Ant community dynamics in lac insect agroecosystems: conservation benefits of a facultative association between ants and lac insects

YOUQING CHEN[1*], SIMING WANG[1], ZHIXING LU[1] & WEI ZHANG[2]

[1]*Research Institute of Resources Insects, Chinese Academy of Forestry (CAF), Kunming, 650224, Yunnan, China*
[2]*School of Conservation Biology, Southwest Forestry University, Kunming, 650224, Yunnan, China*

Abstract: Honeydew is a key resource for ants and a driver of ant community dynamics, yet we know very little about how the quantity of honeydew generates these patterns. This study investigated the effects of an ant-lac insect facultative association on ant community dynamics in a guild of ants attending *Kerria yunnanensis* (Hermiptera: Kerriidae) on its host plant *Dalbergia obtusifolia* in Yunnan Province, China. This was done by manipulating the access of ants to *K. yunnanensis* and the numbers of *K. yunnanensis* available. Regardless of the proportion of lac insect infestation (the ratio of the branch length infested by lac insects to the total branch length), there were more ant species and individuals under the ant-lac insect facultative association, and ant community composition was significantly different from that without this association. The level of branch infestation by the lac insect had a significant influence on the mean frequency of occurrence of ants; a low level of branch infestation by the lac insect led to low ant attendance. Honeydew secreted by the lac insect had a key role in structuring the ant community. There were more co-occurrences of ants under the ant-lac insect facultative association, and co-occurrence of more than three ant species only occurred on trees with lac insect infestation. The significant difference in frequency of month-by-month ant replacement was only observed in the treatment without lac insect hosting, which indicated the positive role of ant-lac insect association in stabilizing ant community. We suggest that the facultative association between ants and lac insects has conservation benefits for ant assemblages and provides a structuring mechanism for the ant community in the lac agroecosystem studied.

Key words: Ant community, ant-lac insect association, co-occurrence, lac insect agroecosystem, replacement.

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Introduction

Most ant-hemipteran associations are generally regarded as facultatively mutualistic, in that neither partner is totally dependent on the other (Buckley 1987a,b; Hill & Blackmore 1980). Studies of ant-scale insect inter-actions typically focus on the benefits for hemipterans, and studies investigating the advantages of the interactions to the ants are rare (Lach *et al.* 2009). Experimental studies of ant-scale interactions typically involve a single ant species (Itioka & Inoue 1996; Rosario *et al.* 1993 other studies summarised in Gullan 1997). However, many hemipteran aggregations

Corresponding Author; e-mail: cyqcaf@126.com

have multiple ant species in attendance (i.e., Buckley & Gullan 1991; Chen *et al.* 2011; Del Claro & Oliveira 1999; Moya-Raygoza & Nault 2000).

Many ant species utilize the same food resource, and competition may occur among them (Apple & Feener 2001; Oliveira *et al.* 1999). Competitive interactions among ants on host plants may potentially shape the community structure of ant assemblages. If competition is a strong determinant of the structure of ant communities, a key question becomes: what allows multiple ant species to coexist in a given habitat (Andersen 2008)? The mechanism for ant species co-existence in the ant-extrafloral nectary (EFN) plant system has been explored (Blüthgen *et al.* 2000; Palmer *et al.* 2000; Young *et al.* 1997). It has been found that in an Amazonian rainforest canopy, competitively superior ants usually dominate the most attractive food items, while ants with lower competitive capacity share the less attractive EFNs. Ant communities that depend on honeydew and EFNs seem to be strongly shaped by competitive hierarchies (Blüthgen *et al.* 2004). Such competitive hierarchies reflect the ranking of abundance of the particular ants in their community (Andersen 1995; Blüthgen *et al.* 2004; Blüethgen & Fiedler 2004; Davidson 1997; Leal *et al.* 2006; but see Xu & Chen 2010). However, little is known of the impact on ant assemblages from aggregations of honeydew-producing hemipterans, especially the pattern of resource utilization in a resource-rich environment (i.e., one lasting at least several months).

Lac insects (*Kerria* spp., Hemiptera: Kerriidae) are scale insects that occur naturally as herbivores on various host plants. They produce lac, a layer of red resin on branches of host-trees on which they settle. Lac resin is natural, biodegradable and non-toxic, and thus is widely used in the food, textile and pharmaceutical industries, in addition to its use as a surface coating in electrical component manufacturing (Chen *et al.* 2010). In China, lac-production and farming have become integrated into an agroforest system. The lac insect agro-ecosystem is a very popular agroforest planting model due to its economical and ecological roles, and is widely distributed throughout mountainous areas of Southwestern China, a region noted for having high conservation values (Chen *et al.* 2010).

We used field manipulation of *Kerria yunnanensis* (Ou et Hong) population levels and a controlled ant-exclusion experiment to test the hypotheses that 1) ant-lac insect facultative inter-action promotes ant richness and abundance, and 2) relative low-level honeydew supply is adequate for maintaining this association, resulting in more ant co-occurrence and fewer ant replacements.

Methods

Study system

This research was conducted in a lac insect agroecosystem in Mojiang county, Yunnan Province, south west of China (101º43′E, 23º14′ N, 999.8–1056 m a.s.l.). The lac insect agro-ecosystem is the main form of cultivation in mountainous regions between 900–1500 m a.s.l., with several lac insect host plant species distributed along or among the semi-arid and semi-humid agricultural plots. The main host tree for lac insects is *Dalbergia obtusifolia* (Prain); other host tree species are distributed randomly among the *D. obtusifolia* plantations. The lac insect *Kerria yunnanensis* is raised on trees from the fifth year onwards, but other honeydew-producing coccid insects can also be found. Many ant species attend lac insects for honeydew rather than eating them. At the same time, these ants influence the natural enemies of lac insect present in the ecosystem, either directly and indirectly (Chen *et al.* 2013). The main crop planted by the farmers under the lac insect host trees or among the patches of trees is corn.

Experimental designs

The main purpose of these designs was to investigate the influence of honeydew quantity on the ant community. In each generation of *K. yunnanensis*, before *K. yunnanensis* was cultivated on *D. obtusifolia*, trees distributed within a 10 ha plot were randomly sampled. The sampling distance between individual trees (10 m) allowed them to be considered independent samples, because most ant species forage only within a few metres of the nest in this study.

Kerria yunnanensis was cultivated on the sampled trees and the population of lac insect was manipulated randomly based on four categories of lac insect load on the tree. Though we randomly manipulated trees into each category, the spatial distribution of treatment trees was interspersed to ensure that individual trees were true replicates.

The four categories of lac insect load were 1) no *K. yunnanensis* on the trees, 2) small number of *K. yunnanensis* on the trees, 3) medium number of *K. yunnanensis* on the trees, and 4) large number of

K. yunnanensis on the trees. The ratio of *K. yunnanensis* numbers among the last three categories was about 6:3:1. Within each category, the number of *K. yunnanensis* on each tree was controlled by measuring the total settlement area of *K. yunnanensis* on all branches of a given tree (same population density on the same tree species, so *K. yunnanensis* population number could be controlled by the area of settlement). For selected trees of a similar size and the same amount of *K. yunnanensis* on the trees, the proportion of branches with lac insects hosting *D. obtusifolia* was similar within each category. Thus in this experiment, the infestation proportions were (i) 0%, (ii) about 10% (ranging from 8% to 12%), (iii) about 30% (ranging from 28% to 32%), and (iv) about 60% (ranging from 58% to 62%). In total, 120 trees were selected, with each category having 30 trees, and four levels of lac insect infestation representing four treatments levels. These treatment levels were maintained throughout the whole life cycle of one generation from May 2009 to October 2009 (the ratio of *K. yunnanensis* population number among the categories was consistent, for the lac insects live a sessile life after settlement). The same experiment was conducted for another generation of *K. yunnanensis* from October 2009 to May 2010. Overall 1440 investigations were conducted.

The size of the lac insect population correlates strongly and positively with the quantity of honeydew produced per tree, and honeydew quality (which would also strongly affect ant attendance) does not differ significantly within each treatment. This is because the selected trees were similar, the lac insect loads for each tree were the same, and the amount of honeydew produced by all lac insects in one tree did not differ significantly within the same treatment at the same time (this assumption was based on personal observations).

The study plot is characterized by an average annual rainfall of 1500–2100 mm (mostly in May to October) and a mean annual temperature of 18.2 ºC (observations in recent three years). *Kerria yunnanensis* is cultivated by farmers on different parts of the host plant throughout the year for lac. On completing its first generation (whose main role is to produce raw lac), *K. yunnanensis* is harvested by cutting the branches on which it feeds and transferring the insect to alternative trees for the second generation (for the production of brood lac).

The experiments were performed in a lac plantation, which is dominated by the lac plant *D. obtusifolia* with a density of 1500 trees per hectare, and the total area is about 10 hectares. On the host *D. obtusifolia*, *K. yunnanensis* settles on the main branch, the shoots and even the leaf stalks. In the larval stage, females and males only produce a little honeydew, but after mating all males die and only females produce honeydew, but the amount is huge. This honeydew can be found on the body of lac insects or on the surface of leaves and branches, and even on the ground. Many ant species including *Crematogaster macaoensis* (Wheeler 1928), *Dolichoderus thoracicus* (Smith 1860), and *Camponotus parius* (Emery 1889) visit *K. yunnanensis* and *D. obtusifolia* for honeydew. In this experiment, 8-year-old *D. obtusifolia* were selected, ranging from 2.5 m to 2.8 m in height and from 5 cm to 7 cm in trunk diameter. The experiments were conducted from March 2009 to December 2010.

Ant assemblage investigation

We randomized the order in which we observed trees, and recorded the species and the individual number of each ant species within the tree. At the same time, we noted where each ant was from. Observations were completed within ca 1–2 minutes per plant, so that each survey represents a random 'snapshot' of simultaneous ant activity on plants, rather than a complete record of the assemblage (Blüthgen *et al.* 2004). The investigated parts of the plant were trunk, main branch, side shoots and leaf stalks, as lac insects can settle on these parts, and attract different ant species. We carried out monthly censuses (09:00–11:00 am) over a 12-month period from May 2009 to May 2010. Ant specimens were identified using keys found in Xu (2002) and Wu & Wang (1995).

Analysis

Though 1440 observations were conducted, only 767 observations were with ants, the observations without ants were listed as Appendix 1.

Individual-based accumulation curves were generated by using EstimateS (Colwell 2009) to evaluate the completeness of sampling. These represented the means of 50 randomized, pooled samples accumulated during 12 samplings from the 120 trees. Expected species richness was calculated by the estimators of ACE (Hortal *et al.* 2006), in order to gauge the completeness of sampling and compare the abundance of ants (Moreno & Halffter 2001). Specimens from the 12
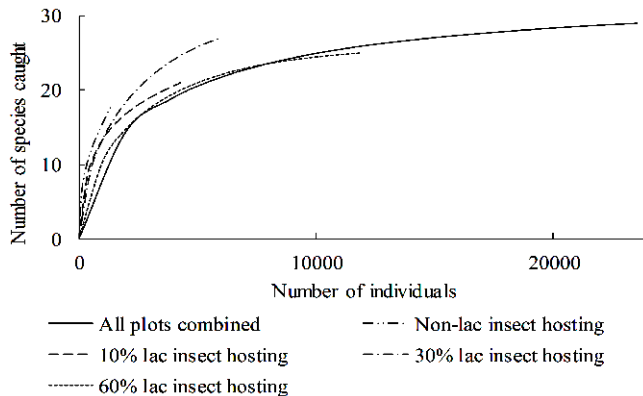
Fig. 1. Individual-based accumulation curves. These represent the means of 50 randomized, pooled samples accumulated during 12 samplings from the 4 treatments.

samplings from each tree were pooled for data analysis of the effect on ant assemblage diversity and structure under different scale insect loads.

Principal Coordinate Analysis (PCA) for ant species was conducted after applying the Hellinger transformation to the abundance data, followed by a calculation of Euclidean distance, using the "decostand", "dist", and "cmdscale" functions of the R language package "vegan". PERMANOVAs were also conducted with R to compare the ant community composition difference among treatments (Oksanen *et al.* 2009; R Development Core Team 2009), followed by multiple comparisons using SPSS 16.0.

The number of ant species that foraged simultaneously on the same individual plant was counted and denoted as 'S'. Ant species co-occurrence was defined as an instance on plant where S>1 and where there were at least two workers for each species. The proportion of visits with co-occurrence was only calculated for those cases where at least two ant workers were present on a plant. MANOVA was used to compare frequency of occurrence (including independent occurrence and co-occurrence with other ant species) and co-occurrence under different experimental regimes (i.e., the 0%, 10%, 30% and 60% branch occupation by lac insects) based on monthly investigation data. The difference of occurrence within the same gradient was analyzed by means of one-way ANOVA; Tukey multiple comparisons were then carried out.

The distribution of co-occurrences between particular ant species was obtained using a reduced dataset, comprising only surveys with at least two ant individuals and species, with a minimum of five observations being considered (following Blüthgen *et al.* 2004). Under these criteria, we obtained 356 samples for 21 ant species overall. Significant deviation from the mean frequency of two or more co-occurring species was tested by a chi-squared test. For a more detailed analysis of patterns in the ant-ant association matrix, a correspondence analysis (CA) was performed using SPSS 16.0 for Windows on the reduced contingency table of associations.

In the MANOVA analysis, the mean frequency of occurrence, co-occurrence and replacement were obtained as follows: within each treatment, the data from 30 trees were divided into three groups randomly, with data from 10 trees as one group, and this partition was consistent for all data during the 12 months. The means were the result of observed months. Besides the analysis above, repeated measures analysis were conducted to test ant attendance, co-occurrence and replacement, but there were trees without ants in different months and different treatments. For the same sampling number in the analysis, we defined the n=12 as the number of treatment levels time the number of replicates; ie. the four treatments, and within each treatment, the three replicates described above.

Results

Ant community structure in the lac insect agroecosystem

Regardless of the degree of lac insect infestation, there were more ant species and individuals when lac insects were present (Fig. 1). In total, 18 ant species were found on *D. obtusifolia* not occupied by *K. yunnanensis,* whereas 21 ant species were found on *D. obtusifolia* with 10% of branches infested, 27 ant species were found on *D. obtusifolia* with 30% of the branches infested, and 25 ant species were found on *D. obtusifolia* with 60% of the branches infested (Table 1). The ratios of the number of captured S to the estimated ant species richness ACE (EstimateS) for the four levels of lac insect infestation were 98.2%, 98.7%, 100%, 96.5%, respectively, which indicated a high level of completeness of sampling. The individual number of ants on the trees with 60% of the branches infested by *K. yunnanensis* represented nearly the entire expected individual pool based on accumulation curves (Fig. 1), indicating the existence of a large number of numerically dominant ant species.

Table 1. Ant species feeding on *D. obtusifolia,* showing with their main nest locations. Typical nest locations: (a) arboreal and (g) ground nests. Numbers are frequencies of occurrences (mean±1SE; n=10) and total number of individuals.

Ant species	Nest location	No branch hosting lac insect	10% of branches hosting lac insect	30% of branches hosting lac insect	60% of branches hosting lac insect
Ponerinae					
Pachycondyla rufipes (Jerdon 1851)	g	–	–	1.00±0.00 (5)	1.33±0.33 (4)
Gnamptogenys bicolor (Emery 1889)	g	1.00 (3)	–	1.00±0.00 (3)	1.00±0.00 (6)
Pseudomyrmecinae					
Tetraponera allaborans (Walker 1859)	a	[AB]2.68±0.30[a] (123)	4.00±0.41[ab] (239)	4.07±0.41[b] (203)	3.38±0.35[ab] (194)
Tetraponera attenuata (Smith 1887)	a	1.00 (1)	1.00±0.00 (2)	1.00±0.00 (22)	–
Myrmicinae					
Cataulacus granulatus (Latreille 1802)	a	[AB]1.71±0.42 (17)	1.09±0.09 (14)	1.33±0.33 (13)	1.36±0.20 (18)
Crematogaster macaoensis (Wheeler 1928)	a	[AB]1.92±0.26[a] (137)	3.06±0.28[a] (1842)	3.09±0.32[a] (2511)	6.67±0.28[b] (8928)
Crematogaster osakensis (Forel 1896)	g	[A]1.00±0.00 (10)	–	1.00(9)	1.00±0.00 (28)
Crematogaster ferrarii (Emery 1887)	g	[B]4.26±0.41[a] (624)	3.09±0.26[a] (909)	3.54±0.33[a] (1558)	1.00±0.00[b] (40)
Pheidologeton affinis (Jerdon 1851)	g	[AB]1.25±0.25 (120)	1.00(30)	1.00±0.00 (353)	1.64±0.31 (685)
Monomorium chinense (Santschi 1925)	g	1.00 (2)	1.00±0.00 (145)	–	1.00±0.00 (48)
Rhoptromyrmex wroughtonii (Forel 1902)	g	–	1.44±0.24[ab] (282)	1.67±0.29[b] (245)	1.00±0.00[a] (438)
Pheidole lighti (Wheeler 1927)	g	1.00 (6)	1.60±0.40 (332)	1.25±0.16 (187)	1.40±0.24 (189)
Pheidole yeensis (Forel 1902)	g	–	–	–	2.00 (58)
Pheidole roberti (Forel 1902)	g	–	1.00±0.00 (17)	1.00 (3)	–
Pheidole pieli (Santschi 1925)	g	[AB]1.50±0.50 (23)	1.67±0.33 (83)	1.00 (70)	1.00±0.00 (41)
Cardiocondyla wroughtonii (Forel 1890)	g	–	1.50±0.50 (11)	1.00 (6)	1.00±0.00 (11)
Aphaenogaster beccarii (Emery 1887)	g	–	1.00(3)	1.00±0.00 (9)	–
Dolichoderinae					
Dolichoderus thoracicus (Smith 1860)	ag	[AB]1.67±0.23 (237)	–	1.91±0.34 (209)	1.50±0.17 (248)

Table 1. Continued.

Ant species	Nest location	No branch hosting lac insect	10% of branches hosting lac insect	30% of branches hosting lac insect	60% of branches hosting lac insect
Iridomyrmex anceps (Roger 1863)	g	–	–	1.00±0.00 (2)	–
Bothriomyrmex wroughtonii (Forel 1895)	g	–	1.00±0.00 (88)	1.00±0.00 (42)	1.00±0.00 (76)
Tapinoma melanocephalum (Fabricius 1793)	ag	1.00 (23)	1.00±0.00 (175)	1.00±0.00 (181)	1.14±0.14 (322)
Tapinoma indicum (Forel 1895)	ag	–	1.00(8)	1.00±0.00 (102)	1.00±0.00 (383)
Formicinae					
Anoplolepis gracilipes (Smith 1857)	ag	1.00 (3)	1.67±0.33 (21)	1.00 (2)	1.33±0.14 (57)
Pseudolasius silvestrii (Wheeler 1927)	g	–	–	1.00±0.00 (118)	1.00(55)
Polyrhachis tibialis (Smith 1858)	a	AB1.14±0.14 (8)	1.10±0.10 (12)	1.50±0.27 (16)	1.22±0.22 (12)
Polyrhachis proxima (Roger 1863)	g	AB1.33±0.33 (9)	1.20±0.13 (14)	1.50±0.26 (19)	1.50±0.16 (26)
Polyrhachis ceylonensis (Emery 1893)	g	–	–	–	1.00(6)
Camponotus parius (Emery 1889)	g	AB1.75±0.28 (27)	1.38±0.24 (26)	1.65±0.21 (47)	2.00±0.21 (40)
Camponotus mitis (Smith 1858)	g	1.00 (1)	–	1.33±0.33 (8)	1.00±0.00 (4)

Significant analysis was applied to those species with≥ 5 observations. The capital letter indicates Tukey multiple-comparison results of different ant species under the same infestation levels of lac insect on branches, and the lower case letter indicates Tukey multiple-comparison results of the same ant species under different infestation of lac insect on branches.

Ant community composition within the four *K. yunnanensis* infestation levels was significantly different (F = 10.435, P = 0.0009, n = 4), with ant community composition on trees without lac insect hosting being different from trees with 10% *K. yunnanensis* infestation (F = 6.346, P = 0.0009, n = 2), to trees with 30% *K. yunnanensis* infestation (F = 10.481, P = 0.0009, n = 2), and to trees with 60% *K. yunnanensis* infestation (F = 25.784, P = 0.0009, n = 2). For trees with lac insect hosting, the ant community composition on trees with 30% *K. yunnanensis* infestation were different from trees with 60% *K. yunnanensis* infestation only (F = 9.291, P = 0.0009, n = 2) (Fig. 2). Several of the ant species identified typically have arboreal nests (in both living and dead plant material), but many ground-nesting species were also involved (Table 1).

The level of branch infestation by the lac insect (expressed as a percentage) had a significant influence on the mean frequency of occurrence of ants (F = 3.73; P < 0.0001; n = 767), low level of branch infestation by the lac insect leading to low ant attendance (Table 1 and Appendix I), but there was no significant difference between larvae stage and adult stage of lac insects (MANOVAs). The frequency of occurrence of ants from trees without lac insect infestation was significantly difference to the other three treatments. However, for the trees with lac insects hosting, a significant difference only existed between 10% and 60% level (repeated measures analysis). On the trees with 60% of the branches infested by *K. yunnanensis*, *Crematogaster macaoensis* was clearly the most common species, while on the trees with 10% and 30% levels of infestation, *C. macaoensis*, *Crematogaster ferrarii* (Emery 1887), and *Tetraponera allaborans* (Walker
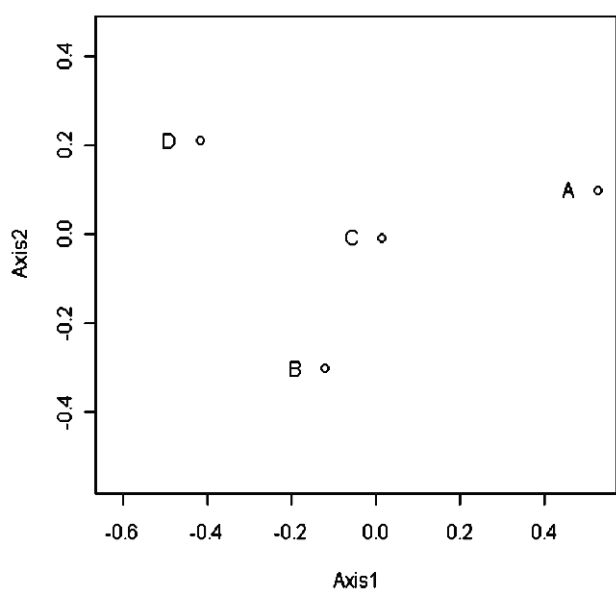
Fig. 2. Principal coordinate analysis for ant species among the four treatments. A-D represent 0%, 10%, 30%, and 60% lac insect infested treatment, respectively.

1859) were the most common ant species. However, on the trees with no *K. yunnanensis* infestation, the most common ant species were *C. ferrarii* and *T. allaborans*. Out of the 29 ant species, only *C. macaoensis* (F = 44.35, P < 0.0001, n = 119), *C. ferrarii* (F = 6.06, P = 0.001, n = 111), *Rhoptromyrmex wroughtonii* (Forel 1895) (F = 3.76, P = 0.035, n = 31), and *T. allaborans* (F = 3.09, P = 0.03, n = 121) had significant differences of frequency of occurrence for the different levels of *K. yunnanensis* infestation (One-way ANOVAs) (Table 1).

Co-occurrence and species replacement

The correspondence analysis indicated that co-occurrences between different ant species on the same plant were common, but not equally common between different pairs of ant species (Table 2). The three most common ants, namely, *C. macaoensis*, *C. ferrarii*, and *T. allaborans*, were found to forage on the same plant, but *C. ferrarii* was mostly on the trunk and main branch, while *C. macaoensis* and *T. allaborans* were mostly on side shoots and even leaf stalks. Other ant species commonly shared the same plants with them. The level of branch infestation by the lac insect had a significant influence on the co-occurrences of ants (F=8.196; P=0.008; n=12), and low levels of branch infestation by the lac insect led to low co-occurrences (Repeated measures analysis). Though
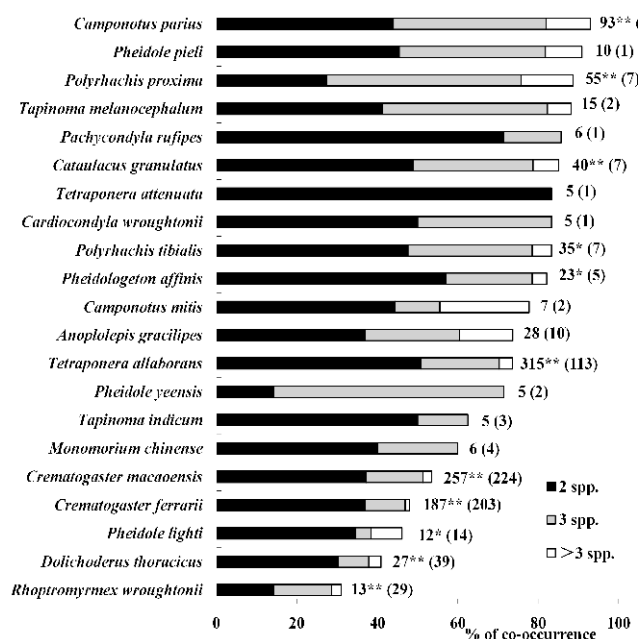

Fig. 3. Frequency of co-occurrence of two, three or more ant species on *D. obtusifolia* by ant species. Only surveys with at least two ant individuals and species with a minimum of five observations were considered; the number of observations in total for each species is displayed after each bar. The numbers in the brackets indicate frequencies of the ant occurring on the trees without any other ants. Significant deviation from the mean frequency of two or more co-occurring species is indicated by * and ** for P < 0.01 or P < 0.001, respectively (χ^2-test, observed against expected frequencies).

the level of infestation by *K. yunnanensis* among the 10%, 30%, and 60% treatments did not have any significant effect on co-occurrence of ants (repeated measures analysis), within the same level of *K. yunnanensis* infestation on branches there was a significant difference respect of co-occurrence of two ant species, with three ant species, and more than three ant species co-occurring, respectively, (One-way ANOVAs) (F = 79.47; P < 0.0001; n = 356); co-occurrence of more than three ant species only occurred on trees with lac insect infestation (Table 3). But there was no significant difference between larvae stage and adult stage of lac insects (MAVONAs).

Of 29 ant species, 21 ant species co-occurred with other ant species (Fig. 3). The three most common ant species had the highest frequencies of co-occurrence with other ant species. Ten ant species also exhibited significant differences between frequency in respect to co-occurrence with

Table 2. The type of ant co-occurrence at different infestation levels of lac insect.

Population number of lac insect	Number of co-occurrence (N)	Frequency of one ant species on plant Mean±1SE (%)	Frequency of two ant species on plant Mean±1SE (%)	Frequency of three ant species on plant Mean±1SE (%)	Frequency of more than three ant species on plant Mean±1SE (%)
Non-branch hosted by lac insect	76	4.64±0.30[a] (47.37)	2.59±0.29[b] (35.53)	1.15±0.10[c] (17.10)	0
10% branches hosted by lac insect	89	4.89±0.33[a] (40.45)	2.94±0.32[ab] (35.96)	1.39±0.14[b] (20.22)	1.00±0.00[b] (3.37)
30% branches hosted by lac insect	89	4.86±0.28[a] (40.45)	3.26±0.25[ab] (38.20)	1.81±0.25[bc] (17.98)	1.33±0.33[c] (3.37)
60% branches hosted by lac insect	102	4.86±0.34[a] (35.29)	3.08±0.28[b] (35.29)	1.78±0.17[bc] (22.56)	1.29±0.18[c] (6.86)

N is the sum of ant co-occurrence in 1440 investigations for each treatment and the Mean is the numbers of ant co-occurrence (one ant on plant or two ant species on plant or three ant species on plant or more than three ant species on plant) per 10 trees at each level of infestation throughout 12 months. A lower case letter indicates Tukey multiple comparison results of frequency under the same infestation level of lac insect.

Table 3. Frequency of replacement of ant species foraging on *D. obtusifolia* under different infestation levels of lac insect on branches (mean±1SE%).

Month	No branch hosting lac insect	10% of branches hosting lac insect	30% of branches hosting lac insect	60% of branches hosting lac insect
Jan.–Feb.	[A]0.00±0.00[a]	2.88±1.82[a]	8.67±4.67[ab]	24.60±0.40[b]
Feb. –Mar.	[AB]4.17±2.08	7.87±3.96	3.59±0.41	10.00±10.00
Mar. –Apr.	[AB]8.33±4.17	5.29±3.22	14.22±3.95	22.02±8.41
Apr. –May	[AB]15.05±10.39	13.23±1.06	12.83±1.74	23.90±8.50
May –Jun	[AB]4.67±2.91	10.77±6.15	8.41±4.81	29.13±3.24
Jun–Jul.	[A]0.00±0.00	5.42±2.92	6.46±3.67	16.39±7.67
Jul. –Aug	[AB]36.35±1.95	26.67±14.53	25.48±1.67	8.68±2.29
Aug –Sep	[AB]23.33±12.02	26.30±8.54	26.44±10.18	9.79±5.03
Sep –Oct	[A]0.00±0.00[a]	13.02±3.60[ab]	22.76±9.01[ab]	10.71±6.19[b]
Oct –Nov.	[AB]5.56±5.56[a]	32.80±5.96[ab]	22.00±6.22[b]	31.49±8.18[b]
Nov. –Dec	[B]47.22±26.50	19.84±1.79	12.49±4.90	16.67±6.67
Dec -Jan.	[AB]20.37±15.16	4.08±1.08	10.83±5.83	10.83±4.58

The data were square root transformed before Tukey multiple comparisons for 10 trees in each treatment. The capital letter indicates Tukey multiple-comparison results of replacement under the same infestation levels of lac insect on branches, and the lower case letter indicates Tukey multiple-comparison results of replacement under the same time.

one ant species, two ant species, and more than two ant species (χ^2-test) (Fig. 3).

The level of branch infestation by the lac insect (expressed as a percentage) had a significant influence on the mean frequency of replacement of ant species (F=1.80, P=0.02, n=144), but there was no significant difference between larvae stage and adult stage of lac insects (MAVONAs). The replacement of ant species between different consecutive months on trees showed significant difference on trees without lac insect infestation, however, there was no significant difference of ant species replacement between different consecutive month on trees with lac insect infestation (Tukey multiple comparisons) (Table 4).

Discussion

Overall, we found that honeydew had a positive effect on the ant community. The number

of ant individuals and species on plants without lac insects were less than those on plants with lac insects. The most common ant species and the community composition on plants without lac insects were different from those on plants with lac insects. With the increase in honeydew, the species richness, number of individuals, the most common ant species, and frequencies of ant co-occurrence were different among levels of treatment. The highest ant richness was found on *D. obtusifolia* with 30% of the branches infested by *K. yunnanensis*, and the individual number of ants on *D. obtusifolia* with 60% of the branches infested by *K. yunnanensis* represented nearly the entire expected individual pool. The percentage of more than three ant species co-occurrence was the highest on the branches with 60% infestation by lac insects (6.86%, 3.37%, and 3.37%, for 60%, 30% and 10%, respectively), and co-occurrence of five ant species could only be found on these trees also. The high ant species richness, abundance and distinctive community composition indicates the importance of honeydew. As indicated by other studies, honeydew is certainly one of the most predictable and stable resources (Jackson 1984a, b; Yanoviak & Kaspari 2000), and represents a key resource for arboreal ant species (Davidson 1997; Davidson *et al.* 2003; Tobin 1995), moreover it can be largely controlled by the ants themselves. Honeydew also benefits for ant communities nesting outside trees, for most of the ant species collected in this study were ground-dwelling species (Table 1).

Ant species have been found to occur in a mosaic spatial distribution in many habitats (reviewed by Rico-Gray & Oliveira 2007), but this pattern was not found here, as more than 70% ant species co-occurred with other ant species in our system. The three most common ant species,, namely *C. macaoensis*, *C. ferrarii*, and *T. allaborans*, were found to forage on the same plant, and had the highest frequencies of co-occurrence with other ant species; other ant species also commonly shared the same plants with them.

Three distinct patterns of honeydew use by ants may contribute to the structure and distribution of ant communities in our study system. The first pattern is ants foraging in partitioned spaces within the plant using different foraging strategies. *Crematogaster ferrarii* preferred to forage on the truck and main branch, while *C. macaoensis* and *T. allaborans* preferred to forage on side shoots and even leaf stalks. The morphological structure of plant parts may itself facilitate ant co-occurrence,

since the lac insect can settle on the trunk, main branch, side shoots and even leaf stalks. These parts represent spatial partitioning of honeydew resources and represent different levels of accessibility. Second is the relative temporal stability of food resources for ants on lac insect host plants. The seasonal partitioning (between larval and adult stage of lac insect) in terms of ant frequency of occurrence, co-occurrence, and replacement was very weak for the ant community foraging on plants with lac insect infestation, with ants continuously active during 12 months (two lac insect life cycles), which further suggests that a relative low-level honeydew supply is adequate for stabilizing ant community structure. The honeydew supply in lac plantations is present in surplus, with many droplets of honeydew abandoned, especially at higher lac insect infestation levels (unpublished data). Third is the monopolization of attractive honeydew sources by dominant ants. The dominance of *C. macaoensis* was more obvious on the trees with 60% of branches infested by lac insects. Many *C. macaoensis* nests could be found on nearby plants, and the number of workers ranged from 10,000 to 100,000 per nest (unpublished data) and some honeydew resources were totally monopolized by them (about 30 branches with lac insect infestation were monopolized by *C. macaoensis*, and no other ant species was found feeding on the honeydew). Honeydew is a relatively nutritious and rewarding resource (Douglas 1993) and numerously dominant species exclusively occupy the most attractive food sources. Food that is lower in attractiveness for dominant species is exploited by opportunistic subordinate species, which also share the resource with other competitively inferior species (Blüthgen *et al.* 2004; Blüthgen & Fiedler 2004). Thus, it is not surprising that trees with 60% of branches infested by lac insects have fewer species.

In conclusion, the ant-lac insect facultative association is beneficial for ant diversity and patterns of honeydew resource usage may provide a major basis for structuring the ant assemblage in the lac insect agroecosystem.

Acknowledgments

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Supporting Information

Additional supporting information may be found in the online version of this article:

Supplementary Table 1. The number of trees without ants in each treatment in every month.

Supplementary Table 2. Frequency of co-occurrence between the ant species on *D. obtusifolia*. Species ordered by total interaction frequency, empty columns removed.