

LARVAL HABITS, HOST-PLANT ASSOCIATIONS, AND SPECIATION IN NEMATINE SAWFLIES (HYMENOPTERA: TENTHREDINIDAE)

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Abstract.—Adaptive radiations consist of two intertwined processes, diversification of species and diversification of their ecological niches, but it is unclear whether there is a causal link between the processes. In phytophagous insects, ecological diversification mainly involves shifts in host-plant associations and in larval feeding habits (internal or external) on different plant parts, and several observations indicate that speciation is facilitated by host shifts. Data on host use in individual species suggest that internal feeders are less likely to colonize new hosts than external-feeding taxa and, consequently, increases in collective host ranges and species numbers should be slowed down in endophagous lineages. We tested these related hypotheses by using phylogenetic information to reconstruct the evolutionary history of larval resource use in the sawfly subfamily Nematinae, a group of 1000 plus species with a broad range of niches: the subfamily's combined host range includes over 20 plant families, and larvae may feed externally on leaves or needles, or internally, for example, in buds, fruits, leaves, or galls. The results show that: (1) Most internally feeding groups have evolved independently from external-feeding ancestors, but several distinct internal habits have appeared convergently multiple times; (2) Shifts among host taxa are clearly more common than changes in larval habits; (3) The majority of host switches have occurred among phylogenetically close plant groups, but many shifts are manifest among distantly related, ecologically proximate hosts; (4) Although external feeding characteristic of the common ancestor of Nematinae is associated with relatively high rates of host-shifting, internal feeders are very conservative in their host use; (5) In contrast, the effect of endophagy on speciation probabilities is more variable: net speciation rates are lowered in most internal-feeding groups, but a striking exception is found in species that induce galls on Salicaceae. The loose connection between collective host ranges and species diversity provides empirical support for theoretical models suggesting that speciation rates are a function of a complex interplay between "intrinsic" niche width and resource heterogeneity.

Key words.—Adaptive radiation, coevolution, insect-plant interactions, larval habits, Nematinae, niche diversification, speciation rates.

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Adaptive radiations consist of two processes, diversification of species and diversification of their ecological niches (Simpson 1953; Schluter 2000; Brooks and McLennan 2002). There is considerable variation in the rates at which these two radiation processes occur in different organismal lineages, but although many traits that lead to elevated net speciation rates have been identified (Farrell et al. 1991; Hodges 1997; Bond and Opell 1998; Hunter 1998; de Queiroz 2002; Bokma 2003), factors that affect rates of niche diversification are far less understood (Hunter 1998; Schluter 2000; Brooks and McLennan 2002). A central question in evolutionary biology is also whether there is a causal link between the processes, because shifts in ecological traits can facilitate speciation (Mitter et al. 1988; Verdú 2002; de Queiroz 2002; Sargent 2004) or, conversely, speciation events may facilitate ecological divergence (Futuyma 1987; Galis and Metz 1998; Nosil and Crespi 2004).

Because of their staggering diversity in terms of both species and niches, plant-feeding insects provide a near-ideal model system for studying adaptive radiations (Farrell 1998; Novotny et al. 2002). Although virtually all plant taxa are fed upon by at least some insects, the resource use of individual species tends to be rather restricted, because most utilize only a small subset of the plant taxa that are available

in their environment (Ehrlich and Raven 1964; Strong et al. 1984; Mitter and Farrell 1991). An additional niche dimension is provided by different plant parts (leaves, flowers, fruits, roots, etc.), and the insect may feed externally, or concealed in the plant tissues in mines or in galls (Mattson et al. 1988, Powell et al. 1998).

Host-plant associations, diet breadths, and feeding habits of insect species are dynamic properties that change over time (Kelley and Farrell 1998; Janz et al. 2001; Percy et al. 2004, Nosil and Mooers 2005), and several observations suggest that the heterogeneity of resources which follows from the divergent properties of different plant taxa has had prime importance in generating the enormous species number of insect herbivores. For example, shifts to phytophagy have on average led to elevated net speciation rates (Mitter et al. 1988) and colonizations of species-rich plant groups tend to lead to the same outcome (Price 1980; Farrell 1998; Farrell et al. 2001; Marvaldi et al. 2002; Morse and Farrell 2005). The underlying cause may be that an initial host-range expansion leads to disruptive selection and the formation of stable sympatric host races, which eventually evolve into reproductively isolated species (Drès and Mallet 2002; Feder et al. 1997; Nosil et al. 2002; Stireman et al. 2005), or that specialization on different hosts by geographically separated populations

lowers the probability of gene flow and population fusion once contact is reestablished (Funk 1998).

Given that shifts among hosts promote speciation, any trait that reduces the likelihood of colonization of new plant taxa should also lead to lower net speciation rates, and at least two patterns suggest that internal feeding could be such a trait. First, insect species that induce galls or mine inside plant tissues on average have narrower host ranges than external feeders (Hering 1951; Price 1980; Mattson et al. 1988; Lewinsohn 1991; Gaston et al. 1992; Frenzel and Brandl 1998), indicating that endophagy reduces the likelihood of oligophagy, a necessary intermediate stage during a host shift (Ross 1972; Drès and Mallet 2002). Second, plant species introduced to North America from Europe are rapidly colonized by external-feeding insects, whereas the accumulation of miners and gallers is slower (Strong et al. 1984). A recent meta-analysis by Denno et al. (1995) also indicated that internally feeding taxa experience increased interspecific competition, which could impede host shifts as a result of ecological preemption (Farrell and Sequeira 2004).

Taken together, the aforementioned observations suggest that on macroevolutionary time scales, increases in collective host ranges and species numbers should be slower in endophagous lineages than in externally feeding taxa (Powell et al. 1998). Unfortunately, phylogenetic studies of herbivorous insect groups have mainly focused on evolutionary patterns in the use of host-plant taxa, while shifts among feeding guilds have received far less attention (Connor and Taverner 1997; Farrell and Sequeira 2004; Pellmyr et al. 2005).

Investigating the macroevolutionary consequences of guild shifts requires insect groups of sufficient ecological and taxonomic diversity to permit multiple comparisons of the association between larval habits, host ranges, and species numbers. One such group, investigated here, is the monophyletic sawfly subfamily Nematinae (Hymenoptera: Tenthredinidae), which comprises over 1000 species spanning a broad range of niche dimensions: nematines collectively use over 20 plant families, with larvae specialized in feeding either externally on leaves or needles, or internally in buds, inflorescences, fruits, berries, catkins, leaves, petioles, or various galls (Smith 1979; Zinovjev and Vikberg 1998). To elucidate the evolutionary history of resource use and species diversification in Nematinae, we used a published phylogeny including 68 nematine taxa representative of the multitude of hosts and feeding modes found in the group. Specifically, we used the phylogenetic information to establish the directions and rates of evolution of different larval habits and host-plant associations, and to test the related hypotheses that internal feeding leads to lowered rates of host-shifting and a concomitant decrease in net speciation rates.

MATERIALS AND METHODS

Study Group, Taxon Sampling, and Phylogenies

At 1000 plus species, Nematinae constitutes one of the largest subfamilies in the sawfly family Tenthredinidae (Goulet 1992). Many nematine species and genera have broad holarctic distributions, and nematines are an ecologically prominent insect group especially in arctic and subarctic habitats (Benson 1962; Smith 1979; Kouki 1999; Viitasaari

2002). The larvae of most nematines feed on deciduous trees or shrubs, but conifers, herbs, and grasses are utilized by some species (Smith 1979; Taeger et al. 1998; Lacourt 1999). Individual species are typically oligophagous on hosts belonging to one or a few related plant genera, but the degree of specialization varies from strict monophagy on single host species to extreme polyphagy on over 10 genera (Smith 1979; Taeger et al. 1998; Lacourt 1999). The larval habits of nematines are equally diverse. Although a majority of the species have larvae that feed externally on leaves or needles, the larvae of others live inside plant tissues. These endophagous groups induce various galls, or mine inside leaves, petioles, buds, inflorescences, fruits, berries, or catkins (Lorenz and Kraus 1957; Smith 1979; Zinovjev 1982; Zinovjev and Vikberg 1998).

The ecological and evolutionary analyses in this study are based on a recent phylogenetic analysis of the Nematinae (Nyman et al. 2006), which employed DNA sequence data from the mitochondrial cytochrome oxidase I (CoI; 810 bp) and nuclear Elongation factor-1 α (EF-1 α ; 777 bp) genes. The analysis included 13 outgroup and 68 ingroup taxa, of which the ingroup species represent all major genera within Nematinae, and most subgenera of the large genera. Consequently, all major host-plant taxa and larval habits are represented, and most "atypical" habits are represented by several species. Four of the missing genera are rare, (near) monotypic, and have unknown hosts (see Appendix available online at: <http://dx.doi.org/10.1554/05-674.1.S1>), and the generic status of some missing taxa with known hosts is disputed (see below). Of the outgroup taxa, *Sterictiphora*, *Diprion*, and *Abia* belong to three other families from the superfamily Tenthredinoidea (Argidae, Diprionidae, and Cimbicidae, respectively), and the 10 nonnematine tenthredinids represent all five other subfamilies in the family Tenthredinidae (Goulet 1992; Taeger et al. 1998).

A detailed description of the phylogenetic analyses is given in Nyman et al. (2006). In short, MrBayes version 3.0b4 (Ronquist and Huelsenbeck 2003) was used to perform a Bayesian phylogenetic analysis in which a separate GTR+I+ Γ_4 model of substitution was allowed for the two genes. One cold and three incrementally heated Markov Chain Monte Carlo (MCMC) chains were run for six million generations while sampling trees from the current cold chain every 100 generations, and 50,000 postburnin trees were used to calculate a Bayesian consensus tree (Figs. 1, 2). Some of the character evolution analyses below require estimates of the relative ages of various nematine groups, so TreeEdit version 1.0a10 (Rambaut and Charleston 2002) was used to ultrametricize the Bayesian tree by nonparametric rate smoothing (NPRS; Sanderson 1997). In the analyses below we have only used the trees obtained from this two-partition Bayesian phylogenetic analysis; although maximum parsimony analyses and Bayesian analyses using different partition schemes resulted in slightly different trees, the main results and conclusions of this study are not affected.

Character Evolution and Diversification Analyses

Larval habits and host-plant associations (genera and families) were coded as unordered multistate characters (Table

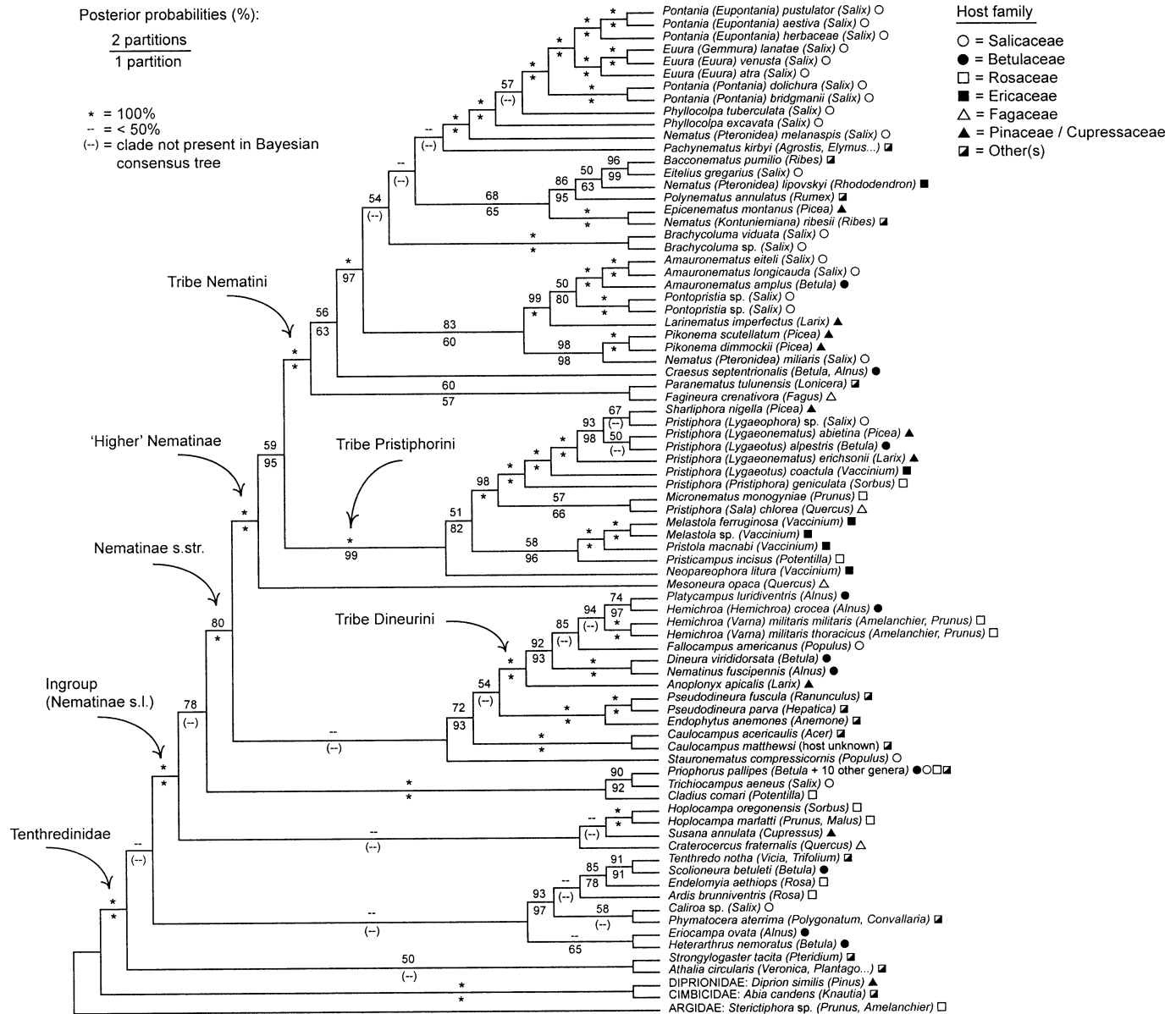


FIG. 1. Phylogeny of the sawfly subfamily Nematinae and selected outgroups from the Tenthredinoidea according to a two-partition Bayesian phylogenetic analysis using a separate GTR+I+I₄ model of substitution for CoI and EF-1 α gene sequences (Nyman et al. 2006). Numbers above branches are posterior probabilities (%) from the two-partition analysis, numbers below branches show posterior probabilities from an analysis in which a single substitution model was used for the whole dataset (i.e., a one-partition analysis). Host genera of each exemplar species are in parentheses after the species names, the six most commonly utilized host families are highlighted by symbols (see legend). Full host ranges of polyphagous species are given in Table 1 (also see Appendix available online).

1) and parsimony-optimized on the phylogenetic tree (Fig. 1) using Mesquite version 1.01 (Maddison and Maddison 2004). Each distinct larval habit was given a unique code (Table 1; 14 character states), and ancestral states were inferred by Accelerated (ACCTRAN) and Delayed (DELTRAN) transformations optimization (Swofford and Maddison 1987) (Fig. 2). Phylogenetic conservatism in the focal characters was assessed with permutation tail probability (PTP) tests (Maddison and Slatkin 1991). Mesquite was also used to reconstruct ancestral feeding modes by maximum-likelihood (ML) methods (Pagel 1994, Schluter et al. 1997), in which we employed a coarser, three-state approach by

coding larval lifestyles either external, intermediate, or internal (Fig. 3). Maximum-likelihood reconstructions were based on both the phylogenetic tree with untransformed branch lengths (Fig. 2) and on the tree with branch lengths ultrametricized by NPRS (Fig. 4). Outgroup taxa were deleted from the trees prior to the analyses following the recommendation of Mooers and Schluter (1999).

To estimate the numbers of changes that have occurred in the focal ecological traits, we used Mesquite to parsimony-optimize hosts and feeding habits on the 50,000 MCMC trees sampled during the Bayesian analysis. This takes into account the phylogenetic uncertainty in the analysis, because each

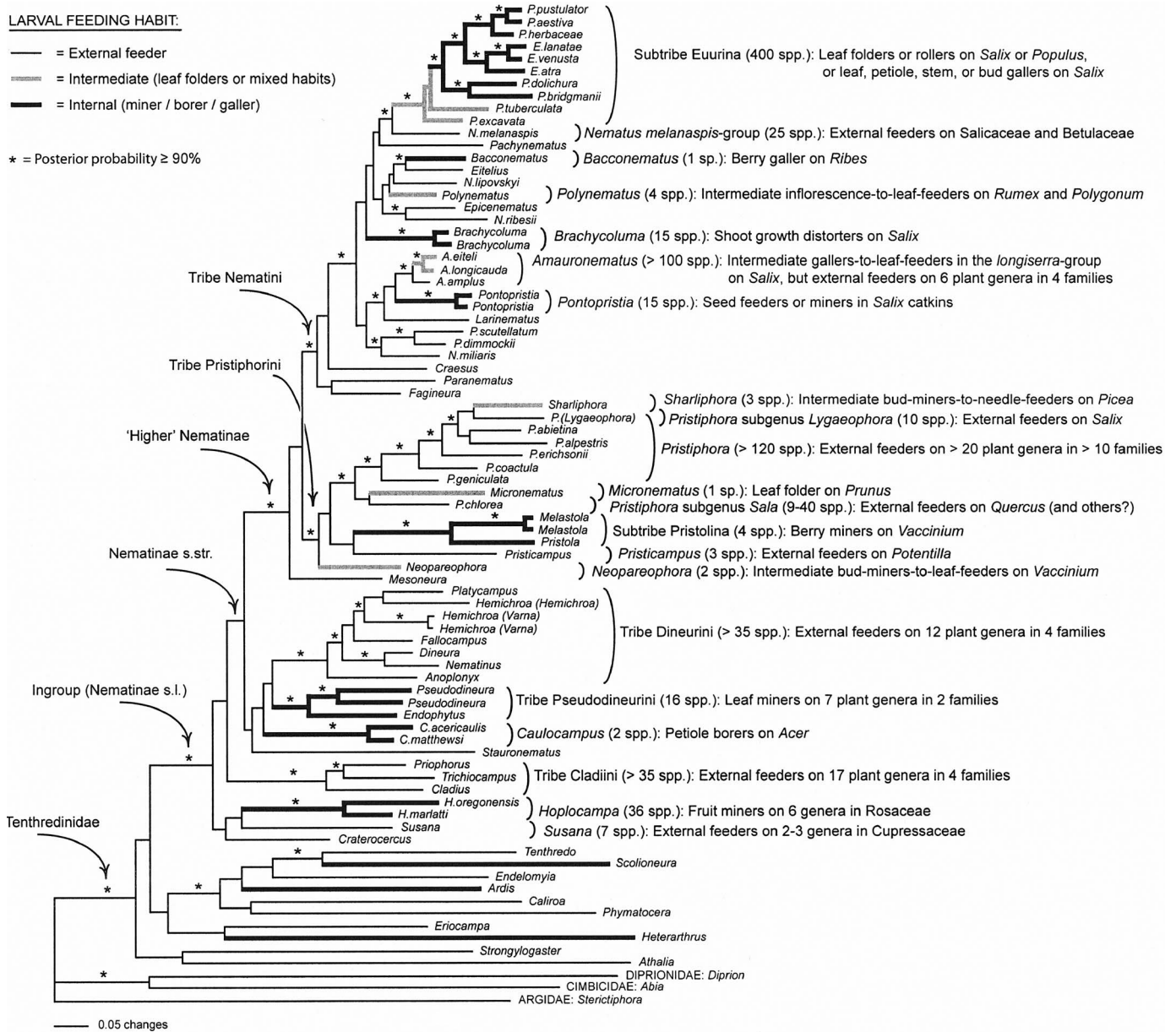


FIG. 2. Reconstruction of the evolution of larval feeding habits in the subfamily Nematinae and selected outgroups from the Tenthredinoidea when habits are coded as unordered (14 character states) and ancestral habits are reconstructed using Accelerated transformations parsimony optimization on the two-partition Bayesian analysis tree (Fig. 1; this tree shows estimated branch lengths). External feeders are indicated by thin black lines, all internal habits by thick black lines, and all intermediate habits by thick gray lines (see legend). Species numbers and host ranges of selected taxa are shown to the right of the tree (see Table 1 and also see Appendix available online); asterisks above branches denote clades with a posterior probability $\geq 90\%$.

estimate of the number of changes that have occurred is automatically weighted by the summed posterior probability of the sampled trees that lead to the estimate (Huelsenbeck et al. 2000). Outgroup taxa were excluded from the analyses by coding their states as unknown. Larval habits were coded as described above (13 character states in ingroup), but in the case of host-plant associations, the presence of oligo- and polyphagous taxa in the analysis makes coding character states problematic (Janz et al. 2001; Morse and Farrell 2005). Consequently, we performed two separate analyses for the host plant data: in the first analysis, the state of each exemplar

species was determined by the main host genus or family of the species (i.e., the first host taxon listed for each species in Table 1; 24 and 13 character states for host genera and families, respectively). This provides a minimum estimate of the number of changes that have occurred, because all nematine species and hosts are not included. In the second analysis, each exemplar species was simultaneously given all states (host families) that are found in the genus/subgenus/species group that the included species represents (20 character states; this analysis could not be performed with host genera because the number of states exceeds the maximum

TABLE 1. Taxa and samples used in the study, their hosts, larval habits, and collection localities. The classification mainly follows Zhelochovtsev (1994), Taeger et al. (1998), and Lacourt (1999), modified according to results by Nyman et al. (2006) for the ingroup. Host plant taxa of the exemplar species are in parentheses, secondary hosts are in square brackets (mainly according to Smith 1979; Goulet 1992; Zhelochovtsev 1994; Taeger et al. 1998; Lacourt 1999). See Appendix available online for all host taxa in the represented sawfly taxa and full list of references. Larval habits are coded as follows: EF, external feeder; FM, fruit or berry miner; PM, petiole miner; LM, leaf miner; IBL, intermediate bud miner—leaf feeder; IBN, intermediate bud miner—needle tier; BG, berry gallier; LF, leaf folder; IIF, intermediate inflorescence feeder; SGD, shoot growth distorter; CF, catkin feeder; GI, gall inducer; IGE, intermediate gall inducer—external feeder; SM, shoot miner (present only in outgroup). Habits of outgroup taxa are given in parentheses because they were not included in the character-evolutions analyses.

Tribe/Group	Genus (Subgenus) species	Sex/stage, hosts	Habit	Location	Collector
Unclassified Basal grade	<i>Susana annulata</i> Smith	♀ (<i>Cupressus</i>)	EF	Butte County, CA, USA	H. J. Jacobson
	<i>Crateroceriscus fraternalis</i> (Norton)	♀ (<i>Quercus</i>)	EF	Hardy County, WV, USA	D. R. Smith
Hoplocampini	<i>Hoplocampa oregonensis</i> (Ashmead)	♀, <i>Sorbus sitchensis</i>	FM	Elk mtn, BC, Canada	T. Nyman
	<i>H. marlatti</i> Rohwer	♀, <i>Malus</i> sp. (<i>Prunus</i>)	FM	Cambridge, MA, USA	T. Nyman
Cladini	<i>Cladius comari</i> Stein	♂ (<i>Potentilla</i>)	EF	Mekrijärvi, Finland	T. Nyman
	<i>Priophorus pallipes</i> (Serville)	Larva, <i>Betula pubescens</i> (<i>Betula</i> , <i>Salix</i> , + 7–14 other genera)	EF	Laboratory colony, Finland	A. Kause
Caulocampini	<i>Trichocampus aeneus</i> (Zaddach)	Larva, <i>Salix phylicifolia</i> (<i>Salix</i>)	EF	Hausjärvi, Finland	V. Vikberg
	<i>Caulocampus acericaulis</i> (MacGillivray)	♀ (<i>Acer</i>)	PM	Ancaster, ON, Canada	B. DeJonge
Pseudodineurini	<i>C. matthewsi</i> Smith	♀ (<i>Acer</i> ?)	PM?	Petersham, MA, USA	T. Nyman
	<i>Pseudodineura fuscata</i> (Klug)	♀ (<i>Ranunculus</i>)	LM	Nana Aseme, Estonia	M. Heidema
Dineurini	<i>P. parva</i> (Norton)	♂ (<i>Hepatica</i>)	LM	Petersham, MA, USA	T. Nyman
	<i>Endophytus anemones</i> (Hering)	Larva, <i>Anemone nemorosa</i> (<i>Anemone</i>)	LM	Etzen, Austria	E. Altenhofer
Dineurini	<i>Platycampus luridiventris</i> (Fallén)	♂ (<i>Alnus</i> [<i>Betula</i> , <i>Corylus</i>])	EF	Joensuu, Finland	T. Nyman
	<i>Anoplonyx apicalis</i> (Bränschke)	Larva, <i>Larix sibirica</i> (<i>Larix</i>)	EF	Janakkala, Finland	V. Vikberg
Dineurini	<i>Dineura viridorsata</i> (Retzius)	Larva, <i>Betula pubescens</i> (<i>Betula</i>)	EF	Janakkala, Finland	V. Vikberg
	<i>Hemichroa (Hemichroa) crocea</i> (Geoffroy)	Larva, <i>Alnus glutinosa</i> (<i>Alnus</i> , <i>Betula</i> [<i>Corylus</i>])	EF	Grimminge, Belgium	J.-L. Boevé
Dineurini	<i>H. (Varna) militaris militaris</i> (Cresson)	♀ (<i>Amelanchier</i> , <i>Prunus</i>)	EF	Petersham, MA, USA	T. Nyman
	<i>H. (Varna) militaris thoracicus</i> (Harrington)	♀ (<i>Amelanchier</i> , <i>Prunus</i>)	EF	Petersham, MA, USA	T. Nyman
Stauronematini	<i>Nematinus fuscipennis</i> (Serville)	♀ (<i>Alnus</i> , <i>Carpinus</i>)	EF	Tohmajärvi, Finland	T. Nyman, M. Vittasaari
	<i>Fallocampus americanus</i> (Marlatt)	♀ (<i>Populus</i>)	EF	Petersham, MA, USA	T. Nyman
Mesoneurini	<i>Stauronematus compressicornis</i> (Fabricius)	Larva, <i>Populus tremula</i> (<i>Populus</i> [<i>Salix</i>])	EF	Suchowola, Poland	H. Roiminen, A. Zinovjev
	<i>Mesoneura opaca</i> (Fabricius)	Larva, <i>Quercus</i> sp. (<i>Quercus</i>)	EF	Overijse, Belgium	J.-L. Boevé
Pristiphorini	<i>Pristicampus incisus</i> (Lindqvist)	Larva, <i>Potentilla fruticosa</i> (<i>Potentilla</i>)	EF	Turenki, Finland	A. Zinovjev, V. Vikberg
	<i>Neopareophora litura</i> (Klug)	♂ (<i>Vaccinium</i>)	IBL	Hardy County, WV, USA	D. R. Smith
Pristiphorini	<i>Pristola macnabi</i> Ross	♀ (<i>Vaccinium</i>)	FM	Mt. Seymour, BC, Canada	T. Nyman
	<i>Melastola ferruginosa</i> Wong	♂ (<i>Vaccinium</i>)	FM	Mt. Seymour, BC, Canada	T. Nyman
Pristiphorini	<i>Melastola</i> sp.	Larva, <i>Vaccinium parvifolium</i>	FM	Burnaby, BC, Canada	T. Nyman
	<i>Micronematus monogyniae</i> (Hartig)	Larva, <i>Prunus</i> sp. (<i>Prunus</i>)	LF	Parikkala, Finland	H. Roiminen
Pristiphorini	<i>Pristiphora (Sala) chlorea</i> (Norton)	♀ (<i>Quercus</i>)	EF	Petersham, MA, USA	T. Nyman
	<i>Pristiphora (Sala) geniculata</i> (Hartig)	♀ (<i>Salix</i>)	EF	Etzen, Austria	E. Altenhofer
Pristiphorini	<i>P. (Pristiphora) erichsonii</i> (Hartig)	Larva, <i>Sorbus aucuparia</i> (<i>Sorbus</i>)	EF	Nuorgam, Finland	T. Nyman
	<i>P. (Lygaeonematus) abietina</i> (Christ)	Larva, <i>Larix sibirica</i> (<i>Larix</i>)	EF	Janakkala, Finland	V. Vikberg
Pristiphorini	<i>P. (Lygaeonematus) alpestris</i> (Konow)	♀ ex larva, <i>Betula pubescens</i> ssp. <i>czerpanovii</i> (<i>Betula</i>)	EF	St. Petersburg, Russia	A. Zinovjev
	<i>P. (Lygaeonematus) coactula</i> (Ruthe)	♀ (<i>Vaccinium</i> (VV, <i>personal observation</i>))	EF	Lab colony, Kevo, Finland	L. Kapari
Nematini	<i>Sharliphora nigella</i> (Förster)	♀ (<i>Picea</i>)	IBN	Nuorgam, Finland	T. Nyman
	<i>Fagineura crenativora</i> Vikberg & Zinovjev	♀ ex larva, <i>Fagus</i> sp. (<i>Fagus</i>)	EF	Vellavere, Estonia	M. Heidema
Nematini	<i>Paramematus tulunensis</i> (Vikberg)	♀ (<i>Lonicera</i> , <i>Symphoricarpos</i>)	EF	Mt. Tanzawa, Japan	A. Yamagami
	<i>Baccanematus pumilio</i> (Konow)	Larva, <i>Ribes nigrum</i> (<i>Ribes</i>)	BG	St. Petersburg, Russia	A. Zinovjev
				Turenki, Finland	V. Vikberg

TABLE 1. Continued.

Tribe/Group	Genus (Subgenus) species	Sex/stage, hosts	Habit	Location	Collector
Nematini (Continued)	<i>Pikonomia scutellatum</i> (Hartig)	Larva, <i>Picea abies</i> (<i>Picea</i>)	EF	Parikkala, Finland	H. Roininen, A. Zinovjev
	<i>Pikonomia dimmockii</i> (Cresson)	♀ (<i>Picea</i>)	EF	Mt. Seymour, BC, Canada	T. Nyman
	<i>Epicnematus montanus</i> (Zaddach)	Larva, <i>Picea abies</i> (<i>Picea</i>)	EF	Parikkala, Finland	H. Roininen, A. Zinovjev
	<i>Pachynematus kirbyi</i> (Dahlbom)	♂ (<i>Agrostis</i> , <i>Elymus</i> , + >10 other genera in Poaceae)	EF	Mekrijärvi, Finland	T. Nyman
	<i>Polynematus annulatus</i> (Gimmerthal)	Larva, <i>Rumex longifolius</i> (<i>Rumex</i>)	IIF	Turenki, Finland	V. Vikberg
	<i>Eitelius gregarius</i> (Marlatt)	Larva, <i>Salix discolor</i> (<i>Salix</i>)	EF	Ithaca, NY, USA	A. Zinovjev
	<i>Craesus septentrionalis</i> L.	Larva, <i>Alnus glutinosa</i> (<i>Alnus</i> , <i>Betula</i> [+ 5–7 other genera])	EF	Grimminge, Belgium	J.-L. Boevé
	<i>Brachycoloma viduata</i> (Zetterstedt)	Larva, <i>Salix myrsinifolia</i> (<i>Salix</i>)	SGD	Parikkala, Finland	H. Roininen
	<i>Brachycoloma</i> sp.	Larva, <i>Salix pentandra</i> (<i>Salix</i>)	SGD	Joensuu, Finland	H. Roininen
	<i>Pontopristia</i> sp.	Larva, <i>Salix myrsinifolia</i> (<i>Salix</i>)	CF	Joensuu, Finland	T. Nyman
	<i>Pontopristia</i> sp.	Larva, <i>Salix candida</i> (<i>Salix</i>)	CF	Churchill, MB, Canada	T. Nyman
	<i>Amauronematus etieli</i> Saarinen	Larva, <i>Salix pentandra</i> (<i>Salix</i>)	IGE	Parikkala, Finland	T. Nyman
	<i>A. longicauda</i> (Hellén)	♀ (<i>Salix</i>)	IGE	Nuorgam, Finland	T. Nyman
	<i>A. amplus</i> Konow	♀ (<i>Betula</i>)	EF	Nuorgam, Finland	T. Nyman
	<i>Nematus (Pteronidea) melanaspis</i> Hartig	♀ ex larva, <i>S. pentandra</i> (<i>Salix</i> , <i>Populus</i>)	EF	Parikkala, Finland	T. Nyman
	<i>N. (Pteronidea) lipovskiyi</i> Smith	♀ (<i>Rhododendron</i>)	EF	Petersham, MA, USA	T. Nyman
	<i>N. (Pteronidea) militaris</i> (Panzer)	Larva, <i>Salix caprea</i> (<i>Salix</i>)	EF	Joensuu, Finland	T. Nyman
	<i>N. (Kontuniemiana) ribesii</i> (Scopoli)	Larva, <i>Ribes rubrum</i> (<i>Ribes</i>)	EF	Ruokolahti, Finland	H. Roininen
	<i>Larinematus imperfectus</i> (Zaddach)	Larva, <i>Larix sibirica</i> (<i>Larix</i>)	EF	Janakkala, Finland	V. Vikberg
	<i>Phyllocolpa excavata</i> Marlatt	Larva, <i>Salix pentandra</i> (<i>Salix</i>)	LF	Kesälähti, Finland	H. Roininen
	<i>P. tuberculata</i> (Benson)	Larva, <i>Salix starkeana</i> (<i>Salix</i>)	LF	St. Petersburg, Russia	A. Zinovjev
	<i>Pontania (Pontania) dolichura</i> (Thomson)	Larva, <i>Salix phlyticifolia</i> (<i>Salix</i>)	GI	Keret, Russia	T. Nyman
	<i>P. (Pontania) bridgmanii</i> (Cameron)	Larva, <i>Salix starkeana</i> (<i>Salix</i>)	GI	Puhos, Finland	T. Nyman
	<i>P. (Eupontania) pustulator</i> Forsius	Larva, <i>Salix phlyticifolia</i> (<i>Salix</i>)	GI	Taivalkoski, Finland	T. Nyman
	<i>P. (Eupontania) aestiva</i> (Thomson)	Larva, <i>Salix myrsinifolia</i> ssp. <i>borealis</i> (<i>Salix</i>)	GI	Kilpisjärvi, Finland	T. Nyman
	<i>P. (Eupontania) herbaceae</i> (Cameron)	Larva, <i>Salix herbacealparlaris</i> (<i>Salix</i>)	GI	Abisko, Sweden	T. Nyman
	<i>Euura (Euura) atra</i> –group sp.	Larva, <i>Salix starkeana</i> (<i>Salix</i>)	GI	Puhos, Finland	T. Nyman
	<i>E. (Euura) venusta</i> (Zaddach)	Larva, <i>Salix aurita</i> (<i>Salix</i>)	GI	Joensuu, Finland	T. Nyman
	<i>E. (Gemmura) lanatae</i> Malaise	♀ ex larva, <i>Salix lanata</i> (<i>Salix</i>)	GI	Kilpisjärvi, Finland	T. Nyman
Outgroup taxa					
Diprionidae: Diprioninae	<i>Diprion similis</i> (Hartig)	♂ (<i>Pinus</i>)	(EF)	Petersham, MA, USA	T. Nyman
Cimbricidae: Abinae	<i>Abia candens</i> Konow	♀ (<i>Knaulia</i>)	(EF)	Suida River, Russia	A. Zinovjev
Argidae: Sterictiphorinae	<i>Sterictiphora</i> sp.	♂ (<i>Prunus</i> , <i>Amelanchier</i>)	(EF)	Petersham, MA, USA	T. Nyman
Tenthredinidae:					
Tenthredininae: Tenthredinini	<i>Tenthredo notha</i> Klug	♂ (<i>Vicia</i> , <i>Trifolium</i>)	(EF)	Joensuu, Finland	A. Zinovjev
Selandrininae: Strongylogastrini	<i>Strongylogaster tacta</i> (Norton)	♀ (<i>Pteridium aquilinum</i> ?)	(EF?)	Petersham, MA, USA	T. Nyman
Allantinae: Athaliini	<i>Athalia circularis</i> (Klug)	♀ (<i>Veronica</i> , <i>Plantago</i> , + > 5 other genera)	(EF)	Parikkala, Finland	A. Zinovjev, H. Roininen
Allantinae: Eriocampini	<i>Eriocampa ovata</i> (L.)	♀ (<i>Alnus</i> [<i>Frangula</i>])	(EF)	Squamish, BC, Canada	T. Nyman, J. Joy
Heterarthrinae: Heterarthrini	<i>Heterarthrus nemoratus</i> (Fallén)	♀ (<i>Betula</i>)	(LM)	Tohmajärvi, Finland	T. Nyman, M. Viitasari
Heterarthrinae: Fenusini	<i>Scolioneura betulleti</i> (Klug)	Larva, <i>Betula pubescens</i> ssp. <i>czerepanovii</i> (<i>Betula</i>)	(LM)	Kilpisjärvi, Finland	T. Nyman, V. Vikberg
Heterarthrinae: Caliroini	<i>Caliroa</i> sp.	Larva, <i>Salix daphnoides</i> ssp. <i>acutifolia</i> (+ > 15 other genera)	(EF)	Salangriva, Latvia	H. Roininen, A. Zinovjev
Heterarthrinae: Caliroini	<i>Endelomyia aethiops</i> (Fabricius)	Larva, <i>Rosa</i> sp. (<i>Rosa</i>)	(EF)	Mekrijärvi, Finland	T. Nyman, A. Zinovjev
Blennocampinae: Blennocampini	<i>Ardis brunniventris</i> (Hartig)	Larva, <i>Rosa</i> sp. (<i>Rosa</i>)	(SM)	Parikkala, Finland	H. Roininen
Blennocampinae: Phymatocerini	<i>Phymatocera aterrima</i> (Klug)	♂ (<i>Polygonatum</i> , <i>Convallaria</i>)	(EF)	Parikkala, Finland	J. Sorjonen



FIG. 3. Maximum-likelihood reconstruction of the evolution of larval habits in the subfamily Nematinae, when feeding modes are coded as either external, intermediate, or internal, and ancestral habits are reconstructed on the ingroup portion of the two-partition Bayesian analysis tree (Fig. 2) using symmetric forward and reverse rates. In the pie diagrams on nodes, the relative likelihood of external feeding is indicated by white, intermediate habits by gray, and internal habits by black (see legend). Species numbers and hosts of nonexternal groups are shown to the right of the tree (see also Table 1 and see Appendix available online); asterisks above branches denote clades with a posterior probability $\geq 90\%$.

allowed in Mesquite). The locations of missing taxa were inferred mainly on the basis of traditional classifications, but whenever large morphologically defined groups (e.g., *Pristiphora* and *Nematus* subgenus *Pteronidea*) turned out to be obviously para- or polyphyletic, the missing taxa and their hosts were assigned to the exemplar species that they resemble morphologically the most (full set of assumptions is available on request). In the placement of missing genera that have known host associations we used the following assumptions: *Kerita* Ross was considered to be the sister taxon of *Pseudodineura* (Ross 1937; Smith 1976a); *Megadineura* Malaise was assumed to be the sister of *Mesoneura* (Wei 1998); and *Moricella* Rohwer was considered a synonym of *Mesoneura* (Benson 1963; Abe and Smith 1991).

We used sister-group comparisons to test the related hypotheses that internal feeding reduces the rates of host range expansion and speciation. The number of times that nematine groups with nonexternal (intermediate/internal) larval feeding modes had fewer host taxa and species than their ancestrally external-feeding sister groups was evaluated against a null hypothesis of no association under the binomial distribution defined by the nonparametric sign test.

We also estimated the rates of host range expansion in external and nonexternal lineages by plotting the collective numbers host families and genera against the inferred ages of the various nematine groups. For this, all maximally inclusive monophyletic clades that are composed exclusively of either external or intermediate/internal feeders were identified from the Bayesian analysis tree (Fig. 2), and the relative age (as % of the maximum) of each of the 36 clades was defined as the time of origin of the common ancestor of the group (Fig. 4). Clade age was used as a predictor of collective diet breadth in separate linear regressions for intermediate/internal and external feeders. Clades originate as single species, so on the y-axis the regression lines were constrained to go through 1, which is the median number of host families of extant species, and the median number of host genera for intermediate/internal feeders. The statistical significance of the direction and magnitude of the slope differences was inferred by randomization tests in which the states (intermediate/internal or external) of the points were shuffled 1000 times, and the observed difference in the slopes was compared to the distribution of slope differences obtained from the randomized datasets.

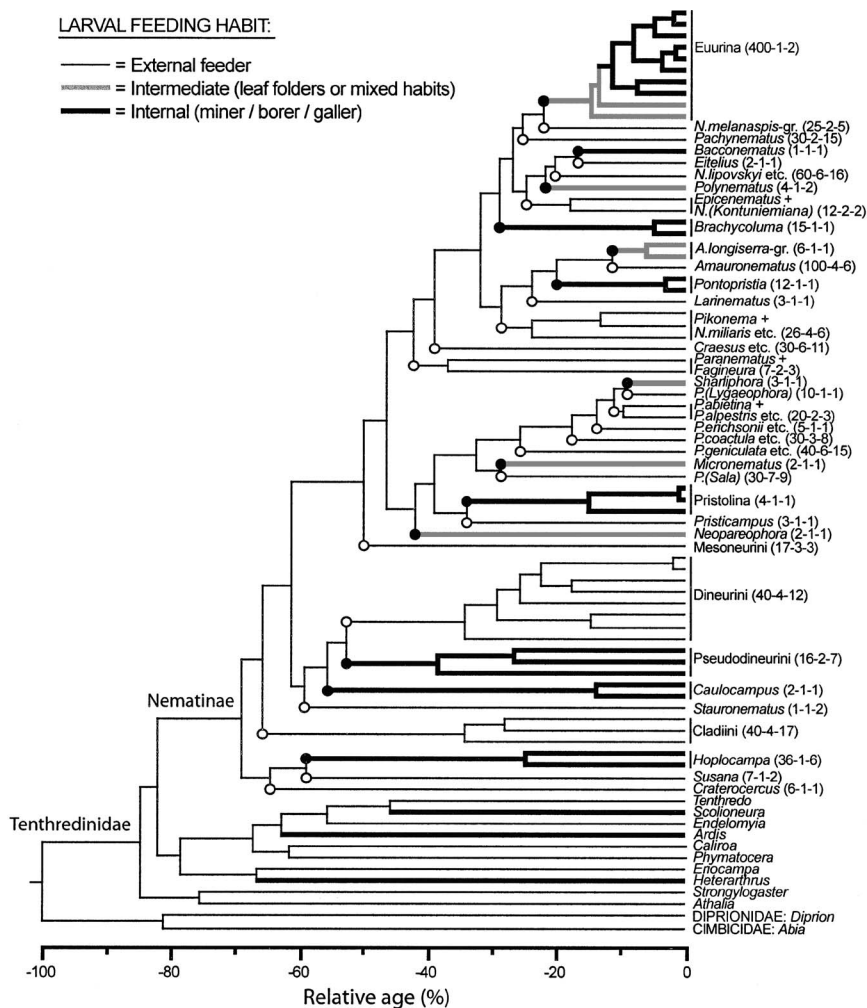


FIG. 4. Phylogeny of Nematinae, with branch lengths ultrametricized using nonparametric rate smoothing (Sanderson 1997; original branch lengths from the two-partition Bayesian analysis tree, Fig. 2). Circles on branches show the relative age estimate points that were used in Fig. 6; open circles refer to external feeders and closed circles to intermediate/internal feeders. Numbers in parentheses after taxon names are in the order: number of species, number of host families, number of host genera.

The estimates of relative clade ages and their species numbers were also used to estimate speciation rates by assuming a pure birth model of diversification. In a pure birth process, the probability distribution of species numbers in clades of a given age is geometric, which makes it possible to estimate speciation rates by maximum-likelihood (Nee et al. 1992; Bokma 2003). A maximum likelihood estimate of the rate of speciation (λ) was fitted separately for the 23 clades including only external feeders and the 13 clades including only intermediate/internal feeders; thereafter, the parameter was estimated for the whole data, and the log likelihood of the complete model was compared to the summed log likelihoods obtained from the partitioned models by using the likelihood ratio statistic $T = 2(L_1 + L_2 - L_0)$, where L_1 and L_2 are the maximum log likelihoods when speciation rates are fitted separately for external and nonexternal feeders, and L_0 is the corresponding value when a single parameter is fitted for the whole data (Bokma 2003). The statistical significance of T was inferred by comparing the value of T to a chi-square distribution with one degree of freedom (Bokma 2003). The

program used for the tests (F. Bokma, unpubl. program) is available on request.

RESULTS

Evolution of Larval Habits and Host-Plant Associations

ACCTRAN and DELTRAN parsimony optimization of larval habits on the Bayesian phylogenetic tree led to identical reconstructions and revealed that feeding modes are conservative: virtually all forms of nonexternal feeding taxa are grouped in strongly supported monophyletic clades that correspond to their specific feeding habits (Figs. 1, 2; PTP tests, $P < 0.001$). Despite the general conservatism in larval guilds, the tree reveals several cases of convergent colonization of distinct intermediate and internal niches such as leaf folding, leaf mining, and mining inside fruits or berries (Fig. 2). In addition, various forms of plant growth manipulation or gall induction have originated on at least five separate occasions.

Ancestral states inferred by maximum likelihood are largely congruent with the parsimony results, but the ML recon-

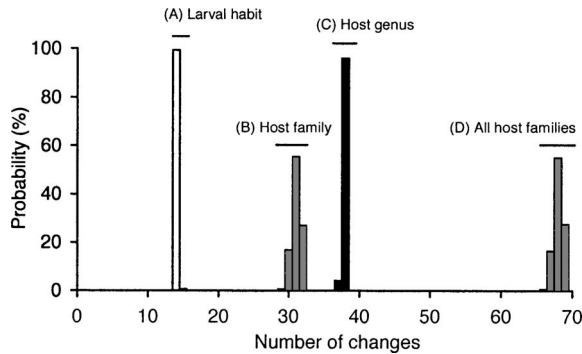


FIG. 5. Probability distributions of the estimated numbers of changes that have occurred in larval habits and host-plant associations in the ingroup, calculated by parsimony optimization of the characters on 50,000 MCMC trees sampled during the two-partition Bayesian phylogenetic analysis: (A), numbers of changes in larval habits; (B) and (C), numbers of changes among host families and genera, respectively, when only the main host taxon of each exemplar species is included; and (D), numbers of steps when each exemplar species is simultaneously coded with all states (host families) present in the nematine taxa that the exemplar represents.

struction indicates a possible reversal from internal to external feeding within the *Caulocampus* + *Pseudodineurini* + *Dineurini* clade (Fig. 3; the analysis based on the ultrametricized tree led to a near-identical reconstruction). However, the result is partly contingent on the coarse coding scheme employed, and given that ML reconstructions are affected by taxon sampling when incomplete phylogenies are used (Mooers and Schluter 1999; Nosil and Moors 2005), the result can only be considered tentative.

Maximum-parsimony optimization of larval habits on the 50,000 trees sampled during the Bayesian analysis indicates that there have most probably been 14 changes in larval habits during the evolutionary history of the Nematinae (Fig. 5), although this is probably a slight underestimate due to missing taxa. We are aware of three species having intermediate larval habits that could not be included in our analysis: *Nematus atriceps* (Marlatt), a facultative flower-feeder on *Trifolium* (Poinar and Smith 2003); *Pristiphora angulata* Lindqvist, a facultative flower bud miner on *Spiraea* (Zinovjev and Vikberg 1998); and *Pristiphora auricauda* Smith, a recently described leaf-roller on *Prunus* in Costa Rica (Smith 2003). Including these missing species would lead to a maximum of 18 inferred habit changes on the trees.

The host-plant associations of nematines have likewise been nonrandom with respect to host families and genera (PTP tests, $P < 0.001$ for both taxonomic levels), but host shifts are clearly more frequent than changes in larval habits (Fig. 5). It should be emphasized that the “absolute minimum” estimate of 29 switches among host families is an unrealistically small number because seven host families are not represented in the taxonomic sample. Even the “maximum” number of 66 to 70 host family shifts is probably a grave underestimate, mainly because the optimization procedure allows unrealistically polyphagous reconstructions for ancestral taxa when exemplar species are simultaneously coded with all states (hosts) that are utilized by the nematine taxa that the exemplars represent, and because multiple changes within these inferred clades cannot be taken into

account. In addition, the estimated difference in the evolutionary rates of habits versus host-plant use is likely to be diminished by the tendency of parsimony to underestimate numbers of changes particularly in fast-evolving characters, in this case host-plant associations (Saitou 1989; Maddison 1994).

Despite the conservative host-plant associations on low taxonomic levels, major host shifts are frequent enough so as to almost completely erase any signs of higher-level congruence between the host and nematine phylogenies, and inferring the ancestral hosts even on intermediate levels on the Nematinae phylogeny is impossible with any degree of certainty (results not shown). Nevertheless, the five plant families most commonly used by nematine species (Rosaceae, Betulaceae, Salicaceae, Fagaceae, and Ericaceae) are all found in multiple locations on the tree (Fig. 1), demonstrating that shifts among these taxa must have occurred frequently. Conifers (mostly *Picea* and *Larix*) have likewise been colonized independently at least six times within the ingroup (Fig. 1; Table 1).

Ecological Diversification and Speciation in Internal and External Feeders

As expected, nematine groups having intermediate or internal larval habits collectively utilize fewer host taxa than their externally feeding sister groups (Table 2; wins one of 10 pairs, three ties; sign test, one-tailed $P = 0.011$). Although some of the groupings are weakly supported, in most cases all putative sister clades have more host taxa, so the end result would be the same regardless of which one was used in the comparisons. Some of the comparisons are complicated because the sister taxa have nonoverlapping host ranges (e.g., *Bacconematus* vs. *Eitellius*), but there are no pairs in which the internal/intermediate taxon would have clearly more host taxa than its external-feeding sister group, and in many cases the host taxa of the concealed feeders are included in the much wider host range of their sister group (e.g., *Pontoprastia* vs. *Amauronematus*).

Plots of clade age against collective host range (Fig. 6B, C) also support the conclusion of slow host range expansion in taxa with nonexternal habits, because the slopes are clearly lower for intermediate/internal feeders both in host families and genera. The observed direction and magnitude of slope differences is statistically significant for both host families (randomization test, $P = 0.007$) and host genera ($P = 0.032$). The slope may be slightly overestimated for the numbers of host genera in the case of external feeders (the median number of host genera for extant free-feeding species is higher than one), and the ages of internally feeding taxa may be overestimated if their actual sister group is not included in the taxonomic sample, but the differences between the internal- and external-feeding nematine groups are so large that they are very likely a result of fundamental differences in the way that these groups experience their niche environment, that is, current and available host taxa.

The sister-group comparisons (Table 2) reveal a correlation between feeding mode and taxonomic diversity: nematine groups that have larvae with intermediate or internal feeding habits tend to include fewer species than their external-feed-

TABLE 2. Sister-group comparisons of host ranges and species numbers when nematine groups having internal or intermediate larval habits are contrasted against their sister groups that have ancestrally free-living larvae (see Figs. 2, 3, 4, and see Appendix available online). Arrows in the last two columns denote the direction and magnitude of the differences (one arrow, "small" difference; two arrows, "large" difference).

Sister group comparison (internall/intermediate vs. external feeders)	Host range	Species (int. vs. ext.)	Host range (int. vs. ext.)	Species (int. vs. ext.)
<i>Hopllocampa</i> vs. <i>Sisana</i>	6 genera in Rosaceae vs. 2–3 genera in Cupressaceae	36 vs. 7	^	^^
<i>Caulocampus</i> vs. <i>Dineurini</i> (+ <i>Pseudodineurini</i>)	<i>Acer</i> vs. 12 genera in 4 families	2 vs. 40	<<	<<
<i>Pseudodineurini</i> vs. <i>Dineurini</i>	7 genera in 2 families vs. 12 genera in 4 families	16 vs. 40	<<	<
<i>Neopareophora</i> vs. rest of <i>Pristiphorini</i>	<i>Vaccinium</i> vs. <i>Vaccinium</i> + 26 other genera in 10 families	2 vs. >100	<<	<<
<i>Pristolina</i> vs. <i>Pristicampus</i>	<i>Vaccinium</i> vs. <i>Potentilla</i>	4 vs. 3		^
<i>Micronematus</i> vs. <i>Pristiphora</i> subgenus <i>Sala</i>	<i>Prunus</i> vs. <i>Quercus</i> + 1–8 additional genera	1–2 vs. 9–40		<
<i>Sharliphora</i> vs. <i>Pristiphora</i> subgenus <i>Lygaeophora</i>	<i>Picea</i> vs. <i>Salix</i>	3 vs. 10		<
<i>Pontopristsia</i> vs. <i>Amauronematus</i>	<i>Salix</i> vs. <i>Salix</i> + 5 other genera in 4 families	12 vs. >100	<<	<<
<i>Amauronematus longiserra</i> -group vs. rest of <i>Amauronematus</i>	<i>Salix</i> vs. <i>Salix</i> + <i>Betula</i> (+ <i>Vaccinium</i> + <i>Potentilla</i> ?)	6 vs. >90	<	<<
<i>Brachycoloma</i> vs. <i>Epicnematus</i> , <i>Pachynematus</i> etc.	<i>Salix</i> vs. <i>Salix</i> + over 30 other genera in 10 families	15 vs. >100	<<	<<
<i>Polynematus</i> vs. <i>Eitelus</i> + <i>N. oligospilus</i> -group	<i>Rumex</i> + <i>Polygonum</i> vs. 17 other genera in 6 families	4 vs. >50	<<	<<
<i>Bacconematus</i> vs. <i>Eitelus</i>	<i>Ribes</i> vs. <i>Salix</i>	1 vs. 2		<
<i>Euurina</i> vs. <i>N. melanaspis</i> -group	<i>Salix</i> + <i>Populus</i> vs. <i>Salix</i> + <i>Populus</i> + <i>Betulaceae</i>	400 vs. 25	<	^^

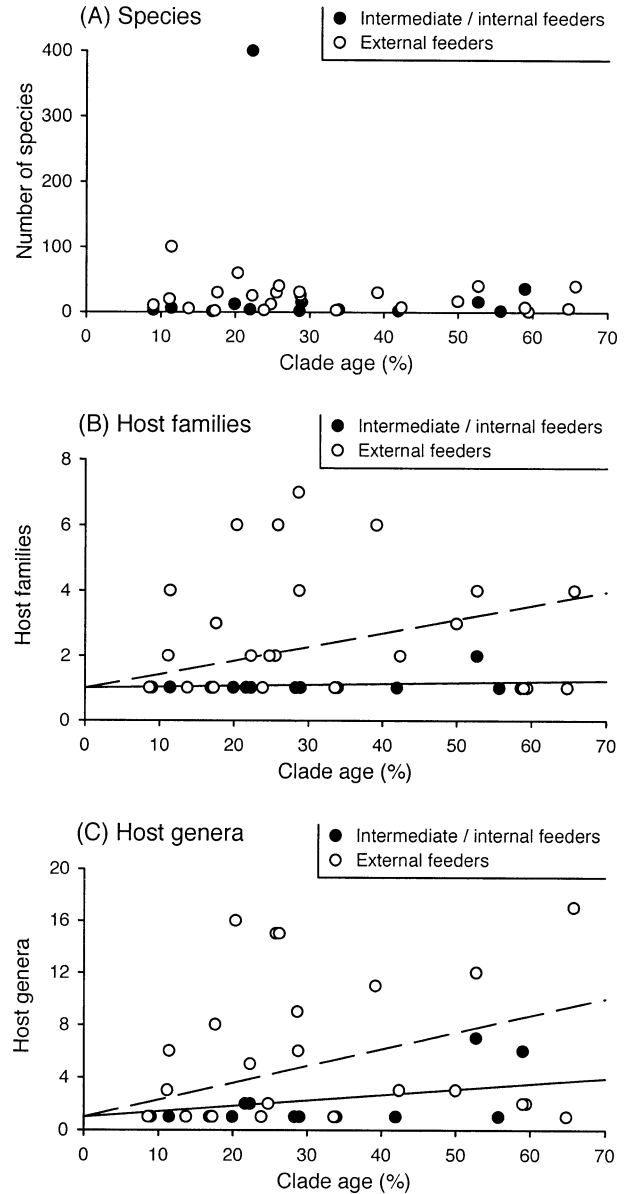


FIG. 6. Effect of clad age and different larval habits on (A) numbers of sawfly species, (B) numbers of host families, and (C) numbers of host genera. In (B) and (C), the broken line shows the linear regression slope for external feeders, and the continuous line shows the slope for intermediate/internal feeders. Regression lines were constrained to go through 1 on the y-axis (see Methods and Results).

ing sister taxa (wins three of 13 pairs; sign test, one-tailed $P = 0.046$). Again, some of the sister-group relationships are weakly supported, but most internal-feeding taxa are so species-poor that nearly all putative exophagous sister groups would have more species. At the same time, it is clear that striking exceptions to the general pattern occur, because the subtribe *Euurina*, which includes about 400 gall-inducing species, is far more species rich than its sister taxon, the "*Nematus melanaspis*-group" (Fig. 2). Estimating diversification rates by maximum likelihood also produces somewhat complicated results: the likelihood ratio test indicates no statistically significant difference in the speciation rates

of the two groups (Fig. 6A; external feeders: $\lambda_1 = 0.13$, $L_1 = -145.28$; intermediate/internal feeders: $\lambda_2 = 0.15$, $L_2 = -77.16$; combined data: $\lambda_0 = 0.14$, $L_0 = -223.11$; $T = 1.31$, $P = 0.25$). However, the result is highly contingent on the subtribe *Euurina*, so that if the *Euurina* point is deleted from the analysis, the difference is highly significant ($T = 24.26$, $P < 0.00001$), and the result remains the same even if the highest point of the external feeders (= *Amauronematus*) is excluded at the same time ($\lambda_1 = 0.11$, $L_1 = -114.18$; $\lambda_2 = 0.06$, $L_2 = -38.05$; $\lambda_0 = 0.09$, $L_0 = -158.20$; $T = 11.95$, $P = 0.00055$).

DISCUSSION

Evolution of Larval Habits

The adaptive radiation of Nematinae has evidently been a dynamic process, during which episodic niche expansions and contractions within individual lineages have led to a slow increase in the group's collective host range, as well as to a proliferation of different larval lifestyles on plants (Figs. 1–3). In contrast to Zinovjev's (1982) hypothesis on the evolution of larval resource use in the subfamily, nematine larvae were originally free feeding, and various internal or intermediate habits have arisen recurrently from external-feeding ancestors (Figs. 2,3). No clear reversals from internal to external feeding can be discerned but, as Zinovjev (1982) pointed out, the intermediate habits of several nematine taxa may represent transitional stages in either direction.

While many larval niches have appeared only once during the radiation of Nematinae (e.g., *Caulocampus*, *Sharliphora*, *Polynematus*, and *Neopareophora*), a number of distinct lifestyles have evolved convergently multiple times. For example, feeding inside fruits or berries has arisen on three separate occasions, and although leaf mining has evolved only once within Nematinae, a minimum of four other origins can be identified in the rest of Tenthredinidae and in other sawfly groups (see below). At least five origins of gall induction have occurred on three different plant genera (Figs. 2,3; Table 1), but the most diverse galls are found on *Salix*: species in the *Amauronematus longiserra*-group induce rudimentary leaf galls, within which the larvae feed during their first instar before becoming external feeders, and oviposition by *Brachycoluma* species leads to the formation of a protective leaf bundle at the tip of the growing shoot (Zinovjev and Vikberg 1998). A particularly diverse willow-associated radiation comprises the about 400–500 species belonging to the subtribe *Euurina* that induce leaf folds or rolls, or various closed galls (Nyman et al. 1998, 2000; Kopelke 2003; Roininen et al. 2005). The recurrent origins of gall induction on *Salix* within the tribe Nematini suggests that the potential to manipulate the growth of salicaceous plants may be based on a physiological mechanism shared by the aforementioned taxa. On the other hand, *Salix* is one of the few plant genera with exceptionally diverse galler faunas (Kennedy and Southwood 1984; Hartley 1992), so the unusual susceptibility of the host taxon possibly also plays a role in creating the pattern.

Larval Habits and Host-Plant Associations

In comparison to larval habits, host-plant associations of nematines are labile (Fig. 5). A similar situation has previously been demonstrated in various beetles (Marvaldi et al. 2002; Farrell and Sequeira 2004; Morse and Farrell 2005) and lepidopterans (Powell et al. 1998; Bucheli et al. 2002), but analogous patterns are present in other parasitic taxa as well (Price 1980; Brooks and McLennan 2002; Farrell and Sequeira 2004). Considering that the inferred numbers of host switches are likely to be severe underestimates, it seems that host shifts have been 10 to 20 times more frequent than changes in larval habits; thus, the relative frequency of guild shifts is comparable to the rate of "major" host shifts among plant families and superfamilies by insect herbivores (cf. Mitter and Farrell 1991).

As in most plant-feeding insects, nematine host switches are most frequent among congeneric or confamilial host plants, punctuated by shifts among different host families (Ehrlich and Raven 1964; Scheffer and Wiegmann 2000; Janz et al. 2001; Wahlberg 2001; Lopez-Vaamonde et al. 2003). Many shifts have apparently occurred back and forth among woody angiosperms in the distantly related families Salicaceae, Rosaceae, Betulaceae, Fagaceae, and Ericaceae (see Angiosperm Phylogeny Group II 2003), indicating that host shifts have been strongly affected by the broadly overlapping geographic distributions and ecological properties of these plant taxa (see also Mitter and Farrell 1991; Dobler et al. 1996; Mardulyn et al. 1997; Ronquist and Liljebblad 2001; Nyman et al. 2002; Bucheli et al. 2002; Lopez-Vaamonde et al. 2003). Switches to conifers have occurred some six to eight times, and seem associated with lower diversity as in other insect groups (Farrell 1998; Farrell et al. 2001), but more thorough taxon sampling in the "Higher" Nematinae is needed for proper testing of this hypothesis.

Diet breadths within particular lineages are correlated with larval feeding habits in plant-feeding insects, so that species and species-groups that feed inside plant tissues or induce galls tend to have more restricted host ranges than external-feeding taxa (Hering 1951; Price 1980; Mattson et al. 1988; Lewinsohn 1991; Gaston et al. 1992; Frenzel and Brandl 1998). However, phylogeny-based contrasts of endo- versus exophagous groups are thus far almost completely lacking, so the possibility of classification biases cannot be excluded in most cases. Within Nematinae, endophagous taxa clearly tend to have narrower collective host ranges than their external-feeding sister groups (Table 2). This apparently results from slow accumulation of new host taxa after the origins of concealed feeding habits (Fig. 6) or, less likely, losses of hosts in endophagous lineages. There are probably many reasons for the restricted host use of internal-feeding groups, but a particularly relevant possibility is that internal feeders face more pronounced structural differences among plant taxa than do external folivores (Nowakowski 1962; Price 1980; Oppenheim and Gould 2002). Origins of internal feeding on comparatively uncommon structures such as catkins or berries may also limit subsequent host shifts to the relatively few plant taxa that bear such structures, but lineage-specific effects are still possible: the various fruit- or berry-mining groups *Hoplocampa*, *Bacconematus*, and the *Pristolina* are

invariably associated with very similar berries of different, particular host taxa (Rosaceae, *Vaccinium*, and *Ribes*, respectively), although shifts between these host berries have probably occurred in *Rhagoletis* flies (Smith and Bush 1997; Berlocher 2000), the curculionid beetle genus *Conotrachelus* (Maier 1990), and in the lepidopteran superfamily Copromorphoidea (Heppner 1987).

The distinct specialization and evolutionary conservatism of internal feeders sometimes leads to cospeciation with host plants (Farrell and Mitter 1990, 1998; Weiblen 2001), but this does not seem to be the case in the Nematinae, as the phylogenetic structure of the *Euurina* gallers is not concordant with the phylogeny of their *Salix* hosts (Roininen et al. 1993; Nyman et al. 2000; Nyman 2002; Roininen et al. 2005). Discordant insect and host phylogenies have also been found in studies on gall-inducing cynipid wasps (Ronquist and Liljeblad 2001), fruit-, leaf-, and cambium-mining flies (Smith and Bush 1997; Berlocher 2000; Scheffer and Wiegmann 2000; Nyman et al. 2002), seed- and leaf-mining moths (Bucheli et al. 2002; Lopez-Vaamonde et al. 2003), and internally feeding beetles (Jones 2001; Farrell and Sequeira 2004; Morse and Farrell 2005). It is interesting to note that whereas a shift to endophagy can severely limit the range of potential hosts, the general evolutionary pattern—phylogenetically nonrandom host shifting—remains the same as in external feeders.

Larval Habits and Speciation Rates

Switches from external feeding to intermediate or internal habits have reduced speciation rates across 10 of 13 sister-group pairs in Nematinae. Although the bias is expected and statistically significant, the result is not unambiguous because a significant difference in diversification under a maximum-likelihood model is found only when the *Euurina* gallers are not included. However, the result in Nematinae seem part of a general pattern across the Tenthredinoidea, where internally feeding taxa are typically species poor and have restricted host ranges: The sister group to the rest of the Tenthredinoidea (> 6000 spp.) are the fern-stem miners in the family Blasticotomidae, which includes only nine known species (Goulet 1993; Vilhelmsen 2001; Schulmeister 2003). The otherwise external-feeding families Argidae (> 800 spp.), Pergidae (> 400 spp.), and Diprionidae (> 90 spp.) each contain less than 10 internally feeding species (Rohwer 1918; Smith 1974, 1979, 1995; Goulet 1992; Connor and Taverner 1997). Within Tenthredinidae, all nonnematine endophagous taxa include fewer than 60 species (Smith 1976b; Goulet 1992; Connor and Taverner 1997) and, with the exception of *Euurina*, all of the about eight gall-inducing sawfly groups are small, none of the groups occurring on more than one plant family (Roininen et al. 2005). The remaining Symphyta are mostly internal feeders (in staminate cones, wood, or shoots; Lorenz and Kraus 1957; Smith 1979), and their collective species number and host ranges are dwarfed compared to the external-feeding Tenthredinoidea (Goulet 1992, 1993). Additional phylogenetic studies are needed for firm conclusions, but such repeated generation of small endophagous groups seems unlikely under a “null model” of equal average

ages and net speciation rates in internal- and external-feeding lineages.

Available data from other insect taxa also point towards the same direction. As Connor and Taverner (1997) showed, decreases in species diversification are associated with the origins of leaf mining across multiple insect orders. On the other hand, a few internal-feeding insect groups are both taxonomically and ecologically diverse (e.g., agromyzid flies and cecidomyiid gall midges; Nowakowski 1962; Gagné 1994), but some of the diverse groups lack external plant-feeding sister groups (e.g., agromyzid flies, cecidomyiid midges, and cynipid gall wasps), making meaningful diversity comparisons more or less impossible at least until the ages of these groups have been determined.

Although endophagous sawfly taxa are generally species poor, the diversity of *Euurina* shows that the relationship between internal feeding and speciation rates is not as straightforwardly negative as the one between internal feeding and host range evolution. It is conceivable that the generation of numerous different gall locations and morphologies within the group (Nyman et al. 1998, 2000; Kopelke 2003) has facilitated speciation by reducing interspecific competition (either direct resource competition, or indirect competition via shared enemies). However, the key factor is probably the unusually restricted host use of these gallers: most *Euurina* species are monophagous on single willow species (Kopelke 2003; Roininen et al. 2005) whereas *Salix*, at over 500 species, constitutes one of the most species-rich plant genera in the Holarctic Region (Argus 1997; Skvortsov 1999; Kouki 1999). Comparable radiations have occurred in cynipid oak gallers (Ronquist and Liljeblad 2001) and agaonid fig wasps (Gibson 1993), in both cases apparently as a result of similar increased “species packing” (sensu Price 1980) on a single, diverse host taxon.

Conclusions

The proliferation of molecular phylogenetic information within the last 10 years has led to a growing appreciation of the role that ecological traits have in directing cophylogenetic patterns between parasitic taxa and their hosts (Weiblen and Bush 2002; Clayton et al. 2004). In Nematinae, changes in one niche dimension (external to internal) are consistently accompanied by severe constraints on shifts in another dimension (host taxa), the result being comparatively slow diversification of collective host ranges in endophagous lineages. It thus seems that the evolution of concealed feeding leads to a more fine-grained view of available plant taxa, which prevents an initial broadening of the host range—the first step in any successful host switch (Ross 1972; Drès and Mallet 2002). This could reflect different morphological or chemical defenses in different host tissues (Cornell 1989; Price 1980; Mattson et al. 1988), but shifts in feeding guilds frequently also change the patterns and relative importance of parasitism, predation, and competition (Hawkins 1994; Denno et al. 1995; Sipura 1999), all of which can influence the probability and direction of host shifts (Bernays and Graham 1988; Denno et al. 1995; Lill et al. 2002; Oppenheim and Gould 2002; Murphy 2004). However, more detailed

information on these forces in individual herbivore species is needed before their importance can be assessed reliably.

Shifts to internal feeding have also led to lowered speciation probabilities in the majority of cases that can be identified on the basis of our phylogeny estimate. Repeated origins of traits that reduce net speciation rates have previously been found in a few plant (Barrett and Graham 1997; Heilbuth 2000) and animal taxa (Wiegmann et al. 1993; Van Valkenburgh et al. 2004). The recurrent appearance of traits leading to low speciation probabilities may seem counterintuitive, but it must be remembered that natural selection operates on relatively short time spans. Consequently, immediate and possibly transient benefits in the form of reduced desiccation, parasitism, etc., can lead to the evolution of a novel larval habit which may nevertheless be harmful from a macroevolutionary perspective (Price et al. 1986; Sagers 1992; Connor and Taverner 1997). It is naturally dangerous to equate evolutionary "success" with species numbers (Thompson 1994; Vogler and Goldstein 1997; Hunter 1998), but narrow collective niches have been demonstrated to increase extinction probabilities (Labandeira et al. 2002; Van Valkenburgh et al. 2004; Kotiaho et al. 2005).

Although data from other nematines as well as other insect groups point to the conclusion that the *Euurina* gallers constitute a rare outlier among internal feeders, the taxonomic diversity of the group also highlights a fundamental complication in both allo- and sympatric models of ecological speciation in herbivorous insects. Colonization of (a) new host(s) can lead either to polymorphism (i.e., oligo- or polyphagy), or to the formation of two or more sexually isolated species due to host-associated disruptive selection (Strong et al. 1984; Funk 1998; Schluter 2000; Groman and Pellmyr 2000; Drès and Mallet 2002; Stireman et al. 2005). Paradoxically, the probability of host colonization and the probability of this leading to disruptive selection may well be inversely correlated; if this is the case, speciation probabilities will be maximized when alternative hosts are at an "intermediate" distance in the multidimensional resource space defined by the various characteristics of plant taxa. Because this distance will depend on the cost of maintaining a wide host range (the "intrinsic" niche width), the effect of endophagy may be contingent both on the taxonomic diversity of the host group and on their similarity to other available plant taxa. If internal feeding evolves on a small, distinct group of plants, a slowdown in speciation rates is expected; but if the habit originates on a common and speciose plant taxon, speciation may be accelerated as a result of the narrow niches. Thus, the rapid radiation of *Euurina* gallers on willows provides empirical support for verbal and mathematical models suggesting that speciation probabilities are a function of a complex interplay between "intrinsic" niche width and the heterogeneity of available resources (Simpson 1953; Price 1980; Tahvanainen and Niemelä 1987; Kouki 1999; Ackermann and Doebeli 2004). Evidently, broad phylogeny-based comparative surveys of the macroevolutionary consequences of guild shifts across multiple herbivorous insect groups could help to tease apart the respective roles of niche-width constraints and resource diversity in determining the rates at which new species are formed in natural ecosystems.

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