



RESEARCH ARTICLE - ANTS

The Ecological Effects of Ant-Aphid Mutualism on Plants at a Large Spatial Scale

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Article History

Edited by

Kleber Del-Claro, UFU, Brazil

Received 25 June 2013

Initial acceptance 22 July 2013

Final acceptance 19 August 2013

Key words

Ant defense, ant-plant interaction, herbivory, exclusion experiment

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Abstract

The protective ant-plant interaction has been considered as a model system in studying mutualistic interactions, but we know little about the ecological effects of the mutualism at relatively larger spatial scales. In this study, by excluding an aphid-tending ant species (*Lasius fuliginosus*) from all host oak trees (*Quercus liaotungensis*) in 20x20 m plots, we evaluated the effects of ants on herbivory, fruit production and leaf toughness of the host tree. Through a two years study, we found that ants have a significant anti-herbivory effect on the host tree, with no effects on fruit production. At the end of the growing season, leaf toughness for plants without ants increased significantly. This suggests that ants are reliable and effective bodyguards for plants at larger spatial scales. For plants, the possible tradeoff between different defensive strategies at larger scale should be focused in further works.

Introduction

Mutualism has been increasingly considered to play an important role in shaping community structure, diversity and ecological functions (Bronstein, 1994; Bruno et al., 2003; Bascompte and Jordano, 2007). The role of mutualistic interactions in nature has been considered as one of the “key gaps in population and community ecology” (Agrawal et al., 2007). Ant-hemipteran interaction is one of the most common mutualistic interactions in nature; in the interaction ants take the honeydew excreted by hemipterans as food and in return, they protect those insects from natural enemies (Del-Claro, 2004; Moreira & Del-Claro, 2005). For a long time, much of the attention has been paid for the effect of the interaction on each other, especially the impacts of ants on hemipteran (Way, 1963; Buckley, 1983; Stadler & Dixon, 2005). More recent studies show that this mutualistic interaction has a wider range of ecological effects, especially for the host plants and related arthropods on foliage (Wimp & Whitham, 2001; Kaplan & Eubanks, 2005; Styrsky & Eubanks, 2007).

The honeydew-collecting ants often benefit plants through their attack and expel on herbivores (Moreira & Del-Claro, 2005; Chamberlain & Holland, 2009; Rosumek et al., 2009; Trager et al., 2010; Romero & Koricheva, 2011; Zhang et al., 2012b). Ants can decrease herbivory through their negative effect on the abundances of herbivores on plants (Zhang et al. 2012b). An important problem to be declared is that the possible effect of spatial scale on the ecological effects of ant-hemipteran interaction has long been ignored. Many biotic interactions can be scale dependent, such as pollination (Leiss & Klinkhamer, 2005; Westphal et al., 2006); herbivory (WallisDeVries et al., 1999), frugivory (Garcia et al., 2011) and seed predation (Curran & Webb, 2000). But to our knowledge, most of the studies on ant-plants interactions conducted at the level of branches or individual plants (Chamberlain & Holland, 2009; Zhang et al., 2012b), the ecological effects of this interaction at larger spatial scales are poorly known.

Considering the complexity of biotic interactions in nature, the conclusions and predictions drawn from a smaller spatial scale may be inconsistent with that got from larger



spatial scale. Therefore studies conducted at even larger spatial scales are needed to fully understanding the ecological effects of the ant-hemipteran interaction in nature.

Theoretical models argue that plant defense should not be redundant (Stamp, 2003). In ant-plant interactions, it has been long assumed that there is a tradeoff between ant defense and the defense of plant itself (such as chemical or physical defense), especially in obligate ant-plant interactions (Janzen, 1966). Mixed evidences for the hypothesis have been found for obligate ant-plant interactions (Heil et al., 2002, Frederickson et al., 2013). But few studies have tested the hypothesis in the facultative ant-plant interactions mediated by hemipteran. Base on the finding that the honeydew collecting ants have significant anti-herbivory effects for plants, we argue that the tradeoff among different defensive strategies can also be existence in the ant-hemipteran-plant system. The exclusion of ants at a relative large scale can facilitate the tradeoff to be shown.

In this study, we evaluated the impacts of aphid-tending ants *Lasius fuliginosus* on the host oak tree *Quercus liaotungensis* by experimentally excluding ants from all oak trees in a plot (20*20 m). We hypothesized that 1) the aphid-tending ants *Lasius fuliginosus* have protective effect on plants at the plot scale 2) the physical defense of plants (leaf toughness) should be stronger when ants were excluded.

Material and Methods

The study area is located in the Beijing Forest Ecosystem Research Station (30°57'29N, 115°25'33E, altitude 1,200-1,400m), a member of the Chinese Ecological Research Network (CERN), about 100 km northwest of Beijing City, China. This area typically has a warm temperate continental monsoon climate with average annual precipitation of 500-650 mm. The mean annual temperature is 5-10°C. It is an oak (*Q. liaotungensis*) dominated, 80-year-old secondary forest with a few birches (*Betula spp.*), maples (*Acer mono*), and shrubs (e.g., *Prunus spp.*, *Vitex negundo* var. *hetertophylla*).

We conducted this experiment during two consecutive growing seasons (2009, 2010) of the oak tree *Q. liaotungensis*, which is the dominant tree species in the study area (Zhang et al., 2006). We selected a slope in a small watershed to conduct the experiment. We chose this area because the previous pitfall trap sampling found that the ant *Lasius fuliginosus* was the only active ant species with high abundance in this area. *L. fuliginosus* is a typical honeydew-feeding ant that has mutualistic relationships with some aphid species (Hopkins & Thacker 1999). In the study area, *L. fuliginosus* was attracted by aphids *Lachnus tropicalis* and *Tuberculatus sp.* in the canopy and *Stomaphis japonica* on the trunk of *Q. liaotungensis*. The aphid was the key factor attracting ants in the canopy of *Q. liaotungensis* in the study site.

In 2009, we set up four pairs of plots (20x20 m) (in 2010, three pairs) with a distance of at least 50 m between

the adjacent pairs. For each pair, one of them was set as the ant exclusion plot and the other as the control plot, with a distance of more than 15 m between each other.

In April of each year (before the growing season), an adhesive ring was smeared around the trunk (about 1 m above the ground, and 5 cm in width) on all trees in the treatment plot to impede the access of ants to aphids on the canopy. The adhesive was made of a polymer resin mixture (Beijing Non-gahaha S & T CO. LTD) and was nontoxic, harmless to plants, and non-attractive to insects. The adhesive was re-smeared every two months during the growing season until the end of the study, it worked effectively through our study. Any bridges that could allow ants to climb onto trees were cut off throughout the study. The differences between tree densities, leaf area index (LAI), and canopy coverage in the treated and control plots were insignificant (Table 1).

From late May to September, the percentage of leaf-area loss was calculated monthly. In each month, we randomly chose ten trees in a plot to evaluate plant herbivory and leaf toughness. The percentage of leaves damaged by herbivores was used as an indicator of plant herbivory.

For each tree, one randomly chosen twig (about 4-5

Table 1 The leaf area index (LAI), cover and tree densities in the treated and control plots.

Year	Variable	Treatment (mean, SE)	Control (mean, SE)	P value
2009	LAI	1.83 (0.09)	1.75 (0.09)	0.69
	Cover	79.2% (1.5%)	77.8% (1.5%)	0.68
	Tree density	38.0 (5.5)	30.5 (4.5)	0.25
2010	LAI	1.64 (0.11)	1.65 (0.12)	0.49
	Cover	75.6% (1.8%)	75.4% (2.3%)	0.55
	Tree density	33.33 (5.5)	24.33 (1.5)	0.13

m high) was cut off. For each twig, from the tip, the first to sixth leaves were collected. All the leaves were scanned by EPSON Perfection 4870 Photo (EPSON America, Inc., USA) and then used to calculate herbivory. For each leaf, the herbivore-damaged parts were repaired using the Adobe Photoshop CS2 (Adobe Systems Inc., USA), according to the expected shape. The original (a) and repaired (b) areas of leaves were calculated with WinFOLIA Basic 2004a (REGENT Instruments Inc., Australia). The percentage of leaf-area loss was calculated as $L = (b-a)/b * 100\%$.

In each plot, we randomly chose ten trees to test leaf toughness. For each tree, a twig 4-5 m high above ground was cut off. Three randomly chosen leaves were used to test toughness using a puncher immediately after the leaves were cut off. Three holes were punched for each leaf. The weight needed to punch the leaf was recorded as the indicator of toughness. This experiment was conducted only in 2010.

In late September (only in 2010), the fruiting season of *Q. liaotungensis*, fruit numbers were recorded by counting the fruit within five 1x1 m small plots in each 20x20 m plot.

Data analysis

Each pair of the plots was treated as a block in data analysis. A mixed effect model was used to test the treatment and year on plant herbivory at first. In this model, treatment, year and their interaction were set as fixed effect; block was set as random effect. Different months were treated as repeated measures, and the type of the covariance structure was selected using the Akaike information criterion (AIC). If the difference for the effect of the two-year was insignificant, data of different years were pooled together for analysis, otherwise the data were analyzed separately. Then, for each year, a mixed effect model was used to test the effects of ants on herbivory. Treatment, month and their interaction were set as fixed effects; the block was set as random factor. Different months were treated as repeated measures, and the type of the covariance structure was selected using the Akaike information criterion (AIC).

This model was also used for evaluating the effects of ants on herbivory. A poisson regression model was used to test the effect of treatment on fruit production. All the analyses were performed with SAS 9.2 with the Mixed and Genmod procedure (SAS Institute 2008).

$P < 0.0001$) and the interaction between treatment and month ($F = 7.90$, $P < 0.0001$). In 2010, the herbivory for plants with and without ants were 6.7% ($n = 907$, $SE = 0.02\%$) and 8.8% ($n = 907$, $SE = 8.8\%$) respectively, with significant differences ($F = 32.59$, $P < 0.0001$). Plant herbivory also showed significant monthly variation in 2010 ($F = 22.48$, $P < 0.0001$), but the interaction between month and treatment on herbivory was not significant ($F = 0.73$, $P = 0.5685$). Further analysis show that in 2009, the anti-herbivory effect of ants was significant only at the earlier of the growth season (May, Jun) (Fig.1), but in 2010, the effect was significant through the growth season except in July (Fig.1). Treatment had significant positive effect on leaf toughness ($F = 11.04$, $P = 0.0009$). Month and its interaction between treatment also showed significant effect on leaf toughness (month, $F = 731.75$, $P < 0.0001$; the interaction between month and treatment, $F = 6.36$, $P < 0.0001$). Further analysis showed that the effect of treatment on leaf toughness was only significant at the end of the growing season (September) (Fig.2). The fruit number in ant exclusive plots (mean = 40.67/m², $SE = 6.46$, $N = 15$) seemed to be higher than that in control plots (mean = 28.73/m², $SE = 4.22$, $N = 15$), but the difference between the two groups was insignificant ($\chi^2 = 2.44$, $P = 0.1184$) (Fig.3).

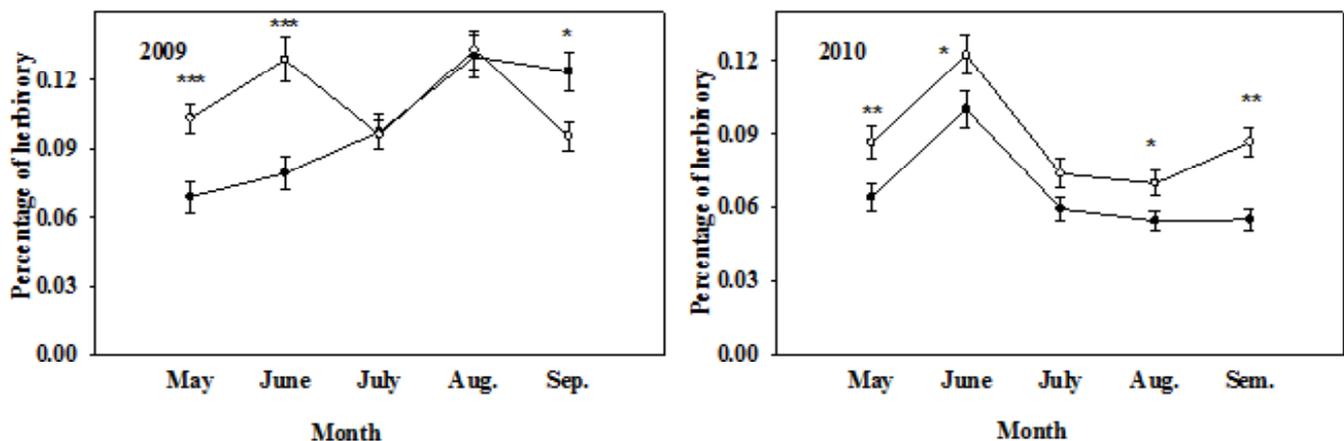


Fig. 1. The monthly variation of herbivory in ant-excluded and control plots (Mean, SE).

Results

In total, 4234 leaves of *Q. liaotungensis* were analyzed for herbivory. For plants without ants, 10.1% ($n = 2105$, $SE = 0.2\%$) of the leaf area were eaten by herbivores, for plants with ants, the value was 8.5% ($n = 2129$, $SE = 0.2\%$), the difference between the two group was significant ($F = 24.73$, $P < 0.0001$). Plant herbivory in 2009 and 2010 were 10.5% ($n = 2420$, $SE = 0.2\%$) and 7.7% ($n = 1814$, $SE = 0.2\%$) respectively, with significant differences between the two years ($F = 5.28$, $P = 0.0216$). Therefore, the data of herbivory for the two years were analyzed separately. In 2009, plant herbivory in treatment plot (mean = 11.1%, $n = 1198$, $SE = 3\%$) was significantly higher than that of plants with ants (mean = 10.0%, $n = 1222$, $SE = 4\%$, $F = 5.35$, $P = 0.0208$). In 2009, plant herbivory was also influenced by month ($F = 9.84$,

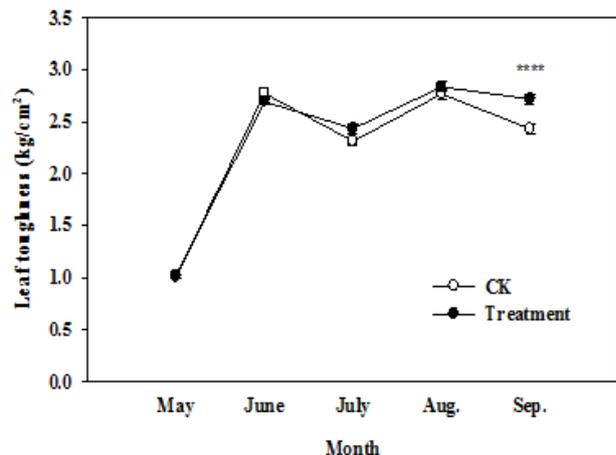


Fig. 2. The monthly variation of leaf toughness in ant-excluded and control plots (Mean, SE).

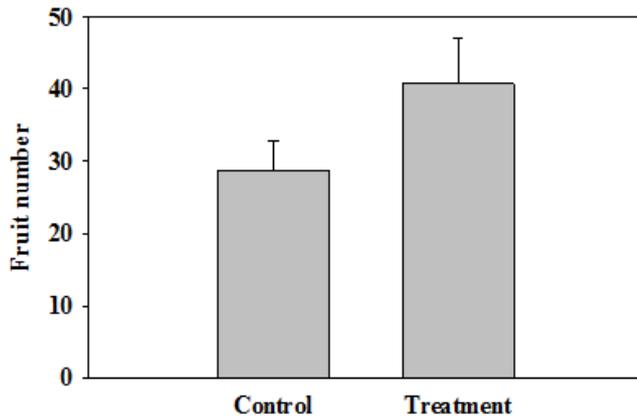


Fig.3. The number of fruit production in ant-excluded and control plots (Mean, SE).

Discussion

Recent meta-analyses found that in general the mutualistic interaction between ants and aphids can benefit plants (Styrsky & Eubanks, 2007; Zhang et al., 2012b), but all the studies used in these meta-analysis were conducted at the individual plant or smaller scale (such as branches or leaves). Whether the conclusions drawn from those smaller scales can still be solid at larger scales is unknown. Here through an experimental treatment at the 20x20 m plot scale, we confirmed the beneficial effect of the aphid-tending ants on plants. The results show that leaf toughness can be an induced defensive trait at a larger scale. These findings are essential for us to evaluate the ecological effect of mutualism in natural communities.

Our studies found that the ecological effect of ant-aphid mutualism is significant for plants beyond the scale of individual plants. Therefore ants can be a reliable bodyguard across different spatial scales. We found that the significant anti-herbivory effect of ants at the scale of individual trees as well as branches in previous work (Zhang et al., 2012a). The strength of the anti-herbivory effect for ants was 1.6% at the plot scale; this value is also within the variation range of the anti-herbivory effect (from 1.38 to 2.96%) at lower scales (Zhang et al., 2012a).

Although biotic interactions are assumed to be scale depended (WallisDeVries et al., 1999; Leiss & Klinkhamer, 2005; Westphal et al., 2006; Garcia et al., 2011), this study indicates that from the point of anti-herbivory, the effect of ants on plants can keep consistent at a wide range of spatial scales. In our study site, both the activity of ants and herbivores varied with the process of the growth season (especially in 2009), this can lead to the variation of the anti-herbivory effects of ants on plants. For different years, conditional outcomes of ant-plant interaction depending on climatic should be considered (Del-Claro & Oliveira, 2000). The climatic variation can lead to the differences of caterpillars as well as herbivory in the two different years. A noticeable result is that the anti-

herbivory effect of ants kept significant at the earlier period of the growth season both in 2009 and 2010. At this period, the *Q. liaotungensis* are expanding their leaves. Considering those young leaves are especially valuable for plants in photosynthesis (Harper, 1989; Pringle et al., 2011), the protective effects of ants at this period can have deep effects on plant growth.

Ants showed significant protective effects for plants but not for the fruit production; this result is also consistent with experiment conducted at smaller scales (Moreira & Del-Claro, 2005; Zhang et al., 2012a). However, there was a trend that plants with ants tended to produce fewer fruits in this work (Fig. 3); the possible negative effects of ants on the flowering of oak should be paid more attention in future researches.

Leaf toughness is an important factor that affects herbivory (Onoda et al., 2011). A recent study found that in obligate ant-plant interactions, there is a tradeoff between ant defense and leaf toughness (Frederickson et al., 2013), but in the facultative ant-plant interactions such as our study system, such examples are rare (but see Korndörfer & Del-Claro, 2006).

Our study indicates in facultative ant-plant interaction, if plants lost ants at a larger spatial scale, they can also increase their leaf toughness to resist herbivore damages. In this study, we found that the leaf toughness in treatment plots was significantly higher than that in control plots only at the end of the growth season. The reason for this monthly variation pattern is unclear, but it is possible that the induced defensive traits has the time lag effects (Agrawal, 2007; Agrawal, 2011). Further studies should pay more attention to the inducible plant defensive (both physical and chemical defense) beyond the scale of individual plants.

In conclusion, this study confirmed that the anti-herbivory effect of the aphid tending ants can also function at a relatively large scale, not limited at the level of branches or individual plants. This suggests that ants are reliable and effective bodyguard for plants regardless across different spatial scales. For plants, the possible tradeoff among different defensive strategies at larger scale should be focused in further researches.

Aknowledgements

This work was supported by National Natural Science Foundation of China (31300368, 31370451). We thank Zhenghui Xu and Gexia Qiao for ant- and aphid-species identification.

References

- Agrawal, A. A. (2007). Macroevolution of plant defense strategies. *Trends Ecol. Evol.*, 22: 103-109.
- Agrawal, A. A. (2011). Current trends in the evolutionary

- ecology of plant defence. *Funct. Ecol.*, 25: 420-432.
- Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Caceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, K. A. Mooney, M. Power, D. Schemske, J. Stachowicz, S. Strauss, M. G. Turner, & E. Werner. (2007). Filling key gaps in population and community ecology. *Front. Ecol. Environ.*, 5: 145-152.
- Bascompte, J. & P. Jordano. (2007). Plant-Animal Mutualistic Networks: The Architecture of Biodiversity. *Annu. Rev. Ecol. Evol. Syst.*, 38: 567-593.
- Bronstein, J. L. (1994). Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.*, 9: 214-217.
- Bruno, J. F., J. J. Stachowicz, & M. D. Bertness. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.*, 18: 119-125.
- Buckley, R. (1983). Interaction between Ants and Membracid Bugs Decreases Growth and Seed Set of Host Plant Bearing Extrafloral Nectaries. *Oecologia*, 58: 132-136.
- Chamberlain, S. A. & J. N. Holland. (2009). Quantitative synthesis of context dependency in ant-plant protection mutualisms. *Ecology*, 90: 2384-2392.
- Del-Claro, K. (2004). Multitrophic relationships, conditional mutualisms, and the study of interaction biodiversity in tropical savannas. *Neotrop. Entomol.*, 33: 665-672.
- Del-Claro, K. & P. S. Oliveira. (2000). Conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, 124: 156-165.
- Frederickson, M. E., A. Ravenscraft, L. M. Arcila Hernández, G. Booth, V. Astudillo, & G. A. Miller. (2013). What happens when ants fail at plant defence? *Cordia nodosa* dynamically adjusts its investment in both direct and indirect resistance traits in response to herbivore damage. *J. Ecol.*, 101: 400-409.
- Garcia, D., R. Zamora, & G. C. Amico. (2011). The spatial scale of plant-animal interactions: effects of resource availability and habitat structure. *Ecol. Monogr.*, 81: 103-121.
- Harper, J. L. (1989). The value of a leaf. *Oecologia*, 80: 53-58.
- Heil, M., T. Delsinne, A. Hilpert, S. Schurkens, C. Andary, K. E. Linsenmair, M. S. Sousa, & D. McKey. (2002). Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos*, 99: 457-468.
- Hopkins, G. W. & J. I. Thacker. (1999). Ants and habitat specificity in aphids. *J. Insect Conserv.*, 3: 25-31.
- Janzen, D. H. (1966). Coevolution of mutualism between ants and acacias in central America. *Evolution*, 20: 249-275.
- Kaplan, I. & M. D. Eubanks. (2005). Aphids alter the community-wide impact of fire ants. *Ecology*, 86: 1640-1649.
- Korndörfer, A. P. & K. Del-Claro. (2006). Ant Defense Versus Induced Defense in *Lafoensia pacari* (Lythraceae), a Myrmecophilous Tree of the Brazilian Cerrado I. *Biotropica*, 38: 786-788.
- Leiss, K. A. and P. G. L. Klinkhamer. (2005). Spatial distribution of nectar production in a natural *Echium vulgare* population: Implications for pollinator behaviour. *Basic Appl. Ecol.* 6: 317-324.
- Moreira, V. S. S. & K. Del-Claro. (2005). The outcomes of an ant-treehopper association on *Solanum lycocarpum* St. Hill: increased membracid fecundity and reduced damage by chewing herbivores. *Neotrop. Entomol.* 34: 881-887.
- Onoda, Y., M. Westoby, P. B. Adler, A. M. F. Choong, F. J. Clissold, J. H. C. Cornelissen, S. Díaz, N. J. Dominy, A. Elgart, L. Enrico, P. V. A. Fine, J. J. Howard, A. Jalili, K. Kitajima, H. Kurokawa, C. McArthur, P. W. Lucas, L. Markesteijn, N. Pérez-Harguindeguy, L. Poorter, L. Richards, L. S. Santiago, E. E. Sosinski, S. A. Van Bael, D. I. Warton, I. J. Wright, S. Joseph Wright, & N. Yamashita. (2011). Global patterns of leaf mechanical properties. *Ecol. Lett.*, 14: 301-312.
- Pringle, E., R. Dirzo, & D. Gordon. (2011). Indirect benefits of symbiotic coccoids for an ant-defended myrmecophytic tree. *Ecology*, 92: 37-46.
- Romero, G. Q. & J. Koricheva. (2011). Contrasting cascade effects of carnivores on plant fitness: a meta-analysis. *J. Anim. Ecol.*, 80: 696-704.
- Rosumek, F., F. Silveira, F. de S. Neves, N. de U. Barbosa, L. Diniz, Y. Oki, F. Pezzini, G. Fernandes, & T. Cornelissen. (2009). Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia*, 160: 537-549.
- Stadler, B. and A. F. G. Dixon. (2005). Ecology and evolution of aphid-ant interactions. *Annu. Rev. Ecol. Evol. Syst.* 36: 345-372.
- Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol.*, 78: 23-55.
- Styrsky, J. D. and M. D. Eubanks. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proc R Soc B-Biol Sci* 274:151-164.
- Trager, M. D., S. Bhotika, J. A. Hostetler, G. V. Andrade, M. A. Rodriguez-Cabal, C. S. McKeon, C. W. Osenberg, & B. M. Bolker. (2010). Benefits for Plants in Ant-Plant Protective Mutualisms: A Meta-Analysis. *PLoS ONE* 5:e14308.
- WallisDeVries, M. F., E. A. Laca, & M. W. Demment. (1999). The importance of scale of patchiness for selectivity in grazing herbivores. *Oecologia*, 121: 355-363.
- Way, M. J. (1963). Mutualism between ants and honeydew-producing homoptera. *Annu. Rev. Entomol.*, 8: 307-344.

- Westphal, C., Steffan-Dewenter, I. & Tschardtke, T. (2006). Bumblebees experience landscapes at different spatial scales: possible implications for coexistence. *Oecologia*, 149: 289-300.
- Wimp, G. M. & T. G. Whitham. (2001). Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. *Ecology*, 82: 440-452.
- Zhang, S., Y. Zhang, & K. Ma. (2012a). Different-sized oak trees are equally protected by the aphid-tending ants. *Arthropod-Plant Interact.*, 6: 307-314.
- Zhang, S., Y. X. Zhang, & K. M. Ma. (2012b). The ecological effects of the ant-hemipteran mutualism: a meta-analysis. *Basic Appl. Ecol.*, 13: 116-124.
- Zhang, Y. X., K. M. Ma, M. Anand, and B. J. Fu. 2006. Do generalized scaling laws exist for species abundance distribution in mountains? *Oikos* 115:81-88.

