Research Article

Defense against Feeding by Spring Aphid Parasitism in the Upper Leaf Parts of Host Plants

Yuuka Murakami, Hisashi Kawai, Akane Shindo, and Eisuke Hasegawa

Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Correspondence should be addressed to Yuuka Murakami; y.mura1217@gmail.com

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In a symbiosis, each participant gains more fitness benefits than is paid in maintenance costs for the symbiosis. The mugwort aphid, Macrosiphoniella yomogicola, is ant-associated, and the host mugwort Artemisia montana is a genet-producing plant that has clonal aboveground shoots. M. yomogicola infests most A. montana shoots from spring to midsummer, and attending ants also repel leaf-eaters of the host plant. However, most aphid colonies become extinct after budding of A. montana inflorescence after early August. A few surviving aphid colonies (1–3 per genet) produce sexuparae in mid-October. The shoots on which the sexuparae emerged lost most of their fitness because the aphids strongly suppress budding and growth of inflorescence. However, as the shoots are genetic clones of each other, the appearance of stem mothers in the next spring may result in early spreading of the aphids and attending ants to clonal shoots, which would protect the host from leaf-eaters. Here, we show that all shoots on a genet with stem mothers are occupied by aphids and ants much faster than those on a genet without stem mothers. The attending ants repel leaf-eaters to unimportant leaves for the host. Our results suggest that, as the shoots of a genet are all clones, sustaining the aphids on a few shoots may be beneficial to a genet as a whole through kin selection.

1. Introduction

In a symbiosis, each participant is expected to pay a fitness cost to maintain the system but gain more fitness benefits than those who did not participate in the symbiosis [1]. If a participant has one-sided costs, they would evolve to counter or compensate for this cost, which may result in the failure of the system [2–6], so all participant relationships will become win-win [7]. Investigating this issue may resolve the mechanism by which the huge biodiversity in nature is maintained.

Some aphids are known to form mutualistic relationships with ants [8–11], and these relationships range from mutualistic to hostile [12]. These relationships, which change depending on environmental conditions [13, 14], are interesting from the perspective of the evolution of biodiversity. Furthermore, symbiotic bacteria coexist in the body of aphids and play an important role in generating intraspecific variation in aphids [15, 16]. Therefore, the interaction between aphids, ants, and other symbionts can be a good model case for the dynamics of biodiversity in a community.

The symbiotic relationship we focused on in this study is a four-way relationship between the mugwort aphid Macrosiphoniella yomogicola, which uses Artemisia montana as a host plant, and the aphid-protecting ants [17] and a leaf-eater, Chrysolina aurichalcea, which feeds on mugwort leaves. Since attending ants repel not only most aphid predators but also leaf-eaters of A. montana, the companion of M. yomogicola may provide an indirect benefit to A. montana through the attending ants. Indeed, using mugwort shoots parasitized by aphids and accompanied by ants, when C. aurichalcea was applied to aphid-parasitized stems at the top of the shoot and to aphid-free stems at the bottom, the ants significantly attacked C. aurichalcea at the top (Kawai and Hasegawa, in submitting).
This indirect benefit has been detected in the symbiosis of a scale insect Coccidae and ants [18].

A. montana forms a genet consisting of multiple shoots (above-ground parts) connected by underground stems. The shoots in a genet are genetically identical clones, and the unit that shares the same gene is called a genet [19, 20]. M. yomogicola infests most shoots from spring to mid-summer. In particular, when the winged form of M. yomogicola appears in mid-June, it infests even genets that previously had no aphids. However, when A. montana inflorescences bud in early August, aphid colonies rapidly die off, and in mid-October, sexuparae are produced on the few shoots where aphid colonies survived, producing overwintering eggs through sexual reproduction [21]. In the shoots where sexuparae are produced, buddings and growth of inflorescence are strongly suppressed, and the shoots lose most of their fitness [22]. Therefore, the decrease in aphids after inflorescence budding in A. montana can be regarded as selfish manipulation by the host plant, but if the survival of a small number of aphid colonies enforces a smaller fitness cost than the fitness gain by sexuparae emergences in a few shoots of the genet in the next spring, the shoot would allow the production of sexuparae on a few shoots. Since the genetic shoots of A. montana are genetic clones of each other, we hypothesized that if survival of some aphids is beneficial, then maintaining aphids at the expense of some shoots fitness a little may increase the overall fitness. This is a kind of kin selection [23]. In other words, Hamilton’s theory means that, in the evolutionary theory of sociality, kin selection in social species is assumed to increase when the recipient’s benefit (b) multiplied by the recipient’s genetic relatedness to the actor (r) is greater than theactor’s cost of reproduction (c), i.e., this assumes that the gene frequency is increased when b × r − c > 0 [24]. Since mugwort shoots are the same clone connected by underground stems, we speculated that aphid infection on some shoots within a genet would be greater than the overall adaptability cost of mugwort.

Because A. montana grows as a community of multiple shoots, photosynthetic efficiency is likely to be high for all leaves when they are short in height in early spring because all leaves receive sunlight to a similar degree, but as they grow, the lower leaves of the shoots in the genet will not receive as much sunlight, so their photosynthetic efficiency will be lower than that of the upper leaves. For this reason, the indirect protection of all shoots in early spring should bring greater benefit through kin selection, even with the loss of fitness in a part of shoots in the previous year.

To initially test this hypothesis, we compared the speed of aphid spreading in genets in which stem mothers did or did not appear. Leaf importance was estimated with season by positions of shoots. Furthermore, differences in the proportions of leaves with and without feeding traces were compared between shoots in each position with and without the presence of aphids. Based on the results, we will discuss whether the persistence of aphid colonies in some shoots may be beneficial to A. montana.

2. Materials and Methods

A. montana genet and M. yomogicola were used as materials. In mid-April 2020, we selected shoots from the field in or near the Sapporo campus of Hokkaido University that had emerged stem mothers and those that had not. In A. montana, the shoots within a genet were almost the same in leaf shape, and we could discriminate genets by the leaf shape difference.

2.1. Observations. In mid-April, we went to check whether the stem mother appeared in mugwort and recorded the date of appearance of the stem mother in the shoot where the stem mother appeared (SM1) and recorded the date of the first confirmation of aphids in the shoot where the stem mother did not appear and aphids spread (SM0). In the shoots with stem mothers (SM1), the number of associated aphids was counted every two to three days, including shoots of the same genet, on which no stem mother appeared (SM0). For the genets where aphids were present, the date of emergence of the winged forms of M. yomogicola was recorded.

2.2. Leaf Feeding Patterns of a Leaf-Eater by Aphid Accompaniments. In late June 2018, we investigated the damage to A. montana leaves caused by leaf-eaters by leaf position on the stems of shoots. First, to determine whether aphid accompaniment reduces feeding by leaf-eaters in A. montana, we selected 4 shoots of A. montana with aphid accompaniment, and we divided each shoot into three equal sections based on height (upper, middle, and lower). We checked the number of leaves that had feeding traces in each section. We compared the proportion of leaves with feeding traces in each section. Second, from 10 of the 20 shoots in a genet, ants and aphids were removed by rubbing Tanglefoot® at the base of the shoots, and after 10 days, the presence or absence of new feeding traces on the upper leaves that are considered important was examined.

2.3. Leaf-Eater Preference in the Presence or Absence of Aphids and Ants. In July 2018, we examined whether a leaf eater, C. aurichalcea, changes the leaf position (preference) by removing the aphids and ants from the shoots. Twenty shoots accompanied by M. yomogicola on a genet were randomly selected. From 10 of the selected shoots, the aphids and ants were removed by rubbing Tanglefoot® at the base of each shoot. Each leaf was photographed before the experiment. A. aurichalcea individual was attached to the lower part of the stem of the shoot, and each shoot was covered by a sweepnet (Sweepnet No. 41, Shiga-Kontyu, Tokyo, Japan) to prevent flying leaf-eaters. A plastic tube (1 mm diameter) was placed over the shoots with the aphid to ensure that the ants remained during the experiment. Three days later, we removed the net, and each leaf was photographed again. The number of feeding traces was counted for each leaf, and the proportions of newly fed upon leaves were calculated for all the leaves. Then, we compared
the proportion of newly fed upon leaves at the upper 1/3 position.

2.4. Statistics. The relationship between the average day when the first aphid had been confirmed on the shoot without stem mothers and the presence/absence of her was examined by a generalized linear model (GLM) using a binomial distribution with a log-link function. The days from the start of observation when aphids were first observed on the shoot was set as the dependent variable, and the genet with stem mothers (1) or not (0) was set as the independent variable in the GLM. In addition, the mean values of the first day of aphid emergence from the beginning of observation of the genets with and without stem mother emergence were tested by Wilcoxon rank-sum test with a correction for ties (Wilcoxon rank-sum test). In addition, the percentage of shoots accompanied by aphids for each genet was compared with Fisher’s exact probability test as of July 6. Differences in the percentage of shoots colonized by aphids were tested by Fisher’s exact probability test, and Bonferroni correction was used in case of multiple comparisons. Feeding trace percentages at different positions were tested by Fisher’s exact probability test with Bonferroni correction for multiple comparisons. The difference in feeding rate of the upper leaves with or without the aphids was tested by Fisher’s exact probability test. All statistical analyses were performed in R (ver. 3.5.2), using the MASS package with the binomial distribution in the GLM and the exactRankTests package for Wilcoxon rank-sum test.

3. Results

*M. yomogicola* was confirmed first on the shoot on which no stem mother appeared at 9 days after the start of the observations in SM1 (*n* = 7) and at 61 days after in SM0 (*n* = 58). We gave a dummy variable of 0 to SM0 and of 1 to SM1 and conducted a GLM by setting the first confirmed days on each shoot as the dependent variable and the dummy variables (0/1) as the independent variables. There was a significant negative regression slope for the first day of aphid confirmation (estimate = −1.2048, S.E. = 0.2721, *z* = −4.428, *p* = 9.5e−06). It was shown that the aphids spread earlier to the surrounding shoots without stem mothers in SM1.

The first date when winged forms of *M. yomogicola* were confirmed was 15 June 2020. On May 24 (before the emergence of the winged forms) and July 4 (after the emergence of the winged forms), the accompanying *M. yomogicola* in each genet was examined. On May 24, aphid accompaniment was confirmed for all 12 shoots in SM1 but for none of the 58 shoots in SM0 (Fisher’s exact probability test, *p* = 9.99e−14). On July 4, 8 out of 12 shoots were accompanied by aphids (several colonies became extinct) in SM1, and 2 out of 58 shoots were accompanied by aphids in SM0 (Fisher’s exact probability test, *p* = 92.095e−08).

In the case of aphids accompanying shoots, the average percentage of leaves with feeding traces was higher in the order lower > middle > upper, and the differences among the positions were statistically significant (Figure 1). When aphids and ants were removed, the percentage of new feeding traces on the upper leaves was significantly higher under the removal treatment than under the control (Figure 2).

4. Discussion

The emergence of stem mothers of *M. yomogicola* showed a more rapid spreading in a genet in early spring than in a genet without stem mothers (see above). In shoots where the stem mothers did not emerge, *M. yomogicola* did not accompany the shoots until the winged aphids emerged on other genets with the aphids. In addition, aphid accompaniment protected the shoots from feeding on the upper leaves, which are considered relatively important compared to the leaves at other positions in this season (early to midsummer). It was also shown that when aphids were artificially removed, the upper leaves received additional feedings. These results suggest that the leaf-eater *C. aurichalcea* was repelled from their preferred leaves (upper) by the presence of the attending ants of *M. yomogicola* in this symbiosis. It was also suggested that the presence of *M. yomogicola* from spring to summer is beneficial to the host plant *A. montana* in terms of
preventing feeding damage by leaf-eaters on relatively important leaves.

Since plants cannot move, they avoid leaf-eating by physical and chemical means. For example, they avoid leaf-eating by having trichomes (fine surface hairs) [25, 26] or by producing alkaloids or repellent hormones. It is also known to eliminate leaf-eaters by accompanying beetles [18]. In the Acacia-Scale insect relationship [27], ants are supplied with nutrients by Acacia, so they drive away leaf-eaters to defend their resources. The same may be true for M. yomogicola and A. montana.

If the cost of aphid feeding in shoots of A. montana is greater than the indirect benefit from the attending ants, the host plant will undergo counterevolution to remove (or reduce) the cost from aphids (arms races [28]). In fact, in M. yomogicola and A. montana, inflorescence budding and growth are strongly suppressed in shoots on which sexuparae are produced in late autumn [22]. When sexuparae of M. yomogicola are produced, the shoots lose most of their fitness [22]. After inflorescence budding, M. yomogicola colonies on most shoots have died off, but some colonies persist until they produce sexuparae [22]. If this persistence is a major fitness cost for a genet of A. montana, then a counterevolution in which the host tries to eliminate M. yomogicola in late autumn should occur, and all M. yomogicola colonies should die off. The reason why this does not occur seems to be that there is an advantage to A. montana in allowing some M. yomogicola colonies to survive. If the accompaniment of M. yomogicola from spring to summer may not incur any fitness cost to A. montana shoots, they would allow accompanying aphids to persist in this season. It will be interesting to explore whether aphid accompaniment incurs fitness costs to A. montana shoots and how the shoots avoid expected costs from aphid sap-sucking if there is no cost.

It may be beneficial for A. montana that the indirect benefit from attending ants prevents leaf-eater feeding on leaves that are relatively important for the shoot. Additionally, in early spring, the shoots have a short height, and all leaves receive sunlight at similar illuminance; therefore, A. montana does not want to eat any part of the leaves during this season, but as the shoot grows, the lower leaves will receive less sunlight as A. montana genets grow as a community, resulting in reduced photosynthetic efficiency of leaves in the lower position. In this period, it is unlikely that feeding damage to the lower leaves would be very costly to A. montana. In fact, A. montana kills its own lower leaves during this period [29, 30].

In addition, the attending ants manipulate the growth rate of the green morph of polymorphic M. yomogicola, which excretes honeydew including nutrients and shows a lower increasing rate than the other red morphs [21]. For ants, the presence of aphid colonies near their nests in the next year will lead to certain resource availability and fitness benefits. The attending ant Lasius japonicus Santschi has been found to prefer green rather than red morphs [20]. However, ants have been found to prefer colonies that are approximately 60% green [31], so it would be interesting to know why they do not eat red aphids, which are probably less nutritious. In a symbiosis with another aphid, L. japonicus Santschi (previously L. niger) selectively predate on aphid individuals with low honeydew secretion [14]. If only reds can resist A. montana manipulations to exclude them after inflorescence budding, then there may be an advantage for ants to maintain reds to secure resources for the following year. These results suggest that the interaction of mugwort, aphids, and ants, as observed, is a win-win situation in which all parties benefit more than when they do not. Hence, this symbiotic system may be maintained. Of course, in order to maintain symbiosis, each party pays a short-term cost of reproduction, but if they abandon it and becomes a cheater, the symbiotic system will decay and the individuals having the cheater allele will not maintain their own genetic line. A win-win relationship like a symbiosis may have a structure in which cheating is not possible. Testing this interesting hypothesis will provide new insights into the maintenance mechanisms of a symbiosis.

Selfish evolution by natural selection seems to realize only the maximization of the increase rate to the next generation [32]. The present synthetic theory of adaptive evolution considers that the optimal trait maximizes the transmission rate of an allele that controls an adaptive trait to the next generation [28]. However, not only in symbiosis but also in one-sided relationships such as predator-prey and host-parasite relationships, if predators have adapted to exploit all the prey, such an allele, they should be selected out as they lose the resource for reproduction. Thus, such excess adaptation in biological relationships may be avoided by another selection mechanism from natural selection [33], but this mechanism would work as a result of selfish evolution based on natural selection.

Especially in a symbiosis, each participant pays a fitness cost to maintain the system, which is thought to be rewarded because the fitness benefit from the symbiosis is greater than the cost [1, 34]. In this case, the relationships among all participants should be win-win and are thought to be optimized for securing long-term benefits (from the persistence of the symbiotic system), not for immediate proliferation. Indeed, it is known that the emergence of cheaters who unilaterally exploit their interaction partners can be eliminated by selection at the community level if it is beneficial for all participants [33, 35]. This is because the presence of a cheater increases the cheater’s short-term gains and lowers the adaptive gains of other participants, causing the system itself to collapse [36]. If the relationship is win-win, only the cheater, being myopic, should have a lower geometric mean fitness than the other honest participants. Selfish evolution by natural selection may finally lead to only win-win relationships where everyone benefits from relationships such as symbiosis. Symbiosis is a good system in which to test this fascinating idea, and further research will bring new insights into adaptive evolution. Further research on biological relationships using the geometric mean fitness view will provide deep insights into symbiosis and the mechanisms that maintain relationships between organisms.
Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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