Phylogeny, Evolution and Biogeography of Gall-Forming Aphids (Insecta: Homoptera): A Case Study from the Eriosomatini

Shin-ichi Akimoto

Division of Environmental Resources, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

ABSTRACT

Gall-forming aphids are highly specific to their host plants, and speciation in galling aphids has proceeded in association with a single species or species group of host plants (primary host). This study firstly aims to revise the associations between galling aphids and their host plants by focusing on historical changes in the distribution of host plants. Aphids of the Eriosomatinae are typically associated with the primary and secondary host plants and alternate host plants seasonally, with sexual reproduction on the primary host plant. However, some species are not host-alternating and are wholly parthenogenetic on the secondary host plant. This study secondly aims to test the possibility of long-term persistence of an aphid species by means of parthenogenesis (relict hypothesis). The eriosomatine genus Colopha is a small aphid group represented by three sexual and three asexual species. The sexual species are associated with two Ulmus sister species distributed disjunctly in Europe and eastern North America. In East Asia, an asexual species, Colopha kansugei, is distributed widely on the secondary host. This study tested whether the relict hypothesis is applicable to C. kansugei by comparing DNA sequences. A high rate of substitution (3.4% at the maximum) was found in the mitochondrial COI sequence between local populations. Available evidence suggests that Colopha kansugei has persisted on the secondary host plant through parthenogenesis, probably following the local extinction of the primary host. Use of molecular techniques might possibly detect much more ancient species among parthenogenetic aphids with disjunct distributions.

Keywords: Asexual, Host alternation, Disjunct distribution, Refuge, Relict

INTRODUCTION

Gall-forming insects, as parasites to plants, are monophagous and highly specific to their host plants. Gall-forming insects have shared an evolutionary history with their host plants, and their life cycles and distribution ranges have been constrained by the host plants. Biogeographic evidence suggests that the associations between gall-forming aphids and their host plants have a long history that traces back to the early Tertiary [1–2]. Throughout the Tertiary and Quaternary, hardwood taxa drastically shifted their distributional ranges depending on global climatic changes, which has resulted in the disjunct distributions of some hardwood taxa in, for example, East Asia and eastern North America [3–5]. Hardwood trees adapted to mild climates are known to have survived glacial episodes in some fragmented refuges and expanded their distribution to the north in interglacial episodes, with the retreat and expansion of their distribution having been repeated during the Quaternary [6–8]. Because the dis-
tribution of gall-forming insects is exclusively affected by the distribution of the host plants, it is necessary to consider past changes in the distribution of the host plants for an understanding of the present distributions of gall-forming insects.

The general pattern in the speciation of gall-forming insects is that a specific clade of gall-forming insects has diversified in association with a specific clade of host plants. Although this phenomenon is likely to be interpreted from the viewpoints of host-parasite coevolution, the most marked pattern is not cospeciation between host plants and insects but accelerated diversification of gall insects on a single plant species. There are a number of examples in which several closely related species of gall-forming insects are associated with the same host species and often coexist on the same host individuals [9–14]. Among gall-forming aphids, examples include Eriosoma on Ulmus [15], Pemphigus on Populus [16], Fordinea on Pistacia [14, 17], Tuberocephalus on Prunus [18] and Nipponaphis on Distylium [19]. This study provides more detailed information about the phylogeny, distribution pattern and host relationship of gall-forming aphids of the Eriosomatini (Aphidoidea: Eriosomatinae) by focusing on historical changes in the host plants of the family Ulmaceae.

APHIDS AND HOST PLANTS

Aphid Life Cycle and Mordvilko's Hypothesis

Aphids of the Eriosomatini are associated with two kinds of plants (primary and secondary hosts) that are distantly related and they seasonally alternate host plants between the primary host, broad-leaved deciduous trees, and the secondary host, mainly herbaceous plants. Most species of eriosomatine aphids consist of sexual and asexual strains. Sexual strains are host-alternating between the primary and secondary host plants, with sexual reproduction on the primary host and asexual reproduction on the secondary host. In contrast, asexual strains persist on the secondary host and reproduce parthenogenetically all year round. In a local population, obligatorily or facultatively asexual strains often coexist with sexual strains on the secondary host [20, 21], and the proportion of asexual strains varies depending on the environmental conditions. Obligatory asexual populations are sometimes distributed beyond the ranges of the primary host plant. A few species are wholly parthenogenetic on the secondary hosts. Mordvilko [22] hypothesized that asexual strains will be distributed outside the range of the primary host if sexual strains became extinct together with local extinction of the primary host due to cold and arid climates but if asexual strains survived in situ on the secondary host. This hypothesis is based on the prediction that secondary host plants, herbaceous and grass species, may be much more cold-tolerant than primary hosts, hardwood species. Mordvilko [22] illustrated this theory with Tetraneura rubra (= T. caerulescens) that is found on the secondary host in Egypt, where the primary host (Ulmus spp.) does not occur at present. If Mordvilko's hypothesis is true, asexual populations may have reproduced parthenogenetically over a long period of time since the extinction of the primary host. In another example, based on molecular phylogeny, von Dohlen et al. [23] inferred that an American Hamamelistes species has lasted on the secondary host by means of parthenogenesis for 2–4 million years.

Some authors [e.g., 20, 24] have criticized Mordvilko's hypothesis on the ground that asexual strains can expand their range by alate migration beyond the range of the primary host where sexual and asexual strains coexist. Several eriosomatine species can disperse over a long distance by means of alate exules that fly from secondary host to secondary host. If this criticism is true, the origins of asexual populations should be more recent events than Mordvilko's hypothesis predicts. The present study makes use of fossil records of the host plants and molecular phylogenetic analysis to evaluate whether or not Mordvilko's hypothesis is true for the origin of some asexual species.

Phylogeny and Biogeography of Ulmaceae

Gall-forming aphids of the Eriosomatini are associated with the genera Ulmus and Zelkova (Ulmaceae; Ulmoideae) as primary hosts. The ancestors of Ulmus and Zelkova originated in the Cretaceous, and in the early Tertiary the elements of the genera were distributed widely in higher latitudes of the Northern Hemisphere around the arctic zone. Ancestors of Ulmus and Zelkova contributed to the so-called Arcto-Tertiary flora that existed in the early Tertiary. Fossil records suggest that with climatic deterioration after the mid Miocene, the distribution of Ulmus and Zelkova gradually shifted southwards, and that the elements survived glacial episodes in some mild refuges, including eastern and western North America, East Asia, the eastern and northwestern Himalaya, the Caucasus, and southern Europe [6]. The present study is based on the present and past distribution of Zelkova and the main clades of
Ulmus, and on a molecular phylogeny of Ulmus [25].

The genus Zelkova, including 5 extant species, is currently distributed disjunctly in East Asia, West Asia (around the Black Sea), Crete and Sicily (Fig. 1), but the fossil record shows that trees of this genus were distributed widely in Eurasia and North America across Beringia during the mid Tertiary. Fossils of Zelkova leaves have been found in sediments from Oregon, North America [26]. The present distribution of Zelkova corresponds to Quaternary refuges, suggesting that local extinction of Zelkova species has occurred frequently outside the refuges.

The genus Ulmus, including 18 extant species, has been divided into five sections. The section Blepharocarpus is composed only of two species, U. americana and U. laevis. Molecular phylogeny reveals that these two Ulmus species are most closely related to each other [25], but they are distributed disjunctly in central Europe (U. laevis) and in eastern North America (U. americana) (Fig. 2). This distribution pattern suggests that the elements of Blepharocarpus have become extinct in Asia and in western North America. Ulmus parvifolia and its related species, characterized by autumnal blooming, occur from southern East Asia to Southeast Asia (Fig. 1). This clade includes evergreen elements and is adapted to moist and warm climates. Molecular phylogeny shows that this clade had branched off the other clades early on [25] and had become restricted to areas around refuges in the Quaternary.

The section of Ulmus with the largest species diversity is Madocarpus, the elements of which are currently very common and distributed continuously and widely over Eurasia from Europe to northern East Asia (Fig. 3). One element of this section occurs in eastern North America. The wide and continuous distribution of Madocarpus suggests that this clade rapidly expanded its range northwards from refuges after the last glacial maximum both in Europe and in Asia; Madocarpus is thus considered to be the most cold-tolerant among clades of Ulmus. The elements of this section are very similar to one another, without clear morphological discontinuities.

![Fig. 1 Distribution of the genus Zelkova (above) and of the parvifolia group of Ulmus (below). Filled squares in the upper figure indicate the collection records of a parthenogenetic aphid species, Gharesia polunini.](image-url)
Fig. 2 The distribution ranges of Ulmus section Blepharocarpus (Ulmus leavis and U. americana; green areas) and the associated gall-forming aphids, the genus Colopha. No elements of Blepharocarpus are distributed in East Asia, but parthenogenetic species Colopha kansugei and C. setaricola are distributed on the secondary hosts, Carex lenta and Setaria chondrachne, respectively, there (yellow area). Another parthenogenetic species Colopha hispanica is distributed in Spain.

Fig. 3 The distribution ranges of Ulmus section Madocarpus.

DIVERSITY AND BIOGEOGRAPHY OF ERIOSOMATINI

The number of species, distribution ranges, and host relationships of the Eriosomatinae are summarized in Table 1. The Eriosomatini is composed of two large and many small genera. Eriosoma and Tetranura are large genera with more than 30 species each. These genera are mainly associated with the Ulmus section Madocarpus as the primary hosts, and, as genera, are distributed widely over Eurasia (and in North America, Eriosoma). On the other hand, three genera closely related to Eriosoma (Aphidiongus, Schizoneurata and Schizoneurella) are all monotypic and associated with U. parvifolia and U. villosa, both of which are adapted to warm climates.
**Table 1** Genera of the Eriosomatinae and their distribution and host plants.

<table>
<thead>
<tr>
<th>Genus</th>
<th>#species*</th>
<th>Distribution</th>
<th>Primary host**</th>
<th>Type of gall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eriosoma</td>
<td>32(1)</td>
<td>Eurasia-N America</td>
<td>U, Madocarpus</td>
<td>leaf roll, open</td>
</tr>
<tr>
<td>E. yangi</td>
<td>1</td>
<td>East Asia</td>
<td>Ulmus parvifolia</td>
<td>leaf roll, open</td>
</tr>
<tr>
<td>Aphidounguis</td>
<td>1</td>
<td>East Asia</td>
<td>Ulmus parvifolia</td>
<td>leaf roll, open</td>
</tr>
<tr>
<td>Schizoneurata</td>
<td>1(1)</td>
<td>Southern N America</td>
<td>SH</td>
<td>—</td>
</tr>
<tr>
<td>Schizoneurella</td>
<td>1</td>
<td>Eastern Himalaya</td>
<td>Ulmus villosa</td>
<td>leaf roll, open</td>
</tr>
<tr>
<td>Tetraneura</td>
<td>32(?)</td>
<td>Eurasia</td>
<td>U, Madocarpus</td>
<td>pouch, closed</td>
</tr>
<tr>
<td>Kaltenbachiella</td>
<td>8(1)</td>
<td>Eurasia-N America</td>
<td>U, Madocarpus</td>
<td>pouch, closed</td>
</tr>
<tr>
<td>Colophina</td>
<td>6(3)</td>
<td>Eurasia-N America</td>
<td>U, Blepharocarpus</td>
<td>pouch, closed</td>
</tr>
<tr>
<td>Paracolopha</td>
<td>2(1)</td>
<td>East Asia</td>
<td>Zelkova</td>
<td>pouch, closed</td>
</tr>
<tr>
<td>Colophina</td>
<td>4(2)</td>
<td>East Asia</td>
<td>Zelkova</td>
<td>pouch, open</td>
</tr>
<tr>
<td>Byrsocriptoides</td>
<td>2</td>
<td>West Asia</td>
<td>Zelkova</td>
<td>leaf roll, open</td>
</tr>
<tr>
<td>Hemipodaphis</td>
<td>3(1)</td>
<td>East Asia-West Asia</td>
<td>Zelkova</td>
<td>leaf roll, open</td>
</tr>
<tr>
<td>Gharesia</td>
<td>1(1)</td>
<td>Himalaya-N America</td>
<td>SH</td>
<td>—</td>
</tr>
</tbody>
</table>

* the total number of species in a genus, with the number of parthenogenetic species found only on the secondary hosts in parenthesis.

** U, Ulmus; SH, recorded from secondary hosts.

---

Schizoneurata is from the secondary host in Florida, North America, and the primary host is unknown. This pattern of species diversity is likely to reflect historical changes in the distributional area of the host plant. It may be hypothesized that the three monotypic genera, Aphidounguis, Schizoneurata and Schizoneurella, represent survivors of ancestral stocks which had a large species diversity before Quaternary glaciation, during which most of the elements became extinct together with the primary host. The low species diversity of galling aphids and their restricted distribution could be explained by assuming a large-scale extinction of ancestral host groups adapted to warm climates during glacial episodes. A similar pattern is found in Tetraneura and its allied genera. Tetraneura is another large genus, and its sister genus Paracolopha is associated with Zelkova and represented by only two species. Zelkova now has a disjunct and restricted distribution, so, as mentioned above, most local populations of Zelkova species may have become extinct since the late Tertiary. Such local extinction of host plants may have been responsible for limited species diversity in aphid genera associated with Zelkova, i.e., Colophina, Byrsocryptoides and Hemipodaphis.

The link between great species diversity of Tetraneura and Eriosoma and a wide and continuous distribution of their Madocarpus host plants seems highly plausible. By being associated with host plants that are cold- and arid-tolerant, Tetraneura and Eriosoma may have evaded extinction, and this may be a reason for their large species diversity.

One Eriosoma species, Eriosoma yangi, is exceptionally associated with Ulmus parvifolia. The association with Ulmus parvifolia in Eriosoma may be ancestral (ancestral host plant, with a subsequent host shift to Madocarpus) or derived (host shift from Madocarpus to U. parvifolia). The peculiar morphology of Eriosoma yangi seems to point to the possibility of an ancestral association with Ulmus parvifolia.

The hypotheses presented here can partly be tested by constructing a molecular phylogeny of Eriosomatinae. If these hypotheses are true, each monotypic genus should have branched off the main branch of the phylogeny early on, and these genera, as a whole, should constitute a paraphyletic group. In contrast, species of Tetraneura and Eriosoma should have diverged rapidly since the late Tertiary, and each of the genera should constitute a monophyletic taxon.

**DISJUNCT DISTRIBUTION AND RELICT APHIDS**

**Possible Examples**

The genus Gharesia is monotypic, and G. polunini has been recorded from Carex spp., originally secondary hosts, at a high elevation of more than 3000 m at three localities: the Himalayan region and two mountainous sites in California, North America (Fig. 1). Based on a morphological comparison, Hille Ris Lambers [27] concluded that the Himalayan and American populations belong to the
same species. The primary host of *Gharesia* is not known. It is a puzzle to understand this long-distance disjunct distribution at high altitude. Mordvilko's hypothesis may explain this distributional pattern. This hypothesis would propose that during the mid Tertiary this aphid species had expanded its distribution from Eurasia to North America across Beringia, together with an unknown host plant of Ulmaceae. After the extinction of the primary host, *Gharesia* may have been left on the secondary host at refuges on different continents and may have persisted as a relict. If this hypothesis is true, *Gharesia polunini* may have originated before the mid Tertiary (at least 12 my ago) when Beringia was covered with deciduous forest for the last time [3]. If this hypothesis is true, a molecular genetic analysis should reveal great genetic differentiation between the Himalayan and California populations.

The genus *Colopha* induces closed-pouch galls on leaves of *Ulmus* species of the section *Blepharocarpus*, that is, *Colopha compressa* on *Ulmus laevis* and *Colopha graminis* and *C. ulmicola* on *Ulmus americana* (Fig. 2). These sexual species are distributed disjunctly in North America and in central Europe, together with the host plants of *Blepharocarpus*. This distribution pattern suggests that the origin of the genus *Colopha* can be traced back to mid Tertiary (at least 12 my ago) for the reason already discussed. In contrast, three parthenogenetic species of *Colopha* are distributed outside the present distribution ranges of *Blepharocarpus*: *Colopha kansugei* and *C. setaricola* in East Asia and *C. hispanica* in Spain (Fig. 2). The origin of these parthenogenetic species could be explained if the primary hosts, *Ulmus* species of *Blepharocarpus*, became extinct from the ranges of these species and if they have persisted on the secondary hosts while maintaining morphological stasis [28]. This hypothesis predicts that there should be large genetic differentiation between local populations or between clones because the asexual species have accumulated genetic changes without genetic recombination since the extinction of the primary hosts. This prediction can be tested by comparing DNA sequences between local populations of an asexual species.

### Test for the Relict Hypothesis

The relict hypothesis was tested using *Colopha kansugei* collected at several localities in East Asia. This species is appropriate for this test because it is common, with a wide distribution range from western Japan to Nepal. Extraction of template DNA from aphid samples, amplification of a 700 bp fragment of the mitochondrial COI gene by PCR, and sequencing are based on Yoshizawa and Johnson [29]. Detailed results are presented elsewhere, and only a synopsis of the results is mentioned here. The relict hypothesis predicts considerable genetic divergence among local parthenogenetic populations of a relict species such as *C. kansugei*. In contrast, the hypothesis predicts less genetic divergence in a species that has been broadly distributed through dispersal by alates. *Tetraneura nigriabdominalis* is broadly distributed over the same range as the ancestral population of *C. kansugei*. Thus, this study compares geographic variation in COI sequences between *C.*

---

**Table 2** Percentage genetic distances (uncorrected p distances) between local populations of *Colopha kansugei* in 700 bp of mitochondrial COI. 1-11, *C. kansugei*; 12, *C. setaricola*. 1 and 5, Chiba prefecture; 2 and 3, Kanagawa prefecture; 6, Fukuoka prefecture; 7-9, Nansei Islands; 10, Wakayama prefecture; 11, Chiangmai, Thailand.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Tateyama</td>
<td>0.143</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Hayama</td>
<td>0.286</td>
<td>0.143</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Yokohama</td>
<td>0.714</td>
<td>0.571</td>
<td>0.429</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Tokyo</td>
<td>0.143</td>
<td>0</td>
<td>0.143</td>
<td>0.571</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Kashiwa</td>
<td>0.286</td>
<td>0.143</td>
<td>0.286</td>
<td>0.714</td>
<td>0.143</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Fukuoka</td>
<td>0.286</td>
<td>0.143</td>
<td>0.286</td>
<td>0.714</td>
<td>0.143</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Okinawa</td>
<td>0.857</td>
<td>0.286</td>
<td>0.143</td>
<td>0.857</td>
<td>0.714</td>
<td>0.143</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Ishigaki</td>
<td>1</td>
<td>0.857</td>
<td>1</td>
<td>1.429</td>
<td>0.857</td>
<td>0.714</td>
<td>0.714</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Iriomote</td>
<td>0.429</td>
<td>0.286</td>
<td>0.429</td>
<td>0.857</td>
<td>0.286</td>
<td>0.143</td>
<td>0.143</td>
<td>0.857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Kii</td>
<td>2.143</td>
<td>2</td>
<td>2.143</td>
<td>2.571</td>
<td>2</td>
<td>2.143</td>
<td>2.143</td>
<td>2</td>
<td>2.286</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---
kansugei and T. nigriabdominalis.

Large genetic differentiation in the sequence of mitochondrial COI was found between some local populations of C. kansugei (Table 2). In particular, the Kii peninsular population differed considerably from other Japanese populations at a level of 2% or more sequence divergence. The Thailand population differed from most of the Japanese populations at more than 3%. A clock for arthropod mtDNA indicates rates of pairwise divergence of about 2% per my for taxa that diverged less than about 3 mya [30–32]. This estimation suggests the Kii and other Japanese populations diverged about 1 mya.

Molecular phylogenies based on the neighbor-joining method and the parsimony method revealed that the Thai population and the Kii population constituted a clade, while the other populations constituted another clade. There were no remarkable differences in morphology between populations of C. kansugei. It is difficult to explain the present distribution of the Thai-Kii clade on the basis of geological changes, but there is a possibility that the Kii population represents an ancestral clone that branched off the main clade early on. In sexually reproducing species, this kind of genetic differentiation is unlikely because of interbreeding between neighboring local populations. However, asexual species could maintain ancestral clones in vicinity to the distribution of derived clones. This pattern of genetic differentiation in C. kansugei contrasted sharply with the differentiation between populations of Tetraneura nigriabdominalis, which is widely distributed from East Asia to Europe on Ulmus species, with the exception of C. kansugei. This comparison suggests that C. kansugei has continued parthenogenetic reproduction for a long time after the extinction of the primary host, so that a large genetic change has accumulated among local populations. This result is consistent with the relict hypothesis.

The evolutionary history of gall-forming aphids is highly constrained by host plants, and ancestral aphid species or ancestral clones may sometimes have survived the Quaternary if their host plants have persisted in refuges. By estimating the phylogeny and by synthesizing geological, biogeographical and phylogenetic information, we can perhaps understand the general pattern of evolution in gall-forming aphids.

ACKNOWLEDGMENTS

I am grateful to Kazunori Yoshizawa and Eisuke Hasegawa for technical advice for constructing a molecular phylogeny. I thank the following people for providing aphid material for molecular phylogeny: Sigeyuki Aoki, Issei Ohsima, Masakazu Sano, and Kazuhiro Sugisima, and also thank anonymous reviewers for comments and criticism. This study was supported by a 21st Century Center of Excellence (COE) Program on “Neoscience of Natural History” (Program Leader: Hisatake Okada) at Hokkaido University, financed by the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

REFERENCES