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The evolution of host plant manipulation by insects: molecular and ecological evidence from gall-forming aphids on *Pistacia*

M. Inbar,[a,*,1] M. Wink,[b] and D. Wool[c]

[a] *Department of Biology, University of Haifa–Oranim, Tivon 36006, Israel*
[b] *Institut für Pharmazie and Molekulare Biotechnologie (IPMB), Universität, Heidelberg, Abt. Biologie, Im Neuenheimer Feld 364, D-69120, Heidelberg, Germany*
[c] *Department of Zoology, Tel Aviv University, Tel-Aviv 69978, Israel*

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Abstract

One of the most striking characteristics of gall-forming insects is the variability in gall position, morphology, and complexity. Our knowledge of the driving forces behind the evolutionary divergence of gall types is limited. Natural enemies, competition, and behavioral constraints might be involved. We present a cladogram, based on sequences of COI and COII (1952 bp), of mitochondrial DNA for the evolution of 14 species of gall-forming aphids (Fordinae). These insects induce five gall types with remarkable morphological variation on *Pistacia* spp. hosts. The parsimony cladogram divides the Fordinae into three lineages, Fordini and Baizongiini, and a third (new) sister group including the previously Fordini member, *Smynthurodes betae* (West). We then use ecological data to trace and explain the evolution of gall morphology. The aphids seem to have evolved gradually towards better ability to manipulate their host plant, induce stronger sinks, and gain higher reproductive success. We suggest that the ancestral gall type was a simple, open, ''pea''-sized gall located on the leaflet midvein. Some Fordini and *S. betae* evolved a two-gall life cycle, inducing a new gall type on the leaflet margin. The Baizongiini improved the manipulation of their host by inducing larger galls near the midvein, with stronger sinks supporting thousands of aphids. Similar gall types are induced at similar sites on different *Pistacia* hosts suggesting control of the aphids on gall morphology and frequent host shifts. Thus, even extreme specialization (specific gall and host) is flexible.
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1. Introduction

The fascinating intimate relationships between gall-forming insects and their host plants attract the attention of ecologists and evolutionary biologists. One of the most striking characteristics in many groups of gall-forming insects is the variability in gall position, morphology, and structural complexity. Galls can be found on many host plant organs, and a given plant organ can bear various gall types (Shorthouse and Rohfritsch, 1992). Leaf galls for example, can develop on the margin, blade, vein, or petiole.

The galling habit probably evolved from related free-feeding insects. In thrips (Crespi and Worobey, 1998) and willow sawflies (Price, 1992; Price and Roininen, 1993), galling was probably preceded by leaf folding. In psyllids, true galls presumably developed from simple pseudogalls (Yang and Mitter, 1994). In oak galls (Stone and Cook, 1998, but see Ronquist and Liljeblad, 2001) and cerataphidine aphids (Fukatsu et al., 1994), ancestral galls are assumed to have a single chamber or cavity, and more recent species induced multi-chambered galls.

The mechanism of gall formation by insects remains unknown. However, the insects seem to control gall formation, subject to suitability and reactivity of the plant tissue (Abrahamson and Weis, 1997; Weis et al., 1988). Phylogenetic studies have suggested a tight linkage between insect relatedness and gall morphology, i.e.,

* Corresponding author. Fax ++ 972-4-983-2167.
E-mail address: minbar@research.haifa.ac.il (M. Inbar).
[1] Former surname for M. Inbar was Burstein.

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closely related insects on distinct host plants usually form similar galls (Crespi and Worobey, 1998; Nyman et al., 2000; Stern, 1995; Stone and Cook, 1998). Galls are therefore described as extended phenotypes of the insects (Dawkins, 1982). The same gall type seems to have evolved convergently several times, suggesting that gall morphology may have an adaptive value (Crespi and Worobey, 1998; Stone and Cook, 1998).

It is possible that the evolutionary divergence of gall types was driven by selection (Crespi and Worobey, 1998). The hypothesis most often cited is that pressure imposed by parasitoids and predators may have been the driving force (Cornell, 1983), because gall size, thickness, toughness, empty air space, color, and spiny surface may decrease predation and parasitism (Abrahamson and Weis, 1997; Stone et al., 2002). Another mechanism that may have caused differentiation in gall traits is competition. Gall formers may compete for galling sites and for limiting nutrients in the same host plant (Inbar et al., 1995; Whitham, 1979), because the ability to better control, draw or intercept nutrients may be site-dependent. Even slight differences in gall position may be critical (Whitham, 1979). Galls are associated with the evolution of social behavior and the appearance of soldiers in aphids and thrips. Gall shape could affect the evolution of sociality (Crespi and Worobey, 1998; Stern, 1995). Finally, an alternative scenario proposes that gall morphologies radiated with no adaptive value for the insects.

The aim of the present study was to combine ecological and phylogenetic information in order to understand the evolution of host association and manipulation in gall-forming aphids (Homoptera: Fordinae) on *Pistacia* (Anacardiaceae). These aphids induce remarkably different galls; some even induce two completely different galls on the same host. We use molecular methods to infer possible phylogenetic relationships among the species. Then we apply our knowledge of the ecology and biology of the species to suggest pathways of the evolution of gall traits and explain the adaptive significance and the driving forces behind the diversity in gall types.

2. Materials and methods

2.1. Gall-formers and host plants

Approximately 16 gall-forming aphids (Fordinae) are found in Israel. Some of them are known in Mediterranean type habitats in Europe, Central Asia, and North Africa (Bodenheimer and Swirski, 1957). These species are divided into two tribes, Fordini and Baizongiini, each species making a characteristic gall on one *Pistacia* host plant. All but the rare species *Rectinasus buxtoni* Theobald and an undescribed species belonging to the genus *Baizongiini*, are included in the present study

Table 1
Life history traits of the Fordinae aphids examined in this study

Gall type[a]	Species	Tribe[b]	Host plant	Aphids/Gall[c]	GenBank Accession No.: COI; COII
Pea/margin	*Smynthurodes betae* (West)	Fordini (Probably a new tribe)	*P. atlantica*	~100	AY227078; AY227104
Pea/margin	*Forda riccobonii* (Stephani)	Fordini	*P. atlantica*	~100	AY227076; AY227101
Pea/margin	*Forda marginata* Koch	Fordini	*P. palaestina*	<50	AY227091; AY227098
pea/margin	*Forda formicaria* von Heyden	Fordini	*P. palaestina*	~100	AY227086; AY227097
Margin	*Paracletus cimiciformis* von Heyden	Fordini	*P. palaestina*	<100	AY227089; AY227102
Margin	Fordini sp.A.	Fordini	*P. atlantica*	<100	AY227089; AY227102
Margin	Fordini sp.B.	Fordini	*P. atlantica*	<100	AY227088; AY227100
Bag	*Asiphonella dactylonii* Theobald	Baizongiini	*P. palaestina*	~300?	AY227084; AY227106
Bag	*Aploneura lentisci* (Passerini)	Baizongiini	*P. lentiscus*	~300	AY227083; AY227092
Spherical	*Geoica* sp[d]	Baizongiini	*P. atlantica*	<1000	AY227075; AY227094
Spherical	*Geoica* sp[d]	Baizongiini	*P. atlantica*	<1000	AY227085; AY227095
Spherical	*Geoica wertheimae* Brown & Blackman	Baizongiini	*P. palaestina*	<1000	AY227080; AY227096
Bud (banana)	*Baizongia pistaciae* L.	Baizongiini	*P. palaestina*	>10,000	AY227079; AY227093
Bud (cauliflower)	*Slavum wertheimae* H.R.L.	Baizongiini	*P. atlantica*	~5000	AY227077; AY227103

The tribal affiliation is according to morphology.

[a] When two galls are formed by the same species, the fundatrix gall is shown first (left).

[b] Division to tribes according to Bodenheimer and Swirski (1957).

[c] Mean number of aphids/gall. Mean number of aphids/pea gall are not shown in the table (see Fig. 4). The number of aphids in the galls of *A. dactylonii* is based on sporadic observations.

[d] On *P. atlantica* we collected aphids from two slightly different *Geoica* galls (with smooth and abrasive surface). The unidentified galls may belong to *G. rungsi* and/or *G. harpazi* (Brown and Blackman, 1994).

(Table 1; photos in Koach and Wool, 1977). The life cycle of the Fordinae includes sexual and asexual reproduction and alternation between the primary host (*Pistacia*) and roots of non-specific secondary hosts (Wool, 1984). Galls are only formed on the primary host. Three *Pistacia* species are hosts for the Fordinae in Israel: *P. palaestina*, *P. atlantica*, and *P. lentiscus*. They occur in the Mediterranean forests and their distributions considerably overlap. Galls are induced in the spring by first instar nymphs (fundatrices) hatching from overwintering eggs. Within each gall, 2–3 generations are produced parthenogenetically. In the fall, winged aphids disperse from the galls and their offspring develop on the roots of the secondary hosts. In the next spring, another winged morph (sexuparae) migrates back to the primary host and give birth to males and females. After mating, the fertilized eggs remain on the host, and the fundatrices hatch from them one-year later (two-year life cycle). Four species induce two types of galls in their life cycle. The fundatrix induces small, pea-shaped ('temporary') galls on the leaflet midvein whereas her offspring induce a different ('final') gall on the leaflet margin (Table 1; Wool and Burstein, 1991b). One species, *Slavum wertheimae*, is monoecious on *P. atlantica*, and completes its life cycle on the primary host in one year.

2.2. Gall characteristics and life history traits

Wertheim (1954) described the Fordinae galls as either open, leaflet-margin galls (pocket galls) or closed (bag) galls. Here we recognize five distinct gall types (Table 1). (1) Pea galls, small (~5 mm long) lentil-shaped, unsealed galls located on the leaflet midvein. (2) Margin galls, unsealed, elongate galls (~20-mm long) located on the leaflet margin. (3) Bag galls, unsealed galls located on the upper (adaxial) leaflet surface, and occupy most of the leaflet surface. The gall openings are near the leaflet midvein. (4) Spherical galls, completely sealed globular galls (volume ~4 cm^3) located on the lower (abaxial) side of the leaflet midvein. (5) Bud galls, the largest, completely sealed galls formed by the Fordinae. Although they are very different in shape (banana-shaped galls of *Baizongia pistaciae* and cauliflower galls of *S. wertheimae*), they are similarly induced on the main vein of young leaflet, and eventually take over the entire bud (Wertheim, 1954; Wertheim and Linder, 1961).

The aphids are phloem feeders and divert plant assimilates to the galls. As a measure of an aphid's ability to manipulate host plant physiology, we ranked the sink strength of different galls for assimilates, on a 5-point scale according to the ability of the gall to import assimilates from distant source leaves (Burstein et al., 1994). (1) The source of assimilates is only part of the galled leaflet. (2) The entire galled leaflet. (3) The entire galled leaf (*Pistacia* leaves are pinnate). (4) Neighboring leaves on the same shoot. (5) Leaves of the entire shoot, and even leaves of neighboring shoots.

2.3. Molecular phylogeny: DNA extraction, PCR, and sequencing

The aphids were collected in the Galilee region, northern Israel (Table 1). Although the aphids were collected only in Israel, they represent all gall types and crucial life history traits that are found in the Fordinae (Wool, 1984). The cotton aphid *Aphis gossypii* Glover that belongs to a distinct non-galling group, the Aphidinae (Bodenheimer and Swirski, 1957; Swirski and Amitai, 1999), served as outgroup. Total DNA was extracted from 20 to 50 aphids, preserved in 80% ethanol. Tissue homogenates were incubated at 37 °C in lysis buffer (10 mM Tris [pH 7.5], 25 mM EDTA, 75 mM NaCl, and 1% SDS) including 1 mg of proteinase K (Merck, Darmstadt) for 48 h, followed by a standard phenol/chloroform protein extraction. DNA was precipitated from the supernatant with 0.8 vol of cold isopropanol, centrifuged, washed, dried and resuspended in TE buffer.

The mitochondrial cytochrome oxidase genes, COI, and COII were amplified by PCR using the following primers: TV-J-1460: TAC AAT TTA TCG CCT AAA CTT CAG CC; C1-J-1718: GGA GGA TTT GGA AAT TGA TTA GTT CC; C1-J-2183: CAA CAT TTA TTT TGA TTT TTT GG; bla1: GCH AAY TCM TCW ATT GAY ATTA; 2993-Stern: CAT TCA TAT TCA GAA TTA CC; A3772-Normark: GAG ACC ATT ACT TGC TTT CAG TCA TCT; L2-N-3014: TCC AAT GCA CTA ATC TGC CAT ATT A; C1-N-2191: CCC GGT AAA ATT AAA ATA TAA ACT TC.

PCR reactions were performed in a 50μl volume containing 0.8 U of Amersham–Pharmacia Biotech *Taq* DNA polymerase, 50 mM KCl, 1.5 mM $MgCl_2$, and 10 mM Tris–HCl (pH 9). After an initial denaturing step for 4 min at 94 °C, 46 cycles were performed with annealing for 60 s at 45 °C, primer extension for 90 s at 72 °C, and denaturing for 60 s at 94 °C.

PCR products were precipitated in 4 M ammonium acetate and 99% ethanol and then cycle sequenced in a 10 μl reaction. Sequence reaction consisted of 2 μl reaction mix with BigDye terminators (according to the BigDye Terminator Protocol; ABI Applied Biosystems), 10 pmol primers (forward either primer 1, 2, 3, 4, or 5; reverse primer 7) and water to obtain a total volume of 10 μl.

The cycle sequencing was carried out in 25 cycles at 96 °C for 10 s, 52 °C for 5 s and 60 °C for 4 min. Sequencing products were purified by precipitation in 1 vol reaction mix, 1/10 3 M NaAcetate (pH 4.6), 2.5 vol ethanol. After centrifugation for 15 min at 13,000 rpm, DNA pellets were washed in 70% ethanol and resus-

pended in 20 μl formamide. The purified products were diluted 1:5 in water and applied to a 16 column automatic capillary sequencer (ABI 3100) using 50-cm capillaries and POP6 polymer or 80-cm capillaries with POP4 polymer. Sequences of COI (1279 nt) and COII (673 nt) were assembled into a datafile consisting of 1952 nt. We found no evidence for polymorphic sites in our sequencing, despite using multiple individuals for the DNA isolation protocol. Sequences have been deposited in GenBank (Table 1) under Accession Nos. AY227075 to AY227091 for COI and AY227092 to AY227106 for COII.

2.4. Phylogenetic and statistical analyses

The data were analyzed using parsimony with PAUP* 4.0.10 (Swofford, 2001). Equally weighted parsimony analyses were performed using "tree-bisection-and-reconnection" (TBR) branch swapping and the heuristic search option (1106 rearrangements). Of 1952 total characters 1285 characters are constant, 211 variable characters are parsimony-uninformative, and 456 are parsimony-informative. For bootstrap calculation, 10,000 replicates were performed using a heuristic search with TBR branch swapping and a single random addition replicate per bootstrap replication. Branch lengths (i.e., number of nucleotide substations) were calculated by the method described by Fitch (1971).

3. Results

3.1. Molecular phylogeny of the fordinae

The parsimony analysis yielded a single shortest cladogram of 1652 steps (CI = 0.530; RI = 0.448; RC = 0.238) that was found under NN random addition replicates. This tree (Fig. 1) indicates that the molecular phylogeny here presented was in general agreement with the taxonomic dichotomy of the Fordinae into two tribes, Fordini and Baizongiini (e.g., Swirski and Amitai, 1999, Table 1). However, unlike current taxonomy, the molecular analysis placed *S. betae* in perhaps a new sister group, most likely in an ancestral position before the speciation into two tribes. Two clades are recognized, the monophyletic Fordini (excluding *Smynthurodes betae*), with 99% bootstrap support, includes three species of *Forda*, *Paracletus cimiciformis* and two undescribed species (*Fordini* sp. A and *Fordini* sp. B). The two latter species are sister taxa. Here further we will refer to the Fordini as a group that does not include *Smynthurodes*. Because of the shared gall types (pea and margin, see below), *S. betae* is probably more closely related to the Fordini rather then the Baizongiini. The Baizongiini cluster (85% bootstrap support) is composed of two subgroups, the *Geoica* and the *Slavum–Baizongia* clades. The position of *Aploneura* and *Asiphonella* within the traditional Baizongiini is still questionable (low bootstrap support). However, morphological analysis (Manheim, 1996) and gall traits (below) suggest that A. *dactylonii* is indeed close to the Baizongiini. Within the Fordini cluster, parsimony placed *P. cimiciformis* as a sister species to *F. formicaria.* Although our analysis could not resolve the ambiguity in the positioning of *P. cimiciformis*, *Aploneura* and *Asiphonella* the molecular phylogeny serves as a useful framework to discuss the evolution of gall types and host plant utilization.

3.2. Host plant association

The clusters in the cladogram of the aphids do not correspond with their host plants specialization (Fig. 1). The Baizongiini make their galls on all three *Pistacia* hosts. Some Fordini induce galls on *P. palaestina* and some on *P. atlantica*. The galls on the two hosts are similar in shape and occupy similar galling sites. We suggest that several host shifts took place between *Pistacia* species during the evolution of the Fordinae (Fig. 2). Shifts between *P. atlantica* and *P. palaestina* took place twice in the *Forda–Paracletus* clade, at least

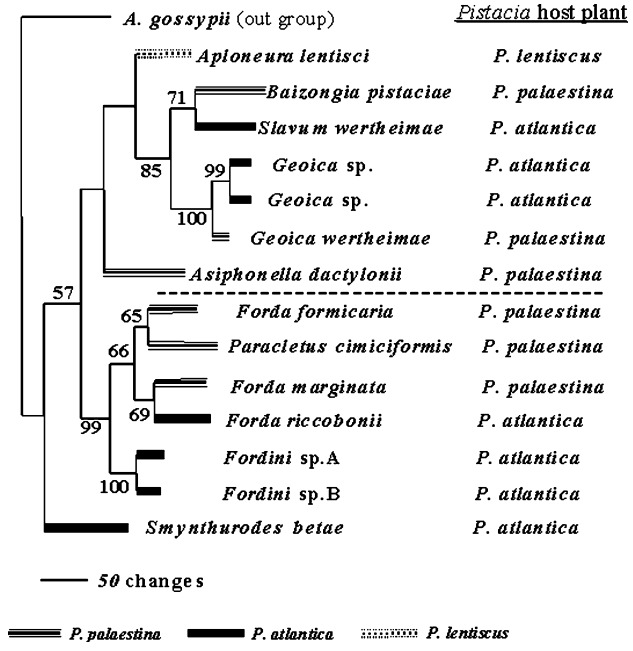

Fig. 1. Single most parsimonious tree of the Fordinae with *Aphis gossypii* (Homoptera: Aphididae) as an outgroup. Bootstrap values (>55) are shown next to the branches. Both *Geoica* spp. were collected from slightly different galls on *P. atlantica.* Dashed horizontal line divides the cladogram to Baizongiini (upper half) and Fordini according to the morphological classification. Note that *S. betae* is placed in a rooted position and therefore maybe considered as a new sister group. Host plant association is listed on right. Note the frequent shifts between *P. atlantica* and *P. palaestina* and a single shift to the distinct host *P. lentiscus.*

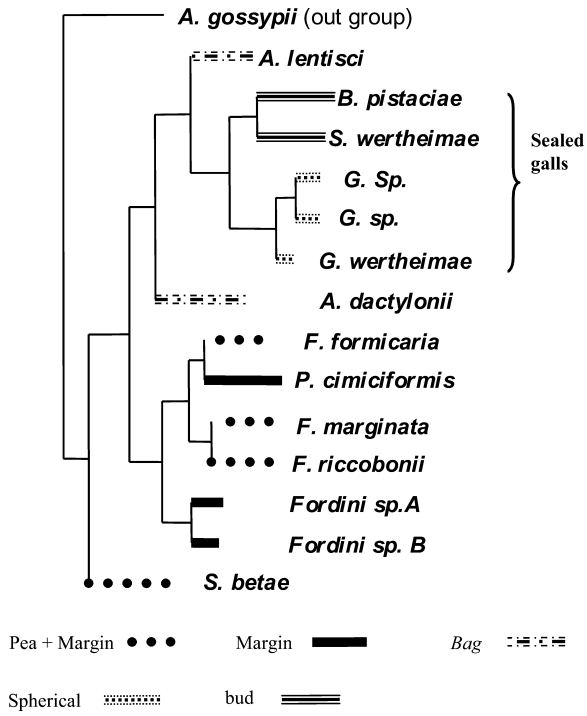

Fig. 2. Gall characteristics mapped on the parsimony phylogram. The galls of the spherical—bud clade in the Baizongiini are also completely sealed. The phylogenetic position of the species with bag galls has low bootstrap support (discussed in the text).

once in the *Geoica* clade, and once in the *Baizongia–Slavum* clade (Fig. 2). Only one species (*A. lentisci*) colonizes *P. lentiscus.* This event seems to have occurred early in the speciation of the Fordinae.

3.3. Phylogenetic patterns in gall types

In contrast with host-plant associations, the cladogram reveals a close association with gall types in the phylogeny of the aphids. Closely related aphids induce similar galls on different hosts, indicating that the insects primarily control gall shape (Figs. 1, 2). In the Baizongiini we find bag galls (*A. dactylonii* and *A. lentisci*) with low statistical support, spherical galls (*Geoica* spp.), and large bud galls of *S. wertheimae* and *B. pistaciae* (Fig. 2). The sealed-gall trait evolved once within the Baizongiini in the root of the bud and spherical branch. It is most likely that the ancestral species of the Fordinae induced a single gall, as is the case in the Pemphiginae. If we accept the parsimony reconstruction placing *S. betae* in a basal position, and the fact that a single gall is an ancestral trait (see Section 4), than the primitive gall type was the simple pea type located on the leaflet midvein. The two-gall life-cycle appeared early in the evolution of the Fordinae with the formation of a second, margin gall in *Smynthurodes* and *Forda*. If this was the case, then the two-gall trait was lost twice in *Paracletus* and the *Fordini* spp. A and B, in which the

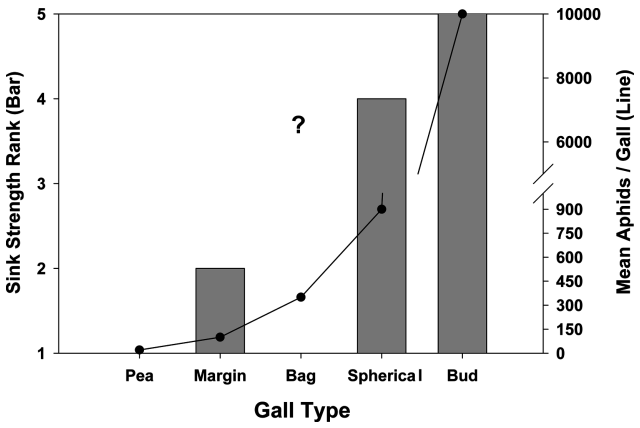

Fig. 3. Gall sink strength and aphid's reproductive success (mean aphids/gall), in the five Fordinae gall types. The sink strength of the bag galls was not measured. It is assumed that the sink strength of this gall type should be ranked three or four. See text for sink rank definition.

fundatrix induces a margin gall. Alternatively, but less likely, the two-gall life cycle as in *S. betae* was the ancestral trait but then was lost once and re-evolved in *Forda* but lost again in *Paracletus*.

3.4. Gall type, host manipulation, and life history traits

There is a clear relationship between gall type, sink strength and the reproductive success of the aphid (Fig. 3, Table 1). The simple, primitive pea galls induce a local, weak sink (Inbar unpublished) that may support ca 20 aphids. Aphids that induce the bud galls however, can manipulate the entire host shoot and produce thousands of offspring. Gall sink strength and aphid reproductive success both increase in the order: pea $<$ margin $<$ bag $\leqslant$ spherical $<$ bud. From the ancestral pea galls we suggest two lines of development. In the *Smynthurodes* and Fordini lines, higher reproductive success was facilitated with the ability of the fundatrix's offspring to emerge from their natal pea galls early enough—when the shoot are still producing new leaves—and induce their own margin galls with slightly stronger sink. The Baizongiini found a way to evolve much stronger sinks and much higher reproductive ability by manipulating the midvein and eventually the entire shoot as in bud galls (Fig. 4, see Section 4). We cannot accurately place the bag galls in the evolution of gall types.

4. Discussion

In general our phylogenetic analyses support the taxonomic division of the Fordinae into two tribes. We show that the two tribes follow different biological pathways of gall types and galling sites. Based on our molecular phylogeny we suggest that *S. betae*, which was previously included in the Fordini (Bodenheimer and Swirski, 1957), may be a new sister group most

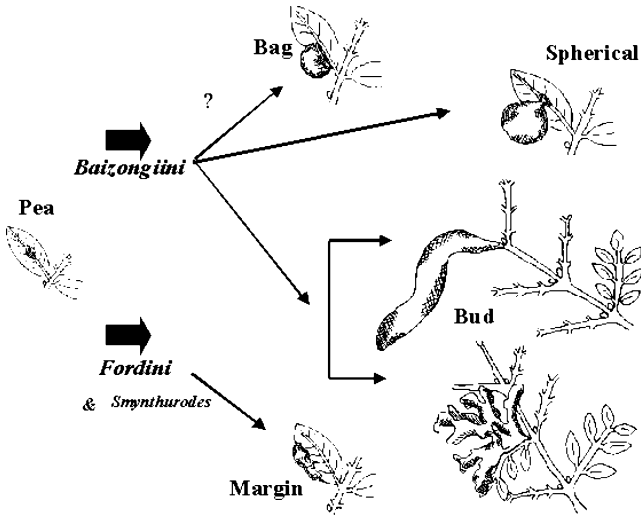

Fig. 4. llustrated evolutionary scenario of the Fordinae gall type. Absolute scaling was not maintained. The pea, margin, bag, and spherical galls are attached to a single leaflet while the large bud galls may reach the banana size. Although not illustrated there is a possibility that within the Baizongiini, the bag gall stands in an intermediate position between the pea and spherical galls. Drawing by Adi Ne'eman.

likely in an ancestral position, but this needs to be tested via congruence with other sources of evidence. The molecular phylogeny also suggests that *Geoica* is monophyletic and nested within the Baizongiini, and not an intermediate group between the two tribes (Davatchi, 1958, cited in Manheim, 1996). Based on gall type and parsimony topology, the position of *Asiphonella* and *Aploneura* (bag galls) as members of the Baizongiini seems reasonable but remains uncertain (Fig. 1).

4.1. Association with the Pistacia host plant

The cladogram of the Fordinae disagrees with their host specialization on *Pistacia* (Fig. 2). Parallel insect-plant coevolutionary cladogenesis is rare (e.g., Ronquist and Liljeblad, 2001). Similarly, sawflies repeatedly and opportunistically colonized willow trees and did not 'track' the host's speciation events (Nyman et al., 1998, 2000). Inbar and Wool (1995) emphasized the remarkable similarities in galling sites between the Fordinae on *P. atlantica* and *P. palaestina*. Each host bears at least one spherical gall, one bud gall, two pea galls and at least three margin galls (Fig. 2). This similarity may be explained by repeated host shifts between the two hosts at least once in each clade (see also Nyman et al., 2000). Each clade forms gall types that were retained during host shifts. Our results are in agreement with phylogenetic analyses of other systems, all claiming insect control of gall morphology (Crespi and Worobey, 1998; Nyman et al., 1998; Stern, 1995; Stone and Cook, 1998). Most host shifts of gall-forming (Ronquist and Liljeblad, 2001; Stern, 1995) and free-feeding insects (Futuyma and McCafferty, 1990) take place between closely related and chemically similar plants (Becerra, 1997). Indeed, *P. Palaestina*, a variant of the European *P. terebinthus*, share similar species of Fordinae (Bodenheimer and Swirski, 1957). Only *A. lentisci* colonized *P. lentiscus* that belongs to distinct section, Eu lentiscus Zoh. (Zohary, 1952).

4.2. Mechanism of horizontal spread and speciation

The horizontal speciation of the Fordinae may have involved two steps: (1) landing on a wrong host and (2) success in inducing a gall on it. The first step was in fact observed. Although the sexuparae did not land on non-*Pistacia* plants, they frequently landed and gave birth on the wrong *Pistacia* species (Wool et al., 1997). Nevertheless, discriminative ability of females for suitable host plants may have little advantage for insects with a short life span, low dispersal capabilities and intimate relationship with the plant. Incorrect landings are common in gall forming cecidomyiids and may be a step toward host shifting (Larsson and Ekbom, 1995). The second step of adopting a new host seems achievable. In a transfer experiment, Burstein and Wool (1993) placed nymphs of *S. betae* on the wrong host-*P. palaestina*. Small typical spindled-shape galls began to develop on the leaflet margins of the non-host (but eventually aborted and the aphids died).

Although the distribution of *P. atlantica* and *P. palaestina* considerably overlap, there are still large regions in the Middle East where they are separated (Zohary, 1952), allowing allopatric speciation. Hybrids between *Pistacia* species can serve as a bridge between hosts. Aphid species characteristically to *P. atlantica* and *P. palaestina* host guilds may be found together on hybrid trees (Koach and Wool, 1977).

4.3. The evolution of gall types

The fundatrix is the most conservative generation in aphids (Moran, 1992). In the vast majority of gall-forming aphids only the fundatrix induces galls. In only few Fordinae species the fundatrix' offspring can also induce galls (Table 1). Because the entire life cycle can be completed in the 'temporary' pea galls (as in *S. betae*, Wool and Burstein, 1991b), it is likely that the ancestral Fordinae aphids had a single gall similar to the midvein pea galls. Interestingly, the galls of the related aphid subfamily, Pemphiginae, are morphologically similar to the pea galls and are often formed on petioles or midveins.

Gall morphology evolved towards greater complexity (Crespi and Worobey, 1998; Fukatsu et al., 1994; Nyman et al., 1998; Stone and Cook, 1998; Yang and Mitter, 1994). For example, in willow-galling sawflies the galls have evolved from marginal leaf folds through leaf margin, leaf petiole to bud galls (Nyman et al., 1998;

Price, 1992, see also Crespi and Worobey, 1998). We suggest the following evolutionary scenario for Fordinae gall morphology (Fig. 4). The ancestral Fordinae had a single, simple open gall located on the leaflet midvein. From this stage the Fordinae evolved in two ways. The Baizongiini have gained the ability to induce larger galls on the midvein, like the open bag galls (*Aploneura*, *Asiphonella*). Next, the completely sealed spherical gall (*Geoica*) and the bud galls (*Slavum*, *Baizongia*) evolved and radiated in parallel. This sequence of events illustrates increasing ability to manipulate the host plant in the vicinity of the midvein, resulting in increased sink strength and reproductive rate (Fig. 3). The Fordini and *Smynthurodes* adopted a different line of gall differentiation. The offspring emerging from the fundatrix pea gall induced their own galls on the leaflet margin (*Smynthurodes*, *Forda*). In this way, a single fundatrix can potentially continue her reproduction through her offspring in 10–20 margin galls (a few hundreds offspring altogether) compensating for the low reproductive output in each gall. This strategy also spreads the risk of gall destruction (Wool and Burstein, 1991b). In some Fordini species (*Paracletus*, *Fordini* spp.), only a single gall is induced (by the fundatrix) that moved to the leaflet margin. The position of the bag gall in this scenario is not clear.

4.4. What drives the evolution of gall morphological divergence?

Although many hymenopteran parasitoids attack aphids, only one, *Monoctonia pistaciaecola* Stary attacks the Fordinae. This wasp attacks the aphids before the galls are closed (Wool and Burstein, 1991a). Aphid predators (dipteran and lepidopteran larvae) consume aphids in the galls of all types (Wool and Steinitz, in preparation). Thus, galls of different shapes do not differ in their vulnerability to natural enemies.

Social behavior, especially the production of sterile soldiers and defenders, has been recorded in gall-forming thrips and aphids (Crespi and Worobey, 1998; Foster and Northcott, 1994). In aphids, the production of soldiers appears to be favored in galls with a small entrance and low surface area/gall volume (Stern, 1995, see also Crespi and Worobey, 1998). Nevertheless, eusociality and soldiers were not discovered among the Fordinae (Inbar, 1998).

Competition is quite frequent among gall-formers, although its role in shaping community structure is questionable (Craig et al., 1990; Inbar and Wool, 1995; Yang and Mitter, 1994). Even when interspecific competition between gall-forming aphids is intense, as among *Eriosoma* species on Japanese elm, selection for shifts in galling sites was not detected (Akimoto, 1988). In the Fordinae there is considerable niche separation that minimizes interspecific competition (Inbar and Wool, 1995). Plant-mediated "sink" competition for assimilates is also possible. Sharing the same leaflet with *G. wertheimae* is lethal for *F. formicaria.* However, only a small fraction of the populations share the same leaflet (Inbar et al., 1995). Overall, interspecific competition among the Fordinae is weak and rare (Burstein and Wool, 1993; Inbar, 1998; Inbar and Wool, 1995; Inbar et al., 1995).

We suggest that shifts in galling site have evolved toward better ability of the aphids to induce stronger sinks, and consequently achieve higher reproductive success (Figs. 3 and 4). Gall formers exploit and modify pre-existing plant physiological processes (Shorthouse and Rohfritsch, 1992), and not every plant tissue is equally reactive (Weis et al., 1988). Baizongiini species that gall the midvein and especially the buds, can better manipulate the hosts than the margin gallers. The bud galls of *S. wertheimae* and *B. pistaciae* induce an active sink that is fed by a well-developed vascular system (Wool, 1997) and can reach impressive sizes and a remarkable reproductive success.

4.5. Extreme specialization: an evolutionary dead end?

Most radiations and speciations of gall-forming insects involved related host plants, with rare shifts to non-related hosts (Ronquist and Liljeblad, 2001; Stern, 1995). Gall-forming insects demonstrate extreme specialization to host species, organs and tissues. In aphids the galls are usually formed by the fundatrix, an extremely specialized stage (Moran, 1992). The proposed trend toward increased specialization of herbivorous insects (usually at the host species level) has led to an ongoing debate whether such specialization would lead to loss of genetic variability and an evolutionary dead-end (Janz et al., 2001; Thompson, 1994). The repeated host shifts in the Fordinae, although between closely related hosts, indicate that specialized ecological interactions are not necessarily dead ends.

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