Phylogenetic evidence that aphids, rather than plants, determine gall morphology

DAVID L. STERN†

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, U.S.A.

SUMMARY

Many diverse taxa have evolved independently the habit of living in plant galls. For all but some viral galls, it is unknown whether plants produce galls as a specialized plant reaction to certain types of herbivory, or whether herbivores direct gall development. Here I present a phylogenetic analysis of gall-forming cerataphidine aphids which demonstrates that gall morphology is extremely conservative with respect to aphid phylogeny, but variable with respect to plant taxonomy. In addition, the phylogeny reveals at least three host plant switches where the aphids produce galls most similar to the galls of their closest relatives, rather than galls similar to the galls of aphids already present on the host plant. These results suggest that aphids determine the details of gall morphology essentially extending their phenotype to include plant material. Based on this and other evidence, I suggest that the aphids and other galling insects manipulate latent plant developmental programmes to produce modified atavistic plant morphologies rather than create new forms *de novo*.

1. INTRODUCTION

Aphids of the tribe Cerataphidini alternate hosts between trees of the genus *Styrax*, the primary host, and a wide diversity of other plants (e.g. Palmae, Zingiberaceae, Graminae, Loranthaceae), the secondary host. An individual aphid species feeds, in general, on a single species of *Styrax*, but may attack several species of secondary host (e.g. several species of Zingiberaceae). This evolutionary conservation of primary host plant is thought to be due, in large part, to the highly specialized feeding requirements of the gall foundress (Moran 1988).

Cerataphidine galls have diverse shapes and sizes, ranging from simple sac-like galls approximately 4 cm long to bizarre, coral-shaped and staghorn fern-shaped galls reaching up to 35 cm long (Docters van Leeuwen-Reijnvaan & Docters van Leeuwen 1926; Stern & Foster 1996). Fukatsu *et al.* (1994), based on examination of gall ontogeny, divided this diversity of cerataphidine galls into two major groups: single-cavity, and multiple-cavity galls. In single-cavity galls, the gall grows around the foundress, enclosing her and her offspring (Aoki & Kurosu 1990; Stern *et al.* 1995). In multiple-cavity galls, the foundress induces many subgalls that do not enclose her. Instead, her offspring move into the subgalls (Kurosu & Aoki 1990*a*, 1991*a*, *b*). The sister tribes Nipponaphidini and Hormaphidini, and most gall-forming aphids, produce single-cavity galls. Therefore, Fukatsu *et al.* suggested that the multiple-cavity gall represents a single derived feature within the Cerataphidini. Here I have tested this hypothesis with a phylogenetic analysis based on mtDNA sequences, and examined the distribution of gall types with respect to host plant taxonomy to test whether galls are a function of the aphids or the host plants.

2. MATERIALS AND METHODS

The methods of specimen collection, DNA preparation and sequencing are described in Stern (1994). Details of collection data for all species are available on request. For 12 species (*Astegopteryx bambucifoliae*, *Cerataphis fransseni*, *Ceratoglyphina styracicola*, *Ceratovacuna japonica*, *Ceratovacuna nekoashi*, *Neothoracaphis yanonis*, *Pseudoregma bambucicola*, *P. koshunensis*, *P. sundanica*, *Tuberaphis styraci*, *T. taiwana*, and *T. takenouchii*), from 758 to 850 base pairs (b.p.) of mtDNA sequence data from the Cytochrome Oxidase I and II regions were analysed. For the remaining eight species ('*Astegopteryx*' *roepkei*, *Astegopteryx* sp., *A. malaccensis*, *Cerataphis vandermeermohri*, *Tuberaphis* sp. 'EDA', *T.* sp. 'SAYA', and *T. sumatrana*) I collected from 393 to 454 b.p. of data from the Cytochrome Oxidase II region. Seven sequences (*Astegopteryx bambucifoliae*, *Ceratoglyphina styracicola* (= *Ceratoglyphina bambusae*), *Ceratovacuna japonica*, *C. nekoashi*, *Neothoracaphis yanonis*, *Pseudoregma koshunensis* and *Tuberaphis takenouchii*) have been published previously (Stern 1994). The remaining sequences are new, and the sequences together with collection data were deposited in GenBank under accession numbers L38295–L38308. Sequences were aligned manually and positions 223–229 at the 3′ end of the CytOxI gene were deleted due to the presence of uninterpretable length variation.

† Present address: Wellcome/CRC Institute and Department of Genetics, Tennis Court Road, Cambridge CB2 1QR, U.K.

The phylogeny was estimated with a preliminary search using maximum parsimony (Felsenstein 1982), as implemented in PAUP (Swofford 1993), and neighbour-joining (Saitou & Nei 1987), as implemented in PHYLIP (Felsenstein, 1993), and a final search with maximum likelihood (Felsenstein 1981, 1982), as implemented in fastDNAml (Olsen *et al.* 1994). Neighbour joining was run using the maximum-likelihood pairwise distances. Bootstrap analysis with 500 replicates was performed for the parsimony and neighbour-joining analyses.

Maximum-likelihood estimation, for both the pairwise distances used in neighbour joining and for the direct estimation of the tree, was run with a transition/transversion ratio of 0.8125 and four categories of relative substitution rates assigned to the first (0.5720), second (0.2235) and third (1.4189) codon positions and the tRNA (0.3943) positions. The relative substitution rates were estimated by using MacClade (Maddison & Maddison 1992) by dividing the number of steps on the parsimony tree by the number of sites for each type of site. The paired-sites test (Kishino & Hasegawa 1989), as implemented in fastDNAml with four categories of substitution rate, was used to test if the most parsimonious trees lacking a monophyletic group of multiple-cavity gall formers (constraint constructed by using MacClade and parsimony trees found using PAUP) were significantly less likely than the maximum-likelihood tree.

The maximum-likelihood tree was rooted with *Neothoracaphis yanonis* (Stern 1994). MacClade was used to reconstruct parsimoniously host plant evolution on the maximum-likelihood tree. Equivocal nodes were resolved to delay changes (DELTRAN).

3. RESULTS

Parsimony analysis found a single most parsimonious tree, and neighbour-joining found a very similar tree (results not shown). Global branch swapping on both the parsimony and neighbour-joining trees with fastDNAml produced a tree similar to both starting trees but with slightly higher likelihood than either starting tree (figure 1).

Twelve most parsimonious trees were found under the constraint of polyphyly of species with multiple-cavity galls. Eight of these trees had significantly lower likelihoods than the maximum-likelihood tree at the 5% level, three trees were significant at the 6% level, and one at the 6.5% level (table 1). Combined with the high bootstrap values for the branch leading to the multiple-cavity gall-forming species (figure 1), these results provide strong support for the monophyly of the multiple-cavity gall-forming species.

The primary-host plants are optimized on the maximum-likelihood phylogeny of the aphids in figure 2. This optimization shows that the aphids have adopted new host plants many times, possibly up to ten times. However, in the absence of a phylogeny of *Styrax* it is not possible to discriminate between the adoption of a new host plant by an aphid species and cospeciation of the aphids and their host plants. The most conservative interpretation of the appearance of aphid species on new host plants is that the aphids and plants cospeciated. However, in four cases a single plant species appears in two disparate parts of the aphid phylogeny (for *Styrax paralleloneura*, *S. japonica*, *S. formosana* and *S. benzoin*). Cospeciation cannot fully explain these patterns. For each pair, at least one of the switches must be due to the adoption of a novel host plant by the aphids.

Three cases (all but *S. paralleloneura*) are particularly useful for dissecting the role of the aphids versus the plants in the determination of gall morphology. In these three cases, the aphids switched to a host plant that already possessed aphids producing galls of a fundamentally different ontogenetic type, either single-cavity or multiple-cavity galls. In all cases the aphids produce galls resembling the galls of their closest relatives, rather than galls similar to those produced by other aphid species already on the host. First, either *Tuberaphis takenouchii* or the common ancestor of *Ceratovacuna japonica* and *C. nekoashi* switched to *Styrax*

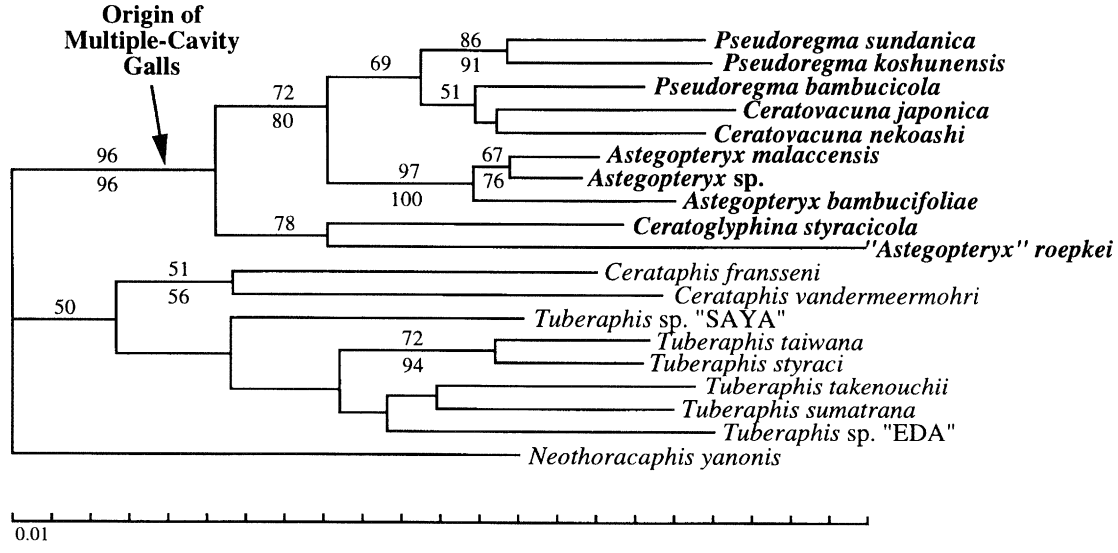

Figure 1. Maximum-likelihood phylogeny for 18 species of the Cerataphidini and *Neothoracaphis yanonis* used as an outgroup to root the tree (Stern 1994). Bootstrap values greater than or equal to 50% are shown for maximum parsimony (above branches) and neighbour joining (below branches). Species producing multiple-cavity galls are in bold type, and the most parsimonious reconstruction of the origin of the multiple-cavity galls is indicated. The scale is marked in units of 0.01 expected substitutions per site.

Table 1. *Kishino & Hasegawa paired-sites tests of maximum-likelihood tree (tree 1), supporting single origin of multiple-cavity galls, versus most parsimonious topologies supporting more than one origin of multiple-cavity galls (trees 2–13)*

tree[a]	LL[b]	ΔLL[c]	s.d.[d]	*p*
1	−4333.84577	—	—	—
2	−4357.99812	−24.15235	12.2142	< 0.05
3	−4358.26851	−24.42274	12.9181	< 0.06
4	−4358.53628	−24.69052	12.5804	< 0.05
5	−4358.82133	−24.97556	13.0814	< 0.06
6	−4359.10708	−25.26131	13.6119	< 0.065
7	−4359.36485	−25.51908	13.2917	< 0.06
8	−4360.91433	−27.06856	12.6306	< 0.05
9	−4361.73361	−27.88784	13.4665	< 0.05
10	−4362.30082	−28.45505	14.2058	< 0.05
11	−4362.60649	−28.76072	13.8964	< 0.05
12	−4363.08643	−29.24066	14.8786	< 0.05
13	−4363.38545	−29.53968	14.5828	< 0.05

[a] Tree 1: ((((AB,(AS,AM)),((PB,(CJ,CN)),(PK,PS))),(AR,CS)),((CV,CF),(((TY,TT),((TK,TU),TE)),TS)),NY).
Tree 2: ((((AB,(AS,AM)),((CJ,(CN,PB)),(PK,PS))),((CV,CF),((((TY,TT),(TU,TK)),TE),TS))),(AR,CS),NY).
Tree 3: (((((AB,AS),AM),((CJ,(CN,PB)),(PK,PS))),((CV,CF),((((TY,TT),(TU,TK)),TE),TS))),(AR,CS),NY).
Tree 4: ((((AB,(AS,AM)),((CJ,(CN,PB)),(PK,PS))),(((CV,CF),(((TY,TT),(TU,TK)),TE)),TS)),(AR,CS),NY).
Tree 5: ((((AB,(AS,AM)),(((CJ,PB),CN),(PK,PS))),((CV,CF),((((TY,TT),(TU,TK)),TE),TS))),(AR,CS),NY).
Tree 6: (((((AB,AS),AM),((CJ,(CN,PB)),(PK,PS))),(((CV,CF),(((TY,TT),(TU,TK)),TE)),TS)),(AR,CS),NY).
Tree 7: (((((AB,AS),AM),(((CJ,PB),CN),(PK,PS))),((CV,CF),((((TY,TT),(TU,TK)),TE),TS))),(AR,CS),NY).
Tree 8: ((((AB,(AS,AM)),(((CJ,PB),CN),(PK,PS))),(((CV,CF),(((TY,TT),(TU,TK)),TE)),TS)),(AR,CS),NY).
Tree 9: ((((AB,(AS,AM)),(((CJ,PB),CN),(PK,PS))),((CV,CF),(((TY,TT),(TE,(TU,TK))),TS))),(AR,CS),NY).
Tree 10: ((((AB,(AS,AM)),(((CJ,PB),CN),(PK,PS))),((CV,CF),(((TY,TT),((TE,TK),TU)),TS))),(AR,CS),NY).
Tree 11: (((((AB,AS),AM),(((CJ,PB),CN),(PK,PS))),(((CV,CF),(((TY,TT),(TU,TK)),TE)),TS)),(AR,CS),NY).
Tree 12: (((((AB,AS),AM),(((CJ,PB),CN),(PK,PS))),((CV,CF),(((TY,TT),(TE,(TU,TK))),TS))),(AR,CS),NY).
Tree 13: (((((AB,AS),AM),(((CJ,PB),CN),(PK,PS))),((CV,CF),(((TY,TT),((TE,TK),TU)),TS))),(AR,CS),NY).
Abbreviations: AB, *Astegopteryx bambucifoliae*; AM, *A. malaccensis*; AR, *A. roepkei*; AS, *A.* sp.; CF, *Cerataphis fransseni*; CV, *C. vandermeermohri*; CS, *Ceratolglyphina styracicola*; CJ, *Ceratovacuna japonica*; CN, *C. nekoashi*; NY, *Neothoracaphis yanonis*; PB, *Pseudoregma bambucicola*; PK, *P. koshunensis*; PS, *P. sundanica*; TE, *Tuberaphis* sp. 'EDA'; TS, *T.* sp 'SAYA'; TY, *T. styraci*; TU, *T. sumatrana*; TT, *T. taiwana*; TK, *T. takenouchii*.

[b] Log likelihood of topology with optimized branch lengths.

[c] Difference in log likelihoods between maximum-likelihood tree and test tree.

[d] Standard deviation of difference in log likelihoods between maximum-likelihood tree and test tree.

japonica. Secondly, *Ceratovacuna nekoashi* and/or *Tuberaphis taiwana* and/or *Tuberaphis takenouchii* switched to *S. formosana*. *S. formosana* and *S. japonica* are probably closely related (P. Fritsch, personal communication) so some of these apparent switches may actually represent lineage sorting. Nevertheless, at least two host switches are required to account for the distribution of species on both *S. formosana* and *S. japonica*. Finally, either *Cerataphis fransseni* or the common ancestor of *Astegopteryx malaccensis* and *A.* sp. switched to *S. benzoin*.

4. DISCUSSION

The conservation of gall morphology with respect to aphid phylogeny and the existence of three host switches where the aphids retained their ancestral gall morphology (figure 2) suggest that gall morphology is determined by aphids (cf. Cornell 1983), rather than being a plant-specific response (cf. Mani 1964) to a generalized gall-inducing signal provided by the aphid. Further support for this conclusion comes from the finding that some gall-producing cerataphidine aphids can invade heterospecific galls and transform the host gall into a morphology more similar to their own galls (Kurosu & Aoki 1990*b*). These results may prove to be general among many gall-inducing organisms. Preliminary evidence from gall-inducing thrips suggests that gall morphology more closely mirrors thrips phylogeny than plant taxonomy (B. Crespi, personal communication).

If the aphids control gall morphology then the gall is essentially an extended phenotype (*sensu* Dawkins 1983) of the aphids, and the aphids should evolve to optimize the morphology and physiology of the gall for their purposes. The physiological changes in plant tissue that accompany gall formation have been studied in several species (see, for example, Bayer 1994). The importance of morphological changes has been given less attention.

For cerataphidine aphids, gall shape and size have two immediate and important consequences. First, the total feeding surface of the gall will probably determine the total number of aphids that can develop within it. It would be interesting to examine whether the evolution of multiple-cavity galls has allowed the evolution of larger colony size. Secondly, colonies in all cerataphidine galls produce reproductively sterile altruistic soldiers (Aoki 1987; Stern & Foster 1996). These soldiers defend the colony by crawling out of openings in the gall onto its surface to attack predators. Foster & Northcott (1994) have discussed the general reasons for the evolution of soldiers within galls. However, the details of gall morphology have two implications for soldier evolution. First, galls with more openings are probably more vulnerable to predators that attack by entering galls (Foster 1990) and more accessible to integral migrants that would compete for resources (cf. Setzer 1980); however, in large galls, multiple openings may allow soldiers rapidly to attack predators. Secondly, the effectiveness of soldier defence is determined to some extent by the surface area to volume ratio of the gall. For example, galls with a small surface area to volume ratio allow a relatively large number of reproductive aphids, within the gall, to be defended with a relatively small soldier force.

How do aphids, and other organisms, induce plants to produce morphologies not normally produced by the plant? Except for the crown gall, which is produced by a virus (Davey *et al.* 1994), the mechanisms of gall induction are unknown for other organisms. The

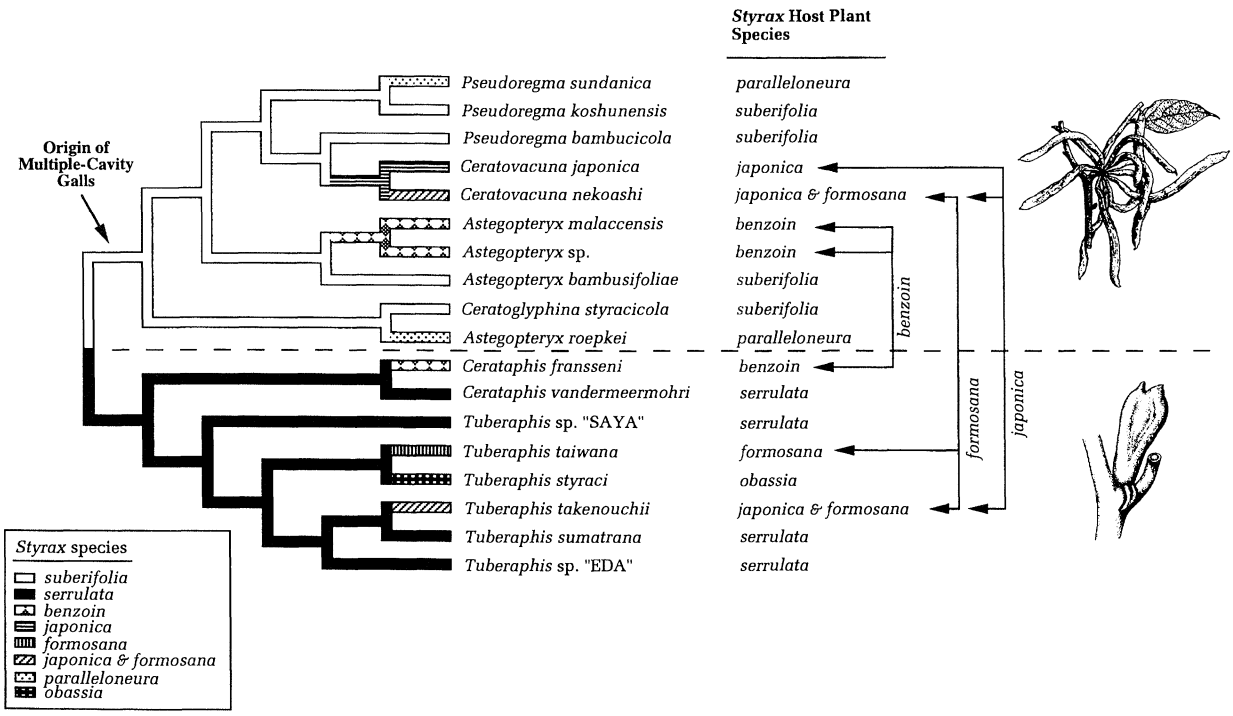

Figure 2. Mapping of primary host plant evolution onto the maximum-likelihood phylogeny for species of the Cerataphidini. The broken line separates the multiple-cavity gall formers (above the line) from the single-cavity gall formers (below). The host switches discussed in the text are indicated with arrows on the right. The multiple-cavity gall of *Astegopteryx* sp. on *Styrax benzoin* (from Docters van Leeuwen-Reijnvaan & Docters van Leeuwen 1926) is illustrated above the broken line, and the single-cavity gall of *Cerataphis fransseni* on *S. benzoin* (from Stern *et al.* 1995) is illustrated below. Other species have galls that appear, superficially, extremely different (see Docters van Leeuwen-Reijnvaan & Docters van Leeuwen 1926; Stern & Foster 1996), but they all share the basic characteristic of being either a single-cavity or multiple-cavity gall.

unusual ontogeny and natural history of cerataphidine multiple-cavity galls provide several clues. All of the multiple-cavity galls mature into, or develop through a phase with, rosettes of subgalls resembling leaves folded over and sutured along the edges, suggesting that the aphids create an atavistic flower composed only of 'leaves'. (Jenkins & Mabberley (1994) have similarly reported apparent atavistic production of a spiny gall resembling a breadfruit by a scale insect on dipterocarps.) Furthermore, the galls of *Ceratovacuna nekoashi*, which grow from stem buds, develop into fully functional flowers if the foundress dies midway through gall development (Kurosu & Aoki 1990*a*). These observations suggest that the aphids induce floral meristem differentiation and then stall normal flower development to produce an atavistic flower containing only leaves. Further modifications, including elongation and folding of the 'leaf', lead to the final gall morphology. Although this is a simplification of the process, this scenario provides testable predictions about the mechanisms underlying gall production. For example, it can be predicted that the aphids first activate meristem identity genes within the plant, such as *LEAFY* OR *APETALA1* (Ma 1994; Weigel & Meyerowitz 1994), causing the meristem to transform towards flower fate. I suggest that the aphids then block some of the organ identity genes (Ma 1994; Weigel & Meyerowitz 1994) to prevent petal, stamen and carpel formation. Mechanisms for unusual flower production are latent within the developmental programme of plants, as shown by recent work on the molecular genetics of flower development (Weigel & Meyerowitz 1994), and may be available as the building blocks for gall inducers.

I thank S. Aoki, B. Crespi, J. Felsenstein, W. Foster, K. Ho and L. Hurst for comments on the manuscript, S. Aoki and U. Kurosu for providing some specimens and considerable help, the Government of Malaysia for permission to undertake research in Malaysia, and the Forest Research Institute of Malaysia. This work was supported by Churchill College, Cambridge and a NSF Doctoral Dissertation Improvement Grant to D. L. S. and Carlos Martinez del Rio.

REFERENCES

Aoki, S. 1987 Evolution of sterile soldiers in aphids. In *Animal societies: theories and facts* (ed. Y. Itô, J. L. Brown & J. Kikkawa), pp. 53–65. Tokyo: Japanese Scientific Society Press.

Aoki, S. & Kurosu, U. 1990 Biennial galls of the aphid *Astegopteryx styraci* on a temperate deciduous tree, *Styrax obassia*. *Acta phytopathol. entomol. hung.* **25**, 57–65.

Bayer, M. H. 1994 Biochemical modification of the phenotype in cynipid galls: cell membrane lipids. In *Plant galls: organisms, interactions, populations* (ed. M. A. J. Williams), pp. 429–446. Oxford: Clarendon Press.

Cornell, H. V. 1983 The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): why and how? *Am. Midl. Nat.* **110**, 225–234.

Davey, M. R., Curtis, I. S., Gartland, K. M. A. & Power, J. B. 1994 *Agrobacterium*-induced crown gall and hairy root diseases: their biology and application to plant genetic engineering. In *Plant galls: organisms, interactions,*

populations (ed. M. A. J. Williams), pp. 9–56. Oxford: Clarendon Press.

Dawkins, R. 1983 *The extended phenotype: the long reach of the gene*. Oxford University Press.

Docters van Leeuwen-Reijnvaan, C. C. & Docters van Leeuwen, W. M. 1926 *Zoocecidia of the Netherlands East Indies, Batavia*. Batavia: Drukkerij de Unie.

Felsenstein, J. 1981 Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. molec. Evol.* **17**, 368–376.

Felsenstein, J. 1982 Numerical methods for inferring evolutionary trees. *Q. Rev. biol.* **57**, 379–404.

Felsenstein, J. 1993 *PHYLIP, Phylogenetic Inference Package*, Version 3.55c. Seattle: University of Washington.

Foster, W. A. 1990 Experimental evidence for effective and altruistic colony defence against natural predators by soldiers of the gall-forming aphid *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). *Behav. Ecol. Sociobiol.* **27**, 421–430.

Foster, W. A. & Northcott, P. A. 1994 Galls and the evolution of social behaviour in aphids. In *Plant galls: organisms, interactions, populations* (ed. M. A. J. Williams), pp. 161–182. Oxford University Press.

Fukatsu, T., Aoki, S., Kurosu, U. & Ishikawa, H. 1994 Phylogeny of Cerataphidini aphids revealed by their symbiotic microorganisms and basic structure of their galls: Implications for host-symbiont coevolution and evolution of sterile soldier castes. *Zool. Sci.* **11**, 613–623.

Jenkins, R. M. & Mabberley, D. J. 1994 A breadfruit amongst the dipterocarps: galls and atavism. In *Plant galls: organisms, interactions, populations* (ed. M. A. J. Williams), pp. 183–199. Oxford: Clarendon Press.

Kishino, H. & Hasegawa, M. 1989 Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. molec. Evol.* **29**, 170–179.

Kurosu, U. & Aoki, S. 1990*a* Formation of a 'cat's-paw' gall by the aphid *Ceratovacuna nekoashi* (Homoptera). *Jap. J. Entomol.* **58**, 155–166.

Kurosu, U. & Aoki, S. 1990*b* Transformation of the galls of *Astegopteryx bambucifoliae* by another aphid, *Ceratoglyphina bambusae*. *Acta phytopathol. entomol. hung.* **25**, 113–122.

Kurosu, U. & Aoki, S. 1991*a* The gall formation, defenders and life cycle of the subtropical aphid *Astegopteryx bambucifoliae* (Homoptera). *Jap. J. Entomol.* **59**, 375–388.

Kurosu, U. & Aoki, S. 1991*b* Incipient galls of the soldier-producing aphid *Ceratoglyphina bambusae* (Homoptera). *Jap. J. Entomol.* **59**, 663–669.

Ma, H. 1994 The unfolding drama of flower development: recent results from genetic and molecular analyses. *Genes Dev.* **8**, 745–756.

Maddison, W. P. & Maddison, D. R. 1992 *MacClade: Analysis of phylogeny and character evolution*, version 3.0. Sunderland, Massachusetts: Sinauer Associates.

Mani, M. S. 1964 *Ecology of plant galls*. The Hague: Dr W. Junk.

Moran, N. A. 1988 The evolution of host-plant alternation in aphids: Evidence for specialization as a dead end. *Am. Nat.* **132**, 681–706.

Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. 1994 fastDNAml: A tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput. Appl. Biosci.* **10**, 41–48.

Saitou, N. & Nei, M. 1987 The neighbour-joining method: A new method for reconstructing phylogenetic trees. *J. molec. Evol.* **29**, 170–179.

Setzer, R. W. 1980 Intergall migration in the aphid genus *Pemphigus Ann. ent. Soc. Am.* **73**, 327–331.

Stern, D. L. 1994 A phylogenetic analysis of soldier evolution in the aphid family Hormaphididae. *Proc. R. Soc. Lond.* B **256**, 203–209.

Stern, D. L., Aoki, S. & Kurosu, U. 1995 The life cycle and natural history of the tropical aphid *Cerataphis fransseni* (Homoptera, Hormaphididae), with reference to the evolution of host alternation. *J. nat. Hist.* (In the press.)

Stern, D. L. & Foster, W. A. 1996 The evolution of soldiers in aphids. *Biol. Rev.* (In the press.)

Swofford, D. L. 1993 *PAUP, Phylogenetic Analysis Using Parsimony*, version 3.1.1. Washington D.C.: Smithsonian Institution.

Weigel, D. & Meyerowitz, E. M. 1994 The ABCs of floral homeotic genes. *Cell* **78**, 203–209

Received 3 January 1995; accepted 26 January 1995