EVOLUTION OF DIFFERENT GALL TYPES IN WILLOW-FEEDING SAWFLIES (HYMENOPTERA: TENTHREDINIDAE)

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Abstract.—The sawflies that feed on the plant family Salicaceae can be divided into eight informal groups based on larval feeding habit or gall type: (1) species with free-living larvae; (2) leaf folders; (3) leaf blade gallers; (4) apical leaf gallers; (5) basal leaf gallers; (6) midrib and petiole gallers; (7) stem gallers; and (8) bud gallers. It has been proposed that the galling habit evolved from free-living larvae via leaf folders, and that the different gall types evolved gradually in the sequence mentioned above. Thus, the galling site would have "wandered" from the leaf margin toward the stem as a result of gradual changes in oviposition site preference. Allozyme data from eight informative loci were used to reconstruct the phylogeny of 18 representative sawfly species. The results suggest that indeed leaf folders seem to be a basal group; leaf blade gallers evolved independently of the other true gallers; apical and basal leaf gallers are not the ancestors of petiole and bud gallers, but they may share a common galling ancestor; bud gallers evolved from midrib/petiole gallers; and stem gallers are polyphyletic. The cause for the observed wandering of the galling site could be intraspecific competition due to a possible "nutrient shading effect" of galls situated closer to the host plant's main vascular system.

Key words.—Allozymes, evolutionary trends, gall types, genetic constraints, Hymenoptera, Nematinae, phylogeny, sawflies.

Recent studies on evolution have commonly focused on the possible constraints that may affect the evolutionary pathways of populations and taxa. It is evident that historical, developmental, and genetical constraints may have an effect on the speed and direction of evolutionary change (Bonner 1982; Futuyma 1988; Wake 1991; Futuyma et al. 1995; Schluter 1996). In some cases, biases in evolutionary transition probabilities exist (e.g., Albrech and Gale 1985; Janson 1992), which can cause evolutionary trends on a larger scale.

A possible trend exists in the evolution of gall types in the nematine gallers that belong to the hymenopteran family Tenthredinidae. Within the large, cosmopolitan family Tenthredinidae, the ability to induce plant galls has apparently evolved twice, in the subfamilies Nematinae and Blennocampinae, of which the latter includes only a few species (Price and Roininen 1993). The tribe Nematini is comprised of hundreds of galling and nongalling species (Viitasaari and Vikberg 1985) that feed exclusively on the Salicaceae, mostly on *Salix* (Price and Roininen 1993). Like their host plants, the nematines are most common in northern latitudes, and in the Arctic they are virtually the only sawflies present (Gauld and Bolton 1988; Price and Roininen 1993).

The number of galling nematine species is probably about 200, and they belong to three genera, *Phyllocolpa, Pontania,* and *Euura* (Price et al. 1994). These genera are recognized on the basis of the gall morphology and galling site on the host plant: *Phyllocolpa* species form simple leaf folds or rolls, *Pontania* species form leaf blade galls, and *Euura* species are leaf midrib, petiole, bud, or stem gallers (Meyer 1987; Price and Roininen 1993). Some authors consider leaf-rolling *Phyllocolpa* species to be a subgenus of *Pontania* (Vikberg 1970; Viitasaari and Vikberg 1985; Zinovjev 1993; Zinovjev and Vikberg 1998); in this study, they are considered separate genera (e.g., Smith 1970; Price and Roininen 1993) for clarity.

The nematine sawflies can be divided into eight informal groups based on the galling site on the host plant and gall morphology: (1) species with free-living larvae; (2) leaf folders or rollers; (3) leaf blade gallers; (4) apical leaf gallers; (5) basal leaf gallers; (6) midrib and petiole gallers; (7) stem gallers; and (8) bud gallers. Each gall type can be found on several willow species, and on each willow species many gall types can coexist (Kontuniemi 1960; Vikberg 1970; Roininen 1991; Price 1992). In all species, the initial gall formation is caused by substances secreted by the female during oviposition (Price and Roininen 1993).

Price (1992) suggested that the different gall types evolved gradually in the sequence mentioned above (Fig. 1; see also Roininen 1991). Thus, the ancestor of all galling nematines would be a *Nematus*-like species with free-living larvae. The next step would be a *Phyllocolpa*-like leaf folder, and after that *Pontania*-like leaf blade gallers would evolve. The galling site would then "wander" on the leaf to be more closely associated with the midrib (apical and basal leaf gallers), and then to the midrib and petiole (*Euura* sp.). The last species to evolve would be the *Euura* stem and bud gallers. According to this hypothesis, radiations to new willow species occurred after the evolution of each gall type. An alternative explanation would be that similar gall types evolved independently on different willow species; however, the process might have been a mixture of both alternatives (Price and Roininen 1993).

The Price-Roininen hypothesis is appealing in its simplicity. The evolution of new gall types would occur gradually as a result of slight changes in behavior and oviposition preference of the females, which would be in concordance with the traditional gradualistic view of evolution. Since gall formation is dependent on exact positioning of the gall-forming substance (McCalla et al. 1962), gall type changes probably require multiple, coexisting changes in behavior and ovipositor shape. In the initial stage of a gall type shift, there must exist a polymorphism in the population, which seems

more probable if the oviposition site of the different forms is relatively similar. These factors might act as a constraint, making large shifts improbable. However, oviposition occurs during the very early development of the willow shoots when different parts of the leaves are in very close proximity. This could enhance the possibility of "oviposition jumps" that appear more drastic than they really are (Price and Roininen 1993).

The purpose of this study was to test the validity of the Price-Roininen hypothesis. This was done by using enzyme electrophoretic data to reconstruct the phylogeny of selected nematine species representing different gall types. The evolution of the gall types could then be inferred by plotting the gall types on the tree and reconstructing the probable ancestral states.

MATERIALS AND METHODS

Sample Collection and Preservation

Four outgroup and 13 ingroup taxa were chosen for the electrophoretic survey (Table 1). In the final analysis, the number of ingroup taxa was 14 because a sample representing *Phyllocolpa leucapsis* included individuals with fixed differences at two loci, which were consequently split into two species. The ingroup taxa were chosen so that all gall types were represented in the sample. Two species were sampled per gall type, except for basal leaf gallers, for which *Pontania pustulator* was the only representative, and for leaf folders for which the final number of taxa was three as a result of the splitting of *P. leucapsis*.

Adults, prepupae, or larvae collected from galls or leaves were used for electrophoresis. The specimens were collected during the summers of 1989–1994, and stored at −80°C, except for some samples that were initially stored at −20°C. The sample collection dates and sites are summarized in Table 1. For each species, only larvae from one willow species were collected, but the collection was made randomly from several willow clones to get a representative sample of the population. Most species were collected at several sites.

Electrophoresis

The electrophoretic survey was performed with standard starch gel electrophoresis as outlined in Vuorinen (1984). Whole adults or larvae were homogenized in 40 μL sample buffer (Ferguson 1980), and the crude extract was used for electrophoresis. The horizontal 13% starch gels were cut into five 2 mm thick slices and stained for specific enzymes.

Nine enzyme loci were scored (Table 2). Sharing of electromorphs was confirmed by side-by-side comparisons. When allele frequencies were calculated, all individuals were treated as diploid, even though hymenopteran males are haploid (Gauld and Bolton 1988). This should not affect the frequencies, however. Allele frequencies are presented in the Appendix. Exact sample sizes could not be calculated, since the larvae could not be reliably sexed. Sample sizes are presented as minimum numbers of haploid genotypes surveyed (N_{min}); these were calculated by giving a weight of two to all individuals that were heterozygous at at least one locus and a weight of one to all individuals that were homozygous
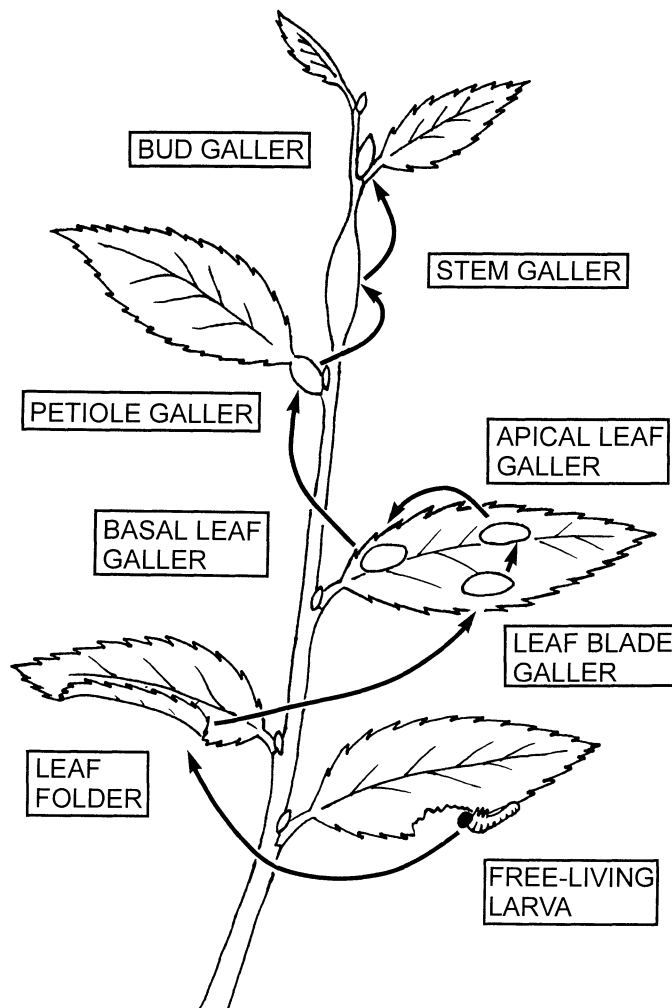

FIG. 1. The Price-Roininen hypothesis of gall type evolution in the nematine sawflies (Price 1992; see also Roininen 1991; Price and Roininen 1993). According to the hypothesis, galling sawflies evolved from species with free-feeding larvae via leaf folders, and new gall types evolved gradually. The result was a trend in galling site toward the more central parts of the host plant.

at all surveyed loci. N_{min} is thus a conservative estimate of the true sample size in each species.

Data Analysis

In the phylogenetic analysis, the loci were treated as characters and allelic combinations as character states, applying step matrices to define the cost of transformations between states (character = locus coding; see Mabee and Humphries 1993; Mardulyn and Pasteels 1994). All detected alleles were included in the study. This is in contrast to, for example, Futuyma and McCafferty (1990), who used a cutoff level of 0.05; alleles occurring with a frequency less than this were coded as absent. Minimum-length trees were found by using PAUP (vers. 3.1.1, Swofford 1993).

Of the nine loci studied, eight were informative in the analysis. Gains and losses were weighted equally, as Mardulyn and Pasteels (1994) recommended. To ensure the finding of the most parsimonious solutions, new (hypothetical

TABLE 1. Species used in the study, their host plants, sample sites, and numbers of individuals sampled.

Gall type[1]	Species	Willow host	Sample sites,[2] year of collection, and number of individuals sampled (in parentheses)
0. Free-living larva (> 200)	*Nematus pavidus* (Lepeltier)	*Salix caprea*	Joensuu (F) 1991 (53), Ruokolahti (F) 1988 (9)
	Nematus salicis (Linnaeus)	*S. fragilis*	Joensuu (F) 1994 (22)
	Pontopristia amentorum (Förster)	*S. phylicifolia*	Joensuu (F) 1994 (5), Parikkala (F) 1994 (24)
	Decanematus viduatus (Zetterstedt)	*S. myrsinifolia*	Parikkala (F) 1994 (23)
1. Leaf fold (11)	*Phyllocolpa puella* (Thomson)	*S. fragilis*	Joensuu (F) 1989 (31), Simpele (F) 1989 (5)
	Phyllocolpa leucapsis (Tischbein) "Phy"	*S. myrsinifolia*	Punkaharju (F) 1990 (11), Parikkala (F) 1994 (11)
	Phyllocolpa leucapsis (Tischbein) "Pon"	*S. myrsinifolia*	Joensuu (F) 1991 (1), 1994 (4), Kerimäki (F) 1990 (5)
2. Leaf blade gall (3–5)	*Pontania bridgmanii* (Cameron)	*S. caprea*	Enonkoski (F) 1991 (6), Joensuu (F) 1991 (14), Parikkala (F) 1994 (18)
	Pontania triandrae (Benson)	*S. triandra*	Tornio (F) 1989 (15)
3. Apical leaf gall (18)	*Pontania arcticornis* (Konow)	*S. phylicifolia*	Björkstugan (S) 1989 (21), Björkliden (S) 1989 (9)
	Pontania aestiva (Thomson)	*S. borealis*	Saana (F) 1989 (17), Tamokdalen (N) 1989 (2), Ivalo (F) 1989 (16)
4. Basal leaf gall (8)	*Pontania pustulator* (Forsius)	*S. phylicifolia*	Joensuu (F) 1991 (10), Kempele (F) 1991 (5)
5. Petiole gall (2–3)	*Euura testaceipes* (Zaddach)	*S. fragilis*	Joensuu (F) 1989 (32)
	Euura venusta (Zaddach)	*S. caprea*	Ruokolahti (F) 1989 (20)
6. Stem gall (4–8)	*Euura lasiolepis* (Smith)	*S. lasiolepis*	Flagstaff (U) 1994 (36)
	Euura atra (Jurine)	*S. alba*	Joensuu (F) 1986 (1), Kaarina (F) 1987 (33)
7. Bud gall (3–11)	*Euura lanatae* (Malaise)	*S. lanata*	Björkliden (S) 1989 (40), Kevo (F) 1989 (2)
	Euura mucronata (Hartig)	*S. lapponum*	Kevo (F) 1989 (41)

[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, since the status of many currently recognized species is uncertain.
[2] F, Finland; N, Norway; S, Sweden; U, USA.

TABLE 2. List of enzymes studied, loci scored, and buffers used for each enzyme.

Enzyme name	E.C. number	Locus	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*Aat-1*[2]	B
		Aat-2	B
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi*	A
Isocitrate dehydrogenase	1.1.1.42	*Idh*	B
Malate dehydrogenase	1.1.1.37	*Mdh-1*[2]	S
		Mdh-2	S
Malic enzyme	1.1.1.40	*Me*	S
Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*[3]	B
Triose phosphate isomerase	5.3.1.1	*Tpi*[3]	A

[1] A = a discontinuous lithium hydroxide-boric acid buffer, pH 8.1 (Ridgway et al. 1970); B = an N-(3-aminopropyl)-morpholine-citrate buffer, pH 6.5 (Clayton and Tretiak 1972); S = a 0.135 M tris-citrate buffer, pH 7.1 (Shaw and Prasad 1970).
[2] Cathodal locus.
[3] Anodal and cathodal alleles.

ancestral) character states were created when needed, as described in Mardulyn and Pasteels (1994). A branch-and-bound search was not feasible because of the computational complexity involved in analyzing step matrix data. Instead, two heuristic searches were performed using different methods (simple addition sequence with TBR branch-swapping, and random addition sequence with 10 replicates and TBR branch-swapping); both produced the same results. A bootstrap analysis (Felsenstein 1985) with 100 replicates and heuristic search was also performed, and the Bremer (1988, 1994) support index was determined for each interior node.

Since the heuristic search does not ensure that the shortest trees are found, an alternative analysis was performed using alleles as characters (character = allele coding; Mickevich and Johnson 1976; Mickevich and Mitter 1981). A branch-and-bound search produced 23 equally parsimonious trees, but all trees were four to seven steps longer than the character = locus trees when evaluated with the character = locus data. These trees were not used for further analyses, because the character = allele approach has been widely criticized (e.g., Swofford and Olsen 1990; Murphy 1993; Meier 1994).

The obtained character = locus trees were rooted by using four taxa with free-living larvae as an outgroup (Table 1). *Nematus pavidus* and *N. salicis* have exophytic larvae that feed on leaves, *Pontopristia amentorum* larvae feed inside willow catkin stems (Viitasaari 1982), and *Decanematus viduatus* larvae initially live inside developing shoot tops, but become free feeders in their later stages of development (Zinovjev and Vikberg 1998). The rooting required paraphyly of the outgroup.

To study the evolution of new gall types, the gall types were plotted on the trees using MacClade (vers. 3.01, Maddison and Maddison 1992). The gall types were coded as unordered multistate characters, that is, all transformations between gall types were considered equally probable.

The trees were also tested against hypothetical trees conveying different evolutionary scenarios. Templeton's (1983; see also Larson 1994) nonparametric test was used to test whether the hypothetical trees are significantly longer than the trees obtained from the phylogenetic analysis.

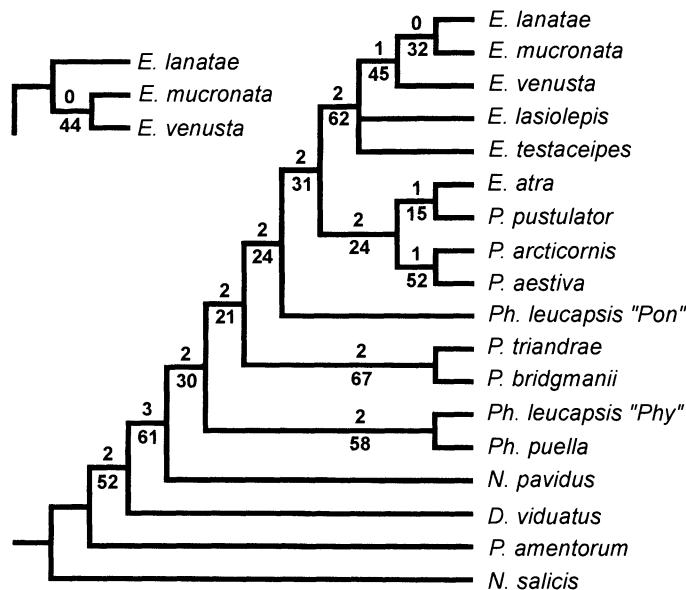Randomization tests (see Liebherr and Hajek 1990) were

FIG. 2. The two equally parsimonious topologies resulting from the character = locus analysis. Numbers above branches are Bremer (1988, 1994) support indices; below branches are the bootstrap proportions from 100 bootstrap replicates. Bremer's (1994) total support index *ti* = 0.26.

used to test whether the observed patterns in gall type evolution are statistically significant. A gall type code was assigned for each species, from 0 (outgroup) to 7 (bud gallers) in the order presented in Table 1. A random dataset was generated by multiplying the original gall types 200 times and shuffling states in the characters (columns) between taxa, but keeping the state of the outgroup species constant (= 0). The length of the original character, when plotted on the trees obtained from the phylogenetic analysis, was then compared with the random character length distribution. The randomization analyses were performed using MacClade (vers. 3.01, Maddison and Maddison 1992).

RESULTS

Trees

The heuristic searches yielded two equally parsimonious solutions for the character = locus data. Both trees (Fig. 2) have a length of 91 steps, and they differ only in the relative position of *Euura mucronata*. Both trees contain a polytomy in the *Euura* (all *Euura* species except *E. atra*) clade. *Euura atra* is included in the *Pontania* clade (*E. atra* plus all *Pontania* species, except for the leaf blade gallers *P. bridgmanii* and *P. triandrae*). Species representing the same gall types generally are monophyletic, and the trees could be rooted so that the ingroup remains monophyletic. Bootstrap proportions were generally low, and Bremer support indices range from one to three (Fig. 2). Bremer's (1994) total support index *ti* = 0.26.

Optimization of Gall Types on the Trees

The polytomy in both character = locus trees was resolved in all possible (three) ways, which led to six optional tree

topologies. To find all possible solutions, the gall types were plotted on each tree using MacClade (Maddison and Maddison 1992), and three optimization options were used (Acctran, Deltran, and show all most parsimonious reconstructions).

The different options resulted in 18 alternative reconstructions. All reconstructions agreed that the first taxa to evolve from nongalling species were leaf-folding *Phyllocolpa* species. Leaf blade galling *Pontania* species evolved independently of the other (leaf galling) *Pontania* species, which may or may not have had a common galling ancestor with the *Euura* group. The alternative reconstructions show little agreement about the most probable gall type at the base of the *Euura-Pontania* clade.

Testing Alternative Trees

The shortest possible tree that would strictly support the Price-Roininen hypothesis has a length of 101 steps, when evaluated with the character = locus data. It is significantly less parsimonious than the character = locus trees ($T_s = 2$, $n = 6$, one-tailed $P = 0.0469$; Rohlf and Sokal 1981).

Similarly, the 32 shortest possible trees that resulted from a phylogenetic analysis where the ingroup species from the same willow host species were forced to be monophyletic are significantly longer than the original trees (100 steps; $T_s = 0$, $n = 5$, one-tailed $P = 0.0312$).

If the ingroup monophyly is disrupted by moving the *Euura* clade to the other side of the outgroup (the galling habit is polyphyletic), the tree has a length of 100 steps ($T_s = 0$, $n = 5$, one-tailed $P = 0.0312$). If the bud gallers are moved to the base of the ingroup, tree length equals 102 steps ($T_s = 0$, $n = 5$, one-tailed $P = 0.0312$). If the *Phyllocolpa* species are moved into the outgroup, so that galling arises directly from nongallers, the trees are at least 100 steps long ($T_s = 2.5$, $n = 7$, one-tailed $P < 0.0391$). Thus, all tested scenarios are statistically significantly less parsimonious than the trees obtained from the phylogenetic analysis (Fig. 2).

Randomization Tests

The purpose of the first randomization test was to ask if gall types change often relative to speciation. If this was the case, gall types would become effectively randomized between taxa on the trees. The six observed trees were tested separately, and the gall types were treated as unordered. The trees require seven to eight gall type changes, and with all six trees the 200 gall type replicates with randomized states required more steps ($P < 0.005$). This shows that gall type is a relatively conservative character. Figure 3 presents the character length distribution of the tree in Figure 4.

The second test was used to see if the gall types change in a pattern that, although not identical, is closer to the Price-Roininen hypothesis than expected by random. Gall types were coded as ordered and gall types were shuffled between groups, while keeping the groups (species representing the same gall type) themselves homogeneous. The six trees required 12 to 14 steps when the gall types were ordered. In the random dataset, 0.5–5.0% of the characters were of the same length or shorter than the original character ($P = 0.005$–0.05). Figure 3 shows the length distribution of the random-
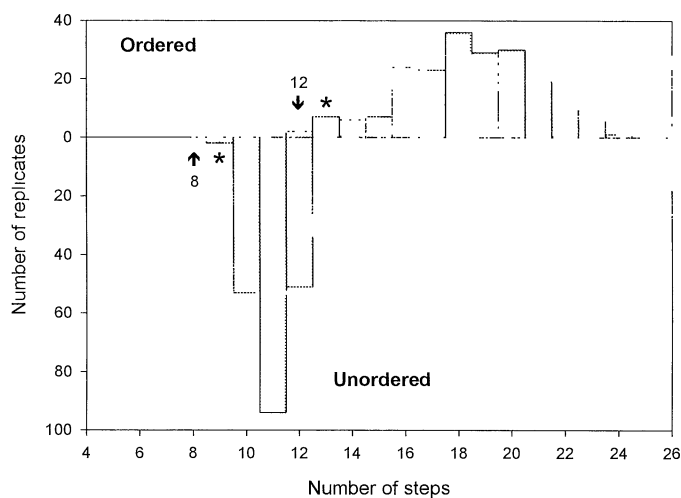
FIG. 3. Length distributions for the 200 replicates of the original gall types, when states have been randomized, and the tree is identical with that in Figure 4. The distribution when the gall types are ordered and states are shuffled between groups (but groups are kept homogeneous) is above the line. The distribution when gall types are unordered and states are randomized among all ingroup taxa is below the line. Lengths of the original gall type characters are shown by arrow with number of steps indicated; alpha < 0.05 level shown by star.

ized characters on the tree in Figure 4. The result of this test shows that gall type switches have occurred in small steps, and that there has been a trend from the leaf edge toward the more central parts of the host plant.

DISCUSSION

When considering gall type evolution in the galling sawflies, two main questions arise: (1) what is the ratio of host shifts compared to evolution of new gall types; and (2) in what sequence have the gall types evolved? If gall type shifts are more common than host plant shifts, species representing a certain gall type would not be monophyletic and, most likely, it would be impossible to infer a distinct sequence in gall type evolution.

Our phylogenetic analysis and the result from the randomization test clearly show that host shifts are more common than the evolution of new gall types. Most groups that were hypothesized to belong together based on gall morphology and location also remained together on the trees obtained from the phylogenetic analysis, although the species occur on different willow species (Fig. 4). The only notable exception is the polyphyly of the stem gallers (*Euura lasiolepis* and *E. atra*). The result is similar to Stern's (1995) finding that in cerataphidine aphids the insects, not their host plants, determine gall morphology. The main question thus becomes, what is the sequence in which the gall types evolved?

The phylogenies obtained from the character = locus analysis support the view that the galling nematines are a monophyletic group with respect to species with free-living larvae. All reconstructions agree that the first species to evolve were the leaf-folding *Phyllocolpa* species. This is in concordance with Smith's (1970) notion that the larvae of *Phyllocolpa* resemble those of the free-feeding *Nematus* species in form

and coloration. The next species to appear would be the leaf blade galling *Pontania*, which, in contrast to the Price-Roininen hypothesis, are not the ancestors of apical and basal leaf-galling *Pontania* species, but are instead of independent origin. Thus, true closed galls apparently evolved independently at least twice. It should be noted that Zinovjev (1993) classifies the *Pontania* leaf blade gallers in the subgenus *Pontania* s. str., which includes both gallers and some leaf rollers; apical and basal leaf gallers belong to the subgenus *Eupontania*, which according to Zinovjev (1993) resemble *Euura* (e.g., by mandible morphology).

The *Euura-Pontania* clade is problematic because of the two equally parsimonious resolutions in the *Euura* clade, and the different options for resolving the polytomy. The most realistic option is probably a tree in which the bud gallers *E. lanatae* and *E. mucronata* are considered to be sister taxa, and *E. lasiolepis* an offshoot from petiole gallers (Fig. 4). Thus, stem gallers would have evolved twice: in the *Euura* group from midrib/petiole gallers and in the *Pontania* clade from leaf gallers. Both *E. lasiolepis* and *E. atra* are currently classified together in the *Euura* (*Euura*) *lasiolepis* group, based on ovipositor shape and pupation in the gall; these features may have been independently derived as a result of convergent galling habits.

An interesting fact is that *E. testaceipes*, which galls midribs and petioles (Price and Roininen 1993), is closer to the *Pontania* clade than *E. venusta*, which galls only petioles (Price and Roininen 1993). This is in concordance with the Price-Roininen hypothesis. The bud galling *Euura* (*Gemmura*) group has clearly evolved late, and thus Smith's (1968, 1970) suggestion that they are intermediates between *Pontania* and *Euura* gallers is incorrect. Smith based his view on similarities in ovipositor shape and pupation on the ground. However, as Price and Roininen (1993) pointed out, the larvae of the petiole galling *Euura* (*Euura*) species also pupate in the soil, and the *Euura* (*Euura*) group is closest to leaf-galling *Pontania* species in oviposition behavior, a trait that can be presumed to be quite complex and slow changing (Price and Roininen 1993).

The ambiguity at the base of the *Euura-Pontania* clade is partly due to the unordered coding method, which assumes all transitions between gall types to be equally probable. Although this assumption minimizes the possibility of circular reasoning, it is not necessarily realistic. The reconstruction in Figure 4 presents the Acctran optimization of an option in which apical and basal leaf gallers are considered to be the same group, which is equivalent to giving the transition between apical and basal leaf gallers a much smaller cost (greater probability) than for the transition between other gall types. This can be justified on the basis that the oviposition behavior of apical and basal leaf gallers is relatively similar to each other. The most probable evolutionary sequence would thus be: (1) evolution of *Phyllocolpa* leaf folders from species with free-living larvae; (2) leaf blade galling *Pontania* species evolve from *Phyllocolpa* ancestors; (3) the common ancestor for (apical/basal) leaf galling *Pontania* and *Euura* species evolves; (4) stem gallers evolve independently from *Pontania* leaf gallers and *Euura* midrib/petiole gallers; and (5) *Euura* (*Gemmura*) bud gallers evolve directly from midrib/petiole gallers, and thus not from stem gallers as the Price-
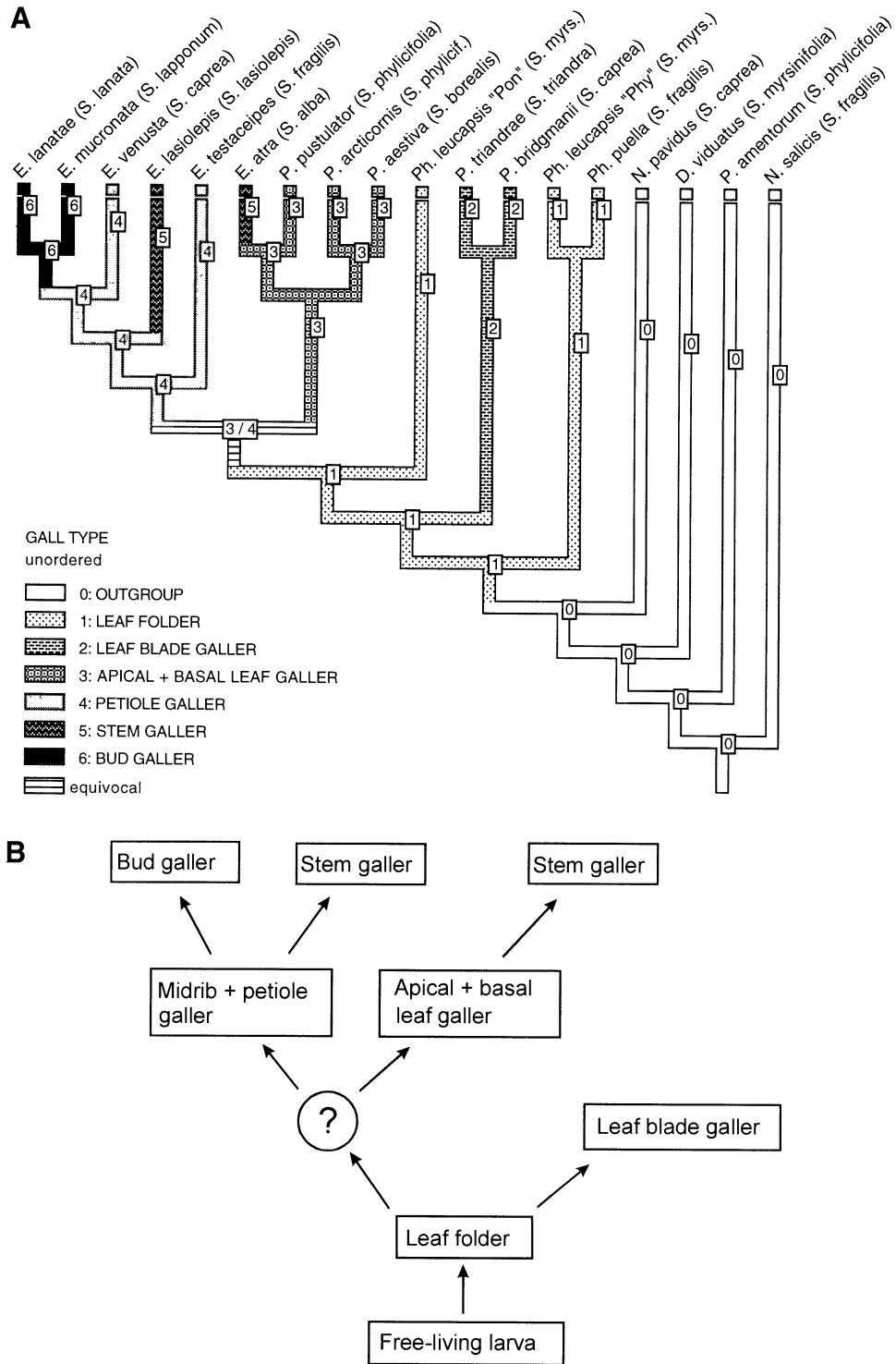
FIG. 4. (A) The preferred phylogeny of the six most parsimonious topologies. The species' host plants are in parentheses. (B) The resulting hypothesis about the sequence in which new gall types evolved.

Roininen hypothesis predicts. Although it is possible that *Pontania* leaf gallers and *Euura* midrib/petiole gallers evolved independently from leaf folders, it is more realistic to assume that they share a common galling ancestor.

Although the dataset in this study is relatively small and the bootstrap analysis yielded low bootstrap proportions for

many groupings, several groups are supported by the beforementioned morphological traits (Smith 1968, 1970; Zinovjev 1993; Zinovjev and Vikberg 1998). Thus, the general outline of the obtained phylogenies should be correct.

The most important implication of the phylogeny is that there is a statistically significant evolutionary trend toward

oviposition in the more basal parts of the growing shoot. If the Price-Roininen hypothesis was correct, there would have been seven gall type transitions during the evolution from outgroup to bud gallers. If the gall types are coded as ordered, the obtained trees require 12 to 13 gall type changes, which is still less than expected by a random process. Since the original state is known (0, i.e., free-living larvae), this shows that the evolution of new gall types occurred in small steps and in the general direction suggested by the Price-Roininen hypothesis.

The gradual evolution of gall types is probably a result of genetical constraints in the oviposition behavior system. Since oviposition and gall formation is probably under the control of coadapted multilocus genetic systems, large shifts are unlikely. In the initial stage of a gall type shift, there must exist a polymorphic condition in the population, which is more probable if the gall types are relatively similar, as in *Euura testaceipes*.

Possible reasons for the observed directionality could include, for example, better control of the flow of nutrients and photosynthetic products, or greater cost of defense reactions for the host, when galls are situated more basally on the leaf. The trend could also be a consequence of intraspecific competition, if there is a "shading effect" in which galls situated closer to the main vascular system block nutrient flow to galls situated in more peripheral parts of the host plant. The effect could be important in *Pontania* species, where one leaf may have many galls (Price et al. 1994). The shading effect has been observed in *Pemphigus* gall aphids (Whitham 1978), but it has not been thoroughly studied in sawflies. Studies on interspecific competition could give an answer, but thus far they have produced ambiguous results (e.g., Fritz et al. 1986; Fritz and Price 1990).

Although evolutionary trends and biases in transition probabilities are often proposed, few cases have been documented. Janson (1992) found unequal transition probabilities between seed dispersal syndromes in Neotropical plants. In the leaf beetle genus *Ophraella*, host shifts are partially constrained by lack of genetical variation (Futuyma et al. 1993, 1995). Liebherr and Hajek (1990) studied habitat preference of carabid beetles, and in only one case (of eight) they found a statistically significant trend. The trend observed in the evolution of the galling nematines is thus interesting, but further studies are needed to gain a better understanding of the genealogical relationships and gall type evolution in the galling nematines.

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APPENDIX

Allele frequencies observed in the studied species, and minimum numbers of haploid genotypes surveyed (N_{min}; see text for explanation) in each locus. Abbreviations: first three letters from generic name plus first three letters from species name, except PPRAME, Pontopristia amentorum. Complete species names are shown in Table 1.

Locus	Allele	NEM PAV	NEM SAL	PPR AME	DEC VID	PHY PUE	PHY LEU "Phy"	PHY LEU "Pon"	PON BRI	PON TRI	PON ARC	PON AES	PON PUS	EUU TES	EUU VEN	EUU LAS	EUU ATR	EUU LAN	EUU MUC
Aat-1	(N_{min})	64	24	41	23	35	4	10	27	6	36	51	26	31	19	5	2	54	43
	−85	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	−89	0.02	1	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0
	−97	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	−100	0.98	0	0	0	1	1	1	1	1	0	0	0	1	1	1	0	1	1
Aat-2	(N_{min})	85	24	29	23	51	23	12	39	15	36	53	54	32	29	20	24	61	44
	113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	110	0	1	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
	100	0	0	0	0	0	0	0	0	0	1	0.97	0.96	0	0	0	0	0.93	0.69
	87	0	0	0	1	0	1	0	1	0	0	0.03	0.04	0	0	0	0	0	0
	85	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	61	0.73	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0.07	0.31
Pgi	(N_{min})	73	24	48	73	51	23	12	39	15	39	44	54	32	29	36	39	61	44
	113	0	0	0	0	0	0	0.20	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	87	0	1	0.64	1	0	1	0.70	1	1	1	1	1	1	1	1	1	1	1
	65	0	0	0.36	0	1	0	0.10	0	0	0	0	0	0	0	0	0	0	0
	53	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	36	0.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idh	(N_{min})	82	24	49	23	50	22	12	39	15	39	53	54	32	29	36	33	61	44
	111	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0.42	0	0	0	0	0	0.96	0	0	0	0	0	0	0.48
	92	0	1	0	1	0	0	0	0	1	1	0	1	1	1	1	1	1	0.36
	72	0	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0
	55	0	0	1	0	0.58	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.16
Me	(N_{min})	90	23	45	23	51	19	12	30	15	38	52	27	32	28	36	29	61	44
	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	133	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0.03	1	0	0	0	0.71	0	0	0	0	0.20	0.24	0.07
	86	1	0	0	0	0	0.97	0	0	0	0	0.29	0	0	0	0	0.76	0.69	0.93
	80	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
	70	0	1	0	1	0	0	0	1	1	1	0	1	1	1	0	0.04	0.07	0
	60	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mdh-1	(N_{min})	89	24	45	23	51	23	12	39	15	39	53	54	32	29	36	41	61	44
	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mdh-2	(N_{min})	89	24	46	23	51	23	12	39	15	39	53	54	32	29	36	35	61	44
	312	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	260	0	0	0	0	0	0	0	0	0	0.23	0	0	0	0	0	0	0	0
	206	0	0	0	0	0	0	0	0	0	0.77	0	0	0	0	0	0	0	0
	168	0	1	1	0.96	0	0	0	1	0	0	0.93	1	0	0	0	0	0	0
	164	0	0	0	0.04	0	0	0	0	0	0	0.07	0	0	0	0	0	0	0
	156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	0
	92	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0.33
	80	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0.67
	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

APPENDIX. Continued.

Locus	Allele	NEM PAV	NEM SAL	PPR AME	DEC VID	PHY PUE	PHY LEU "Phy"	PHY LEU "Pon"	PON BRI	PON TRI	PON ARC	PON AES	PON PUS	EUU TES	EUU VEN	EUU LAS	EUU ATR	EUU LAN	EUU MUC
Pgdh	(N_{min})	66	24	31	23	46	22	12	38	15	32	44	54	32	29	36	25	55	44
	250	0	0	0	0	0	0	0	0	0	0	0.19	0	0	0	0	0	0	0
	200	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0
	150	0	0	0	0	0	0	0	0	0	0	0.73	0	0	0	0	0	0	0
	147	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	1	0	1	1	1	1	0.07	1	1	1	1	1	1	1
	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0.23	0	0	0	0	0.93	0	0	0	0	0	0	0	0	0	0	0	0
	25	0.77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	−27	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	−50	0	0	0	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0
Tpi	(N_{min})	69	24	44	23	51	23	12	39	15	39	44	53	32	29	32	39	55	43
	100	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0.29	1	1
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.71	0	0
	26	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	−38	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0