

EVOLUTION OF GALL MORPHOLOGY AND HOST-PLANT RELATIONSHIPS IN WILLOW-FEEDING SAWFLIES (HYMENOPTERA: TENTHREDINIDAE)

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Abstract.—There are over 200 species of nematine sawflies that induce galls on willows (*Salix* spp.). Most of the species are mono- or oligophagous, and they can be separated into seven or eight different groups based on the type of gall that they induce. We studied the evolution of different gall types and host plant associations by reconstructing the phylogeny of five outgroup and 31 ingroup species using DNA sequence data from the mitochondrial cytochrome *b* gene. Maximum-parsimony and maximum-likelihood analyses resulted in essentially the same phylogeny with high support for important branches. The results show that: (1) the galling species probably form a monophyletic group; (2) true closed galls evolved only once, via leaf folders; (3) with the possible exception of leaf rollers, all gall type groups are mono- or paraphyletic; (4) similar gall types are closer on the phylogeny than would be expected by a random process; (5) there is an apparent evolutionary trend in galling site from the leaf edge towards the more central parts of the host plant; and (6) many willow species have been colonized several times, which excludes the possibility of parallel cladogenesis between willows and the gallers; however, there are signs of restrictions in the evolution of host use. Many of the patterns in the evolutionary history of nematine gallers have also been observed in earlier studies on other insect gallers, indicating convergent evolution between the independent radiations.

Key words.—Coevolution, convergent evolution, evolutionary trends, extended phenotypes, gall morphology, gradualism, insect-plant relationships, Nematinae, phylogeny.

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The habit of galling has evolved many times independently in various organisms (Meyer 1987). In insects alone, gall induction has evolved in at least seven different orders, and in most of them several radiations have arisen (Meyer 1987; Dreger-Jauffret and Shorthouse 1992). The multiple origins of galling makes it possible to search for convergent features in these independent evolutionary histories. Indeed, recent phylogenetic analyses of gall-inducing aphids (Stern 1995), wasps (Stone and Cook 1998), and thrips (Crespi and Wobeser 1998) have shown that common features exist in separate radiations. For example, in all these taxa gall morphology is determined mainly by the galler, not the host plant, and thus the gall can be considered an extended phenotype (*sensu* Dawkins 1982) of the galler.

The nematine sawflies that induce galls on willows (*Salix* spp.) also offer a good model system for the study of evolutionary questions, especially questions about the evolution of different gall types and host plant associations (Price 1992). These sawflies belong to the tenthredinid subfamily Nematinae, which is comprised of hundreds of galling and nongalling species (Smith 1979; Gauld and Bolton 1988). There are about 200 species of galling nematines, most of which are mono- or oligophagous (Smith 1970; Price et al. 1994). Like their host plants, these sawflies are common and have a mainly holarctic distribution (Smith 1979; Gauld and Bolton 1988).

The nematine gallers have traditionally been treated as a separate tribe within the Nematinae, the Euurina (Vikberg 1982; Viitasaari and Vikberg 1985). The species have been divided into three genera, mainly on the basis of the type of gall that they induce: *Phyllocolpa* species form leaf folds or rolls; *Pontania* species are leaf blade gallers or apical/basal leaf gallers; and *Euura* species induce petiole, stem, or bud galls (Meyer 1987; Price and Roininen 1993). In all, about

eight different gall types can be recognized. Thus, the structural diversity of nematine galls rivals that of the cynipid wasp galls; however, the internal structure of the galls has remained relatively uniform and simple. Each gall type can be found on several species of willow, and several types of gallers can coexist on each willow species (Kontuniemi 1960; Roininen 1991).

Price (1992; see also Smith 1970; Roininen 1991) suggested that the different gall types evolved gradually, that is, the evolutionary sequence would have been: (1) leaf folders/rollers; (2) leaf blade gallers; (3) apical leaf gallers; (4) basal leaf gallers; (5) petiole gallers; (6) stem gallers; and (7) bud gallers. Thus, there would have been a slow, gradual process with radiations to new willow species after the evolution of each new gall type. In addition, there would have been an evolutionary trend toward earlier oviposition in the more central parts of the host plants and a more intimate relationship between galler and host (Price and Roininen 1993).

The gradualistic view is indeed tempting: In all species the galls are initiated by substances secreted by the ovipositing female into the oviposition scar (Price and Roininen 1993), and exact positioning of the egg is necessary for the gall to develop normally (McCalla et al. 1962). A change in gall type would probably require simultaneous changes in ovipositor morphology and the oviposition behavior of females. Thus, small changes in the site of gall induction may be considered to be more likely than large changes.

In a previous paper (Nyman et al. 1998), we used enzyme electrophoretic data to test the Price-Roininen hypothesis. We were able to show that there are signs of constraints on the evolution of gall types, and that gall type changes may be gradual. However, the limited taxonomic sample (18 species) and the low support for many of the groupings in the resulting phylogeny prevented us from reaching definite conclusions.

TABLE 1. Species used in the study, their host plants, and sample sites.

Gall type ¹	Species	Willow host ²	Sample site ³ /date
0. Outgroup	<i>Tenthredo arcuata</i> (Förster)	—	Joensuu (F), 1996
	<i>Nematus salicis</i> (Linnaeus)	<i>Salix fragilis</i> (S)	Joensuu (F), 1996
	<i>Nematus capreae</i> (Linnaeus)	<i>S. myrsinifolia</i> (V)	Kilpisjärvi (F), 1997
	<i>Pontopristsia amentorum</i> (Förster)	<i>S. borealis</i> (V)	Kilpisjärvi (F), 1997
	<i>Amauronematus eiteli</i> (Saarinen)	<i>S. pentandra</i> (S)	Parikkala (F), 1998
1. Leaf fold/roll (11)	<i>Phyllocolpa nudipectus</i> (Vikberg)	<i>S. phyllicifolia</i> (V)	Joensuu (F), 1996
	<i>Phyllocolpa leucosticta</i> (Hartig)	<i>S. caprea</i> (V)	Puhos (F), 1997
	<i>Phyllocolpa puella</i> (Thomson)	<i>S. fragilis</i> (S)	Joensuu (F), 1997
	<i>Phyllocolpa excavata</i> (Marlatt)	<i>S. pentandra</i> (S)	Joensuu (F), 1997
	<i>Phyllocolpa coriacea</i> (Benson)	<i>S. aurita</i> (V)	Mekrijärvi (F), 1997
2. Leaf blade gall (3–5)	<i>Phyllocolpa anglica</i> (Cameron)	<i>S. dasyclados</i> (V)	Krasnojarsk (R), 1993
	<i>Pontania proxima</i> (Lepeletier)	<i>S. alba</i> (S)	Joensuu (F), 1991
2? Sausage gall (4–10)	<i>Pontania triandrae</i> (Benson)	<i>S. triandra</i> (S)	Keminmaa (F), 1997
	<i>Pontania dolichura</i> (Thomson)	<i>S. phyllicifolia</i> (V)	Paanajärvi (R), 1996
3. Apical leaf gall (18)	<i>Pontania dolichura</i> (Thomson)	<i>S. glauca</i> (C)	Kilpisjärvi (F), 1997
	<i>Pontania glabrifrons</i> (Benson)	<i>S. lanata</i> (V)	Kanin Peninsula (R), 1994
	<i>Pontania samolad</i> (Malaise)	<i>S. lapponum</i> (V)	Paanajärvi (R), 1996
	<i>Pontania reticulatae</i> (Malaise)	<i>S. reticulata</i> (C)	Kolguev Island (R), 1994
	<i>Pontania pedunculi</i> (Hartig)	<i>S. caprea</i> (V)	Lebed-Ozero (R), 1996
	<i>Pontania nivalis</i> (Vikberg)	<i>S. glauca</i> (C)	Kakhovskiy Bay (R), 1994
	<i>Pontania hastatae</i> (Vikberg)	<i>S. hastata</i> (V)	Björkstugan (S), 1989
	<i>Pontania arcticornis</i> (Konow)	<i>S. phyllicifolia</i> (V)	Paanajärvi (R), 1996
	<i>Pontania</i> sp.	<i>S. schwerinii</i> (V)	Ussuri Reserve (R), 1996
	<i>Pontania</i> sp.	<i>S. gracilistyla</i> (V)	Kedrovaja Pad Reserve (R), 1996
4. Basal leaf gall (8)	<i>Pontania aestiva</i> (Thomson)	<i>S. myrsinifolia</i> (V)	Paanajärvi (R), 1996
	<i>Pontania lapponica</i> (Malaise)	<i>S. lapponum</i> (V)	Kilpisjärvi (F), 1997
	<i>Pontania pustulator</i> (Forsius)	<i>S. phyllicifolia</i> (V)	Joensuu (F), 1994
	<i>Pontania polaris</i> (Malaise)	<i>S. polaris</i> (C)	Kilpisjärvi (F), 1997
5. Petiole gall (2–3)	<i>Euura testaceipes</i> (Zaddach)	<i>S. fragilis</i> (S)	Joensuu (F), 1989
	<i>Euura venusta</i> (Zaddach)	<i>S. caprea</i> (V)	Härskää (F), 1989
6. Stem gall (4–8)	<i>Euura atra</i> (Jurine)	<i>S. alba</i> (S)	Simpele (F), 1988
	<i>Euura atra</i> (Jurine)	<i>S. lapponum</i> (V)	Kilpisjärvi (F), 1997
	<i>Euura lasiolepis</i> (Smith)	<i>S. lasiolepis</i> (V)	Flagstaff, Arizona (U), 1997
6? Multilarval stem gall (1)	<i>Euura amerinae</i> (Linnaeus)	<i>S. pentandra</i> (S)	Joensuu (F), 1998
7. Bud gall (3–11)	<i>Euura mucronata</i> (Hartig)	<i>S. phyllicifolia</i> (V)	Kilpisjärvi (F), 1997
	<i>Euura lanatae</i> (Malaise)	<i>S. lanata</i> (V)	Kilpisjärvi (F), 1997

¹ Numbers in parentheses indicate numbers of European species according to Price and Roininen (1993), Kopelke (1994), and pers. obs. The numbers are only approximate because the status of many currently recognized species is uncertain.

² Letters in parentheses indicate the willow subgenus to which the host belongs: S, *Salix*; C, *Chamaetia*; V, *Vetrix*.

³ F, Finland; R, Russia; S, Sweden; U, United States.

The purpose of the present study was to obtain a more reliable view of the evolution of the galling nematines. For this, we used DNA sequence data (678 bp) from the mitochondrial cytochrome *b* gene coupled with a broader taxonomic sample (36 species). The analysis yielded a robust phylogeny that answered many questions left open in the previous study and corrected some apparent errors. The new phylogeny was used to infer the evolutionary history of gall-type changes and host-plant relationships in the nematine gallers.

MATERIALS AND METHODS

Sample Collection and Preservation

Five representative outgroup species and 31 ingroup species were selected for the study (Table 1). Each gall type was represented by from one to 10 species. Adult sawflies or larvae were collected between 1988 and 1998 from Finland, Sweden, Russia, and the United States. Sample sites and dates are summarized in Table 1. Samples were stored in 100% ethanol at -20°C .

DNA Extraction, Polymerase Chain Reactions, and Sequencing

Total DNA was extracted from larvae or adult sawflies as described in Garnery et al. (1991). The DNA was dissolved in 20–50 μl H_2O , depending on the size of the sample.

For the polymerase chain reactions (PCR), we used primers CB-J-10933 and TS1-N-11683 (Simon et al. 1994), which amplify an approximately 750-bp fragment in the cytochrome *b*-tRNA-Ser region. The amplification conditions in a 25- μl reaction were: 0.4 μM of each primer, 0.1 mM of each dNTP, 2.5 mM MgCl_2 , 1 \times reaction buffer (Promega, Inc., Madison, WI), and 1 unit of *Taq* DNA polymerase (Promega). Typical thermal cycling conditions were a denaturation step (94°C for 2 min) followed by 25 cycles of 94°C denaturing for 45 sec, 50°C annealing for 45 sec, and 70°C extension for 1 min; the last cycle was followed by an extra extension step (70°C for 5 min).

The resulting double-stranded product (40 μl) was purified using the QIAquickTM (Qiagen, Valencia, CA) kit and sequenced using the ABI PRISMTM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City,

CA) following the manufacturers' instructions. Sequences were analyzed on an ABI 310 automated sequencer. The primers for the initial PCR reactions were used in the sequencing reactions, and both strands were sequenced to enhance accuracy. All sequences have been deposited in GenBank under accession numbers AF209932–AF209967.

Data Analysis

Sequence electropherograms were read, edited, and aligned using the ABI Sequence Navigator (Applied Biosystems, Inc., Foster City, CA). No insertions or deletions were observed, so the sequences could be aligned unambiguously. Plots of substitutions against sequence divergence indicated that the number of transitions levels off after approximately 10–15% divergence; because no such effect indicating saturation (Swofford et al. 1996) was observed in transversions, we performed the maximum-parsimony (MP) analyses using a 3:1 weighting of transversions over transitions (216 informative characters), and transversions only (123 informative characters). MP analyses were performed using PAUP* version 4.0b1 (Swofford 1998). For both MP analyses we used a heuristic search with 100 random addition sequences and tree bisection and reconnection (TBR) branch swapping. Branch support was determined by bootstrapping (Felsenstein 1985) 1000 times over all characters (heuristic search with random addition sequence and TBR branch swapping). Decay indices (Bremer 1988, 1994) for interior branches were determined using AutoDecay version 4.0 (Eriksson 1998) in conjunction with PAUP*.

For the quartet puzzling maximum-likelihood (ML) analysis we used PUZZLE version 4.0.1. (Strimmer and von Haeseler 1999). The analysis used 10,000 puzzling steps and incorporated an HKY model of substitution (Hasegawa et al. 1985). Nucleotide frequencies were estimated from the dataset (A = 36.6%, C = 14.9%, G = 9.4%, T = 39.2%). The following parameters were estimated using a neighbor-joining tree: proportion of invariable sites (0.45); gamma distribution parameter for variable sites (1.01, with eight rate categories); expected transition:transversion ratio (3.06).

All trees were rooted by using five species with different larval habits as outgroups. *Tenthredo arquata* is a large distantly related tenthredinid sawfly with larvae feeding on *Trifolium repens* (Kontuniemi 1960). The other outgroups are nematine sawflies with larvae feeding on *Salix* species: *Nematius salicis* and *N. capreae* have free-feeding larvae; *Pontoprastia amentorum* larvae live inside willow catkins; and *Amauronematus eiteli* larvae live originally inside a small procecidium, but become free-feeders at a later stage (Zinovjev and Vikberg 1998).

The evolution of different gall types and host plant associations was inferred by plotting the characters on the MP tree using MacClade version 3.01 (Maddison and Maddison 1992). Each gall type was given a code from 0 (outgroup) to 7 (bud galls) as shown in Table 1. The gall types were treated as unordered, and ancestral states were reconstructed using accelerated transformations (Acctran) optimization.

Statistical significance of the inferred evolutionary patterns was determined by ordered and unordered permutation tests (Liebherr and Hajek 1990; Maddison and Slatkin 1991). To

TABLE 2. The numbers of galler species observed to use *Salix* species in the three Eurasian willow subgenera (H. Roininen, A. G. Zinovjev, and T. Nyman, unpubl. data). Occurrences of galler species were calculated for each willow species and summed separately for each willow subgenus and galler group (basal taxa = *Phyllocolpa* folders/rollers + *Pontania* leaf blade/sausage galls; *Eupontania* = apical/basal leaf galls; *Euura* = petiole, stem, and bud galls). Possible oligo- and polyphages were treated as multiple monophagous species. The galler groups differ significantly in their pattern of use of willow subgenera ($\chi^2 = 17.9$, $P = 0.001$).

Willow subgenus	Galler group		
	Basal taxa	<i>Eupontania</i>	<i>Euura</i>
<i>Salix</i>	20	4	9
<i>Chamaetia</i>	19	27	4
<i>Vetrix</i>	118	75	32

test whether similar gall types are closer on the tree than would be expected by a random process, the gall types were treated as ordered (0–7) and gall type codes were shuffled between groups while keeping the outgroup state constant (0). The statistical significance of the observed situation was inferred by comparing the length of the original ordered gall type character to the character length distribution from 1000 randomized characters created with MacClade 3.01.

The phylogenetic analysis also included species not mentioned by the Price-Roininen hypothesis: *Pontania dolichura* induces sausage-shaped double galls along the midrib of leaves, and *Euura amerinae* induces strongly distorted multilarval stem galls. *Pontania dolichura* galls were coded as leaf blade galls (2), because the internal structure of *P. dolichura* galls closely resembles the structure of galls induced by *Pontania proxima*, for example, in having an inner green layer (Zinovjev and Vikberg 1998). *Euura amerinae* galls were coded as stem galls (6). The permutation tests were performed both including and excluding the extra gall types.

The phylogeny of the genus *Salix* is still largely unknown (Argus 1997). However, the genus can be divided into three or four subgenera (Skvortsov 1968; Argus 1997). To test whether the use of host plants is conservative, we plotted the willow subgenera *Salix*, *Chamaetia*, and *Vetrix* (Table 1) on the MP tree and performed an unordered permutation test using 1000 randomized replicates of the original character. Only ingroup species (excluding *Phyllocolpa anglica*) were used for this, because most of them are known to be mono- or oligophagous (Smith 1970).

Constraints in host use were further studied by using galler host records on 138 willow species mentioned in Skvortsov's (1968) study of willows in Europe and the former Soviet Union (H. Roininen, A. G. Zinovjev, and T. Nyman, unpubl. data). The numbers of observed use of host species in different willow subgenera were calculated separately for the basal group consisting of *Phyllocolpa* rollers/folders and *Pontania* leaf blade/sausage galls, the genus *Euura*, and the subgenus *Eupontania* (apical and basal leaf galls; Table 2). Presumed oligo- and polyphages were treated as multiple monophagous sibling species, as has been demonstrated, for example, in the cases of the 'polyphagous' *Euura atra* (Roininen et al. 1993) and *P. dolichura* (Kopelke 1990, 1994). Equality of the three galler groups' distributions on the three

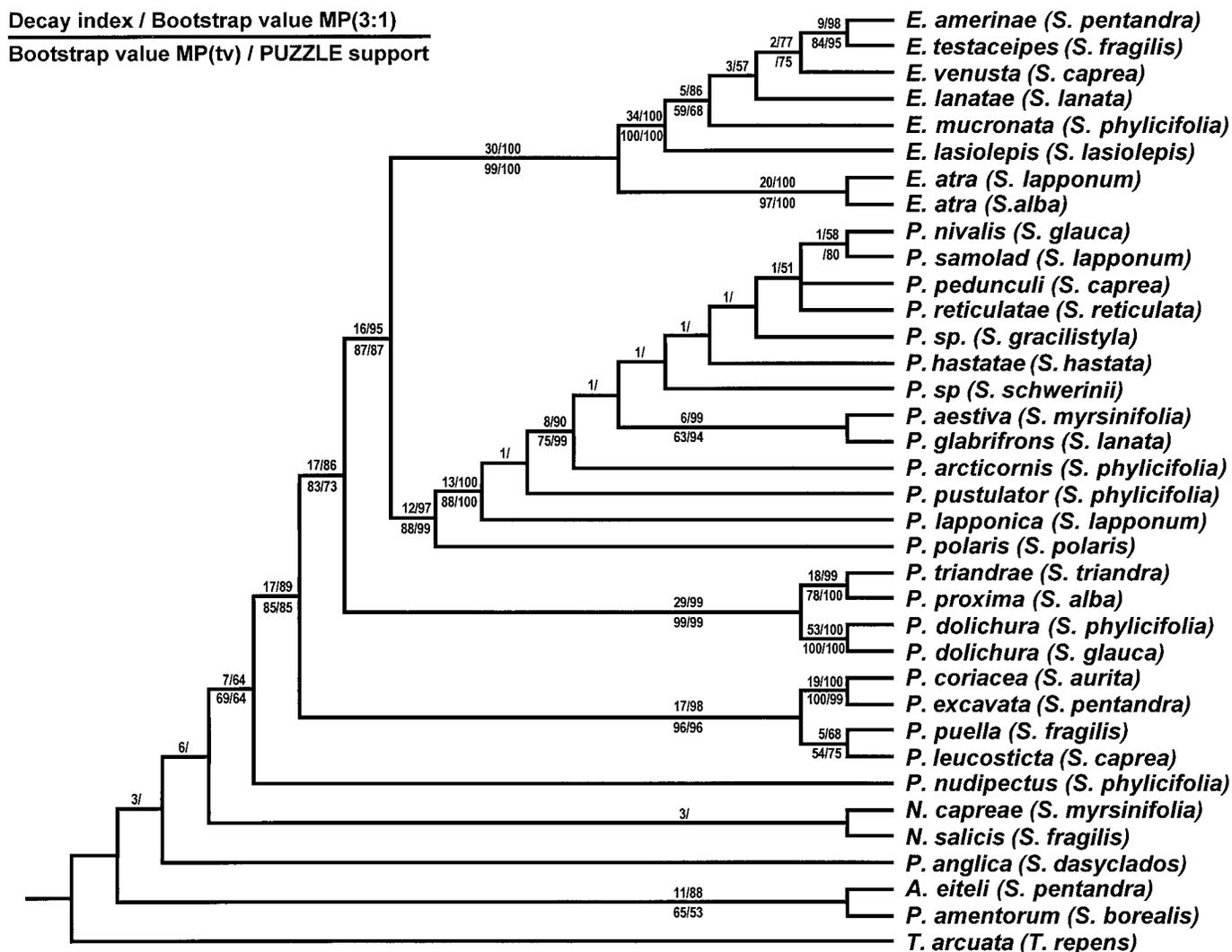


FIG. 1. The single most-parsimonious tree when transversions are weighted 3:1 over transitions (tree length = 1526; CI excluding uninformative characters = 0.436; RI = 0.744). Above branches are decay indices (Bremer 1988, 1994) and bootstrap values from 1000 replications. Below branches are bootstrap values from 1000 replications when only transversions are used and PUZZLE maximum-likelihood support values.

willow subgenera was tested with the chi-square test using the SPSS version 8.0 software (SPSS, Inc., Chicago, IL).

RESULTS

Trees

The MP analysis with a 3:1 transversion:transition weight produced a single most parsimonious tree (MP[3:1]) with a length of 1526 steps (Fig. 1; CI excluding uninformative characters = 0.436, RI = 0.743). The MP analysis using only transversions produced six MP trees (MP[tv]) with a length of 321 steps (CI excluding uninformative characters = 0.461, RI = 0.786). The MP(tv) trees have two polytomies, in the genus *Euura* and the *Pontania viminalis* group (apical leaf galls), and differ in the relative position of *Pontania pustulator* and *P. lapponica*. There are also minor differences in the structure of the outgroup. The differences between MP(tv) and MP(3:1) trees are minimal and the MP(3:1) tree can be

obtained from the strict consensus of the MP(tv) trees by resolving the polytomies. In both analyses, decay indices and bootstrap values of important branches in the ingroup are high.

The ML tree also differs only slightly compared to the MP(3:1) tree. In the ML tree, *P. lapponica* is closer to the *P. viminalis* group than *P. pustulator* is. Otherwise, the MP(3:1) tree can be obtained from the ML tree by resolving the polytomies in the *Euura* and *P. viminalis* clades and the outgroup. None of the differences between the trees have any effects on the inferences about gall type evolution. PUZZLE support values are generally close to the MP(3:1) bootstrap values.

The MP and ML trees could not be rooted so that the ingroup remains monophyletic because *Phyllocolpa anglica* is grouped within the outgroup. However, this grouping is not strongly supported; with both parsimony analyses, ingroup monophyly can be enforced without the trees becoming significantly less parsimonious. For example, with the MP(3:

1) data the constraint tree is 10 weighted steps longer than the original tree (Kishino-Hasegawa test, $P = 0.45$; Templeton's test, $P = 0.63$). We note that the exact position of *P. anglica* does not alter the inferences about the evolution of gall types in the ingroup.

Comparisons to the Allozyme Tree

The general outlines of the present phylogeny and the allozyme tree of Nyman et al. (1998) are similar, but there are some clear differences. For example, the genus *Euura* is not polyphyletic, as the allozyme tree suggested, and also closed galls have evolved only once. The allozyme MP tree can be forced to be like the MP(3:1) tree without the tree becoming significantly less parsimonious when tested with the allozyme data (Templeton's test, $P = 0.083$). However, it is not possible to change the MP(3:1) tree to be like the allozyme MP tree without the tree becoming statistically significantly less parsimonious. Thus, we consider the present phylogeny to be more reliable than the allozyme tree.

Gall-Type Evolution and Host-Plant Use

The phylogeny gives a relatively clear picture of the evolution of different gall types in the nematine sawflies (Fig. 2): (1) gallers evolved via leaf folders or rollers; (2) species forming true closed galls are monophyletic; (3) with the possible exception of leaf rollers, all gall type groups are mono- or paraphyletic, that is, each gall type has evolved only once; and (4) similar gall types are closer to each other than would be expected by a random process (ordered permutation test, $P = 0.007$; if *P. dolichura* and *E. amerinae* are included, $P = 0.005$); this indicates constraints in transition probabilities and a trend toward galling in the more central parts of the host plants. The evolution of host-plant relationships is not as clear, but some patterns are evident. Most importantly, the phylogeny excludes the possibility of strict cospeciation of the gallers and their willow hosts. Several willow species have been colonized independently by nematine species representing different gall types (Fig. 1). The phylogeny also excludes the possibility of escape-and-radiate type of coevolution.

The permutation test found no statistically significant conservatism in the use of willow subgenera (unordered permutation test, $P = 0.17$). However, the ingroup can be divided into three groups, formed by the basal taxa (leaf folders/rollers + leaf blade gallers/sausage gallers), the subgenus *Eupontania*, and the genus *Euura*. These groups differ significantly in their use of the three willow subgenera (Table 2; chi-square test, $P = 0.001$), which can be interpreted as evidence for phylogenetic inertia in the utilization of host plants.

DISCUSSION

Gall-Type Evolution

It seems very likely that species forming true closed galls evolved via leaf folders or rollers (Fig. 2). In these species, the folding of the leaf is caused by the ovipositing female (Smith 1970; Zinovjev and Vikberg 1998), and it can be hypothesized that this ability to induce abnormal growth on

leaves was a preadaptation that led to the evolution of galls. This is also supported by the observation that some leaf-rolling species induce small vestigial procecidia at the site of oviposition (Price and Roininen 1993; Zinovjev and Vikberg 1998). Interestingly, leaf folding also seems to be the initial step preceding galling in thrips that induce galls on *Acacia* species (Crespi and Worobey 1998).

Although the next step seems to be more obscure, it was probably leaf blade galling, something like the galls induced by species in the *P. proxima* group or the *P. dolichura* complex. The gall structure of these species is simple, with an inner green layer seen in the vestigial galls induced by some *Phyllocolpa* rollers (Zinovjev and Vikberg 1998). *Pontania dolichura* has also retained the *Phyllocolpa*-like habit of multiple insertions of the ovipositor during egg-laying (Kopelke 1985, 1998). *Pontania dolichura*, and occasionally *P. proxima*, oviposit through the midrib, which is more typical for the subgenus *Eupontania* (Kopelke 1998; Zinovjev and Vikberg 1998).

In the subgenus *Eupontania*, females oviposit only once through the midrib. Females may make multiple oviposition attempts, but they do not inject gall-inducing substances until the egg is laid (Meyer 1987, p. 143). The galls are typically located along the midrib. The apical leaf gallers form a monophyletic group within the *Eupontania* (Fig. 2).

The main macroevolutionary transition in the phylogeny seems to be at the base of the genus *Euura*, because stem gallers are clearly ancestral in the group. Other transitions within *Euura* can be considered minor, because in both bud gallers and petiole gallers oviposition occurs through the petiole base (Smith 1968; Kopelke 1998). The polyphyletic origin of stem galling is not necessarily surprising, because Smith (1968) divided the Nearctic stem gallers into two groups and noted that the *E. exiguae* complex is morphologically close to the European *E. amerinae* and petiole galling *Euura* species.

The most important result is that gall morphology mirrors the phylogeny of the nematine gallers, not that of their host plants. Thus, the gall can be considered an extended phenotype (sensu Dawkins 1982). This interpretation is identical to the results from earlier phylogenetic studies concerning gall-inducing pemphigine aphids (Stern 1995), cynipid wasps (Stone and Cook 1998), and Australian gall thrips (Crespi et al. 1997; Crespi and Worobey 1998). To date, the only exception seems to be in forinine aphids, because Wool (1997) suggested that the plants (*Pistacia* spp.) may have a larger role than the insects in determining gall morphology. However, a more thorough phylogenetic analysis of this group is needed.

The permutation tests show that similar types of galls are closer to each other than would be expected by a random process. This indicates that gall types evolve gradually in small steps, which is in agreement with the traditional gradualistic view of evolution. This pattern has also been observed in gall wasps and thrips (Crespi and Worobey 1998; Stone and Cook 1998). Taken together, these studies indicate that there are constraints on the possible transitions in gall types.

The constraints may be a result of the fact that in the initial stage of a shift in gall type the population must be poly-

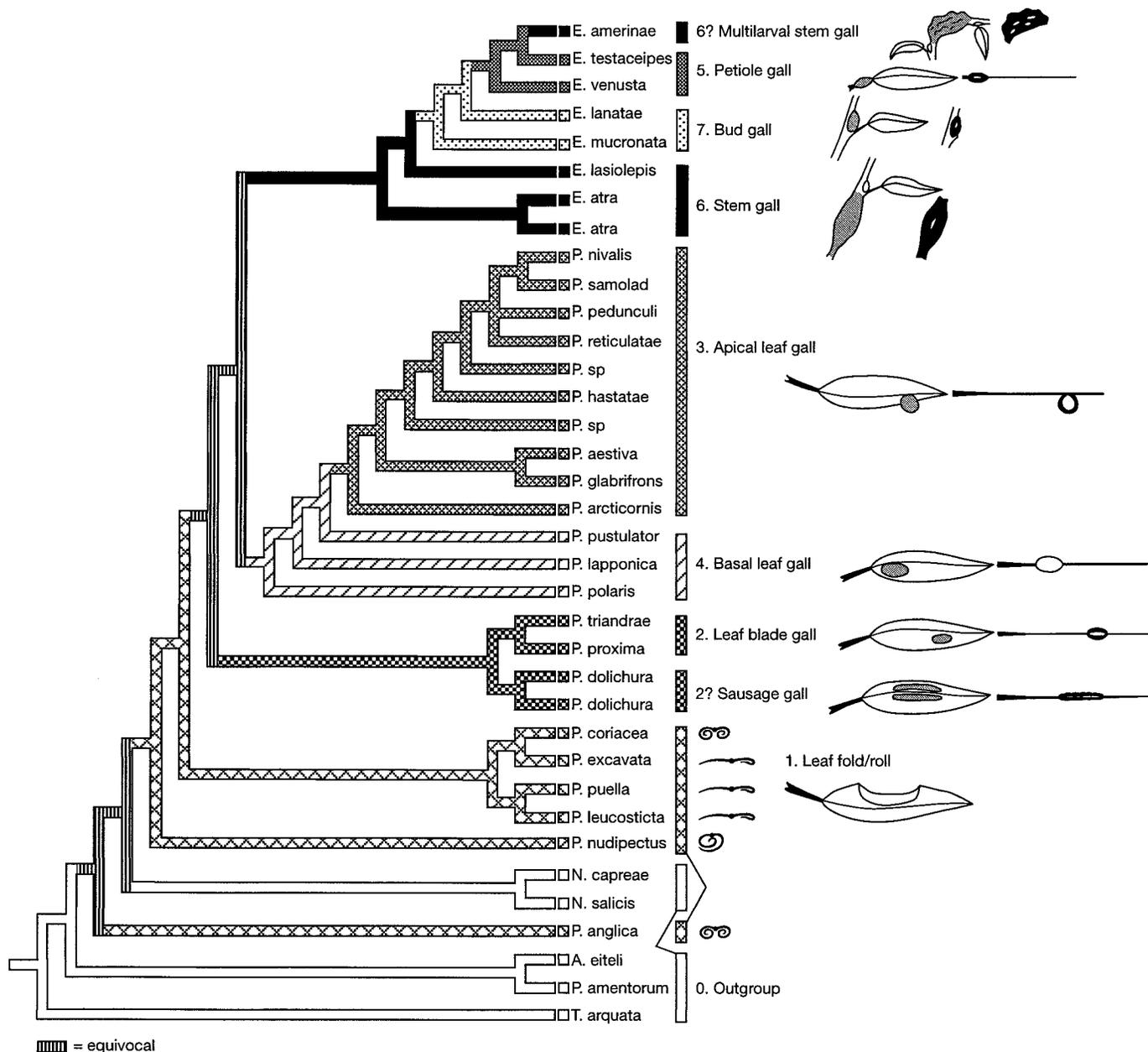


FIG. 2. The hypothesis of gall type evolution when gall types are treated as unordered and ancestral states are reconstructed on the MP(3:1) tree using accelerated transformations optimization. Figures of gall types with respective longitudinal or cross-sections are shown to the left of the tree.

morphic with respect to the type of gall; this, in turn, seems more probable if the gall types induced by the insect species are relatively similar. In cases where species have been shown to be polymorphic, intraspecific variation mirrors the variation observed between species (Crespi and Worobey 1998).

The result of the permutation tests combined with the fact that the ancestral state (nongalling) is known suggests that there has been a trend toward gall induction in the more central parts of the host plant. This is intriguing, because although evolutionary trends have often been proposed, few real cases have been documented (McShea 1998).

Although the reasons for changes in gall type can be adap-

tive or nonadaptive, the apparent trend indicates that adaptive explanations may be needed. There are at least three mechanisms that could favor oviposition in more central positions on the host plant: (1) Evasion of abscission reactions; cottonwoods (*Populus* spp.) can selectively abscise leaves with aphid galls (Williams and Whitham 1986). (2) Better control of nutrient flow in the plant: As Larson and Whitham (1991) showed, galls behave as sinks for nutrients and photosynthetic assimilates in plants. In aphid leaf galls the strength of the sink effect depends on the position of the gall: A difference of 12 mm caused a fourfold difference in the amount of resources drawn to the gall from adjacent leaves

(Larson and Whitham 1991). (3) Asymmetric intraspecific competition: Competition could lead to biased transition probabilities if galls can intercept nutrient flow to other galls situated in more distal positions. The suggestion is not entirely speculative: In pemphigine aphids, females fight for the most basal parts of the leaves (Whitham 1979), where reproductive success is higher (Whitham 1978). In the oak leaf galler *Cynips divisa*, gall location in relation to other galls and the midrib affects gall size and growth (Sitch et al. 1988; Hartley 1998) and galler mortality (Gilbert et al. 1994). Fritz et al. (1986) demonstrated the possibility of asymmetric competition between the stem galler *E. lasiolepis* and three other species representing different gall types on *Salix lasiolepis*.

Host-Plant Relationships

The phylogeny of willows is still largely unknown, in spite of their great importance in boreal and arctic ecosystems (Argus 1973, 1997; Leskinen and Alström-Rapaport 1999). However, there is agreement that the genus *Salix* can be divided into three (Skvortsov 1968) or four (Argus 1997) subgenera, of which the subgenus *Salix* is the most primitive.

The uncertainty about the phylogeny of willows makes it difficult to draw conclusions about the host plant use of nematine gallers, but some patterns are evident. Most importantly, the pattern of host shifts excludes any coevolutionary hypotheses in the sense of gallers "tracking" speciation events in willows (Fig. 1). For example, *S. lapponum* has been colonized repeatedly three times by species representing different gall types, and *S. phylicifolia* and *S. pentandra* at least two times each. Considering available host plant records of Eurasian galler species not included in this study, it seems likely that the numbers of independent colonizations for many *Salix* species are considerably larger. Furthermore, the phylogeny is not concordant with the escape-and-radiate model of coevolution (Ehrlich and Raven 1964), because there cannot be any concordant clades in the willow and galler phylogeny (Thompson 1999).

There is also no clear connection between the phylogeny of the nematine gallers and the chemical similarity of willows (Julkunen-Tiitto 1986, 1989; Shao 1991). Similar results were found by Roininen et al. (1993) in the *Euura atra* complex. This is not surprising because the chemical properties of galls can differ drastically from that of the host plants (Hartley 1992, 1998; T. Nyman and R. Julkunen-Tiitto, unpubl. data).

The permutation test using willow subgenera did not indicate any statistically significant conservatism in host use. However, this is partly a result of the limited sampling of species feeding on the willow subgenera *Salix* and *Chamaetia*. Possible restrictions in host use exist, for example, in the subgenus *Eupontania*, which is almost exclusively restricted to the willow subgenera *Chamaetia* and *Vetrix* (Zinovjev 1993, 1995). Furthermore, the host plant data of the three galler subgroups on the willow subgenera (Table 2) clearly show that there are differences between the galler groups in the utilization of different willow subgenera. This can be taken as an indication of phylogenetic inertia and constraints in host use. A similar combination of conservatism and pro-

miscuity in host use has been observed in galling thrips (Crespi et al. 1997).

It seems that the nematine gallers and their willow hosts offer an exceptionally promising system for the study of insect-plant relationships. The phylogeny demonstrates the existence of three to five independent radiations on the genus *Salix*; separate phylogenetic analyses of these clades would facilitate a comparative analysis of radiation patterns in the groups. In particular, the genus *Euura* and the subgenus *Eupontania* are promising for further studies.

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