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Costs and constraints in aphid-ant mutualism

Abstract While many studies have demonstrated that ants provide beneficial services to aphids, Bristow (Ant-plant interactions, Oxford University Press, Oxford, 104–119, 1991) first questioned why so few aphid species are ant-attended. Phylogenetic trees have demonstrated multiple gains and loss of ant-attendance in the course of aphid-ant interactions, implying that mutualisms easily form and dissolve. Several studies have reported the factors that influence the formation and maintenance of aphid-ant interactions. Examples include the physiological costs of ant attendance, competition for mutualistic ants, ant predation on aphids, the influence of host plants, and parasitoid wasps. Recent physiological techniques have also revealed the chemical component of aphid-ant mutualisms. The honeydew of ant-attended aphids contains melezitose (a trisaccharide), which has an important role in aphid-ant interactions. Studies of cuticular hydrocarbons on aphids and ants have clarified the underlying mechanisms of ant predation on aphids. Attending ants also reduce aphid dispersal ability, causing the formation of fragmented aphid populations with low genetic diversity in each population. The reduced aphid dispersal could be partly explained by higher wing loading and reduction of flight apparatus due to ant attendance. Whether ant attendance is associated with the range of host plants of aphids or genetic variation in microorganism in aphids remain to be explored.

Keywords Aphid-ant mutualisms · Cost of ant attendance · Honeydew · Dispersal · Flight apparatus

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Introduction

Mutualisms have been traditionally recognized as stable reciprocal interactions, in which beneficial services are exchanged between mutually participating species. However, some studies have proposed that mutualisms should be viewed as a cost-benefit balance model, in which the interaction lasts as long as the benefits exceed the costs to at least one of the two species (Bronstein 1994; Sachs et al. 2004; Leigh 2010). The stability of the cost-benefit balance model depends on changes in the abiotic/biotic environment surrounding participating species; consequently, mutualisms could dissolve conditionally (Bronstein 1994; Herre et al. 1999). It is necessary to quantify the costs and benefits of a mutualism to understand the evolution of species interactions. Furthermore, comparisons across related taxa using phylogenetic trees have provided useful information on how the cost-benefit balance leads to different evolutionary outcomes; specifically, the persistence or loss of mutualistic partnerships (Herre et al. 1999; Sachs and Simms 2006).

The mutualistic interaction between aphids and ants was well-documented in the scientific literature more than 50 years ago by Way (1963). In the 1980s, researchers focused on establishing the benefits of ant attendance to aphids, whereas the costs were overlooked. However, technological advancements in biochemical and genetic assays in the 1990s facilitated research on the costs of ant attendance to aphids and related studies on honeydew production (Stadler and Dixon 2008). During this period, researchers also began to focus on the trophic interactions among aphids (including other homopteran), ants, and plants (Cushman 1991; Ito and Higashi 1991; Breton and Addicott 1992b; Gaume et al. 1998). In the 2000s, molecular phylogenetic trees were used to conduct species comparisons between ant-attended and non ant-attended aphids, which helped to develop a comprehensive understanding about aphid-ant mutualisms.

Benefits of ant attendance to aphids

Aphids suck the phloem sap from host plants and produce honeydew that is rich in sugar but poor in amino acids (Mittler 1958). Ants attend aphid colonies to forage the honeydew and repel aphidophagus predators that enter aphid colonies. Many studies have demonstrated that ants provide beneficial services to aphids, such as protection from predators and the prevention of sooty mold infestation (Ascomycete fungi), which grows when honeydew accumulates (Way 1963; Stadler and Dixon 2008). Although ant protection enhances the survival rate of aphid colonies, ants may not always be attracted to colonies even in ant-attended aphid species. In a study using the aphid *Aphis asclepiadis* Fitch collected from five populations and the ant *Formica podzolica* Francour, Mooney (2011) demonstrated that the number of ants attending aphids varied between populations, implying that genotypic variation in aphids regulates whether ants are attracted to aphid colonies. It is known that the genotypes of aphids differentially affect the abundance of predators and parasitoids (Hazell and Fellowes 2009; Mooney 2011). Thus, variation in the attractiveness of aphid colony to ants might evolve in each aphid population.

Sanitation services by ants may be considered as protection against the spread of fungi in aphid colonies, and might contribute towards strengthening aphid-ant interactions. Honeydew removal by ants prevents the buildup of honeydew, maintains good hygiene, and clears passageways for walking in aphid colonies (Wimp and Whitham 2001). Matsuura and Yashiro (2006) showed that the eggs of the aphid *Stomaphis hirukawai* Sorin are carried by the ant *Lasius productus* Wilson to ant nests, where the eggs are protected from pathogenic microorganisms by ant grooming behavior. In the mutualism between *A. asclepiadis* and *F. podzolica*, aphids infected with pathogens are quickly removed from ant-attended aphid colonies, indicating that ants perform sanitizing and quarantining behavior to reinforce aphid-ant mutualisms (Nielsen et al. 2010).

Why are so few aphids ant-tended?—the question raised by Bristow (1991)

Since ants commonly occur in many habitats and exhibit aggressive behavior, it would be advantageous for aphids to employ ants as bodyguard. However, Bristow (1991) showed that only 117 out of 479 (24.4 %) aphid species are associated with ants in the Rocky Mountain region of the United States. Hence, the author raised the question of why do three-quarters of aphid species forego the potential benefits of associating with ants. After Bristow raised this question, a number of studies have reported the negative impacts of ants on ant-attended aphids. Existing studies clearly show that aphid-ant interactions are conditionally formed, maintained,

and subject to unstable relationships (Addicott 1978; Pontin 1978; Sakata 1994; Fischer et al. 2001; Wimp and Whitham 2001). The evolutionary course of aphid-ant interactions also provides evidence that mutualism is unstable. Molecular phylogenetic studies using sequences of cytochrome oxidase subunit I (COI) in mitochondria have revealed that multiple gains and losses of ant mutualism have occurred at least five times for two genera of aphids, *Chaitophorus* and *Tuberculatus* (Shingleton and Stern 2003; Yao 2011). Evidence that ant-attended aphid species do not form a monophyletic group indicates that aphid-ant interactions are not fixed relationships but fragile connections. Potential costs of ant attendance that have been identified so far include the physiological costs of ant attendance through honeydew production, ant predation on aphids, competition among aphid species for mutualistic ants, the mediation of host plants, and the parasitism by wasps.

Costs of ant attendance to aphids

Stadler and Dixon (1998) first revealed that *Lasius niger* L. ants have a negative influence on the physiological and developmental status of the aphid *Aphis fabae cirsiiacanthoides* Scopoli. For instance, these ants cause a decrease in soma and gonad dry mass, delayed development time, and a lower mean relative growth rate of aphids. Following this first report, other studies also reported the costs of ant attendance on other aphid species. For instance, the ant *Formica yessensis* Forel caused the rate of honeydew excretion by the aphid *Tuberculatus quercicola* (Matsumura) to increase two-fold compared to the ant-exclusion treatment. In this case, ant attendance led to the poor assimilation of phloem sap, along with a decrease in the body size of the aphids and the number of embryos (Yao et al. 2000; Yao and Akimoto 2001, 2002). In the mutualism between *Aphis craccivora* Koch and *L. niger*, the ants suppressed the development of the aphid colony (Katayama and Suzuki 2002), probably due to an increase in honeydew excretion (Takeda et al. 1982). In contrast, it has been reported that ant attendance increases the lifetime fecundity and rate of offspring production of two myrmecophilous aphid species, *Symydobius oblongus* (von Heyden) and *Metopeurum fuscoviride* Stroyan, compared to ant exclusion (Stadler and Dixon 1999; Flatt and Weisser 2000).

The costs and/or benefits of ant attendance are not always in a steady state for the various forms of aphid-ant mutualisms, and are influenced by spatial and temporal scales. Because aphids increase their numbers parthenogenetically during the summer months, the effects of ants on aphids are expected to vary with aphid colony growth. Breton and Addicott (1992a) showed the presence of density-dependent mutualism in aphid-ant interactions under natural conditions; whereby higher rates of colony growth of the ant-attended aphid *Aphis*

varians Patch were recorded in small colonies compared to large colonies. Recently, studies on the density-dependence of mutualism have been developed in the laboratory, in which the number of aphid-attending ants is controlled. It has been hypothesized that the costs of ant attendance on aphid performance vary with respect to tending intensity (i.e., the ratio of ants to aphids in an aggregation) or across generations. For instance, in the mutualism between the aphid *Chaitophorus populicola* Thomas and the Argentine ant *Linepithema humile* Mayr, the reproductive rate of aphids minimally changed in response to low ratios of tending intensity but decreased at higher levels of tending intensity. This observation indicates that tending intensity had a non-linear effect on aphid performance (Yoo and Holway 2011). Tegelaar et al. (2013) demonstrated that the costs of ant attendance vary over generations, by observing the interaction between *A. fabae* and *L. niger* over 13 aphid generations in the laboratory. At the start of the experiment, the performance of ant-attended aphids decreased; however, the costs of ant attendance disappeared within four generations of interaction. This study revealed that transgenerational effects, where offspring phenotypes are adjusted by maternal influences, are important in aphid-ant interactions.

Competition and predation within mutualistic relationships

Aphid-ant interactions are often interfered-with or terminated-by another aphid species. Addicott (1978) showed that high numbers of *Formica integroides* Creighton ants attend *Aphis farinosa* Gmelin aphids on willow trees during the early part of summer, but that these numbers declined to zero by mid-summer. This change arose because of the decline in the number of *A. farinosa* and increase in the number of *Aphis varians* Patch on fireweed adjacent to the willow. Similar results have been observed in the relationships among aphid species feeding on tansy *Tanacetum vulgare* L. Fischer et al. (2001) demonstrated that *L. niger* abandoned the colonies of the less preferred species (*A. fabae*) as soon as colonies of higher-ranking species (*Me. fuscoviride* and *Brachycaudus cardui* L.) were available on neighboring tansy plants. Furthermore, if two aphid species were present in a mixed colony on the same shoot, the population size of the preferred species increased, whereas that of the less preferred species decreased due to predation by *L. niger*. These results indicate that ants might be able to recognize which aphid species produce honeydew the most efficiently, and to change their foraging site to colonies with greater resources.

In some myrmecophilous aphids, ants often prey on aphids in the colonies that they attend. Therefore, ant predation on aphids might be a factor that limits the formation of associations between aphids and ants. It is thought that ant predation on aphids is determined by the balance of ant requirements for carbohydrates (honeydew) and proteins (aphid body) (Pontin 1978; Sakata 1994) (an overview of the chemical mechanism underlying ant predation on aphids is provided in "Effects of ant cuticular hydrocarbons on aphids"). Furthermore, ant predation on aphids is assumed to regulate total aphid numbers to maintain optimal conditions under which aphids feed on phloem sap efficiently (Ivens et al. 2012b). The two aphid species, *Pleotrichophorus utensis* Pack and Knowlton and *Uroleucon escalantii* Knowlton, are occasionally attended by the ant *Formica obscuripes* Forel. In both mutualisms, the ants simultaneously protect and prey on aphids. This relationship indicates that selection to both appease ants and to gain their protection represent important forces leading to the formation of aphid-ant mutualisms (Billick et al. 2007). Sakata (1995) demonstrated the density-dependent predation of *L. niger* on two ant-attended aphids *Lachnus tropicalis* (van der Goot) and *Myzocallis kuricola* (Matsumura). As the density of *L. tropicalis* increased, the ant exhibited an increase in predation pressure on *M. kuricola* compared to *L. tropicalis*. This behavior suggests that when given access to honeydew of two aphid species, ants prey on the aphid that produce less honeydew.

Mediation of host plants on aphid-ant interactions

Extrafloral nectaries (EFNs) are nectar-secreting plant glands that develop on plant structures other than flowers, such as the stem. Ants frequently feed on the secretions of EFNs because they produce sugar-rich secretions (Engel et al. 2001). Therefore, it is thought that aphids compete more often with EFNs for ant partners, rather than with other aphid species. The two ant species, *Lasius japonicus* Santschi and *Tetramorium tsushimae* Emery, use the EFNs of the plant *Vicia faba* L. when *A. craccivora* aphid density is low; however, the ants preferentially use honeydew when aphid density is high (Katayama and Suzuki 2003). Later, Katayama and Suzuki (2010) demonstrated that, even when *A. craccivora* aphid density is low, the larvae of a predatory ladybird beetle, *Coccinella sepetempunctata* L., remain in aphid colonies for shorter residence times, due to their being attacked by EFN-visiting ants. This observation indicates that aphids in small colonies gain indirect benefits from EFNs.

Aphids depend on the phloem sap of plants during their lifetime. However, the quality of host plants deteriorate during summer due to a decrease in water content and amino acid concentration and an increase in leaf toughness (Shibata et al. 2001), which has a major impact on aphid performance (Yao 2004). Thus, the deterioration in host plant quality is expected to influence interactions between aphids and ants. Collins and Leather (2002) discovered that some nymphs of the black willow aphid *Pterocomma salicis* L. are carried to high quality host plants by *L. niger*, indicating that ants are able to detect host plant quality via the nutritional

level of the honeydew produced by aphids. In a rearing study of three different degrees of ant-attended aphid species and one non ant-attended aphid species living on poor and high quality plants, Stadler et al. (2002) demonstrated that the fitness costs of ant attendance depend on the degree of myrmecophily (i.e., positive interactions of ants with the other species) and host plant quality. For instance, obligatory ant-attended Me. fuscoviride was negatively impacted by low quality plants and ant absence. In contrast, low plant quality had no effect on intermediate ant-attended B. cardui, facultative ant-attended A. fabae, and non ant-attended Macrosiphoniella tanacetaria Kaltenbach. These observations indicate that host plant quality regulates the performance of aphids, with high reproductive rates under ant attendance.

Over the last 10 years, a number of studies have shown that the formation of aphid-ant interaction is limited by host plant genotype. Along the Weber River in Utah (USA), the ant Formica propinqua Creighton attends the aphid C. populicola on two species of poplar tree, Populus fremontii S. Watson and Populus angustifolia James. However, aphids inhabiting hybrids of the two host plants attract fewer ants compared to those on the two host plant species (Wimp and Whitham 2001). Johnson (2008) demonstrated that, even in conspecific host plants, some plant genotypes of the common evening primrose, Oenothera biennis L., influence the interactions between the aphid Aphis oestlundi Gillette and several species of ants. It was found that some plant genotypes have direct and indirect effects on ant abundance and aphid density, respectively. In the mutualisms between F. podzolica ants and two aphid species, A. asclepiadis and Myzocallis asclepiadis (Monel), the genotypes of the milkweed plant Asclepias syriaca L. indirectly influence ants through changes in the number of ants recruited per aphid (Mooney and Agrawal 2008). These examples indicate that aphids inhabiting plant hybrids or certain plant genotypes might not attract ants because they fail to produce good quality honeydew.

Parasitoid wasps

The parasitoid wasps of aphids make the outcomes of interactions among aphids, ants, and aphid predators challenging. Although parasitoid wasps are often repelled by ants, once wasps successfully oviposit in aphids, these aphids also receive protection by ants, resulting in higher emergence rates of adult parasitoid wasps (Völkl 1992; Kaneko 2002). The outcomes of interactions between parasitoid wasps and aphid-ant mutualisms differ with ant species (Kaneko 2003), in addition to the number of days after the establishment of aphid-ant interactions in the field (Tegelaar et al. 2012). These studies demonstrate that beneficial protection services from ants may, under certain circumstances, raise the costs of ant attendance to aphids.

Chemical aspects of aphid-ant mutualisms

Over the last two decades, advances in studies about the interactions between aphids and ants have been facilitated by the acquisition of increased information about the physiology of both partners. Ant-attended aphids produce sugar-rich honeydew, which contains glucose, fructose, trehalose, sucrose, melezitose, and other sugars. Melezitose is a trisaccharide that is hydrolyzed to glucose and turanose (isomer of sucrose). This sugar has been detected in phloem-feeding insects, but not the host plants. Therefore, the role of melezitose in insects has received much focus from various scientific fields. Examples of such research include osmoregulation (Kennedy and Fosbrooke 1972; Downing 1978; Fisher et al. 1984; Walters and Mullin 1988; Rhodes et al. 1997; Wilkinson et al. 1997; Woodring et al. 2007), nutritional recycling between aphids and host plants via biological nitrogen fixation (Owen and Wiegert 1976; Owen 1978; but see Petelle 1980), and anti-predator strategies against parasitoid wasps (Wäckers 2000). There is increasing evidence that melezitose found in honeydew is critical in aphid-ant interactions. In addition, the regulation of ant predation behavior on aphids by the cuticular hydrocarbons (CHCs) produced by ants has been extensively researched.

Melezitose in the honeydew of ant-attended aphids

Kiss (1981) was the first researcher to link melezitose to interactions between aphids and ants. The author demonstrated that L. niger selectively prefer honeydew containing melezitose. This observation led to the hypothesis that aphids have evolved to synthesize melezitose from glucose and sucrose in the phloem sap to attract ants. Völkl et al. (1999) simultaneously reared four aphid species (also described in "Mediation of host plants on aphid-ant interactions" in relation to the study by Stadler et al. 2002) on the host plant tansy T. vulgare, and compared the composition of carbohydrates in the honeydew of the four species. Melezitose was detected in the honeydew of the two higher ranking of ant-attended species (Me. fuscoviride and B. cardui) but not in the two lower ranking ant-attended species (A. fabae and Ma. tanacetaria) and host plants. These observations clearly supported the findings of Kiss (1981). However, studies over the last 10 years have reported that there is variation in the synthesis of melezitose by A. fabae and its subspecies (Fischer et al. 2005; Vantaux et al. 2011b). The relationship between melezitose and ant attendance has also been documented by Fischer and Shingleton (2001), in which melezitose accounted for about 50 % of the honeydew composition of two obligatory ant-attended Chaitophorus populeti Panzer and Chaitophorus populialbae Boyer De Fonscolombe, but accounted for < 5 % in that of a non ant-attended aphid, Chaitophorus tremulae Koch. For ants, aphid-synthesized

melezitose acts as a signal sugar for the workers. In other words, the detection of this substance indicates the presence of aphid colonies (Detrain et al. 2010). Recently, Vantaux et al. (2011a, b) observed clonal mixing and interclone differences in the melezitose of wild populations of the facultative ant-attended aphid *A. fabae*. Although the presence or absence of ant attendance to each clone was not correlated to differences in melezitose, the results indicated the presence of cheaters in aphid colonies, which do not produce melezitose. This finding led to the hypothesis that group selection (natural selection acting between groups of organisms, rather than between individuals) occurs in interactions between *A. fabae* and ants (Vantaux et al. 2012).

Effects of ant cuticular hydrocarbons on aphids

In some myrmecophilous aphids, ants often prey on aphids in the colonies that they attend. It has been suggested that ants prey on aphids as a source of protein when there is excess honeydew in ant nests (Sakata 1994; Offenberg 2001). Sakata (1994) first hypothesized that the duplicitous behavior of ants depends on whether chemical markings containing CHCs are present or absent on aphids. Using the ant *Lasius fuji* Radchenko and the aphid *Stomaphis yanonis* Takahashi, Endo and Itino (2012) verified that the CHCs of ants are transferred to the body surface of aphids through ant attendance, and are used by ants to chemically discriminate between aphids with and without experience of ant attendance. When ants prey on aphids, aphids are confronted with two alternatives: to continue to receive protection by ants or to hide themselves from ants with chemical camouflage. Endo and Itino (2013) showed that *S. yanonis* produces CHCs that resemble those of *L. fuji* after exuviation to prevent predation by ants. Chemical marking by ants and the chemical mimicry of aphids imply that aphid-ant mutualisms might have evolved from predator–prey interactions.

Effects of ant attendance on aphid dispersal and flight apparatus

It has been reported that, in addition to life history traits, the dispersal of aphids is also influenced by ant attendance (Kleinjan and Mittler 1975; Kindlmann et al. 2007; Oliver et al. 2007). For example, ants cause a delay in the timing of migration of ant-attended aphids, a decrease in the walking speed of apterous (wingless) aphids, and an increase in the production of apterous aphid morph types. In France, Kindlmann et al. (2007) investigated the number of ant-attended and non ant-attended aphid species trapped by six suction traps over a 22-year period. The surveys revealed that, while there was no significant difference in the annual number of the two types of aphid species, the ant-attended species exhibited delayed migration time compared to the non

ant-attended species. The delay in the timing of dispersal suggests that ant-attended species have a delayed opportunity to feed on host plants with high quality phloem sap, possibly leading to a decrease in embryo numbers. The authors suggested that a delay in the timing of dispersal represents a possible cost of ant attendance. Oliver et al. (2007) demonstrated that semiochemicals produced by *L. niger* decreased the walking speed of the ant-attended aphid *A. fabae*, but did not affect that of the non-attended aphid *Acyrthosiphon pisum* (Harris). Hence, semiochemicals might play an important role in whether ants monopolize aphid colonies. In another study, it was reported that the ant-mandible extract of the ant *Formica fusca* Wheeler increased the percentage of *A. fabae* aphids that developed into apterae, indicating that the aphid dispersal is prevented by ant attendance (Kleinjan and Mittler 1975). The production of these chemical materials by ants might have developed during the evolutionary course of various types of aphid-ant mutualisms.

Genetic structure of ant-attended aphid populations

Where habitats have become fragmented by man-made development or natural disaster, the gene flow of animals and plants that have low migration capabilities are limited to areas where only a small number of dispersers are able to migrate among fragmented populations. Such fragmented populations are termed "meta-populations" (Hanski and Gaggiotti 2004). As bottlenecks, inbreeding, and genetic drift are more likely in small populations compared to large populations, the number of alleles in organisms rapidly decreases or becomes fixed in fragmented populations, leading to a subsequent increase in homozygous alleles.

As shown in the introduction of this section, examples showing that the dispersal of ant-attended aphid species is limited by ants are increasing. Therefore, populations of ant-attended aphid species are expected to be increasingly fragmented. Consequently, it is expected that genetic diversity defined by an averaged heterozygosity would be lower in ant-attended aphid species compared to non ant-attended aphid species that inhabit the same host plants. Using microsatellite markers and field surveys, Yao (2010) compared the extent of genetic diversity between ant-attended *T. quercicola* and two non-attended species, *Tuberculatus paiki* Hille Ris Lambers and *Tuberculatus japonicus* Higuchi, which occur sympatrically on *Quercus dentata* Thunberg. In addition, the author also assessed the extent of genetic diversity between an ant-attended *Tuberculatus* sp. A and other non-attended *Tuberculatus* species, which occur sympatrically on *Quercus crispula* Blume. A number of genetic diversity indices were examined, which showed that ant-attended species had lower numbers of alleles per locus and lower clonal diversity, along with a higher inbreeding index and higher genetic differentiation among fragmented popu-

lations, compared to non ant-attended species. In addition, field-trap studies of wild populations indicated that the effective population size of *T. quercicola* and sp. A, compared to that of the two non ant-attended species, was extremely small because of limited gene flow. Yao and Kanbe (2012) extended the collection sites to the whole of Japan, and compared the haplotype diversity of ant-attended *T. quercicola* and non ant-attended *T. paiki* that were collected, focusing on COI regions. Haplotype diversity was higher in *T. quercicola* compared to *T. paiki*. Analysis of molecular variance (AMOVA) showed that the molecular variance was higher in *T. quercicola* compared to *T. paiki*. These two studies indicate that the low dispersal capacity of ant-attended aphids has generated unique haplotypes at each site, whereas the widespread dispersal of non ant-attended aphids has promoted gene flow and prevented speciation in subpopulations. Vantaux et al. (2011a) investigated the level of clonal mixing and relatedness within colonies in the facultatively ant-attended aphid *A. f cirsiiacanthoides*, and showed that relatedness remains constant throughout the season, but that relatedness and ant association were not linked.

Until recently, studies about the genetic diversity of aphids have focused on free-living aphids in above-ground populations. Ivens et al. (2012a, b) investigated the genetic diversity of four ant-attended subterranean root aphid species (*Geoica utricularia* (Passerini), *Tetraneura ulmi* (L.), *Forda marginata* Koch, and *Forda formicaria* von Heyden) that are attended by the ant *Lasius flavus* (Fabricius), and found that most chambers of ant nests farmed only a single clone of each aphid species. The authors suggested that the observed low genetic diversity at the colony level might be attributed to the absence of primary host plants or the culling of aphids by ants to farm a single clone of aphid in ant nests.

Reduction of aphid flight muscle through ant attendance

It is known that costs are incurred by the formation and maintenance of flight apparatus (e.g., wings and flight muscle) (Dixon et al. 1993). In a comparison of long-winged and wingless individuals from three aphid families, it was found that the development of flight apparatus prolongs the time required for maturation into adult, and resulted in a 20 % reduction in gonad size (Dixon et al. 1993). Wing dimorphism has been documented in many insect species, whereby wingless or short-winged morphs exist in the same species, providing evidence for the trade-off between flight and fecundity (Roff and Fairbairn 1991; Zera and Denno 1997; Zera and Brink 2000; Zera 2004; but also see Guerra and Pollack 2009). Although species in the genus *Tuberculatus* have neither wingless nor short-winged morphs, ant-attended species might allocate more of their body resources to reproduction rather than to the wings. This hypothesis was confirmed by Yao and Katagiri (2011),

who found that ant-attended *T. quercicola* had a larger body volume, higher fecundity, and higher wing loading (i.e., the ratio of body volume to wing area) compared to non ant-attended *T. paiki*, which had a smaller, slender-shaped body, lower fecundity, and lower wing loading. Flight muscle development was significantly lower in *T. quercicola* (40.3 %) compared to *T. paiki* (51.4 %). These results indicate that the additive effect of higher wing loading and the lower amount of flight muscle development in *T. quercicola* might increase the physical difficulty of flight, and hence might be responsible for the lower dispersal ability of this species. The trade-off between fecundity and dispersal documented in wing-dimorphic insects might, therefore, be applicable to *T. quercicola*, which has fully developed wings.

Although it has been reported that ants increase the percentage of apterous aphids and prevent aphid dispersal (Kleinjan and Mittler 1975), it remains unclear as to whether flight apparatus is directly affected by ant attendance. Yao (2012) investigated whether the components of flight apparatus differ between aphids attended by ants and not attended by ants. Pair comparison experiments were conducted on *T. quercicola* aphids that were reared on *Q. dentata* trees in the wild. The results showed that ant attendance had a negative influence on aphid flight apparatus, indicating that aphids produce honeydew at the expense of resource investment in flight apparatus. Since the dispersal of *T. quercicola* is limited by ant attendance, the reduction in flight apparatus might precede a decrease in body size. Existing research has shown that, when *T. quercicola* aphids are attended by ants, aphids increase honeydew excretions by up to twofold compared to aphids under ant-exclusion conditions, causing an increase in the total concentration of amino acids in the honeydew (Yao and Akimoto 2001, 2002).

Comparative phylogenetic methods

Scientific approaches based on species comparisons have also provided examples supporting the hypothesis that ants restrict aphid dispersal. Closely related species share many characteristics as a consequence of their common ancestry. Thus, similarity between lineages is often influenced by phylogenetic relatedness, rather than by independent evolution (Felsenstein 1985; Harvey and Pagel 1991). Comparative analysis of independent contrasts uses independent comparisons of various components of a phylogeny, with each comparison being made at a different node in the phylogeny (Purvis and Rambaut 1995).

A literature survey of 112 European aphid species conducted by Stadler et al. (2003) showed that ant-attended aphid species on woody trees tend to form colonial aggregations. This phenomenon might be attributed to ants being able to access more honeydew from aggregated individuals more efficiently. In addition to forming colonies, tree-dwelling aphids have a long

proboscis to penetrate the thick cell wall to feed on phloem sap. However, when feeding on phloem sap, aphids are unable to escape quickly from attack by predators, as time is required to remove the long proboscis from the plant. Under such conditions, the protective presence of ants would be advantageous to aphids. Shingleton et al. (2005) used phylogenetic independent contrast analysis to compare 15 *Chaitophorus* aphid species that feed on poplar, and found that proboscis length is positively associated with both escape time and ant attendance. Hence, the formation of associations with ants may have led to the evolution of a long proboscis in highly ant-attended aphids. Yao (2011) assessed the correlation between ant association and the wing loading of 20 *Tuberculatus* species distributed across Japan that mainly feed on *Quercus* trees, of which nine aphid species were ant-attended and 11 were non ant-attended. The construction of comparative analyses based on the neighbor-joining, most parsimony, and maximum likelihood phylogenetic trees showed that increased wing loading is positively correlated with ant associations, indicating that ant-attended aphids allocate more resources to their body compared to their wings, resulting in a low dispersal capacity.

Perspectives

Since aphids spend their entire life feeding on phloem sap, the exploitation of phloem sap containing idiosyncratic secondary metabolites plays a critical role for aphids in the process of acquiring novel host plants. While non ant-attended aphid species only feed on phloem sap to benefit their own metabolism, ant-attended aphid species need to exploit phloem sap for their own nutritional requirements and to provide enough honeydew to meet the demands of attending ants. This difference might affect the process of the adaptation of aphids to novel host plants and determine the range of host plants available for ant-attended species. Furthermore, whether ant-attended aphids feed on woody plants or herbaceous plants might influence the range of host plants used. Comparison of the range of host plants between congeneric ant-attended and non ant-attended aphid species would contribute towards understanding the coevolution between herbivorous insects and host plants.

As shown in "Costs of ant attendance to aphids", some aphid species increase in the frequency of honeydew excretion under ant attendance. Enhanced honeydew excretion would affect both the physiological status of aphids and nitrogen recycling regulated by *Buchnera*, the microorganism found in aphids, because essential amino acids are synthesized from unessential amino acids in the phloem sap via nitrogen recycling (Sasaki et al. 1991). Thus, the efficiency in phloem sap assimilation and, hence, the degree of dependence on *Buchnera* is expected to differ between ant-attended and non ant-attended aphid species. It is hypothesized that relationships between hosts and *Buchnera* are more intimate in ant-attended aphid species compared to non ant-attended aphid species. This hypothesis could be tested by comparing the genetic variation of *Buchnera* in ant-attended and non ant-attended aphid species. Future studies should focus on the range of host plants, the microorganism *Buchnera* in aphids, and phylogenetic comparative methods between ant-attended and non ant-attended aphid species. The integration of such research would contribute towards providing a more comprehensive understanding about the evolution of mutualism between aphids and ants.

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