

EVOLUTION OF THE GALL WASP–HOST PLANT ASSOCIATION

FREDRIK RONQUIST^{1,2} AND JOHAN LJLJEBLAD^{3,4}

¹Department of Systematic Zoology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden

²E-mail: fredrik.ronquist@ebc.uu.se

³Department of Zoology, University of Stockholm, SE-106 91 Stockholm, Sweden

⁴Department of Entomology, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden

Abstract.—Gall wasps, or cynipids, form the second largest radiation of galling insects with more than 1300 described species. According to current views, the first cynipids were phytophagous and developed in herb stems of the Asteraceae without modifying plant growth or development. The first galls were supposedly multichambered stem swellings, and subsequent trends involved increase in gall complexity and reduction in the number of larval chambers. Gall wasps also have many of the features believed to be characteristic for phytophagous insects radiating in parallel with their host plants. We tested these hypotheses by mapping characters onto a recent estimate of higher cynipid relationships from a morphology-based analysis of exemplar taxa, controlling for phylogenetic uncertainty using bootstrapping. Characters were also mapped onto a metatree including all gall wasps, assembled from phylogenetic analyses as well as recent classifications. The results contradict many of the current hypotheses. The first cynipids with extant descendants were not Asteraceae stem feeders but induced distinct single-chambered galls in reproductive organs of herbaceous Papaveraceae, or possibly Lamiaceae. There has been a general trend toward more complex galls but the herb-stem feeders evolved from ancestors inducing distinct galls and their larval chambers are best understood as cryptic galls. Woody hosts have been colonized only three times, making the apparently irreversible transition from herbs to woody hosts one of the most conservative features of the gall wasp–host plant association. The evolution of host plant preferences is characterized by colonization of preexisting host-plant lineages rather than by parallel cladogenesis. Cynipids are mono- or oligophagous and host-plant choice is strongly phylogenetically conserved. Yet, the few major host shifts have involved remarkably distantly related plants. Many shifts have been onto plant species already exploited by other gall wasps, suggesting that interspecific parasitism among cynipids facilitates colonization of novel host plants.

Key words.—Coevolution, galls, host shifts, insect-plant relationships, parasitism, phylogeny.

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With 1300 described species (Liljebblad and Ronquist 1998; Ronquist 1999), the gall wasps (Cynipidae) constitute the second largest radiation of galling insects after the gall midges, and some of the most complex and well-organized galls are induced by gall wasps (Cornell 1983). The most familiar cynipid galls are those found on roses and oaks, but there is also a significant number of herb-galling cynipids (Fig. 1a). Among the herbs, plants in the Asteraceae, Lamiaceae, Rosaceae, and Papaveraceae are particularly favored as hosts (Fig. 1b).

Cynipid galls are found on all plant organs (i.e., flowers, leaves, buds, stems, twigs, and roots) and may contain from one to more than 100 larval chambers. The larva feeds on a layer of particularly nutritious plant cells lining its chamber (Bronner 1992; Rohfritsch 1992). A hard layer of sclerenchyma, presumably serving to protect the larva from natural enemies, encloses this nutritive tissue and externally delimits what is referred to as the inner gall. Outside the sclerenchyma is a zone of parenchyma cells supplying the inner gall with water and nutrients. Externally, many galls are covered with specialized hairs or other structures foreign to the attacked plant organ (Figs. 2a, b) but characteristic of the gall-inducing species. Many of these structures are readily interpretable as devices by which the gall wasp protects itself against natural enemies, particularly parasitic wasps that oviposit into the gall (Cornell 1983). Even when complex surface structures are absent, the gall usually causes conspicuous swelling of the attacked plant tissue (Figs. 2c, d). However, there are also some cynipid species whose larvae develop inside stems or twigs, usually of herbs, without causing any visible external deformation of the plant (Fig. 2e).

About 200 cynipid species do not induce galls; instead, their larvae feed on the gall tissue inside the galls of other cynipids. These so-called *inquilines* form a monophyletic group, which is deeply nested among gall-inducing cynipid lineages (Ronquist 1994). The inquilines are gallers that have lost the ability to initiate gall development but retain a remarkable capacity of modifying the host gall (Shorthouse 1980; Brooks and Shorthouse 1998). The inquilines mainly attack rose and oak gallers but also are found in other galls on woody host plants.

Alfred Kinsey, one of the founders of the Society for the Study of Evolution, devoted the early part of his scientific career to the study of the systematics and evolution of gall wasps (Kinsey 1919, 1920, 1923, 1930, 1936, 1937). In a pioneering paper (Kinsey 1920), he presented the first phylogenetic analysis of higher gall-wasp relationships, based on three morphological and seven biological features, long before the advent of cladistic methodology. Kinsey considered oak and rose gallers to be derived from herb-galling lineages and thought that the first cynipids were “plant-tissue inhabiting, not gall-making insects” (Kinsey 1920, p. 400). These early cynipids left extant descendants in the genera *Aulacidea* and *Phanacis*, which develop inside the stems of various herbs belonging to the Asteraceae, sometimes without causing external deformation of the plant (Fig. 2e) and sometimes causing conspicuous, multichambered stem swellings. The subsequent evolution of cynipid galls supposedly involved irreversible trends from structurally simple to complex galls, from multi- to single-chambered galls, and from integral to detachable galls (Kinsey 1920). Other early work-

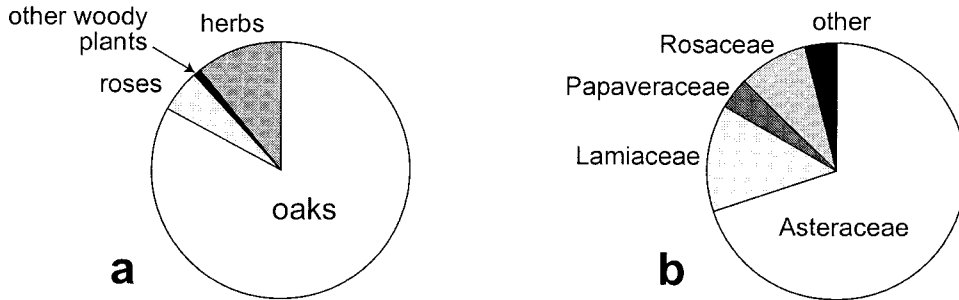


FIG. 1. The proportion of gall wasp species attacking different types of plants (a), and the proportion of herb-galling species attacking plants in different families (b).

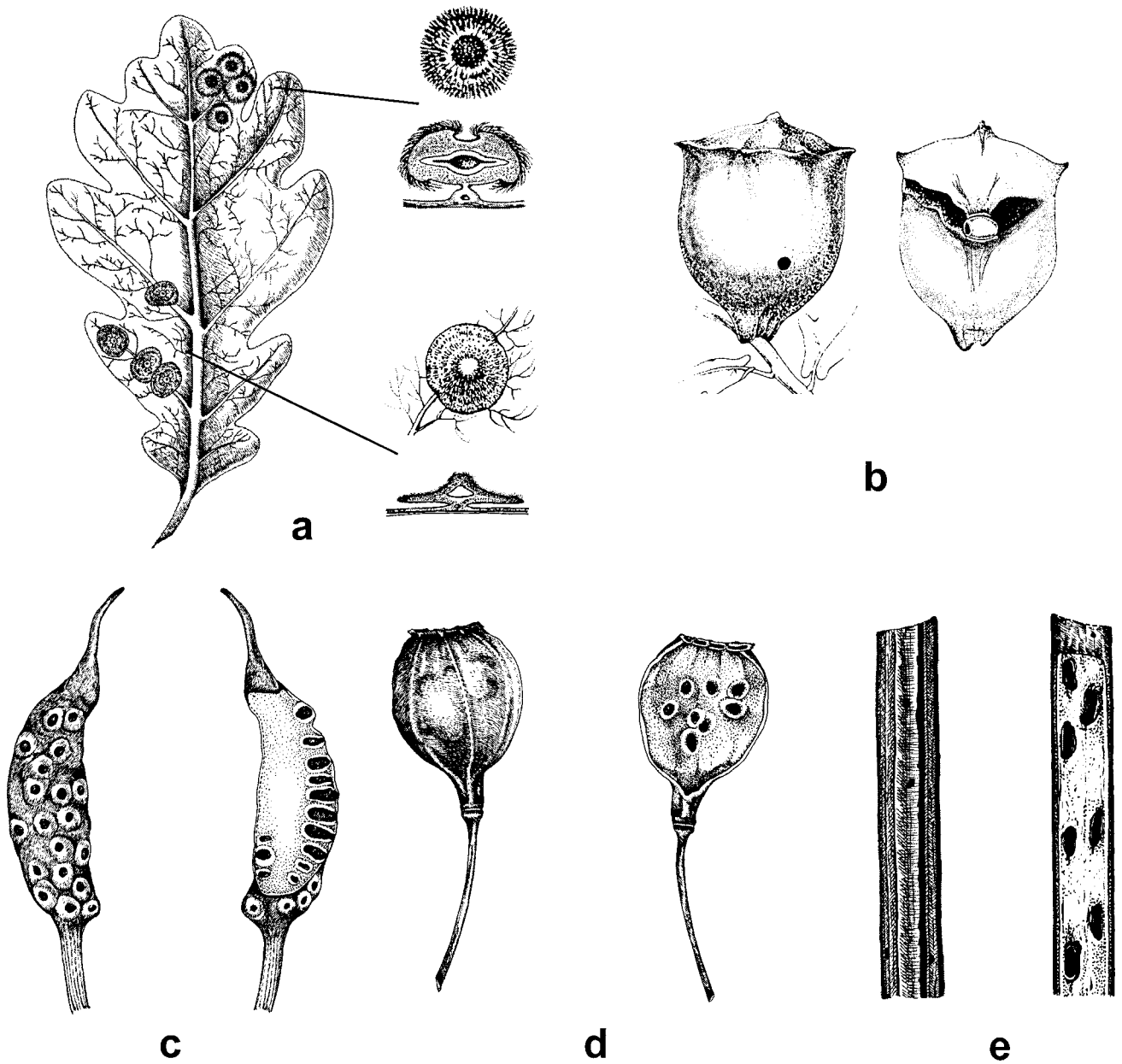


FIG. 2. Different types of cynipid galls: (a) complex, detachable, single-chambered leaf galls (top: *Neuroterus numismalis*, bottom: *N. quercusbaccarum*, both on *Quercus*); (b) complex, detachable, single-chambered bud gall (*Andricus quercustozae* on *Quercus*); (c) simple, integral, multichambered twig gall (*Diastrophus rubi* on *Rubus*); (d) simple, integral, multichambered gall in seed capsule (*Aylax papaveris* on *Papaver*); (e) single-chambered stem gall causing no external deformation of the plant (*Phanacis lucidulus* on *Centaurea*).

ers similarly speculated on the first cynipid galls being multichambered stem swellings, and subsequent evolution leading to an increase in gall complexity (Cockerell 1890; Wells 1921). Kinsey did not explicitly speculate on the geographical origin of cynipids, but the species he considered most similar to the first cynipids are all Nearctic species of the genus *Aulacidea* (Kinsey 1920).

Malyshev (1968) is the only worker that has seriously challenged Kinsey's ideas. Malyshev argued that the first cynipids were more likely to have been associated with oaks than with Asteraceae, because the latter represent a more recent radiation, and proposed that the gallers evolved from seed rather than stem feeders, the first galls being induced in reproductive buds or developing seeds. However, most subsequent authors have accepted Kinsey's rather than Malyshev's scenario as being more probable. For instance, Roskam (1992) argued that an ancestral association with the Asteraceae is possible because the gall wasps constitute a recent radiation, consistent with Kinsey's view that cynipids are not much older than the Oligocene (Kinsey 1919). It is not until recently, however, that modern phylogenetic analyses of higher gall wasp relationships (Ronquist 1994; Liljeblad and Ronquist 1998) have made it possible to rigorously test Kinsey's and Malyshev's scenarios.

The past decade has seen an explosion in the number of empirical studies of phylogenetic patterns in the evolution of insect-plant associations. Gall wasps are interesting in this more general context for several reasons. The host-plant record of cynipids is exceptionally complete because the adult specimens are usually obtained through rearing: Host-plant records are currently lacking for only 28 of 1366 described species. Also, there is the possibility of parallel cladogenesis between gall wasps and their host plants. An emerging consensus from empirical studies is that only a few insect-plant associations show significant patterns of insect-plant codivergence. Factors that are believed to favor such patterns include: (1) intimate dependence on the host plant; (2) monophagy; (3) internal feeding, particularly leaf mining or galling; (4) sedentary adults feeding on the same host plant as the larvae or not feeding at all; (5) long-lived host plants; (6) intense competition in the insects for limited plant resources; and (7) use of host-derived toxins in the defense system of the insects (Mitter and Farrell 1991; Crespi et al. 1997; Farrell and Mitter 1998; Berenbaum and Passoa 1999). Gall wasps fulfill most of these criteria. They are intimately dependent on their host plant because of the intricacies of gall induction, they usually attack only one or a few closely related plant species (only nine cynipid species—less than 1%—have been reported from more than one host plant genus, and some of these records may be due to erroneous determination of the host plant), they feed internally, the females presumably often oviposit into the same plant individual they galled as larvae (this is particularly evident in some wingless oak gall wasps), most of their host plants are long-lived (this is certainly true for oaks and roses, but most of the attacked herbs are also perennial), and successful gall induction appears to be dependent on oviposition into the right plant tissue at the right time, circumstances that may lead to intense competition. Indeed, fighting between ovipositing females has been observed in one species of *Dias-*

trophus (Pujade i Villar 1984) and may occur more widely in cynipids. Considering their biology, gall wasps are prime candidates for cospeciation with their host plants.

Gall wasps are also an interesting group with respect to the influence of plant secondary compounds on insect host-plant preferences. Many workers argue that plant chemistry is more important than plant relationships as a determinant of macroevolutionary patterns in insect-plant associations, particularly in dietary specialists (e.g., Ehrlich and Raven 1964; Mitter and Farrell 1991; Miller 1992; Menken 1996; Becerra 1997; Janz and Nylin 1998; Becerra and Venable 1999). Secondary plant metabolites may shape both the frequency and nature of insect shifts between host plants. For instance, the fact that tree-feeding butterflies more readily colonize new host plants than herb-feeding butterflies has been attributed to qualitative differences in defensive compounds between herbs and trees (Janz and Nylin 1998). Gall wasps differ from most other phytophagous insects in that their larvae feed on plant tissue (the nutritive layer of the gall), which is devoid of secondary compounds (Cornell 1983; Price et al. 1987). Thus, gall wasps may provide an important control group in tests of hypotheses concerning the role of secondary plant chemistry in shaping the evolution of host-plant preferences in phytophagous insects.

The phylogenesis of the gall wasp–host plant association has been studied previously in two distantly related cynipid genera, *Isocolus* (herb gallers of *Centaurea*, Asteraceae; Baumann and Brandl 1993) and *Andricus* (oak gallers; Stone and Cook 1998). Here, we address larger-scale patterns in the evolution of the entire gall-wasp family by mapping biological and distribution characters onto a recent estimate of higher cynipid relationships based on analysis of an extensive morphological dataset (Liljeblad and Ronquist 1998). Because this estimate was associated with considerable uncertainty concerning basal cynipid relationships and the ancestral cynipid state was essential in testing several of the hypotheses, we used bootstrapping to control for the effect of phylogenetic error on our character mappings.

MATERIALS AND METHODS

Estimates of Phylogenetic Relationships

We used two different trees for inferring cynipid evolution, both derived from the recent analysis of higher-level gall wasp relationships by Liljeblad and Ronquist (1998). This analysis was based on 164 morphological and two biological characters, coded for 37 exemplar species representing almost all genera in all cynipid tribes except the large and species-rich Cynipini (i.e., the oak gall wasps), which is likely to be a monophyletic group (Kinsey 1920; Roskam 1992; Ronquist 1994; Liljeblad and Ronquist 1998).

One of our trees, the exemplar tree (Fig. 3b), was based on the character matrix of Liljeblad and Ronquist (1998) complemented with the nine biological and distributional characters examined here (Table 1; the host-plant family character was already used in the original analysis). We also added a single morphological character, the presence or absence of a basal articulation in the female ovipositor. This is one of the primary synapomorphies for the Figitidae, the sister group of the gall wasps (Ronquist 1999). It was not

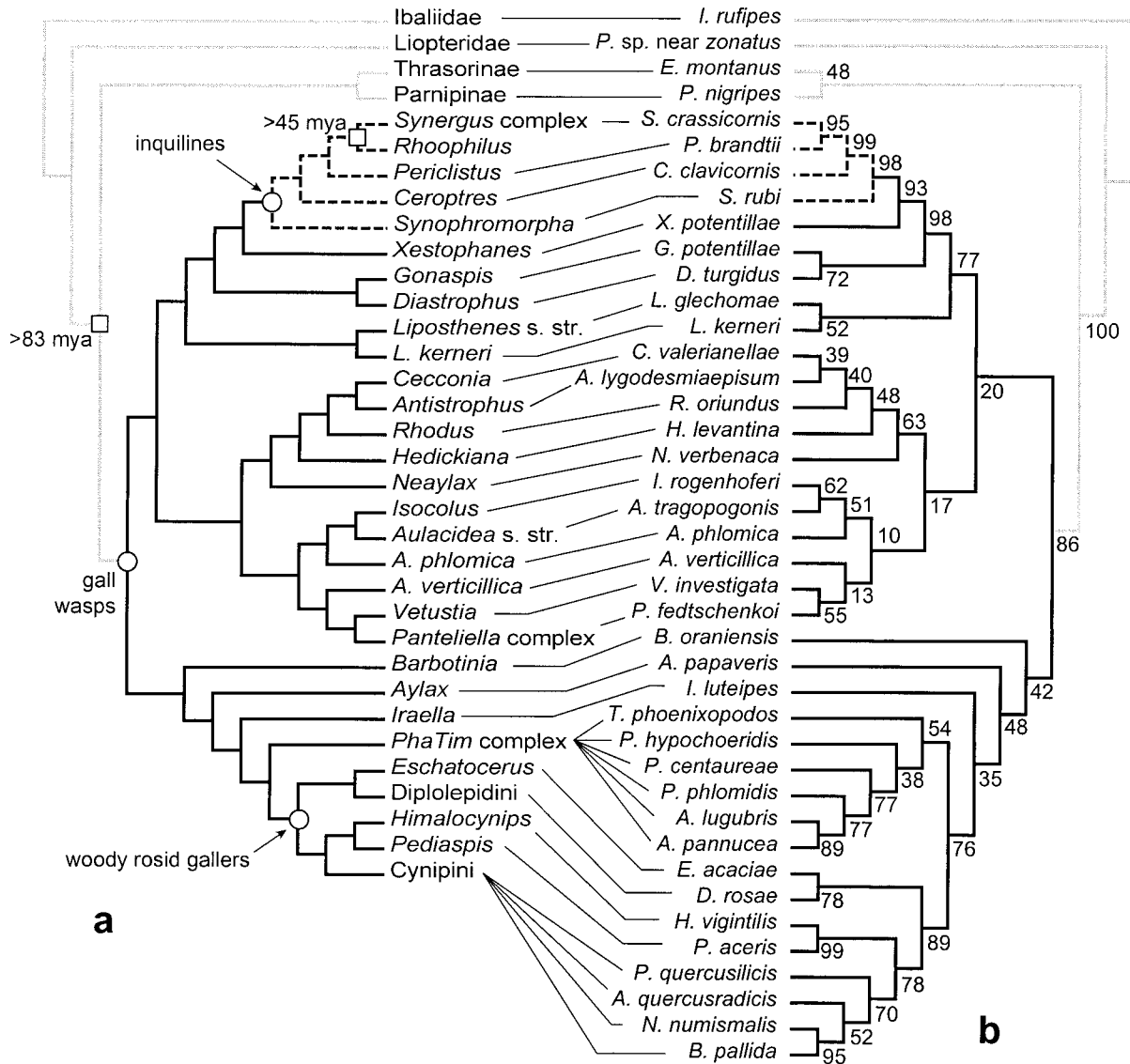


FIG. 3. Two phylogenetic trees used in mapping gall wasp traits. The gall wasp part of both trees is bold (drawn in black) with the inquiline portion dashed. Two important datings based on fossil evidence (Ronquist 1999) are indicated in the left tree. The metatree (a) was derived from the exemplar tree by adding all the remaining cynipid species according to the best estimate of their phylogenetic position. The resultant tree included all 1378 described cynipid species but was only resolved basally; several of the terminal clades represented unresolved polytomies of a large number of species, particularly the oak galls (Cynipini, 977 described species) and the rose galls (Diplolepidini, 53 species). The metatree allowed us to reconstruct overall patterns in the evolution of the Cynipidae and control for the effect of taxon sampling in the exemplar analysis. The exemplar tree (b) is based on an analysis of 175 morphological and biological characters (shortest tree from multiple random addition sequences with bootstrap support values on branches). Note the poor support for many basal branches in the gall wasp part of the tree. A set of bootstrap trees generated from this dataset was used in assessing the influence of uncertainty concerning cynipid relationships on the evolutionary inferences.

included in the analysis of Liljebblad and Ronquist (1998) because the state of *Parnips* was unknown at the time, rendering the character uninformative in the context of the taxon sample. It has since been shown that the articulation is present in both *Parnips* and *Euceroptres* (Ronquist 1999). The articulation is known to be absent in all the remaining taxa in the exemplar tree except that the state is still unknown for some rare cynipids (the species coded with question mark in character 159 in Liljebblad and Ronquist [1998]).

The resultant character matrix (available via www.ebc.uu.se/systzoo/staff/ronquist.html) and TreeBASE ([\[harvard.edu/treebase\]\(http://harvard.edu/treebase\); accession numbers S648, M1017\), had 175 characters and was analyzed with PAUP 4.0β4a \(Swofford 1998\). All the characters added to the original matrix were treated as independent and unordered \(nonadditive\) in the total-evidence analysis. The shortest tree \(hit 100 times in 100 random addition-TBR searches\) from the total-evidence analysis was identical to the shortest tree in the original analysis \(Liljebblad and Ronquist 1998\). Bootstrap support values were calculated based on 10 random addition-TBR searches on each of 1000 replicates of the dataset \(Fig. 3b\). The exemplar tree allowed us to assess the degree to which uncertainty in the phy-](http://www.herbaria.</p>
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TABLE 1. Biological data traced on the supertree and on the exemplar tree. Unless the exemplar species is entered in the table, it has the same state as the terminal taxon it represents or the underlined state of the terminal taxon if the latter is polymorphic.

		Plant					Gall ²		
Taxon	Species	Distribution ¹	Family	Genus	Form	Structure	Position	Chambers	Attachment
Ingroup (Cynipidae)									
<i>Synergus</i> complex†	125	Eur, NA, EP	Fagaceae	<i>Quercus</i>	woody	—	—	—	—
<i>Rhoophilus</i>	1	S, Africa	Anacardiaceae	<i>Rhus</i>	woody	—	—	—	—
<i>Periclistus</i>	17	NA, EP, Eur	Rosaceae + ³	<i>Rosa</i>	woody	—	—	—	—
<i>Ceroptres</i>	25	NA, EP, Eur	Smilacaceae	<i>Smilax</i>	woody	—	—	—	—
<i>Synophromorpha</i>	5	NA, EP	Fagaceae	<i>Quercus</i>	woody	—	—	—	—
<i>Xestophtanes</i>	4	NA, EP	Rosaceae	<i>Rubus</i>	woody	—	—	—	—
<i>Gonaspis</i>	1	Europe	Rosaceae	<i>Potentilla</i> + ⁴	herb	swelling	stem	single & many	integral
<i>Diastrophus</i>	8	North America	Rosaceae	<i>Potentilla</i>	herb	swelling	stem	single	integral
	7	NA, Europe	Rosaceae	<i>Rubus</i>	woody	swelling	stem	many	integral
	1	North America	Rosaceae	<i>Potentilla</i> + ⁵	herb	swelling	stem	many	integral
<i>Liposthenes</i> s. str.	3	Europe, EP	Smilacaceae	<i>Smilax</i>	woody	swelling	stem	many	integral
<i>L. kernerii</i>	1	Europe	Lamiaceae	<i>Nepeta</i>	herb	swelling	leaf	many	integral
<i>Cecconia</i>	2	Europe	Lamiaceae	<i>Nepeta</i>	herb	swelling	inflorescence	single	detachable
<i>Antistrophus</i>	8	N. America	Valerianaceae	<i>Valerianella</i>	herb	swelling & cryptic	inflorescence	single	detachable
			Asteraceae	<i>Stiphium</i> + ⁶	herb	swelling & cryptic	stem & inflorescence	many	integral
<i>Rhodus</i>	1	Europe	Lamiaceae	<i>Sabia</i> + ⁷	herb	swelling	bud	many	integral
<i>Hedickiana</i>	1	Europe	Lamiaceae	<i>Sabia</i>	herb	swelling	stem	many	integral
<i>Neaylax</i>	4	Europe	Lamiaceae	<i>Sabia</i>	herb	swelling	inflorescence	single	detachable
<i>Isocolus</i>	17	Europe	Asteraceae	<i>Centaurea</i> + ⁸	herb	swelling & cryptic	inflorescence & stem	single & many	integral
<i>Aulacidea</i> s. str. ⁹	48	Eur, NA, EP	Asteraceae	<i>Tragopogon</i> + ¹⁰	herb	swelling & cryptic	inflorescence & stem & leaf	single & many	integral
<i>A. phlomisica</i>	1	EP	Apiaceae	<i>Eryngium</i>	herb	cryptic	stem	single	integral
<i>A. verticillata</i>	1	Europe	Lamiaceae	<i>Phlomis</i>	herb	cryptic	stem	single	integral
<i>Vetustia</i>	1	Europe	Lamiaceae	<i>Sabia</i>	herb	swelling	inflorescence	single	integral
<i>Panteliella</i> complex‡	3	Europe, EP	Lamiaceae	<i>Phlomis</i>	herb	swelling	inflorescence	single	integral
	3	Europe, EP	Lamiaceae	<i>Phlomis</i>	herb	swelling & cryptic	leaf & stem	single	integral
<i>Barboitina</i>	1	Europe	Asteraceae	<i>Serratula</i>	herb	cryptic	inflorescence	single	integral
<i>Aylax</i>	3	Europe	Papaveraceae	<i>Papaver</i>	herb	swelling	inflorescence	single	integral
<i>Iraella</i>	1	Europe	Papaveraceae	<i>Papaver</i> + ¹¹	herb	swelling	inflorescence	many	integral
<i>PhaTim</i> complex ¹²	34	Europe, EP	Papaveraceae	<i>Papaver</i>	herb	cryptic	stem	single	integral
			Asteraceae	<i>Centaurea</i> + ¹³	herb	cryptic & swelling	stem & leaf	single & many	integral
<i>T. phoenixopodos</i>	2	EP	Apiaceae	<i>Eryngium</i> + ¹⁴	herb	swelling	stem	single	integral
<i>P. hypochoeridis</i>	1	Europe	Lamiaceae	<i>Phlomis</i>	herb	cryptic	stem	single	integral
<i>P. centaureae</i>		Europe	Asteraceae	<i>Lactuca</i>	herb	swelling	stem	many	integral
<i>P. phlomisidis</i>		Europe	Asteraceae	<i>Hypochoeris</i>	herb	swelling	stem	many	integral
<i>A. lugubris</i>		Europe	Asteraceae	<i>Centaurea</i>	herb	swelling	stem	single	integral
<i>A. pannucea</i>		EP	Lamiaceae	<i>Phlomis</i>	herb	cryptic	stem	single	integral
<i>Eschatocerus</i>	3	EP	Lamiaceae	<i>Phlomis</i>	herb	? ?	? ?	? ?	? ?
<i>Diptolepidini</i>	53	S, America	Fabaceae	<i>Acacia</i> + ¹⁵	woody	swelling	stem	many	integral
		NA, Eur, EP	Rosaceae	<i>Rosa</i>	woody	swelling & complex	all	single & many	integral & detachable
<i>Himalocynips</i>	1	EP	? ?	? ?	? ?	? ?	? ?	? ?	? ?
<i>Pediopsis</i>	1	Europe	Aceraceae	<i>Acer</i>	woody	swelling	leaf & root	single	integral

TABLE 1. Continued.

Taxon	Species	Distribution ¹	Plant				Gall ²					
			Family	Genus	Form	Structure	Position	Chambers	Attachment			
Ingroup (Cynipidae)												
Cynipini	977	NA, Eur, EP	Fagaceae	<i>Quercus</i> + ¹⁶	woody	all	all	all	single & many	integral & detachable		
<i>P. quercusilicis</i>		Europe	Fagaceae	<i>Quercus</i>	woody	swelling	leaf & inflorescence	many	many	integral		
<i>A. quercusradicis</i>		Europe	Fagaceae	<i>Quercus</i>	woody	swelling	stem & root	many	many	integral		
<i>N. numismalis</i>		Europe	Fagaceae	<i>Quercus</i>	woody	swelling & complex	leaf	single	single	integral & detachable		
<i>B. pallida</i>		Europe	Fagaceae	<i>Quercus</i>	woody	swelling & complex	bud & root	many	many	integral		
Outgroups												
Parnipinae (Figitidae)	1	Europe	Papaveraceae	<i>Papaver</i>	—	—	—	—	—	—		
Thrasorinae (Figitidae)	3	NA	Fagaceae	<i>Quercus</i>	—	—	—	—	—	—		
	8	SA ¹⁷	Fabaceae + ¹⁸	<i>Mimosa</i> , <i>Acacia</i> + ¹⁹	—	—	—	—	—	—		
Liopteridae	170	SE Asia ²⁰	—	—	—	—	—	—	—	—		
Ibalidae	20	NA, Eur, EP	—	—	—	—	—	—	—	—		

¹ The area with most species given first; obvious recent introductions are omitted. Abbreviations: EP, East Palaearctic; NA, North America; Eur, Europe; SA, South America.

² Data only given for gall inducers, not for inquiline or parasitoids.

† Including *Synergus*, *Saphoneurus*, and *Synophrus*.

³ One species on *Smilax* (Smilacaceae).

⁴ Also on *Sibbaldia* (Belizim 1959).

⁵ One species on *Fragaria*.

⁶ Also on *Chrysothamnus* and *Lygodesmia*. The old uncertain record on *Lactuca* or *Mulgedium* (Bassett 1900) excluded, as well as the apparently erroneous record on *Stephanotis* (Weld 1952).

⁷ Galls on *Salvia*. The original record from *Phlomis cretica* (Quinlan 1968) is based on misidentification of the host plant (Pujade 1997).

⁸ A few species on *Cirsium*, *Cousinia*, *Inula*, and *Serratula*.

⁹ Including some Asteraceae-galling species currently placed in *Aylax*.

¹⁰ Also on *Acerpilton*, *Ambrosia*, *Arnica*, *Artemisia*, *Cacalia*, *Centaurea*, *Cousinia*, *Echinops*, *Hieracium*, *Hypochoeris*, *Koeleria*, *Lactuca*, *Mulgedium*, *Phaeopappus*, *Prenanthes*, *Scorzonera*, *Serratula*, *Silybum*, *Solidago*, *Sonchus*, and *Stemmacantha*.

‡ Including *Panteliella* and *Parapanteliella*.

¹¹ Also on *Hypecoum*.

¹² Including *Phanacis*, *Timaspis*, *Asiocynips*, and *Zerovia*. All species except three attack Asteraceae.

¹³ Also on *Carthamus*, *Chondrilla*, *Cichorium*, *Cousinia*, *Crepis*, *Echinops*, *Eplasia*, *Heracleum*, *Hypochoeris*, *Lactuca*, *Lapsana*, *Picris*, *Sonchus*, and *Urospermum*.

¹⁴ Also on *Heracleum*.

¹⁵ Also recorded on *Prosopis*.

¹⁶ Three species gall other genera of Fagaceae: one on *Lithocarpus*, one on *Castanea*, and one on *Castanopsis*.

¹⁷ Also in Australia and North America.

¹⁸ Also on Myrtaceae (*Eucalyptus* and *Blepharocalyx*).

¹⁹ Also on *Eucalyptus* and *Blepharocalyx* (Myrtaceae).

²⁰ Also occurs in North and South America, Eastern Palearctic, Australia, and Africa.

logenetic estimate of cynipid relationships affected our evolutionary conclusions (see below).

We included all examined characters in our phylogenetic estimate because we believe that character exclusion leads to weaker phylogenetic hypotheses and suboptimal evolutionary inference (de Queiroz 1996; Luckow and Bruneau 1997). For the benefit of the readers who take a different stance in this controversial issue and worry about circularity, we report that analysis of the matrix stripped from the mapped characters resulted in two shortest trees, the strict consensus of which did not conflict with the shortest tree reported here (Fig. 3b). The bootstrap support values were comparable but slightly lower than those for the tree derived from the total-evidence matrix.

We also mapped characters on a tree obtained by adding all remaining cynipid genera and species to the exemplar tree according to the best current estimate of their relationships. The term supertree is commonly used for the conglomeration of multiple quantitative phylogenetic analyses (e.g., Sander-son et al. 1998), but our tree is a more general summary of existing phylogenetic hypotheses, including both taxonomic revisions (Zerova et al. 1988; Nieves Aldrey 1994) and preliminary phylogenetic analyses of the position of particular taxa (Ronquist 1994; Liljeblad and Ronquist 1998), and we suggest that *metatree* might be an appropriate name for this type of summary. Our metatree (Fig. 3a) contained all 1378 described cynipid species but was only resolved basally. It enabled us to examine character evolution across all gall wasps and assess the influence of the particular sample of taxa chosen by Liljeblad and Ronquist (1998) on our inferences. Thus, the metatree includes more information and gives a more complete picture than the exemplar tree, even though it is more difficult to quantify the robustness of particular subclades in it. The accuracy of the metatree of course rests on the reliability of the existing classification, but we believe that cynipid taxonomy is currently good enough that the metatree mappings represent a valuable complement to the exemplar analyses.

Two cynipid species were excluded from the metatree. *Aylax onobrychidis* was described from larvae in stem galls on *Onobrychis* (Fabaceae; Dalla Torre and Kieffer 1910) and was tentatively placed in the genus *Aylax*. The gall has not been found since and adults are unknown, making it impossible to determine the correct phylogenetic position of the species, if it is a cynipid at all. *Aylax spirorhynchusi* was described based on specimens reared from galls in the stem of *Spirorhynchus sabulosus* (Brassicaceae; D'yakonchuk 1990). The species was not included in the analysis of Liljeblad and Ronquist (1998) but J. Liljeblad later had the opportunity to examine dry-mounted specimens in the Schmalhausen Institute of Zoology in Kiev. Several characters suggest that the species belongs to the *Isocolus-Neaylax* lineage (Fig. 3a), but its exact position will remain uncertain until it can be dissected and examined for a large number of characters with scanning electron microscopy and included in a formal analysis.

Dealing with Phylogenetic Uncertainty

Statistical testing is increasingly used in macroevolutionary studies but uncertainty in character mappings and in phy-

logenetic estimates is usually not given enough attention. The former source of error particularly affects parsimony estimates of ancestral states for rapidly evolving characters, and it can be dealt with using maximum-likelihood methods (Schluter et al. 1997). However, there were at least two problems with likelihood estimation of mapping errors in our study. First, branch lengths either have to be assumed to be equal across the tree, which is clearly unrealistic, or estimated from the data, which would have required the development of model-based methods of phylogenetic inference from morphological and biological characters. Second, the exemplars were chosen to maximize the representation of gall-wasp diversity and this could interfere with error estimation. For example, mapping of host-plant family shifts onto our 37-species exemplar tree with parsimony revealed 13 shifts, whereas our current understanding of cynipid relationships strongly suggests that inclusion of all 1366 cynipid species would only increase that number to 20. If there is a general shifting rate in cynipids and this rate was estimated by maximum likelihood from the exemplar tree, the taxon sampling bias would result in a gross overestimate, suggesting that the reconstruction of ancestral host plants is more uncertain than it really is.

Uncertainty in phylogeny reconstruction is likely to be a more serious source of error in many studies (Huelsenbeck et al. 2000). In our case, the phylogenetic estimate was uncertain concerning the correct resolution of some parts of the gall-wasp tree, particularly close to the base (Fig. 3), which was potentially problematic because we were interested in drawing inferences about the state of the most recent common ancestor of cynipids. Fortunately, this type of uncertainty is easier to control for. Here, we used parsimony bootstrapping to produce a set of trees describing uncertainty in our phylogenetic estimate and drew evolutionary inferences from each of them.

We saved the trees generated in 1000 bootstrap replications of the analysis, performed as described above. Zero-length branches were not collapsed because of the difficulties involved in mapping characters onto polytomous trees. In other words, polytomous trees were represented by all possible binary resolutions and each binary tree was given equal weight. The characters of interest were mapped onto each of the trees and inferences about ancestral states and transformations were drawn from these optimizations, weighting the results from each tree according to $1/nb$, where n is the total number of bootstrap replications and b is the number of equally parsimonious trees in the particular replication producing the tree. In drawing inferences about the state of an ancestral node, the few bootstrap trees that did not include the node of interest were omitted. Because no existing programs compute these statistics from character mappings, we used software developed by F. Ronquist specifically for this purpose (available from him on request).

Mapping of Geographical Distribution and Gall Features

On the gall-wasp phylogeny we traced the geographical distribution of the wasps, the growth form of the host plant, and the evolution of the structure and location of the gall (Table 1). The data were compiled from several key refer-

ences (Dalla Torre and Kieffer 1910; Zerova et al. 1988; Nieves Aldrey 1994) and from original species descriptions. The variation in the analyzed characters naturally fell into a small number of distinct states and the coding of individual taxa was rarely problematic. MacClade 3.07 (Maddison and Maddison 1992) was used for optimizing the discrete characters onto the metatree. Unless noted otherwise, characters were assumed to be unordered (optimized using Fitch parsimony) and terminals were coded as polymorphic when members had several states.

The distribution of gall wasps was coded in terms of six major areas (Europe, Eastern Palearctic, North America, South Africa, South America, and Australia). Obvious recent introductions were omitted (see footnotes to Table 1). Dispersal-vicariance analysis (Ronquist 1997) as implemented in DIVA 1.1a (Ronquist 1996) indicated that the biogeographic history of gall wasps was dominated by dispersal rather than successive vicariance events. Because such patterns are adequately described by Fitch optimization of areas and dispersal-vicariance analysis could not readily be applied to our bootstrap set of trees, we used Fitch optimization to assess the effect of phylogenetic uncertainty with regards to the center of origin of gall wasps.

Cynipid galls fall naturally into three classes of structural complexity: cryptic chambers, distinct swellings, and complex galls. The cryptic chambers are the cavities formed around the feeding larva inside stems or twigs, usually of herbs, without any external modification of the plant (Fig. 2e). The distinct swellings are conspicuous outgrowths of the attacked plant part, but the external structure of the gall is similar to that of the plant organ forming it (Figs. 2c, d). Finally, the complex galls are those galls with an external structure entirely different from the attacked plant organ (Figs. 2a, b, formed from leaves and buds, respectively). The gall complexity character was mapped both as unordered and ordered in the sequence cryptic chambers ↔ swellings ↔ complex galls.

Gall attachment was coded as a binary character with the states integral and detachable. Integral galls are those that cannot be easily separated from the attacked plant organ (Figs. 2c–e). Detachable galls can easily be separated from the plant with minimal damage to the latter (Figs. 2a, b). Seed galls that fall to the ground earlier than normal seeds were regarded as detachable.

Galls were divided into two classes with respect to the number of larval chambers: single-chambered (monothalamous; Figs. 2a, b, e) and multichambered (polythalamous; Figs. 2c, d). Many single-chambered galls are found in large aggregations on the attacked plant part, but multichambered galls are different in that the inner galls abut and form a single outer gall. Intraspecific variation suggests that, as a rule, gall wasps consistently produce either mono- or polythalamous galls, whereas the exact number of chambers in polythalamous galls can vary considerably.

The attacked plant organ was classified as being: (1) a stem, twig, or runner (Figs. 2c, e); (2) a growing root tip; (3) a reproductive organ (flower head, bract, seed, or seed capsule; Fig. 2d); (4) a leaf (Fig. 2a); or (5) a bud (Fig. 2b). Leaf and bud galls are similar but a bud galler oviposits earlier and the gall develops before the leaves have been formed, whereas

a leaf gall develops initially from one or more normal leaflets, even though subsequent gall growth may sometimes completely obscure the original leaflets.

Mapping Host Plant Preferences

In mapping host-plant preferences, we deleted a few unconfirmed, old host-plant records from those reported in the literature (see footnotes to Table 1). The record of galls on *Smilax* (a monocot vine) caused by an American species of *Diastrophus* (Ashmead 1896) was previously doubted (Weld 1952; Burks 1979) but has recently been confirmed (G. Mélika, pers. comm.). Host-plant preferences were mapped both with the insect-parasitic outgroups attacking hosts inside plants coded as having state unknown for the host plant characters or coded for the host plants of their victims. The rationale for the latter strategy was that the parasitoids are likely to recognize both host plant and host insect cues when they search for oviposition sites, and the host plants attacked by their victims might therefore be informative about the original host plants of gall wasps.

With respect to host-plant growth form, we distinguished between woody hosts (trees, bushes, vines) and herbaceous hosts (annuals or perennials). There are so few cynipids attacking annual herbs that it made little sense to separate these from perennial herbs.

We examined whether there was any evidence of cospeciation between the gall wasps and their host plants by fitting the gall-wasp metatree to a tree of relationships among host-plant families (Fig. 10) derived from a recent analysis of higher angiosperm relationships based on *rbcL* and *atpB* sequences of 357 taxa (Savolainen et al. 2000). The insect and plant trees were fitted together using parsimony-based tree fitting under the four-event model (Page 1995) as implemented in TreeFitter 1.0 (Ronquist 2000). Event costs were set either to maximize cospeciation (cospeciations –1, other events 0; cf. Ronquist 1998) or to TreeFitter defaults (cospeciations 0.0, duplications 0.0, sortings 1.0, switches 2.0). The latter combination of cost-event assignments is less sensitive than maximum cospeciation to duplications, increasing the chances of finding significant cospeciation when duplications are common. The prevalence of cospeciation was tested by comparing the observed number of cospeciations with that expected by chance, as indicated by 1000 permutations of the terminals of the gall-wasp tree. The plant tree was fitted to the gall-wasp tree both with all plant lineages included and with plant lineages not hosting gall wasps excluded. Pruning of the nonhosts does not affect the results of maximum-cospeciation analysis but increases the chances of finding significant codivergence under the default event costs in TreeFitter.

Finally, we examined host-plant colonization patterns by mapping host plant families and genera (Table 1) onto both the metatree and the exemplar tree using Fitch optimization. We also mapped host plants onto the cynipid phylogeny based on a step matrix taking both plant relationships and growth form into account. The idea was to include all available information into a combined estimate of the ancestral host plants of various gall-wasp lineages, giving each piece of information the same weight. Thus, in the step matrix, shifts

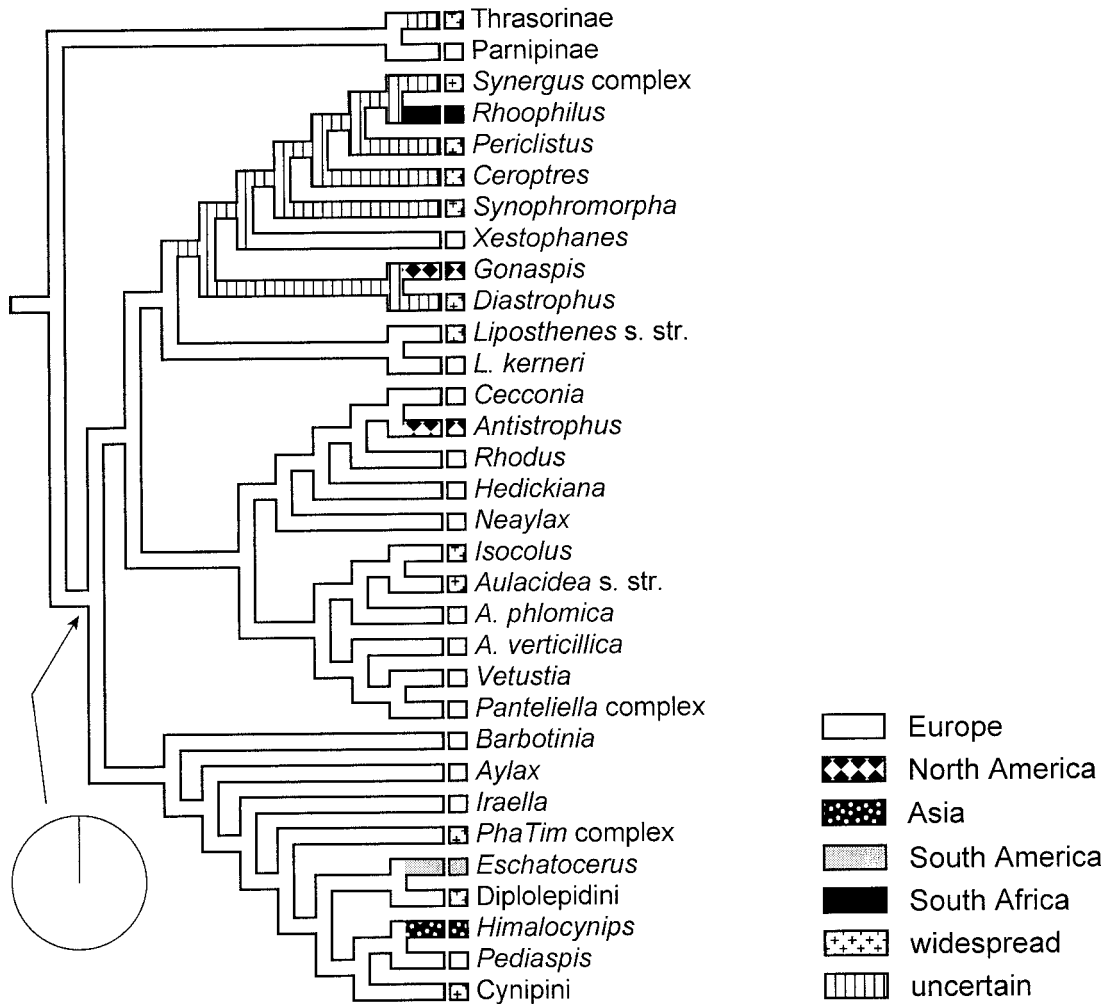


FIG. 4. Biogeographic history of the Cynipidae. The mapping is a Fitch-parsimony optimization of ancestral cynipid distributions onto the metatree; dispersal-vicariance analysis gave essentially identical results. The reconstruction indicates that the origin and early radiation of gall wasps took place in Europe. North America was colonized independently by several lineages, some of which, notably the oak gallers and their inquiline, underwent spectacular radiation there. The pie chart describes the uncertainty concerning the ancestral distribution of cynipids, as indicated by Fitch optimization onto each of the bootstrap trees resulting from analysis of the exemplar dataset. Almost the entire probability falls on Europe being the geographic origin despite the uncertainty concerning basal cynipid relationships.

between genera in the same family cost 1, shifts between families within rosid or asterids cost 2, shifts between asterid and rosid families cost 3, and shifts between more distant families cost 4. In addition, shifts between herbaceous and woody hosts bore an additional cost of 1. Distant shifts were weighted more heavily based on the assumption that they were likely to be less common than shifts between closely related hosts. Estimation of uncertainty concerning ancestral host plants was based on simple Fitch optimization of host-plant families onto the set of bootstrapped exemplar trees.

RESULTS

Mapping of traits onto the metatree and the exemplar tree produced similar results. Here we consistently present mappings onto the metatree as our best point estimate of the evolution of the studied characters in the Cynipidae.

Geographical Origin

Both Fitch-parsimony optimization and dispersal-vicariance analysis indicated Europe as the likely ancestral area for gall wasps, and this conclusion is not affected by uncertainty concerning basal cynipid relationships (Fig. 4). Many of the basal cynipid lineages live exclusively or predominantly in the Mediterranean region and/or around the Black Sea, suggesting an origin in this area. The genera *Eschatocerus* and *Rhoophilus* apparently spread later to South America and South Africa, respectively. North America has been colonized from Europe or the eastern Palearctic independently by at least five groups of gall wasps: the inquiline and their relatives (*Diastrophus* and *Gonaspis*), the genus *Antistrophus*, some species in the genus *Aulacidea*, the rose gallers, and the oak gallers (Fig. 4). Some of these groups, particularly the oak gallers and their inquilines, have sub-

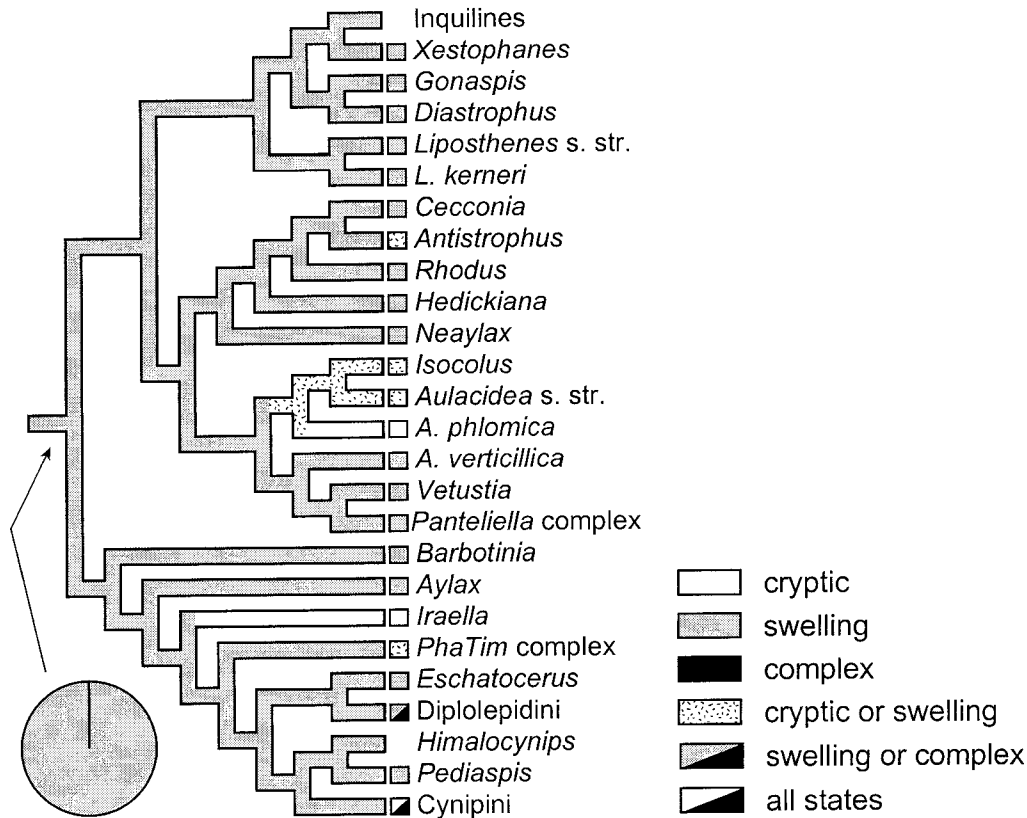


FIG. 5. Evolution of gall structure in the Cynipidae. The ancestral cynipid induced galls that were simple but distinct swellings (almost all bootstrap trees had swellings as the ancestral state, as shown by the pie chart). Galls formed in the pith of herb stems without any associated external swelling of the plant were derived independently several times. Galls with complex, species-specific surface structures evolved independently in oak gallers (Cynipini) and rose gallers (Diplolepidini).

sequently undergone spectacular radiation in North America, but North America is unlikely to be the center of origin of gall wasps.

Gall Type and Position

The phylogenetic character mappings suggest that the ancestral cynipid gall was a distinct swelling without secondary surface adornments (Fig. 5), was induced in reproductive organs (Fig. 6), had a single larval chamber (Fig. 7), and was integral (Fig. 8). The species developing inside herb stems without causing any visible external deformation of the plant (cryptic chambers) were apparently derived from lineages forming typical stem galls (Figs. 5, 6). The uncertainty analyses indicate that the ancestral gall might possibly have been a stem gall rather than a seed gall (Fig. 6), but in either case it is likely to have been a distinct swelling rather than a cryptic larval chamber embedded in normal plant tissue (Fig. 5). The inference concerning ancestral gall complexity was identical regardless of whether this character was treated as ordered or unordered. There was not a single bootstrap tree in which an irreversible increase in complexity (cryptic chambers → distinct swellings → complex galls) was among the most parsimonious optimizations of the ordered gall complexity character.

Host Plant Growth Form

All basal cynipid lineages are herb gallers and the gall-wasp ancestor is therefore likely to have been an herb galler, too (Fig. 9). Uncertainty concerning basal gall-wasp relationships does not affect this conclusion. Woody hosts have apparently only been colonized three times in the evolution of gall wasps: once in the genus *Diastrophus* (assuming that the eight *Rubus* gallers form a monophyletic subgroup within the genus), once in the ancestor of the woody rosid gallers, and once in the ancestor of the inquilines. There have been no shifts from woody to herbaceous hosts.

Cospeciation

Except for a single species of *Diastrophus* attacking the monocot *Smilax*, all gall wasps are associated with eudicots but they are only found on a few distantly related lineages, as indicated by the large number of nodes separating the hosts in the eudicot tree (Fig. 10). Nevertheless, cospeciation between gall wasps and their host plants could be important if extant gall wasps represented relicts of an ancient radiation or if gall wasp–host plant coevolution were characterized by tight host tracking with occasional cospeciation. In either case, the gall wasp and host plant phylogenies should be more congruent than expected by chance. Using maximum-cospe-

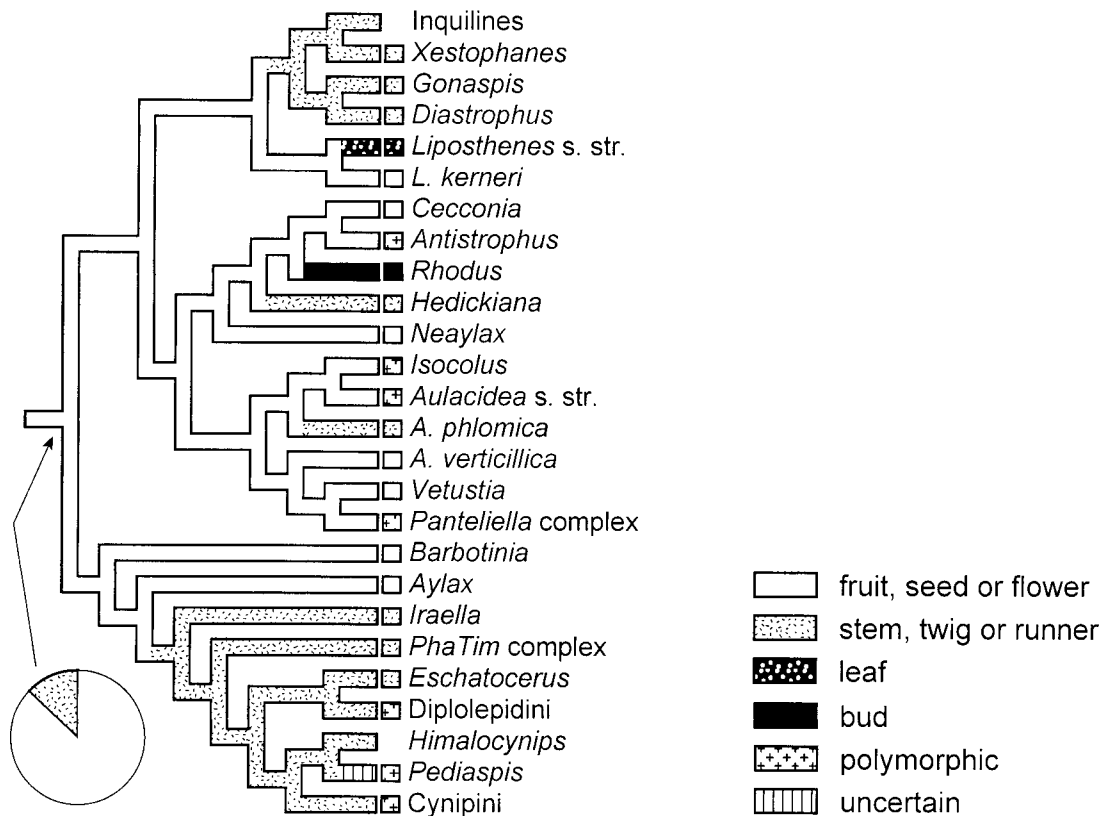


FIG. 6. Evolution of gall position in the Cynipidae. Early cynipids most probably induced galls in reproductive structures (seeds, seed capsules, or flower-heads) of their host plants but the analysis of phylogenetic uncertainty (pie chart) also placed some weight on the ancestral gall location being stem, twig or runner.

ciation analysis and the recent option for treating widespread terminals (Ronquist 2000), there were 11 or 12 gall wasp–host plant cospeciations depending on whether the gall-associated figitids were included in the analysis. A large proportion of the randomly permuted gall-wasp trees had at least this many codivergence events under the maximum-cospeciation criterion, indicating a lack of fit between the wasp and plant trees ($P = 0.76$ for the cynipid tree, and $P = 0.79$ for the cynipid + figitid tree).

Under the default event-cost assignments of TreeFitter, permutation tests revealed significant phylogenetic constraints in the host-plant use of gall wasps. None of the 1000 randomly permuted gall-wasp trees had a lower total cost than the observed tree when fitted to the pruned plant tree (excluding nonhosts), whether or not the figitids were included. The optimal reconstruction had five gall wasp–host plant cospeciations. As expected, these codivergence events were better supported by the data than the 11 events found by maximum-cospeciation analysis. However, the observed number of codivergence events was still not significantly higher than expected by chance ($P = 0.22$). The phylogenetic constraints were instead due to a high frequency of duplication events and a low frequency of host shifts (none of the 1000 permutations had more duplications or fewer shifts than the observed data). When all lineages were included in the plant tree, the default event cost assignments of TreeFitter produced an optimal solution with only duplication and switching events, identical to Fitch optimization of the host

plant families onto the gall wasp tree. This pattern was highly significant ($P < 0.001$) both with respect to the overall cost and to the low number of switching and high number of duplication events.

In summary, parsimony-based tree fitting shows that there is no evidence for parallel cladogenesis between gall wasps and their host plants, particularly when the large number of sorting events that such a scenario requires is taken into account. Nevertheless, host plant use is strongly phylogenetically conserved in gall wasps. When gall wasps speciate, daughter lineages tend to remain associated with plants in the same plant clade (family) used by their common ancestor, and shifts between major host clades (families) are rare. This pattern lends itself well to study by Fitch optimization of hosts onto the wasp phylogeny.

Colonization Patterns

Tracing host-plant families and genera onto the cynipid metatree using Fitch parsimony unambiguously identified the host plant of most ancestral cynipid lineages and the shifts between them. Incorporating information about plant relationships (genus, family), major plant clade (rosids, asterids, other) and growth form (woody, herbaceous) into a step matrix, as described in Materials and Methods, increased the precision by selecting a subset of the most parsimonious optimizations of the unordered plant family character. We present the step-matrix optimization, including figitids, as our

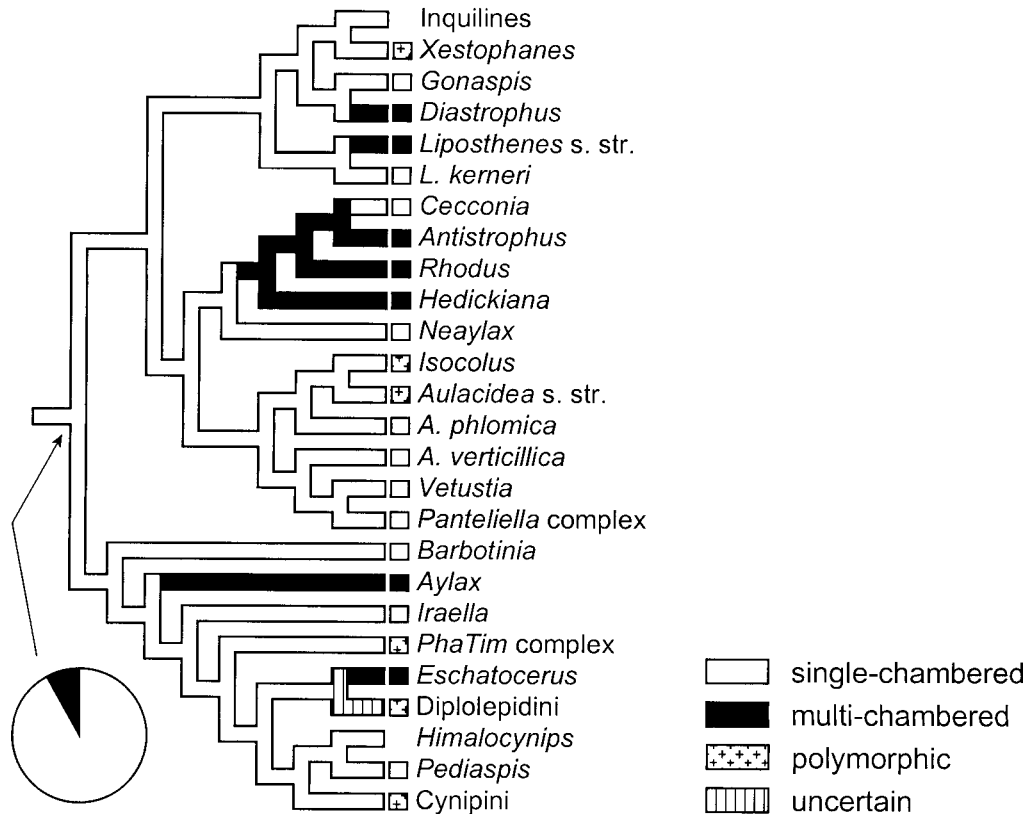


FIG. 7. Evolution of the number of gall chambers in the Cynipidae. The first cynipid gall was single-chambered; multichambered galls then evolved repeatedly in different lineages. The pie chart gives the relative probabilities of different ancestral cynipid states, as indicated by bootstrap analysis.

best estimate of the evolution of host-plant use in the Cynipidae (Figs. 11, 12).

Analysis of the uncertainty in these mappings, based on simple Fitch optimization of host-plant families onto the bootstrapped exemplar trees, indicates that the first gall wasps were associated with Papaveraceae (65%) or possibly Lamiaceae (27%), and then shifted onto other plant groups (Fig. 11). The ancestral host plant is unlikely to have belonged to the Asteraceae (5.1%) or the Fagaceae (2.6%). When figitids were excluded from the analysis, the results shifted such that the Lamiaceae became the most likely ancestral hosts (57%), followed by Papaveraceae (24%) and Asteraceae (17%), with other host plants being ancestral in 1% or less of the bootstrap trees.

Mapping of host-plant genus (not shown here, but see Ronquist 1998) onto the cynipid metatree suggests that gall wasps may show high fidelity even at this level. For instance, the Lamiaceae feeders in the *Neaylax-Cecconia* clade are all restricted to the genus *Salvia*, whereas Lamiaceae feeders in the *Isocolus-Panteliella* clade mostly feed on *Phlomis* (Fig. 11; Table 1). Members of the genera in the *Barbotinia-Iraella* grade are almost all found on *Papaver*, and most or all members of the genera *Diastrophus*, *Gonaspis*, and *Xestophanes* are associated with *Potentilla*. Generic mappings using simple Fitch optimization suggest that, if the ancestral host belonged to one of the genera hosting gall wasps today, the most likely genera would be *Papaver* (36%), *Phlomis* (35%), and *Salvia* (15%; analysis with figitids excluded).

The metatree allowed us to estimate the rate of shifts between different host-plant genera and host-plant families in gall wasps. For the entire family tree, the genus shift rate is 0.07 and the family shift rate is 0.01 per lineage splitting event. There are important rate differences between inquiline, woody rosid gallers, and the remaining species, mostly herb gallers: the latter are considerably more labile in their host-plant choice (0.07 family and 0.39–0.46 genus shifts per speciation [lineage split], the variation in genus shift rates depending on the resolution of terminal polytomies in the metatree) than inquilines (0.02 family and 0.02 genus shifts per speciation) and woody rosid gallers (0.003 family shifts and 0.007 genus shifts per speciation).

Considering that their host-plant preferences are so conservative, cynipids have shifted between remarkably distant hosts (Fig. 12). Some of the most noticeable examples of distant shifts include the ones from Papaveraceae to Lamiaceae, from *Rubus* or possibly *Potentilla* or *Fragaria* (Rosaceae) to the monocot *Smilax* (Liliaceae) and from Lamiaceae (asterids) to *Potentilla* (Rosaceae, rosids). Nevertheless, the major shifts tend to be more common among relatively more closely related plant families. We showed this by examining the shifts between host plant families indicated by Fitch optimizations, which treated all families as equal without taking host-plant relationships into account, onto the exemplar tree. Ten of the 13 shifts on the exemplar tree could be unambiguously classified as being either between families within the asterids or rosids (seven shifts) or between more

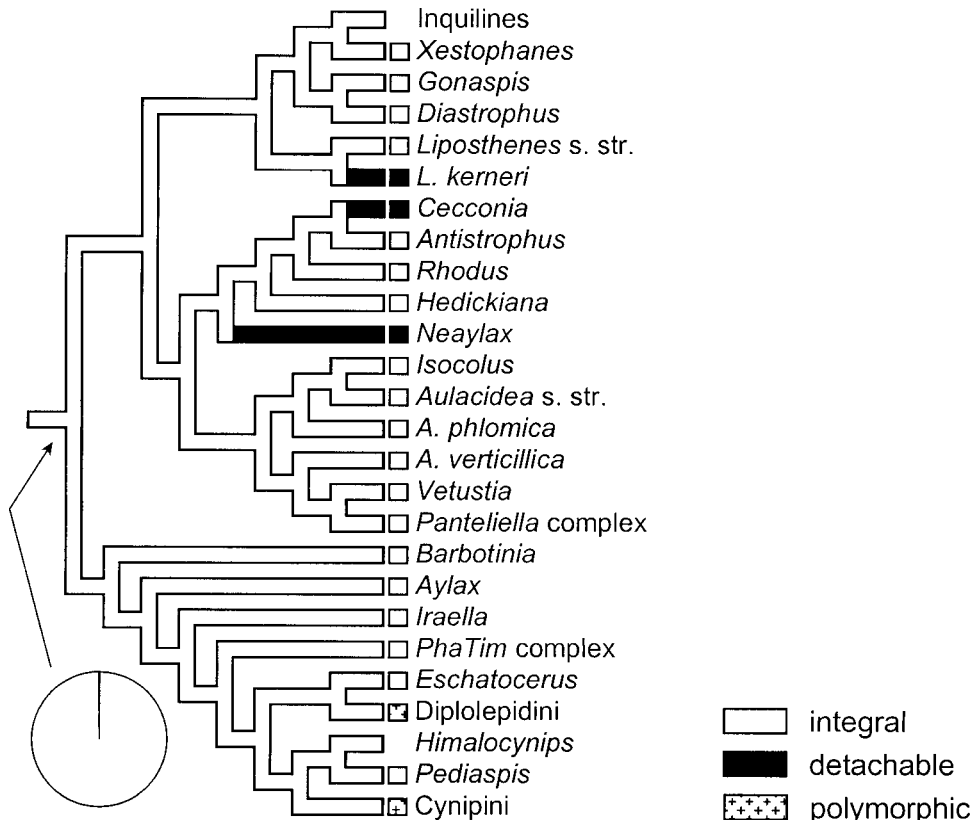


FIG. 8. Evolution of the gall attachment in the Cynipidae. In the ground plan, the cynipid gall was integral. Detachable galls have then evolved independently in the oak gallers (Cynipini) and rose gallers (Diplolepidini), as well as in a few herb-galling lineages. The pie chart gives the relative probabilities of different ancestral cynipid states, as indicated by bootstrap analysis.

distantly related families (three shifts). We then randomly drew 10 species pairs attacking different host-plant families from the terminal taxa in the exemplar tree, and calculated the probability of obtaining seven shifts or more between plant families within the rosids or asterids. The probability was estimated to be $P = 0.0093$, indicating a highly significant tendency of major host-plant shifts to be between relatively more closely related plant families. These results are particularly convincing because Fitch optimization is likely to underestimate the prevalence of shifts between relatively more closely related plant families.

Convergent Colonization of the Same Host Plants

When the host-plant preferences are examined at a more detailed level, it is evident that gall wasps only use a few distantly related genera and species in each plant family they attack. Despite this, gall wasps have repeatedly converged upon the same host plant species. Some of these convergences are explained by the inquiline tracking their hosts among the woody rosoid gallers, but most of the convergences involve pairs or sets of herb-galling species.

We examined the convergent patterns in more detail for the *Phanacis-Timaspis* (PT) complex and the *Aulacidea-Isocolus* (AI) complex, the latter comprising *Aulacidea* s. str., *A. phlomica*, and *Isocolus* (Fig. 3). Most of the species in these complexes are gallers of the Asteraceae, but the two groups are only distantly related and independently came to

be associated with this host plant family (Fig. 11). Nevertheless, to a large extent they use the same plant genera and species as hosts. In the western Palearctic, 22 genera are used as hosts by the AI complex and 19 genera by the PT complex. Of these genera, eight (*Centaurea*, *Cousinia*, *Echinops*, *Eryngium*, *Hypochoeris*, *Phlomis*, *Silybum*, and *Sonchus*) are attacked by species from both groups. This is a remarkable degree of overlap, considering that the genera belong to three different plant families (Asteraceae, Apiaceae, and Lamiaceae). In the Asteraceae, the six doubly galled genera belong to three different subtribes in the tribe Cardueae and two different subtribes in the tribe Lactuceae (Bremer 1994). Thus, the convergences are not due to chance colonization of a set of closely related genera; each of the generic convergences appears to represent an independent evolutionary event.

The convergences do not only involve the same plant genera, but also the same plant species. For instance, there are only three cynipids associated with the Apiaceae. Yet, there is one member of each complex galling the same *Eryngium* species in the Caucasus region (D'yakonchuk 1984). The ancestors of the PT complex have probably never been associated with hosts in the Lamiaceae (Fig. 11). Yet, there is a single species in the complex, *Phanacis phlomidis* (one of the species in our exemplar tree; Fig. 3), developing in stems of *Phlomis tuberosa*, the very same species that hosts *A. phlomica* in the AI complex, as well as several cynipid species

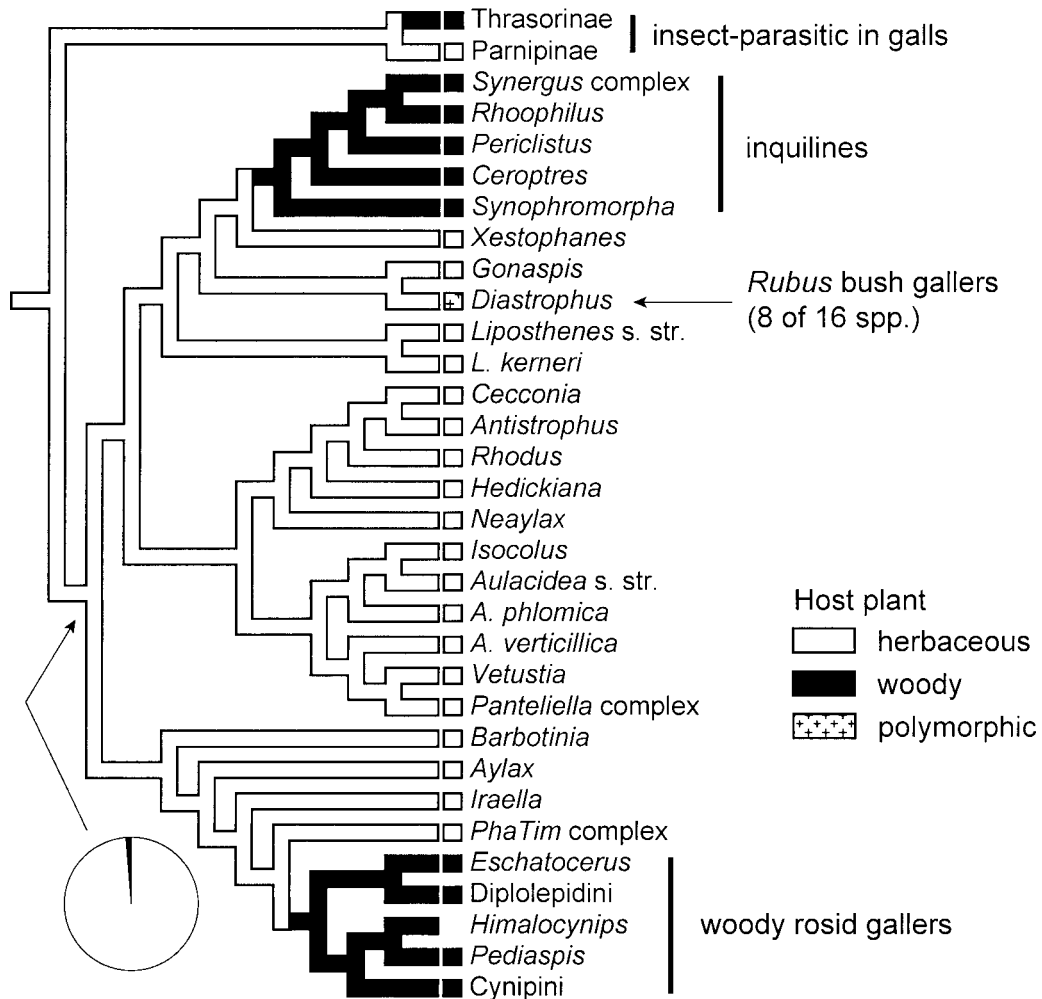


FIG. 9. The growth form of cynipid host plants. Gall wasps were originally herb gallers. Woody hosts were colonized three times: once in the ancestor of the woody rosid gallers, once in the ancestor of the inquilines, and once in the genus *Diastrophus* (assuming that the eight species in this genus inducing galls on *Rubus* bushes form a monophyletic subclade within the genus). The pie chart gives the relative probabilities of different ancestral cynipid states, as indicated by bootstrap analysis.

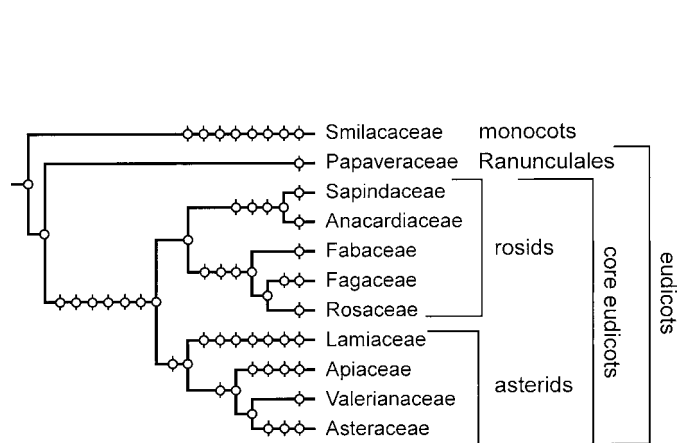


FIG. 10. Relationships among families galled by cynipids according to a recent molecular analysis of angiosperm relationships based on *atpB* and *rbcL* sequences (Savolainen et al. 2000, fig. 6). Nodes on the branches represent the minimum number of different nonhost plant lineages that attach to these segments in the angiosperm phylogeny.

that are closely related to the AI complex. Other species of *Phlomis* apparently do not host gall wasps.

On the doubly attacked plants, the galls induced by the PT species and the AI species are more similar than expected by chance. To show this, we compiled data on the structure and location of the galls induced by species in the PT and AI complexes (Table 2). For simplicity, we omitted the species associated with *Centaurea*, because some *Centaurea* species are galled by several AI and PT species and some AI and PT species have rather wide host ranges within the genus *Centaurea*, making it difficult to exactly identify associated species pairs. Among the remaining species for which we had gall data, there were five pairs of associated AI and PT species. In four of these cases the species induced the same type of gall (types defined as in Table 2); in the fifth, the AI gall was a single-chambered leaf midrib swelling, whereas the PT gall was a multichambered stem swelling (galls on *Hypochoeris*). It is unlikely that four of five AI-PT species pairs drawn randomly from the non-*Centaurea* associated Palearctic species would have similar galls ($P = 0.012$, cal-

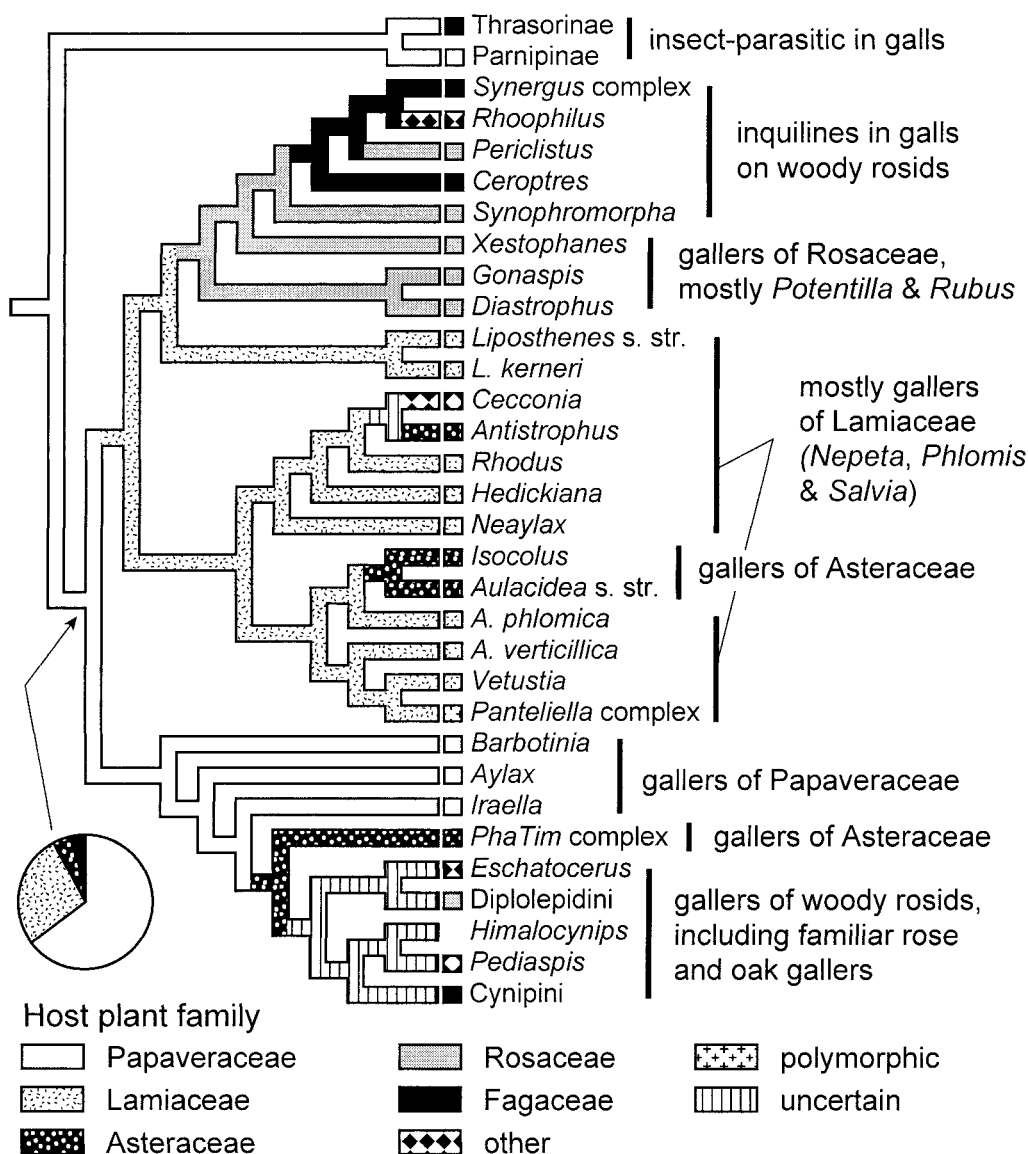


FIG. 11. Inferred ancestral host plants of cynipids. The reconstruction is based on host-plant family optimization incorporating information about plant relationships (major plant clade and genus) and growth form. Simple Fitch optimizations of plant families gave identical results, except that they left the host plant unresolved for a few additional nodes among the inquilines and in the immediate ancestry of the woody rosid galls. Uncertainty concerning the ancestral cynipid host plant (pie chart) is based on simple Fitch optimization of host-plant family onto the trees resulting from bootstrap analysis of the exemplar dataset.

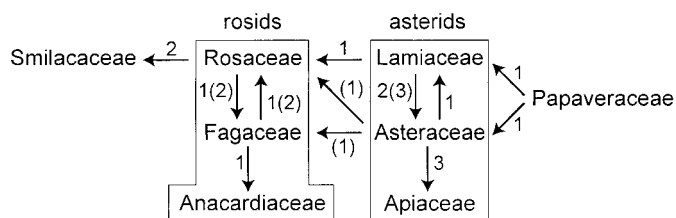


FIG. 12. Shifts between host-plant families in cynipids according to the reconstruction in Figure 11. Numbers without parentheses refer to the number of unambiguous shifts; numbers in parentheses to the maximum number of shifts including ambiguous events. Note that shifts tend to be between families within major plant clades (rosids and asterids).

TABLE 2. Diversity of galls induced by species in the *Aulacidea-Isocolus* (AI) and the *Phanacis-Timaspis* (PT) complex, excluding the species associated with *Centaurea*.

Type of gall	Number of species	
	AI complex	PT complex
Stem, swelling	7	8
Stem, no swelling	3	5
Inflorescence, swelling	7	1
Leaf, swelling	2	1
Root/stolon, swelling	1	0
Inflorescence, no swelling	3	0

culated from the frequency of different gall types in the AI and PT complexes as specified in Table 2).

DISCUSSION

The Origin of Cynipids

The results reported here suggest that many of the earlier ideas concerning the origin and early evolution of cynipids are erroneous. The most recent common ancestor of extant cynipids is not likely to have been associated with the Asteraceae or the Fagaceae but with the Papaveraceae or possibly the Lamiaceae. It was an herb galler but its larvae did not develop in cryptic larval chambers inside stems, causing no deformation of the plant. Instead, the first galls are likely to have been simple but distinct seed or fruit capsule swellings. The cynipids originated in Europe and not in North America; the Nearctic herb-stem gallers cannot be traced back directly to the earliest cynipids but instead represent a few derived lineages that separately colonized the New World.

Whereas some workers have regarded the Cynipidae as a recent radiation dating back no further than the Oligocene (Kinsey 1919; Roskam 1992), recent findings suggest that their evolutionary history stretches back well into the Cretaceous. Amber fossils of the Figitidae are known from the Santonian (upper Cretaceous, about 83–86 million years ago; Kovalev 1994; Ronquist 1999), showing that the most recent common ancestor of figitids and cynipids, which is likely to have been associated with galls (Ronquist 1999), must be at least this old. This dating is consistent with the recent discovery that one of the oldest cynipid fossils, a Baltic amber fossil from the Eocene (45 million years ago) described as *Aulacidea succinea* (Kinsey 1919), belongs to the *Synergus* complex of inquilines, a deeply nested group within the cynipid tree (Fig. 3; Ronquist 1999). It also agrees well with biogeographic evidence from more basal cynipoid groups suggesting that cynipids and figitids diverged from their relatives in the earliest Cretaceous (Ronquist 1995; Nordlander et al. 1996, 1999). Definite cynipid galls are not known until the Oligocene and Miocene, although cynipid-like galls appear much earlier (Scott et al. 1994).

Considering the controversy surrounding the age of cynipids, our finding that they may have originally been associated with the Papaveraceae, perhaps one of the oldest families of eudicots, is intriguing. With a few other families, Papaveraceae belong to the Ranunculales, the sister group of all other eudicots (Angiosperm Phylogeny Group 1998). The basal eudicot branchings must be dated to at least 100 million years ago (the mid-Cretaceous) based on fossil evidence (Crane et al. 1995) and a recent molecular analysis (Savolainen et al. 2000) suggests that the Papaveraceae may have separated from their closest relatives earlier than any other eudicot family. The pollen record of Papaveraceae is poor and does not go far back, but Cretaceous fruits of North American Papaveraceae have recently been discovered (Smith 1996), suggesting that the family may indeed be as old as indicated by the molecular evidence.

Recent discoveries of undescribed cynipid galls on *Fumaria* in the Mediterranean region (F. Ronquist and J. L. Nieves Aldrey, unpubl. data) and on *Corydalis* in the Himalayas (F. Ronquist, unpubl. data) imply that gall wasps may

be more diverse on the Papaveraceae than expected from current data. This indicates that the ancestral host plant of cynipids could have been any member of the Papaveraceae lineage, rather than specifically *Papaver* as suggested by mappings of host-plant genera onto the metatree. It is remarkable that *Barbotinia oraniensis*, the single representative of one of the earliest cynipid lineages (Fig. 3), has retained many of the features concluded here to be characteristic of the most recent common ancestor of cynipids. *Barbotinia* lives in the Mediterranean region and induces distinctly swollen, single-chambered galls inside the seed capsules of poppies (*Papaver* spp.). Based on phylogenetic evidence, *Barbotinia* may thus be described as a living-fossil cynipid (Ronquist and Nieves Aldrey, in press).

The Evolution of Cynipid Galls

Kinsey (1920) and others have hypothesized that cynipid gall structure evolved from multichambered to single-chambered, from integral to detachable, and from simple to complex. Our results indicate that Kinsey was wrong about the larval chambers. The first cynipid galls were single-chambered and most subsequent changes went in the opposite direction to that proposed by Kinsey, namely from single-chambered to multichambered galls (Fig. 7). Kinsey was probably right, however, in that detachable galls evolved from integral galls. Detachable galls are mainly found among the oak and rose gallers and only occasionally among the more basal cynipid lineages (Fig. 8). Similarly, the complex cynipid galls with species-specific external structures are obviously derived in the context of the entire family: They are only found within the rose gallers (Diplolepidini) and the oak gallers (Cynipini; Fig. 6).

Our mappings clearly indicate that the cryptic larval chambers inside herb stems are derived from more typical galls causing abnormal swelling of the attacked plant organ. They are not likely to represent the ancestral feeding mode in the Cynipidae, as proposed by Kinsey. In fact, detailed examination reveals that the cryptic chambers are very similar to the inner gall of normal cynipid galls. There is a well-structured concentric layer of nutritive tissue around the developing larva, as well as a surrounding zone of sclerenchyma (F. Ronquist, unpubl. data). The only significant difference between the cryptic chambers and normal cynipid galls is that the former are embedded in normal plant tissue rather than being associated with a distinct outer gall. Thus, the cryptic chambers may easily have evolved from more conspicuous galls by suppression of signals causing the outer gall to develop. They may best be understood as cryptic galls and it is tempting to speculate that they evolved to reduce the attack from natural enemies of gall wasps, which may have experienced difficulties in locating galls without associated external modifications of the host plant.

Except for the evolution of cryptic galls, our results agree well with the early notion (Cook 1902; Kinsey 1920; Wells 1921) that galls tend to evolve toward increasing complexity. Both the evolution of detachable galls from integral ones and the elaboration of the outer gall in the Cynipini and Diplolepidini to form features protecting the gall inhabitant from attack by natural enemies can be interpreted as trends toward

increased gall complexity. More detailed studies of oak and rose gallers will be necessary to reveal the extent to which this trend is reversible in cynipids. Although there has been a general increase in gall complexity in cynipids, it should be pointed out that, compared to most other insect galls, even the simplest cynipid galls are complex with several distinct, well-organized circular zones of differentiated plant tissue entirely enclosing the feeding larva. Even the galls of *Barbotinia* follow this cynipid blueprint.

The reason for the macroevolutionary trend toward increased gall complexity, which also has been documented in galling aphids, thrips, and sawflies (Fukatsu et al. 1994; Nyman et al. 1998; Morris et al. 1999, 2000), can probably be sought in phylogenetic constraints on gall formation mechanisms. Although galls may be considered the extended phenotype of the insects producing them, mastering gall development obviously poses a major challenge to the insect, causing a phylogenetic lag between the appearance of selective factors driving sophistication of gall structure and the invention of mechanisms in the insects providing the appropriate effect on host-plant development.

It is striking that the complex galls with species-specific outer structure are restricted to the two major gall-wasp radiations, the oak and the rose gallers. Can the spectacular radiation of these lineages be explained, at least in part, by their ancestors taking the art of forming plant galls to a new level? More detailed studies pinpointing the origin of complex galls in the rose and oak gallers should be able to shed light on this possible correlation between gall complexity and net speciation rate.

Host Plant Longevity and Growth Form

One of the more striking features of the host-plant preferences of cynipids is their fondness of long-lived host plants. Even the herb gallers are predominantly associated with perennial hosts. The preference for long-lived hosts appears to be a general phenomenon in galling insects (Crespi et al. 1997) and may occur either because long-lived plants suffer less severe fitness consequences from galling, and thus are not heavily selected for resistance against gallers, or because the longer generation time prevents them from responding efficiently to novel galler traits, putting them at a selective disadvantage in a defense/counter-defense arms race. Nonetheless, there are several cynipid gallers that apparently do well on annual hosts, such as *Cecconia valerianellae* inducing seed galls on *Valerianella* and an undescribed galler making seed galls on *Fumaria*. Furthermore, it is intriguing that some of the apparently most archaic cynipid lineages, *Barbotinia* and *Aylax*, are associated with annual rather than perennial species in the genus *Papaver*. If their association with annual hosts goes back a long time, they may be interesting contrasts in attempts to untangle the factors favoring the use of long-lived host plants in most insect gall inducers.

An extremely conservative feature of the gall wasp–host plant association is the host-plant growth form. This agrees well with patterns observed in butterflies (Janz and Nylin 1998), although association with woody hosts is derived and apparently irreversible in gall wasps but ancestral and transient in butterflies. The tendency of phytophagous insects to

remain on plants with a similar growth form when they shift host plants may be related to significant differences between woody hosts and herbs in biotic and abiotic environmental factors important to phytophagous insects. Apparently, these differences affect both external foliage feeders like the Lepidoptera and internal gallers like the Cynipidae.

Host Plant Relationships

Our data fail to demonstrate significant codivergence between gall wasps and their host plants. These findings contribute to an emerging consensus that parallel insect-plant cladogenesis is rare, even in insect groups that have been regarded as prime candidates for such tight coevolution with their hosts. Rather than by cospeciation, the evolution of cynipid host preferences appears to be characterized by a high degree of conservatism mixed with rare shifts between distantly related hosts. It is true that the lack of congruence between basal cynipid divergences and plant relationships does not preclude the existence of cospeciation in the evolution of terminal groups, such as the radiation of *Isocolus* onto *Centaurea* or the oak gall wasps onto oaks, but we suspect that these terminal clades are characterized by a similar mixture of conservatism and rare shifts as we have documented at the larger scale. This expectation is slightly contradicted, however, by an early study of the association between *Isocolus* and *Centaurea*, which did find some evidence for a single basal cospeciation event between the gall wasps and their host plants (Baumann and Brandl 1993). Obviously, the macroevolutionary patterns of terminal cynipid radiations deserve more attention.

Most phytophagous insects make a major host shift (onto a new host plant family) once or twice per 10 speciation events (Mitter and Farrell 1991). In this perspective, cynipids are extremely conservative in their host plant use with their 20 shifts between plant families in approximately 1300 species, an average rate of only 0.01 major host shifts per speciation event. Gall wasps may actually be the most conservative group of phytophagous insects studied thus far for macroevolutionary patterns of host use. The cynipid oak gallers, with their approximately 1000 described species, constitute the largest radiation of a phytophagous insect group onto a single host-plant genus. Only the fig wasps (Agaonidae) come close, with about 650 species of gall inducers and phytophages in *Ficus* fruits (Goulet and Huber 1993).

Gall wasps associated with woody plants (inquilines and woody rosid gallers) have shifted more rarely between host-plant families than those associated with herbs. This is in stark contrast to butterflies, in which host shifts are more common in tree feeders than in herb feeders (Miller 1992; Janz and Nylin 1998). This is probably due to the differences in the role plant secondary compounds play in these associations. For butterflies, it is thought that shifts to novel host plants are facilitated in tree feeders because trees tend to have less diverse and acutely poisonous secondary compounds than herbs (Feeny 1976). Gall wasps, in contrast, feed on plant tissue devoid of secondary compounds and should be far less sensitive to the secondary metabolites of herbs. It is still unclear, however, why gall wasps should be more conservative when associated with woody hosts. This pattern

may be wholly or partly due to the spectacular diversification of gallers and inquiline on oaks and roses. When the species associated with oaks and roses are excluded, host shift rates are comparable in herb-associated and tree-associated cynipids.

Considering the conservatism in their host plant use, it is difficult to understand how gall wasps were able to make some of the distant host plant shifts documented by their phylogeny, such as that between Rosaceae and Smilacaceae or that between Papaveraceae and Lamiaceae (Figs. 10–12). If they are able to shift between hosts that are so remarkably distantly related, why do they not shift hosts more often? The confusion hypothesis (Larsson and Ekbom 1995) provides one possible explanation. The idea is that short-lived specialist insects gain little from being choosy when their host plant is rare in the environment, particularly if they can deposit most of their eggs on the eclosion plant. Thus, the rare distant shifts may be the effect of rather frequent indiscriminant gall-wasp oviposition into nonhost plants filtered by a low probability that these attempts will result in successful gall induction.

There are few studies of cynipid oviposition preferences, but the available data suggest that gall-wasp females are much more specific than predicted by the confusion hypothesis. For instance, Harrison (1922) exposed females of *Diplolepis rosae* to 16 species of *Rosa* and found that oviposition only took place on members of one section of *Rosa*. We think that the explanation for the mixture between conservatism and distant shifts is more probably related to the mechanism of gall induction. If gall wasps manipulate highly conserved plant traits, like morphogenetic control mechanisms (cf. Weis et al. 1988), they should be able to colonize any eudicot host with only slight modifications to their gall induction system. If, at the same time, there were a requirement for a precise fit between gall-inducer and plant traits, shifts would tend to be rare even though they could occur between distantly related plants.

Convergent Colonizations

A remarkable feature in the evolution of the gall wasp–host plant association is the convergent colonization of a small set of unrelated plant species, particularly because there are no obvious links among the attacked plants in terms of secondary compounds, morphology, plant community, or phytophagous insect community. For instance, among the few cynipid-hosting genera of Asteraceae, belonging to several unrelated tribes, there are both plants with and without latex canals. Within large plant genera, such as *Phlomis*, *Potentilla*, and *Salvia*, it is commonly found that cynipids only attack a few, apparently randomly selected species or groups of species. Although other factors may contribute, the only plausible mechanism that seems to be able to explain the extreme tendency to converge onto the same, small set of host-plant species is that interspecific parasitism among cynipids is involved in many of the host-plant shifts.

Intense inter- or intraspecific competition is common in gall-inducing insects. The competition may be for favorable gall-induction sites (Ngakan and Yukawa 1996), but may also involve attempts to take over young galls because gall ini-

tiation is prone to fail or requires substantial energy investment (Crespi et al. 1997). Cynipid inquilines apparently originated from such a competitive interaction, in which one species lost the ability to initiate galls and became an obligate usurper (Ronquist 1994; F. Ronquist, unpubl. data). The inquilines are specialized on cynipids galling woody rosids, and therefore convergently infest the same host plants.

Suppose that, in many species of herb gallers, there are individual females that are facultative parasites of other young galls of their own species. A female arriving late at a favorable oviposition site may be better off ovipositing into a young gall, and thus killing the larva in that gall, than searching for another favorable oviposition site. Such parasitic individuals may develop a capability to search for young galls using cues that are similar across cynipids. If so, they may sometimes end up on the wrong host plant, attacking an individual of an unrelated cynipid species. Because the victim has initiated gall development, the offspring of the usurper has a considerably better chance to survive than if the usurper had tried to initiate a gall in the foreign plant *de novo*. In this way, occasional interspecific parasitism may facilitate the adoption of a new but distantly related host plant already attacked by other gall wasps. Unfortunately, oviposition behavior has never been studied in detail in herb-galling cynipids so the prevalence of intraspecific gall parasitism is unknown. However, communal oviposition and female fighting for favorable oviposition sites are known to occur in *Diastrophus* species galling *Rubus* bushes (Jones 1983; Pujade i Villar 1984). Interspecific parasitism among herb gallers is rare enough that it has not yet been reported, but it does not have to be common to be evolutionarily significant.

The hypothesis that gall initiation in cynipids involves injection of a symbiotic virus or some other transferable genetic element into the host plant (Cornell 1983) raises an interesting possibility for convergences in host-plant preferences. If females or larvae of parasitic individuals were able to take up the foreign virus or gall-initiation element of their victims, the parasites could shift easily to the host plant harboring the victim. If gall structure were determined by the symbiont, then the galls of the parasite on the new host plant would be similar to those of the original gall inducer. The structural similarity between the galls induced by associated herb galler species pairs lends some support to this idea (Table 2). However, it is also possible that this similarity is simply due to the fact that species making similar galls on the same host plant organ are more likely to be involved in interspecific interactions. In either case, the high degree of convergence in host-plant use among cynipids is remarkable and warrants further study.

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Corresponding Editor: K. Shaw