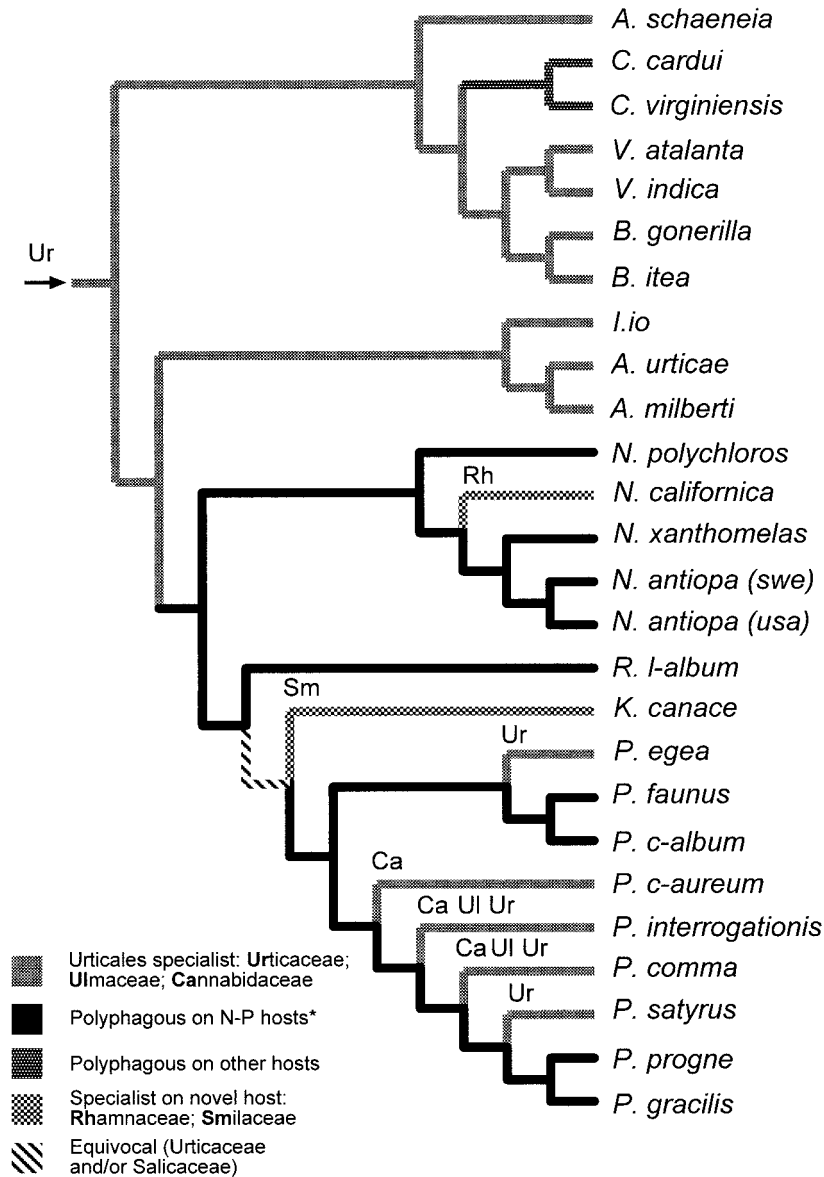


FIG. 2. Host use in part of the tribe Nymphalini, simplified for ease of interpretation. The phylogeny is one of the two most parsimonious reconstructions, differing only in the reversed positions of *Polygonia interrogationis* and *P. c-aureum*. The phylogeny that is shown here is the one that is favored by successive weighting (Nylin et al. 2001). The figure shows results from several independent optimizations of each plant family on the butterfly phylogeny. Note that "polyphagy" does not refer to a single state, it represents a number of combinations of host use involving more than one plant family. N-P hosts refer to the *Nymphalis-Polygonia* hosts. Apart from the families in Urticales, these hosts are: Salicaceae, Betulaceae, Ericaceae, Grossulariaceae, and Rosaceae.

tions of internal branches as having no host-plant association at all. However, in a relatively small dataset as in this study, this can fairly easily be dealt with by manual inspection of the nodes. Therefore, we have in this paper used binary optimizations with each plant family as an individual character. In the rare occurrences of a branch being reconstructed as having no host-plant association, we have treated it as equivocal. The third problem is potentially even more difficult, and has no easy solution. As already stated, host-plant use is the result of the long-term interaction between oviposition preference and larval performance, and it is difficult to evaluate the relative importance of the two traits and their interaction. Furthermore, there is no available optimization

technique that allows for tracing a complex character of this kind. Our approach involves trying to separate the two traits, optimizing them separately, and analyzing the differences.

Host-Plant Data and Larval Establishment Tests

Host-plant data were gathered in two ways. First, host-plant information was collected for all species from several literature sources (Field 1971; Common and Waterhouse 1972; Smart 1975; Johnston and Johnston 1980; Scott 1986; DeVries 1987; Ackery 1988; Larsen 1991; Parsons 1991; Corbet and Pendlebury 1992; Ebert 1993; Bascombe et al. 1999). Because host-plant records are not always reliable,

we have been conservative in our acceptance of a host plant into the repertoire of a butterfly. Atypical records have only been included if they were independently corroborated by at least two sources.

We also conducted a series of larval rearing experiments in which we tested the ability of larvae of different species to feed and survive on a number of plants (cf., Scriber 1988; Futuyma et al. 1993, 1994). The plants were selected so that they represented as large part as possible of the combined host range of the Nymphalini. In addition, we tested a number of plants outside the normal repertoire of the clade to control for the possibility that the larval capacities to feed on non-hosts were randomly distributed over the plant phylogeny (Appendix 1). Not all butterfly species were tested on all plants, due to lack of butterfly and plant material; our first priority was to try rearing larvae from all butterfly species on select representatives of the most commonly used host-plant groups: Urticaceae, Ulmaceae, Cannabidaceae, Salicaceae, Ericaceae, Grossulariaceae, Rosaceae, Betulaceae, and Asteraceae. Of these families, Asteraceae was considered least crucial (because it is only used by the genus *Cynthia*, outside the focal group of the study), so when necessary this family was omitted in the establishment tests. Consequently, the reconstruction of potential host-plant use is less reliable for Asteraceae than for the rest of the plant families mentioned above. Successful establishment and growth of larvae were coded from 0 to 6, where 0 means no success at all and 6 means survival to pupation. In-between these endpoints larvae were given a code (1–5) depending on the larval stage they managed to reach on the plant in question. Successfully reaching the second instar (code 2) was used as an indication of larval feeding capacity in the optimizations. Reaching the second instar means that they have to complete a full molt on the plant, which we take as an indication of some ability to feed on the plant, an ability that may conceivably be more developed in other genetic variants of the species. Reaching the second instar typically meant surviving on the plant for 5–7 days, and molt in these butterflies is not possible without prior feeding and growth.

Typically, five larvae of each species were tested on each plant. This is a much too small number to achieve any reliable screening of feeding capacity, but it was a necessary compromise, allowing us to test the larvae on more plant species. In addition, in most cases only one representative of each plant family was tested. Accordingly, failures to feed and survive on a plant do not necessarily mean that the species has no capacity to feed on the plant family in question. Any positive result, however, is a reliable indication that such a capacity does exist in the species. In some rare cases, the establishment failed on a plant from a family that a butterfly species is known to use as a host (perhaps because the Swedish plant species used was not the species present in the butterfly's native area). This further illustrates that a score of 0 in the establishment test cannot be taken as evidence against a capacity to feed on the plant family in question. For this reason, establishment data are only used to add to the actual host use in the expanded dataset, not to delete an association.

To test whether the successful establishments were biased toward plants that are used by other members of the Nym-

phalini tribe, we analyzed the establishment data with Fisher's exact test on a 2×2 contingency table (success/failure \times host/nonhost). Again, reaching the second larval instar was classified as a successful establishment.

Tracing Host-Plant Range

We first used the literature data to trace actual host plant use on the butterfly phylogeny as binary characters using parsimony. To assess whether there is a phylogenetic signal in the host-plant data on this level of resolution, we compared the consistency index (CI) of the actual host-plant data on the given phylogeny with 100 optimizations where the actual character states had been randomly reshuffled among butterfly taxa.

The results of the optimization of actual host-plant use were compared with optimizations of the same data extended with the results from the larval establishment tests (ideally, potential host-plant use). It is hardly likely that loss of a host-plant association requires as much evolutionary modification as colonization of a new plant. Under some circumstances, host-plant losses require no genetic modification at all, but can be entirely attributable to external factors, such as changes in plant and insect distribution patterns. Consequently, weighting gains and losses equally may not give a fair reconstruction of the patterns of host-plant use. We therefore performed an additional set of optimizations of the same host-plant data, where gains were given twice the transformation weight than losses. Admittedly, transformation weights that correspond to the actual costs of gains and losses are difficult to obtain, and our scheme was chosen rather arbitrarily. It is only intended to serve as an illustration of the impact that the transformation-weighting scheme has on the optimizations. See Wahlberg 2001 for a more thorough discussion of this issue.

In addition, we tested the hypothesis of specialization as a dead end. If there is such a universal directionality in host range evolution toward increasing specialization, polyphagy should be ancestral, whereas specialization should show a more apical distribution in the phylogenetic tree. Kelley and Farrell (1998) used a modified version of the PTP test (Faith and Cranston 1991) to demonstrate that monophagy had an apical distribution in *Dendroctonus* bark beetles. Unfortunately, this method would require us to force host range variation into a dichotomous character, which would discard much of the dynamics in our dataset. A simple but direct alternative way to test for directionality in the process of host range evolution is to compare the total number of host-plant gains (colonization) and losses (typically corresponding to specialization) in the phylogeny. If there is a trend toward increasing specialization, there should be more host-plant losses than gains. However, gains need not necessarily lead to host range expansion if the original host is immediately lost from the repertoire (i.e., on the same branch in the phylogeny). If host range is not to be extended, the number of colonizations directly followed by exclusions (host shifts) should outnumber the number of colonizations without exclusions (widening of the host-plant range). In this analysis, we used optimizations of both the original data matrix with

TABLE 2. Summarized results of the larval establishment tests (see Appendix 1 for details). Establishments were coded from 0 to 6, where 0 means no establishment and 6 survival to pupation. The codes 1–5 correspond to the larval stage the larvae succeeded to reach. Combinations of butterflies and plants that were not tested are marked with a dash. Successful establishments (to any degree) are marked with bold, establishments on plants that are not actually used as hosts are marked with underlined bold. Table entries marked with an asterisk indicate establishment failures on plant families that the butterfly species is known to use in the field. *Nymphalis antiopa* was sampled from Stockholm, Sweden (marked “Swe”) as well as from Mount Rainer, Washington State (marked “USA”).

	Urticaceae	Ulmaceae	Cannabidaceae	Salicaceae	Betulaceae	Grossulariaceae	Ericaceae	Rosaceae	Asteraceae
<i>Araschnia levana</i>	6	0	0	0	0	0	0	0	0
<i>Bassaris itea</i>	6	0	0	0	0	0	0	—	—
<i>Cynthia cardui</i>	6	1	0	0	0	0	0	2	6
<i>Vanessa atalanta</i>	6	<u>0</u>	0	0	0	0	0	<u>0</u>	0
<i>Vanessa indica</i>	6	1	0	6	0	0	0	0	—
<i>Aglais milberti</i>	6	<u>0</u>	0	<u>0</u>	0	0	0	0	0
<i>Aglais urticae</i>	6	0	4	0	0	0	0	0	0
<i>Inachis io</i>	6	0	<u>6</u>	0	0	0	0	0	0
<i>Nymphalis antiopa</i> (Swe)	4	6	0	6	6	1	—	3	0
<i>Nymphalis antiopa</i> (USA)	5	1	0	6	5	<u>0</u>	0	<u>0</u>	0
<i>Nymphalis polychloros</i>	4	6	—	6	0	1	5	6	—
<i>Nymphalis xanthomelas</i>	1	1	1	6	0	0	<u>0</u>	0	—
<i>Kaniska canace</i>	<u>0</u>	<u>0</u>	<u>0</u>	0	0	0	0	0	—
<i>Polygonia c-album</i>	6	6	6	6	6	6	6	0	0
<i>Polygonia c-aureum</i>	6	0	6	0	0	0	<u>0</u>	0	—
<i>Polygonia egea</i>	6	0	0	0	0	0	0	0	0
<i>Polygonia faunus</i>	6	0	0	1	1	6	6	—	—
<i>Polygonia gracilis</i>	<u>0</u>	0	0	0	0	6	0*	0	0
<i>Polygonia interrogatilis</i>	6	6	0*	0	0	0	0	0	0
<i>Polygonia satyrus</i>	6	0	<u>6</u>	0	0	0	0	0	0

only literature data, and the extended matrix that included larval feeding capacities from our establishment tests.

RESULTS

Host-Plant Data and Larval Establishment Tests

Although the tribe Nymphalini is associated with more than a dozen plant families, the dominating theme is definitively Urticales, especially Urticaceae (Table 1, Fig. 2). This is also by far the most likely ancestral host-plant association for this group. The other plants have gradually been added to the repertoire by a number of host range expansions, most notably in the closely related genera *Nymphalis* and *Polygonia* (and the immediate ancestor to these genera) and in the very polyphagous genus *Cynthia* (Fig. 2). Results from the larval establishment tests are summarized in Table 2 (and are shown in detail in Appendix 1). There were 15 successful establishments of plants that are not used as hosts by the species in question. Among these, there was a significant bias toward plants that are used as hosts elsewhere in the Nymphalini (Fisher's exact test, $P = 0.045$). In fact, only the extremely polyphagous *Cynthia cardui* was able to initiate growth on any of the plants outside the normal repertoire of the tribe (it successfully reached pupation on *Ranunculus acris* in Ranunculaceae). There were other patterns in what plant species that did support larval growth. The most noticeable result was that all butterflies tested managed to feed successfully on *Urtica dioica* (Urticaceae), with the exceptions of *Kaniska canace* and *Polygonia gracilis* (Table 2). The latter has apparently fulfilled a complete switch from the dominating Urticaceae theme to *Ribes* (Grossulariaceae), whereas *K. canace* represents the most dramatic shift in host-plant use in this clade, now feeding exclusively on monocotyledons (Smilacaceae). However, as mentioned before, establishment fail-

ures should be treated with caution in this analysis because the sample of larvae was very small. The two other families in Urticales, Ulmaceae and Cannabidaceae, also turned out to be accepted for feeding by a wider range of butterflies than actually use them as hosts (Table 2). The results of the establishment tests align well with previous findings by Futuyma et al. (1995) that colonizations are constrained by genetic variation in host use.

Another interesting result is that *Polygonia c-album* was able to survive until eclosion on *Vaccinium myrtillus* (Ericaceae). Ericaceae is a plant family that is used by most of its close relatives (however, the actual host genus is *Rhododendron*), but is not believed to be used as a host plant by *P. c-album*. Some results were unexpected, such as the ability of *Vanessa indica* to feed on *Salix* (Salicaceae) and the ability of *N. polychloros* to feed on *Vaccinium* (Ericaceae) and *Ribes* (Grossulariaceae).

Tracing Host-Plant Range

Optimizing actual host-plant use onto the butterfly phylogeny as binary characters suggests several independent colonizations of most major host-plant families used in Nymphalini (Table 3), sometimes reaching numbers as high as five to nine colonizations, as with Ulmaceae. The 12 plant families have been independently colonized 29 to 37 times in this tribe, according to the unordered optimization of actual host-plant data. Nevertheless, there is still a clear phylogenetic signal in host-plant data within Nymphalini. The optimization of actual host use data produced a higher consistency index than optimizations resulting from randomly reshuffled host-plant data, this is, the butterfly phylogeny is a good predictor of host-plant use (Fig. 3). Still, considering the conservatism of host-plant use on higher taxonomic lev-

TABLE 3. Independent gains and losses of host-plant families, when optimizing host use, either according to literature data only or with an extended data set that includes results from the larval establishment tests, using either equal or biased transformation weights. U, unordered transformation costs; W, weighted transformation costs (gains twice as high as losses).

Plant family	Independent gains				Independent losses			
	Actual		Potential ¹		Actual		Potential ¹	
	U	W ²	U	W ²	U	W ²	U	W ²
Urticaceae	2–5	0–2	1–3	0–1	2–5	5–9	3–5	5–7
Ulmaceae	5–9	3	4–7	2	0–4	7	0–3	6
Cannabidaceae	4	3–4	4–5	3–4	1	1–3	1–2	2–4
Salicaceae	2–4	1–2	3–5	2–3	0–2	2–4	0–2	2–4
Betulaceae	4	4	4	4	0	0	0	0
Grossulariaceae	2	2	2	2	0	0	0	0
Ericaceae	2	2	3	3	0	0	0	0
Rosaceae	3	3	3	2–3	0	0	0	0–2
Asteraceae	1	1	1	1	0	0	0	0
Malvaceae	1	1	1	1	0	0	0	0
Rhamnaceae	2	2	2	2	0	0	0	0
Smilacaceae	1	1	1	1	0	0	0	0
Total:	29–37	23–27	29–36	23–27	3–12	15–23	4–12	15–23

¹ Includes results of the larval establishment test.

² Gains weighted twice as high as losses.

els, the high number of colonizations seen in this study is somewhat remarkable. It seems highly unlikely that they really represent independent events, especially because some of these plant groups do not occur as hosts in the close relatives of Nymphalini. Adding the results from the larval establishment tests did not change this picture much. In some cases it merely added to the pattern of a suspiciously high number of colonizations (Table 3), probably because more species are now marked as capable of using the plants, sometimes in disparate positions on the phylogeny. Weighting gains twice as expensive as losses reduced the total number of colonizations to between 23 and 27, but even this number is remarkably high (Table 3).

We found no support for directionality toward increasing specialization in the Nymphalini. First, the ancestor of the clade was probably a specialist on plants in Urticaceae (Fig.

2); thus, polyphagy is likely to be the derived state. Second, there were much fewer host plant losses than gains in the clade when both types of transformations were given equal weights (Table 3). Third, the number of colonizations without an associated loss of the ancestral plant significantly outnumbered the number of direct host shifts (Table 4). Thus, in the overwhelming majority of colonizations these were not followed by an immediate loss of the ancestral plant (i.e., a host shift), but have instead lead to a host range expansion. If anything, the trend in this clade is toward increased polyphagy, not specificity.

According to the reconstructions, the buildup of polyphagy in Nymphalini is a gradual process. Moving up the tree from the ancestral Urticaceae association, plants are incorporated into the host range by colonization at several occasions, gradually increasing the host range. Some recent species have subsequently lost one or more of these plant groups from its repertoire, a few of them becoming true specialists. Others have retained (or regained) all of these plants, sometimes even added to the host range. Interestingly, most of the specializations have been on the ancestral Urticales hosts (Fig. 2).

DISCUSSION

The tribe Nymphalini is closely associated with plants in Urticales, especially Urticaceae, and it is highly likely that the ancestor of this tribe was a specialist on this family. This becomes even clearer in the light of the larval establishment tests, where almost all tested species showed some ability to grow on nettles (*Urtica dioica*), even if they do not use these plants as hosts in the field (Table 2). This host-plant association has an even longer history that dates back far beyond the Nymphalini. Urticales is a member of a larger rosoid clade, which also contains Fabaceae and Rosaceae, probably the ancestral host-plant association for the butterflies as a whole (Janz and Nylin 1998). Urticales itself is also used in the closely related tribe Kallimini, including the outgroup genus

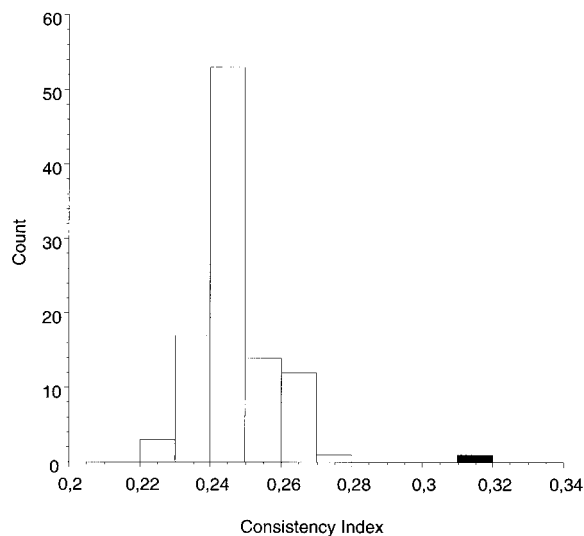


FIG. 3. Histogram of consistency index of the actual host-plant characters (black) compared with indices derived from random re-shuffling of the character states ($n = 100$).

TABLE 4. Number of plant colonizations leading to host range expansions and host shifts.

	Actual host use ¹		Potential host use ²	
	ACCTTRAN ³	DELTRAN ⁴	ACCTTRAN ³	DELTRAN ⁴
Expansions	24	34	26	32
Shifts	5	3	3	3
<i>P</i> (χ^2)	<0.001 (12.44)	<0.001 (25.97)	<0.001 (18.24)	<0.001 (24.03)

¹ Literature data only.

² Data matrix extended with results of the larval establishment test.

³ Accelerated transformations: equivocal tracings resolved as to accelerate changes.

⁴ Delayed transformations: equivocal tracings resolved as to delay changes.

Hypolimnas. The widespread capacity to feed on *Urtica* was astonishing, considering that successful establishments were rather rare, and suggests that ancestral host associations sometimes can be retained for long periods of time as part of the larval repertoire.

Although association with Urticales is a strong conservative feature, the optimizations of host-plant use show a very dynamic pattern, with a large amount of colonizations of a heterogeneous group of plants. Even if gains of plant families are weighted twice as high as losses, the optimization suggests numerous colonizations and losses of the plant families used in the tribe. In total, there were between 23 and 37 independent colonizations of 12 plant families within this clade of 30 butterfly species, depending on weighting scheme (Table 3). Moreover, the vast majority of these colonizations have occurred within the two genera *Nymphalis* and *Polygonia* (Fig. 2). This seemingly opportunistic pattern of host use is intriguing. Such a high rate of colonization as seen here would lead to a tremendous diversification in host-plant use over time, something that is not reflected at all in higher-level studies (e.g., Ehrlich and Raven 1964; Jermy 1984; Janz and Nylin 1998), where conservatism in host use is very strong.

However, there are reasons to believe that many of the host-plant colonizations within the clade are not truly independent. A majority of the gains and losses involved the same set of plant families. There were 29–37 independent colonizations in the dataset with actual host use and equal weighting. Of these, 11–17 colonizations involved plants in Urticales (Urticaceae, Ulmaceae, and Cannabidaceae) and another 13–15 colonizations involved the other typical hosts of *Nymphalis* and *Polygonia* (Salicaceae, Betulaceae, Grossulariaceae, Ericaceae, and Rosaceae). Most of these plant families are colonized repeatedly, and together these eight families appear to have been colonized 24–32 times in the *Nymphalis-Polygonia* clade (including also *Roddia* and *Kaniska*). Moreover, there was a bias among successful larval establishments on nonhosts toward plants used by other butterflies in the clade (Table 2), also hinting at nonindependence between these colonization events.

Interestingly, the few examples of extreme host shifts in the clade (the use of Smilacaceae by *Kaniska canace*, Rhamnaceae by *Nymphalis californica*, and several novel plant families in *Cynthia cardui*) have all taken place in the most polyphagous sections of the tribe. Oviposition mistakes are well known to occur among egg-laying insects (e.g., Chew 1977; Feldman and Haber 1998) and have been argued to provide a path to novel plant associations (Larsson and Ekbohm 1995).

The task of correctly evaluating and discriminating between possible host plants will be increasingly difficult as the host range increases (e.g., Bernays 1996, 1998), and polyphagous insects have been shown to make more oviposition errors than specialists do (Stanton 1984; Janz and Nylin 1997; Nylin et al. 2000). Thus, by increasing the oviposition error rate, polyphagy may facilitate radical host shifts.

As expected, doubling the weights of gains over losses reduced the number of independent colonizations. However, many plant families are still optimized as being colonized several times in the clade. One example is Grossulariaceae, which is not used at all in the Nymphalid clades close to Nymphalini (e.g., Melitaeini, Kallimini, and Coloburini), indeed it is hardly used at all by butterflies outside of *Polygonia*. Yet, this plant group has been colonized twice within *Polygonia*, according to the optimizations. At closer inspection, it turns out that there are indications of use of this plant group in several other species in the *Nymphalis-Polygonia* group: One note claims that “*N. vau-album*” (*Roddia l-album*) uses *Ribes* in eastern Europe (König and Niculescu 1970), *N. polychloros* showed a limited ability to feed on these plants in our tests (Table 1), and older larvae of *P. interrogationis* and *P. comma* are able to feed on *Ribes* (S. Nylin, pers. obs.). Under these circumstances, it does not seem likely that there have been several wholly independent colonizations of this unusual plant family within *Polygonia*, even though this is what all optimizations suggest. Assuming that the phylogenetic reconstruction is correct (trees that reduce the number of colonizations of this group to one are about 10 steps longer) there are three other possible explanations that seem more likely: (1) some other shared characteristic of this genus (or possibly the *Nymphalis-Polygonia* clade) has preadapted them to colonizing Grossulariaceae, that is, a case of parallel evolution (Fig. 4A); (2) an early colonization of the family has been followed by several losses, leaving two disparate sections of *Polygonia* with this host-plant association today (Fig. 4B); and (3) an early association with Grossulariaceae may have been lost by one of the *Polygonia* sections and then recolonized by some of its members (Fig. 4C). These scenarios would neatly explain the general dynamics of host use in Nymphalini, with much apparent colonization of shared host-plant families. Larval establishment tests, together with optimizations of actual versus potential host-plant use, could potentially provide evidence for these scenarios, and, with enough detail, help choose among them (cf., Scriber 1988; Futuyma et al. 1993, 1994). Unfortunately, our limited samples did not allow us to answer these questions with any certainty. In addition, the positive results

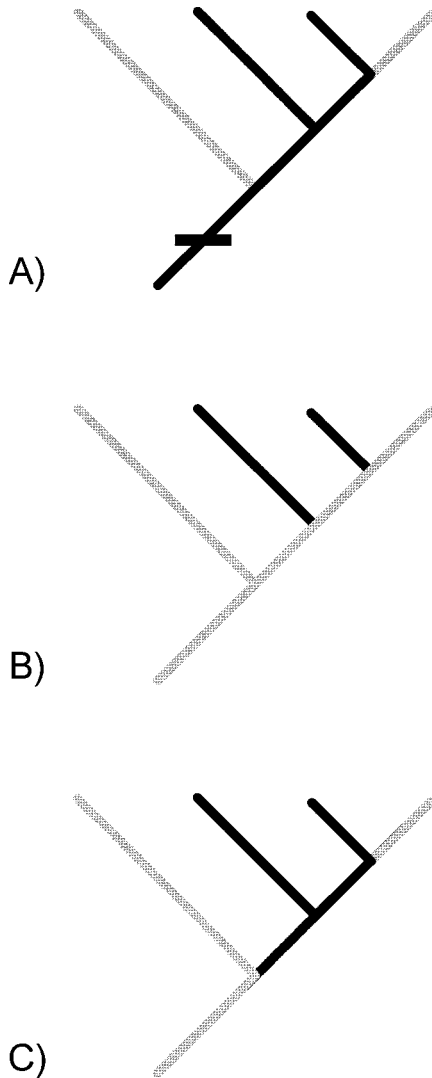


FIG. 4. Three alternative scenarios that explain the pattern of multiple apparent independent colonizations of the same plant taxon within a small insect clade. (A) Shared characteristic (preadaptation) followed parallel evolution. The shared characteristic makes the colonization of the plant taxon more likely in the descendant species. (B) One colonization, followed by several losses. This scenario may be more likely if gains of host plants are more expensive than losses. (C) One colonization, followed by losses and recolonizations. Genetic variation for the capacity to feed on the lost host plant remains in the species, making it more likely to be colonized again.

we did get were ambiguous. On one hand, we have examples of abilities to feed on ancestral host plants such as the almost universal ability to feed on Urticaceae and the ability of *P. c-album* to feed on *Vaccinium* in Ericaceae, a family used by other *Polygonia* and that optimizations show to have been used in the lineage leading to *P. c-album*. These results support the third scenario described above: The shared host-plant may have been colonized at an early stage and then lost from the repertoire, but kept in the potential host range of the larvae and recolonized at a later stage. On the other hand, there are other positive results that instead fit the parallel evolution scenario: *Vanessa indica* showed an unexpected ability to

feed on Salicaceae (a *Nymphalis-Polygonia* host), and *Nymphalis polychloros* was able to feed on Ericaceae (a *Polygonia* host). Of course, these scenarios are not mutually exclusive, so the ambiguous results should perhaps not be too surprising.

By adding the results from the larval establishments to the host use matrix, we constructed a potential host range for the butterflies. According to our second prediction in the introduction, there should be fewer colonization events when these data are optimized onto the phylogeny, compared to the data matrix with actual host use. This was, however, not the case (Table 3). For example, there were fewer colonizations of Urticaceae, but more of Salicaceae and Ericaceae. This prediction, corresponding to scenario 3 above, could thus only be corroborated for Urticaceae, the most ancient host-plant association of the tribe.

Host range evolution in Nymphalini has apparently been very dynamic, with numerous expansions and reductions of the host-plant range. There is no support for the hypothesis that specialization should be derived within this tribe (cf. Kelley and Farrell 1998). Optimizations suggest that the ancestor of the tribe was a specialist on Urticaceae (Fig. 2). There were many more gains than losses in the clade, as long as these events were given equal weights (Table 3), instead indicating a trend toward polyphagy. Furthermore, colonizations have typically not lead to an immediate shift, that is, where the old host-plant is lost on the same branch of the phylogeny. Instead, colonizations in the Nymphalini have typically led to host range expansions, as the number of expansions greatly (and statistically significantly) outnumbered shifts, also supporting a general trend toward increasing host ranges. Many host-plant expansions have taken place in relatively derived positions, within the genera *Polygonia* and *Nymphalis* or in their immediate common ancestor. Both these genera have a predominantly temperate distribution, which has been shown by Scriber (1973) to be correlated with increasing host ranges among butterflies in the Papilionidae (but concerning Lycaenid butterflies, see Fiedler 1998). However, the sister clade to the *Nymphalis-Polygonia* group (*Aglais + Inachis*) also has a temperate distribution, and the species in this clade can all be considered specialists. Still, there are circumstances in parts of the temperate regions that could favor host range expansion, by relaxing the selection for specialization (Nylin 1988; Scriber and Lederhouse 1992; Janz et al. 1994).

Interestingly, the relatively polyphagous *Nymphalis-Polygonia* clade is much more species rich (at least 18 species) than the sister clade (*Aglais + Inachis*, probably three species). The current dataset is too small to statistically test for a relationship between host range expansion and speciation, but it is an interesting observation that deserves to be more thoroughly examined (cf. Orr and Smith 1998). Theoretically, cause and consequence in such a link could go both ways. Either polyphagy facilitates fragmentation into subpopulations that eventually leads to speciation or a polyphagous feeding habit is itself a result of such fragmentation. Such a relationship could imply that the diversification of plant feeding insects is driven more by oscillations in host range than by specialization in itself and is something that deserves closer investigation.

Results of previous studies on the issue of a directionality

in host range evolution have been mixed (Moran 1988; Wiegmann et al. 1993; Futuyama et al. 1995; Dobler et al. 1996; Thompson et al. 1997; Kelley and Farrell 1998). If anything, the trend in Nymphalini is toward polyphagy, but a more important deduction from this study and previous studies is that host range is a very dynamic feature of most insect-plant associations. Over time, insect clades will pass through phases of host range expansion and contraction. Whether we find a trend toward increased or decreased specialization in any particular clade will depend on the evolutionary phase that this particular clade happens to be in at the moment. Given the predominance of specialists over generalists among butterflies and many other groups of plant-feeding insects, polyphagy is likely to be an evolutionary transient phase, occasionally punctuating the general theme of specialization. Therefore, even if specialization appears to be a general trend, this trend is probably repeatedly interrupted by host range expansions, possibly coupled with diversification. Consequently, however dominant the theme of specialization is in insect-host plant associations, it is not a path of no return.

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APPENDIX 1

Host plant associations of the species in Nymphalini that are included in this study. Sources are abbreviated as follows: A, Ackery 1988; B, Bascombe et al. 1999; DV, DeVries 1987; E, Ebert 1993; L, Larsen 1991; CW, Common and Waterhouse 1972; J, Johnston and Johnston 1980; Sc, Scott 1986; CP, Corbet and Pendlebury 1992; PO = personal observation.

Species name	Host-plant families	Source
<i>Symbrenthia hypselis</i>	Urticaceae	A
<i>Mynes geoffroyi</i>	Urticaceae	A, CW
<i>Hypanartia lethe</i>	Urticaceae, Ulmaceae	A, DV
<i>Antanartia schaeneia</i>	Urticaceae	L
<i>Bassaris gonerilla</i>	Urticaceae	A, F
<i>Bassaria itea</i>	Urticaceae	A, F
<i>Vanessa atalanta</i>	Urticaceae	A, F
<i>Vanessa indica</i>	Urticaceae	A, F
<i>Cynthia cardui</i>	Urticaceae, Asteraceae, Malvaceae, Boraginaceae, Fabaceae, Rosaceae, Chenopodiaceae, Hydrophyllaceae, Lamiaceae, Verbenaceae, Solanaceae, Convulvaceae, Plantaginaceae, Brassicaceae, Polygonaceae, Apiaceae, Cucurbitaceae, Rhamnaceae, Ulmaceae, Rutaceae, Poaceae	A, F, Sc
<i>Cynthia virginiensis</i>	Urticaceae, Asteraceae, Malvaceae, Boraginaceae, Fabaceae, Balsaminaceae, Scrophulariaceae	A, F, Sc
<i>Araschnia levana</i>	Urticaceae	
<i>Inachis io</i>	Urticaceae, Cannabidaceae	
<i>Aglais milberti</i>	Urticaceae	Sc
<i>Aglais urticae</i>	Urticaceae	A, E
<i>Nymphalis antiopa</i> (Swe)	Salicaceae, Betulaceae	A, E
<i>Nymphalis antiopa</i> (USA)	Salicaceae, Betulaceae, Ulmaceae, Cannabidaceae, Rosaceae	Sc
<i>Nymphalis polychloros</i>	Ulmaceae, Salicaceae, Rosaceae	A, E
<i>Nymphalis xanthomelas</i>	Ulmaceae, Salicaceae	A
<i>Nymphalis californica</i>	Rhamnaceae	Sc
<i>Roddia l-album</i>	Ulmaceae, Salicaceae, Betulaceae	A, Sc
<i>Kaniska canace</i>	Smilacaceae	A, CP, B
<i>Polygonia egea</i>	Urticaceae	A, PO
<i>Polygonia c-album</i>	Urticaceae, Ulmaceae, Cannabidaceae, Salicaceae, Betulaceae, Grossulariaceae, Corylaceae	A, E, PO
<i>Polygonia faunus</i>	Salicaceae, Betulaceae, Grossulariaceae, Ericaceae	Sc
<i>Polygonia c-aureum</i>	Cannabidaceae	A, B, J
<i>Polygonia interrogationis</i>	Urticaceae, Ulmaceae, Cannabidaceae	Sc
<i>Polygonia comma</i>	Urticaceae, Ulmaceae, Cannabidaceae	Sc
<i>Polygonia satyrus</i>	Urticaceae	Sc
<i>Polygonia gracilis</i>	Grossulariaceae, Ericaceae	Sc
<i>Polygonia progne</i>	Grossulariaceae, Ericaceae, Betulaceae	Sc

