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A trophic cascade induced by predatory ants in a fig–fig wasp mutualism

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Summary

1. A trophic cascade occurs when predators directly decrease the densities, or change the behaviour, of herbivores and thus indirectly increase plant productivity. The predator–herbivore–plant context is well known, but some predators attack species beneficial to plants (e.g. pollinators) and/or enemies of herbivores (e.g. parasites), and their role in the dynamics of mutualisms remains largely unexplored.

2. We surveyed the predatory ant species and studied predation by the dominant ant species, the weaver ant *Oecophylla smaragdina*, associated with the fig tree *Ficus racemosa* in southwest China. We then tested the effects of weaver ants on the oviposition behaviour of pollinating and non-pollinating fig wasps in an ant-exclusion experiment. The effects of weaver ants on fig wasp community structure and fig seed production were then compared between trees with and without *O. smaragdina*.

3. *Oecophylla smaragdina* captured more non-pollinating wasps (*Platyneura mayri*) than pollinators as the insects arrived to lay eggs. When ants were excluded, more non-pollinators laid eggs into figs and fewer pollinators entered figs. Furthermore, trees with *O. smaragdina* produced more pollinator offspring and fewer non-pollinator offspring, shifting the community structure significantly. In addition, *F. racemosa* produced significantly more seeds on trees inhabited by weaver ants.

4. *Oecophylla smaragdina* predation reverses the dominance of the two commonest wasp species at the egg-laying stage and favours the pollinators. This behavioural pattern is mirrored by wasp offspring production, with pollinators' offspring dominating figs produced by trees inhabited by weaver ants, and offspring of the non-pollinator *P. mayri* most abundant in figs on trees inhabited by other ants.

5. Overall, our results suggest that predation by weaver ants limits the success of the non-pollinating *P. mayri* and therefore indirectly benefits the mutualism by increasing the reproductive success of both the pollinators and the plant. Predation is thus a key functional factor that can shape the community structure of a pollinator-plant mutualistic system.

Key-words: competitive release, fig wasp, mutualism, predation, predator-exclusion experiment, trophic cascade

Introduction

Predators can have a profound effect on the structure and function of ecological systems (Rosenheim, Wilhoit & Armer 1993; Moran & Hurd 1997). Predators may regulate herbivore populations directly (Dieckmann, Marrow & Law 1995; Mougi 2012a,b), but can also indirectly alter primary productivity by influencing plant–herbivore interactions (Schmitz & Suttle 2001; Schmitz 2003). These indirect effects on primary production are termed trophic cascades and represent an important class of nonlinear ecological interactions (Pace *et al.* 1999). Understanding

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the forms and mechanisms of the cascading effects is critical for predictions of changes in ecosystem dynamics and for ecosystem management (Pace *et al.* 1999; Heithaus *et al.* 2008).

Predators may directly reduce herbivore population densities (density-mediated indirect interaction with primary productivity, DMII) or change the morphology, phenology, physiology or behaviour (trait-mediated indirect interaction with primary productivity, TMII) of herbivores, leading to cascading effects on plant growth, plant biomass and associated ecosystem functions (Peacor & Werner 2001; Griffin & Thaler 2006; Yoshie & Yusa 2011). Trophic cascade theory has been applied previously to the predation of antagonistic herbivores that inflict costs onto plants (Polis 1999), but can also be applied to the predation of beneficial mutualists of plants. For instance, pollinating insects may be prey items of certain predators (Muñoz & Arroyo 2004; Dukas 2005), which may also attack parasites of herbivores. Such cases provide an opportunity to extend trophic cascade theory beyond the traditional emphasis on predator–herbivore–plant chains and to gain a wider insight into the role of trophic cascades in the dynamics of a range of natural systems.

The fig tree–fig wasp mutualism supports a wider community of herbivorous and parasitic wasp species, and provides an excellent model for exploring factors that influence species interactions and community structure (Compton & Hawkins 1992; Kerdelhue, Rossi & Rasplus 2000; Segar *et al.* 2013a). Fig trees (*Ficus* spp.) and their host-specific pollinating wasps (Hymenoptera: Agaonidae) form a remarkable plant–insect obligate mutualism. The wasps are the trees' only pollen vectors, and in return, the trees provide food and space within their characteristic enclosed inflorescences ('figs') for wasp larvae to develop (Weiblen 2002). Each fig species also hosts non-pollinating wasp species (NPFWs), including both herbivorous gall makers that feed on fig tissue and parasitoids that feed on other fig wasps. Together, these form a fig wasp community that may consist of up to 30 species associated with a single fig species (Compton & Hawkins 1992). Both fig-pollinating wasps and other gall-making non-pollinating fig wasps lay their eggs in female fig flowers (Kerdelhue, Rossi & Rasplus 2000), with a single galled flower supporting only a single wasp larva (Kerdelhue, Rossi & Rasplus 2000).

Individual figs go through several developmental stages over a period of several weeks or months (Galil & Eisikowitch 1968). Each species of wasp lays its eggs at a particular stage (or stages) of fig development (Kerdelhue, Rossi & Rasplus 2000; Ranganathan, Ghara & Borges 2010; Segar *et al.* 2013b). The pollinating wasps enter receptive figs through a specialized entrance (the ostiole) to lay their eggs in female flowers from within the fig, whilst most NPFW species use their long ovipositors to lay eggs through the fig wall from the outside. Because NPFW species that gall fig flowers have herbivorous larvae, they may inflict costs on both mutualists by competing with the pollinators for oviposition sites and reducing fig seed production (Wang & Zheng 2008; Wang, Yang & Wiggins 2014). Furthermore, various parasitoid species develop at the expense of pollinators (Segar & Cook 2012), or NPFWs, which may also affect wasp community structure and fig seed production. NPFWs can reach high densities within individual figs (West & Herre 1994; Kerdelhue & Rasplus 1996; West *et al.* 1996) and thus have the potential to affect both pollinating wasp and fig seed production (Wang *et al.* 2005; Segar & Cook 2012; Wang, Yang & Wiggins 2014).

Fig trees are often closely associated with ants, which are well known as predators of fig wasps (Bronstein 1988; Schatz *et al.* 2008; Bain, Harrison & Schatz 2013). Ants associated with *F. carica* prey mostly on pollinators (Schatz & Hossaert-Mckey 2003), whilst on other fig species, ants also prey on NPFWs or disturb NPFW oviposition behaviour when the wasps are on the outer surface of figs (Compton & Robertson 1988; Schatz *et al.* 2006; Harrison 2013). Both (lethal) predation and (non-lethal) disturbance of oviposition by ants may reduce the negative effects of NPFWs on the mutualists. Although the potential effects of predatory ants have long been recognized (Schatz & Hossaert-Mckey 2003; Wei *et al.* 2005; Schatz *et al.* 2006), studies that measure all stages of the interaction, and thereby quantify the outcomes for wasp community structure and fig seed production, are lacking.

In this study, we evaluate the effects of predatory ants on the interactions and relative abundance of members of the fig wasp community of *Ficus racemosa* in Xishuangbanna, China. We first surveyed the diversity and abundance of ant species inhabiting *F. racemosa* trees in the study area. We next observed ant behaviour and recorded predation of wasps in the field. We then used a manipulative exclusion experiment to test the effects of predatory ants on pollinator entry to figs and the oviposition behaviour of NPFWs. Finally, we made use of natural variation in ant occupancy to compare the abundance and community structure of fig wasps on trees with and without the dominant predatory ant species.

Methods

STUDY SITE

This study was carried out in and around the Xishuangbanna Tropical Botanic Garden (XTBG), located in Xishuangbanna (21°41′N, 101°25′E), Yunnan province, southwest China. The altitude is ~600 m, and average annual precipitation is 1557 mm.

NATURAL HISTORY

Ficus racemosa (subgenus *Sycomorus*; pollinated by *Ceratosolen fusciceps* Mayr) is a monoecious fig species (Kobmoo *et al.* 2010), whose figs contain about ~4000–5000 flowers (Wang *et al.* 2011). Each fig emits volatiles when the female flowers are receptive to pollen (Hossaert-McKey, Gibernau & Frey 1994), attracting female pollinating wasps (foundresses) that then enter the fig through a bract-lined tunnel, the ostiole (Proffit *et al.* 2009). Foundresses then spread pollen from their natal tree whilst laying their eggs individually into some flower ovaries, which subsequently develop into wasp galls. The foundresses die inside the fig they have entered and the wasp offspring then develop in synchrony with fig maturation. The new generation of adult wasps emerges into the fig cavity when the seeds and male flowers mature. The wingless males emerge first, mate with the winged females, and excavate an exit hole through the fig wall. The fertilized females collect pollen actively before dispersing through exit tunnels to find another tree with a crop of receptive figs to start the cycle anew (Janzen 1979; Weiblen 2002).

At Xishuangbanna, five non-pollinating fig wasp species reproduce in the figs of *F. racemosa* (Yang *et al.* 2005): three species of *Platyneura* (*P. mayri*, *P. testacea* and *P. agraensis*) and two *Apocrypta* species (*Apocrypta* sp. and *A. westwoodi*). *P. mayri* and *P. testacea* independently gall fig flowers and have herbivorous larvae, whereas *P. agraensis* is parasitoid of the pollinators. *Apocrypta* sp. and *A. westwoodi* are parasitoids of *P. testacea* and *P. mayri,* respectively (Wang & Zheng 2008). Each NPFW oviposits at a specific stage of fig maturation from the outer surface of the fig, but all emerge from their galls at the same time as the pollinating wasps, and use the exit holes made by male pollinators to disperse from their natal fig.

Field survey of ant species

We identified 33 *F. racemosa* trees within or near to XTBG, each with trunk diameter at breast height >40 cm (Appendix S1). Each tree stood at least 50 m from any other *F. racemosa* tree. Ants were collected from tree branches and foliage with small tweezers by the same person for 40 min for each tree (Gotelli *et al.* 2011). These ants were stored in 75% ethanol in a 50-mL centrifuge tube, returned to the laboratory, and then sorted by morphology to enable identification. This was done with the aid of a stereomicroscope at 10–40× magnification.

Survey of ants and non-pollinating wasps on figs at different developmental stages

The results of the ant survey (see above) showed that *Oecophylla smaragdina* was the most common species in Xishuangbanna. In our next survey, we recorded the number of *O. smaragdina* ants on each fruit cluster and NPFWs on the surface of figs at different developmental phases, categorized as bud, pre-female, receptive, inter-floral and male floral after Galil & Eisikowitch (1968). We did not record ants on figs in their post-floral phase, because by this time wasps have already dispersed.

All observations for our second survey were performed on four (of the original 33) trees, and on three of these, we also recorded predation of fig wasps by *O. smaragdina* on receptive figs. Pollinators only visit receptive figs, so this is the only stage at which a comparison of the predation of pollinators and NPFWs can be made. Previous work has shown that one NPFW species, *P. mayri*, also attacks figs at this stage of development (Wang, Sun & Zheng 2010). For each tree, the numbers and species of fig wasps captured by *O. smaragdina* were observed and recorded on one branch for 1 hour. All the observations were conducted between 0930 and 1130, the time of maximum activity of both ants and wasps (Schatz *et al.* 2006). These data were recorded between the 21st May and the 27th August 2012.

Effects of ants on NPFW and pollinator oviposition. Three trees dominated by *O. smaragdina* were selected to evaluate the effects of the ant on fig wasp community structure. For each tree, six to eight fruit clusters that had figs in the bud phase and were easy to reach were selected, and two adjacent fruit clusters were randomly assigned to be either controls or to receive experimental treatment. The proximal part of each cluster (closest to the main trunk of the tree) was surrounded by a circular filter paper (12 cm diameter), which was fixed to the branch using staples. For the treatment, approximately 5 mL flavourless, colourless rat stop glue (Yiwu Xinqi Super Glue Product Factory, Zhejiang, China) was smeared on the surface of the filter paper to exclude ants. Control clusters were surrounded only by filter paper. Fruit clusters were checked daily.

Additional glue was smeared onto filter papers approximately every 3 days throughout the experiment, which began when the figs were buds. For each tree, the NPFWs on the surface of each fig were counted between 0930 and 1130 on ten rain-free days within a 15-day period when figs were in the pre-female and receptive phases. This experiment was conducted from the 25th May to the 7th August 2012, and the figs were collected 1 week after pollination. They were then dissected to reveal the number of foundresses inside the fruits, after external inspection of the number of oviposition scars made by non-pollinating wasps (Harrison 2013). These scars result from *P. mayri* piercing the fruit wall to lay eggs using their long ovipositors.

Association between O. smaragdina presence and wasp community and fig seed production

Of the 33 trees we surveyed, six were excluded because no figs were present. The remaining 27 trees were categorized into two groups: trees with *O. smaragdina* present (13 trees) and trees without *O. smaragdina* (14 trees). Six trees were randomly selected from each group, to investigate the association between ant species and wasp community structure from May to October 2012. For each tree, 10 near-mature figs (with yellow or orange colour) without exit holes were collected and put into fine mesh bags. The wasps that emerged into the bags were killed in 75% ethanol. All the wasps were sorted out by species and sex, identified and counted. The figs were cut into four equal parts; seeds from one quarter (chosen randomly) were scraped free and counted. The total number of seeds per fig was estimated by multiplying the quarter segment count by four (Kerdelhue, Rossi & Rasplus 2000).

DATA ANALYSIS

We calculated three different standard diversity indeces for the ant species from each tree: (i) Shannon–Wiener index, (ii) Pielou's evenness and (iii) Fisher's logarithmic Alpha.

The number of ants on each fig cluster was recorded repeatedly among the different fig development phases. Observations were made for three trees that each had figs developing in synchrony. Because the number of figs in each fruit cluster varied, average ant number per fig was used for analysis. The average numbers of ants and NPFWs per fig were not distributed normally. Hence, we compared the number of ants/wasps with a nonparametric rank-based analysis of longitudinal data with ANOVA-type statistic (ATS), with 'tree' as a between-subject factor and developmental phases (five repeated observations) as a within-subject factor (Noguchi *et al.* 2012). Multiple comparisons with Bonferroni adjustment were used to compare the difference between developmental stages (Noguchi *et al.* 2012).

In the ant-exclusion experiments, some figs were aborted by the tree before the observation period finished. Data from these figs were thus excluded from the analysis. Because wasp numbers on the fig surface were measured repeatedly and not distributed normally, the nonparametric rank-based analysis for longitudinal data was used, with trees and treatment (ant exclusion vs. control) as between-subject factors and time (ten times of observations) as a within-subjects factor (Noguchi *et al.* 2012). The effects of weaver ants (fixed factor, two levels: branches with or without *O. smaragdina*) and tree (random factor) on foundress number and NPFW oviposition scar number were fitted to a mixed-effects model (Pinheiro *et al.* 2013). Two species of fig wasp were observed as prey of *O. smaragdina* during the receptive phase. Their numbers were compared using a chi-square test to see whether there was biased predation between them.

We also tested for associations between the presence of weaver ants and both wasp community structure and fig seed production. For each tree, wasp numbers (per species) were summed for over the ten figs sampled per tree to represent its wasp community composition. Cluster analysis was used to classify wasp communities into different groups based on Bray–Curtis distances, which range from 0 (similar) to 1 (dissimilar). The clustering strategy used was the unweighted pair-group method, using arithmetic averages (UPGMA) (Krebs 1999).

To examine how weaver ant presence affected wasp community structure at the tree level, we used analysis of similarity (ANOSIM). This uses a nonparametric permutation/randomization procedure to test for significant differences between different groups (trees with and without *O. smaragdina*) based on a similarity matrix (Clarke 1993; Palkovacs & Post 2009). The ANOSIM was based on Bray–Curtis distances and run for 999 permutations. We also calculated similarity percentages (SIMPER) to identify which wasp species contributed most to similarity/dissimilarity between groups. SIMPER performs pairwise comparisons of groups and finds the average contributions of each species to the average overall Bray–Curtis distance (Clarke 1993; Palkovacs & Post 2009).

Finally, we also used nonmetric multidimensional scaling (NMDS), which is a nonparametric dimension reduction technique, used here to graphically represent wasp community differences (Bray–Curtis distance) in two dimensions (Clarke 1993). The stress value summaries overall fit of the NMDS to the observed distance matrix, with a smaller stress value representing a better fit (Anderson 1971; Kenkel & Orloci 1986). Before analysis, we performed a square-root transformation and then used Wisconsin double standardization to normalize the data. The numbers (sum total of the 10 figs of each tree) of wasps and seeds from trees with and without ants were compared using Welch two-sample t-tests. All the data were analysed using R 3.0.2 and its 'nparLD', 'VEGAN', and 'nlme' add-on packages (Noguchi *et al.* 2012; Oksanen *et al.* 2013; Pinheiro *et al.* 2013; R Development Core Team 2013).

Results

ANT SPECIES

Thirty-three species of ants were identified (Appendix S1), including species from several genera: *Aphaenogaster*, *Camponotus*, *Cataulacus*, *Crematogaster*, *Dolichoderus*, *Monomorium*, *Oecophylla*, *Pachycondyla*, *Paratrechina*, *Polyrhachis*, *Polyrhachus*, *Pristomyrmex*, *Technomyrmex*, *Tetramorium*, *Tetraponera*. Overall, *O. smaragdina* was the most common ant species and was present on 13 of 27 trees accounting for >70% of all individual ants in 10 of the sample trees. Ant species diversity and evenness per tree were low (Table 1). For some trees (T15, T16, T20, T26), only one ant species was present, and in most trees (18 of 27 trees), there were fewer than four.

NUMBER OF O. SMARAGDINA AND NPFW ON THE SURFACE OF FIGS IN DIFFERENT PHASES

The number of *O. smaragdina* differed among trees (ATS = 26·82, d.f. = 1·8, $P < 0{\cdot}001$) and developmental phase (ATS = 43·14, d.f. = 2·85. $P < 0{\cdot}001$). The effect of tree on the number of NPFWs was not significant (ATS = 0·8, d.f. = 3, $P = 0{\cdot}5$), but the number of NPFWs differed significantly among figs of differing developmental phase (ATS = 56·32, d.f. = 2·54, $P < 0{\cdot}001$). In the bud phase, almost no ants and NPFWs were observed on the surface of figs. NPFW numbers increased from bud to receptive phase, as did the total number of *O. smaragdina*. Both NPFW and ant numbers peaked on figs in the receptive phase. There were then decreases in numbers of NPFWs (significant) and ants (not significant) after the receptive phase, and in the male floral phase, almost no NPFWs were observed on the surface of figs (Fig. 1).

In total, we observed 159 *P. mayri* and *C. fusciceps* being caught by *O. smaragdina*. Twenty-nine (18%) of these were pollinators and 130 (82%) were *P. mayri* gallers. *O. smaragdina* therefore captured far more gall-making wasps than pollinating wasps (Pearson chi-square, $\chi^2 = 64{\cdot}15$, $P < 0{\cdot}01$).

EFFECTS OF ANTS ON NPFW AND POLLINATOR OVIPOSITION

The number of NPFWs on the surface of figs in both treatments increased as figs aged, but much less on the control fig clusters than on ant-excluded ones. The effects of tree (ATS = 14·38, d.f. = 1·8, $P < 0{\cdot}001$) and treatment (ATS = 49·97, d.f. = 1, $P < 0{\cdot}001$) on the number of NPFWs present were both significant. Time (ten sets of observations) also had a significant effect on the number of NPFWs (ATS = 3·88, d.f. = 6·81, $P < 0{\cdot}001$) (Fig. 2a). Ant-excluded figs had more NPFW oviposition scars (Fig. 2b, t = 3·45, df = 70, $P < 0{\cdot}01$), and fewer pollinator foundresses, (Fig. 2b, t = −4·85, df = 70, $P < 0{\cdot}001$) than control figs.

Table 1. Diversity indexes of ant species from each *Ficus racemosa* tree in Menglun Xishuangbanna

Diversity indexes	Trees 1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	19	20	24	25	26	27	28	29	30	32	33
Shannon–Wiener index	0·49	0·85	0·19	0·67	1·31	0·49	0·26	0·53	0·84	0·22	0·07	0·02	0·33	0·00	0·00	0·08	0·05	0·00	0·06	0·06	0·00	0·06	0·07	0·19	0·91	0·50	1·01
Pielou's evenness	0·49	0·85	0·19	0·67	1·31	0·49	0·26	0·53	0·84	0·22	0·07	0·02	0·33	0·00	0·00	0·08	0·05	0·00	0·06	0·06	0·00	0·06	0·07	0·19	0·91	0·50	1·01
Fisher's logarithmic Alpha	1·82	0·88	0·40	0·46	2·35	0·31	0·74	0·53	1·31	0·55	0·48	0·29	0·86	0·14	0·15	0·31	0·28	0·14	0·31	0·50	0·13	0·32	0·31	0·71	1·15	0·32	0·94

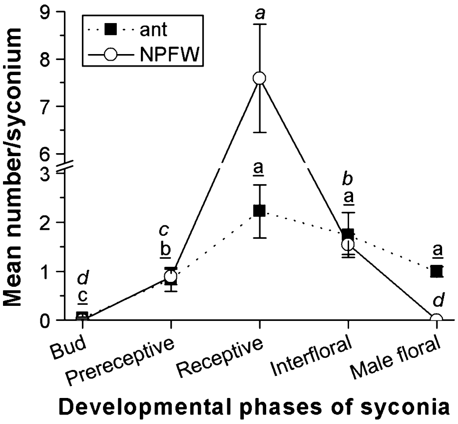

Fig. 1. Number of non-pollinating fig wasps and ants on the surface of figs in different developmental phases. Points and bars show mean ± SE. Data with the same letter are not significantly different at $\alpha = 0{\cdot}05$ (Bonferroni adjustment test). Underlined letters show differences of ant numbers, and italics show differences of wasp numbers.

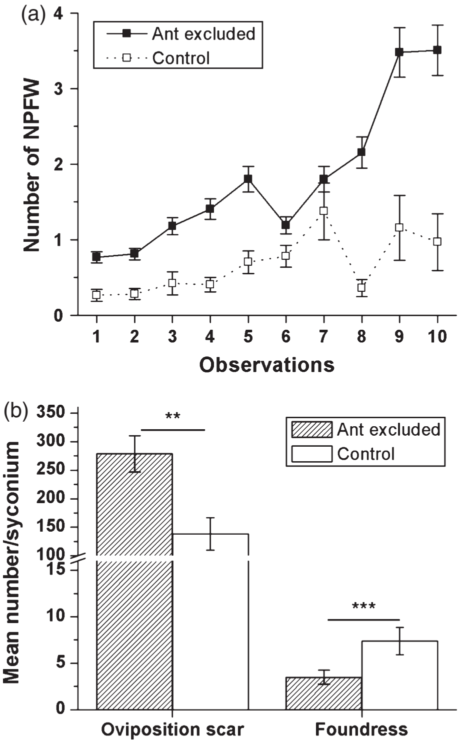

Fig. 2. Mean number (±1SE) of non-pollinating wasps on the surface of figs (a), foundress and non-pollinating fig wasp oviposition scars (b) in control (with *Oecophylla smaragdina*) and ant-excluded figs. Mixed-effects model, with treatment (figs with *O. smaragdina* or ant excluded) as fixed factor, tree as a random factor, **($P < 0{\cdot}01$), ***($P < 0{\cdot}001$).

ASSOCIATION BETWEEN ANT PRESENCE AND WASP COMMUNITY STRUCTURE AND FIG SEED PRODUCTION

There were significant differences in the wasp community structure between trees inhabited by *O. smaragdina* and

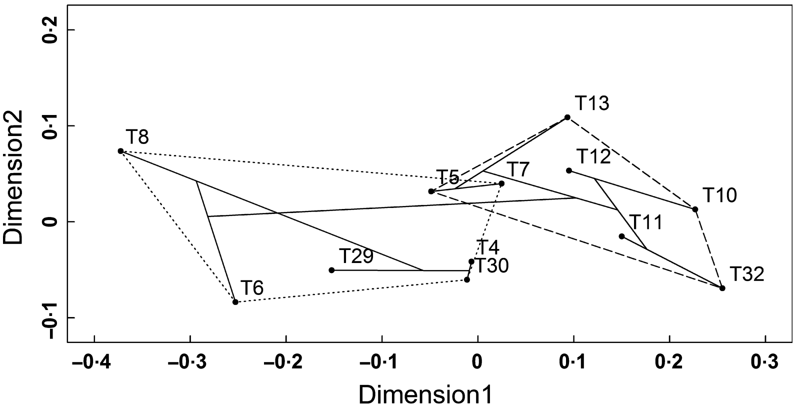

Fig. 3. Nonmetric multidimensional scaling (ordination) of wasp community structure from trees with (dotted line) or without weaver ants (dashed line). The solid line shows the dendrogram based on distance calculated from wasp community data using Bray–Curtis measures of dissimilarity. Each data point represents a different tree. T4, T6, T7, T8, T29, T30 represent trees inhabited by weaver ants, and T5, T10, T11, T12, T13, T32 represent trees without weaver ants.

those inhabited by other ants (ANOSIM, $R = 0·69$, $P < 0·01$). In the dendrogram, tree 4 and tree 30 had the lowest dissimilarity index (0·05), and trees 6, 8 and 29 join this cluster next. All these trees are inhabited by *O. smaragdina* (Fig. 3).

The pollinating wasp, *C. fusciceps*, contributed most (28%) to wasp community dissimilarity. The gall-making wasp, *P. mayri*, contributed 9%, whilst other species played minor roles (<6%). NMDS comparisons of wasp communities on different trees are shown in Fig. 3 (stress value 0·02, and $R^2 = 0·998$). For trees with *O. smaragdina* present, the pollinator was the dominant wasp species (66% of all wasps) developing in figs, followed by *P. mayri* (17%), *P. testacea* (6%), *P. agraensis* (6%), *A.* sp. (4%) and *A. westwoodi* (1%). In contrast, for trees that harboured other ant species, *P. mayri* was the dominant wasp species (41%) followed by pollinators (25%), *P. testacea* (17%), *A.* sp. (9%), *P. agraensis* (5%) and *A. westwoodi* (3%) (Fig. 4a).

Figs from trees inhabited by *O. smaragdina* contained more seeds ($t = -5·05$, df = 8·01, $P < 0·001$) and pollinating wasp progeny, *C. fusciceps* ($t = -3·54$, df = 5·66, $P < 0·05$) than figs inhabited by other ants (Fig. 4b). Furthermore, figs with *O. smaragdina* had fewer progeny of *P. mayri* ($t = 2·73$, df = 8·21, $P < 0·05$) and *P. testacea* ($t = 3·52$, df = 9·99, $P < 0·01$). The wasp progeny numbers of *P. agraensis* ($t = -1·67$, df = 8·50, $P = 0·13$), *A. westwoodi* ($t = 1·02$, df = 9·07, $P = 0·33$) and *A.* sp. ($t = 0·41$, df = 9·7, $P = 0·69$) did not differ significantly between trees with different ant species.

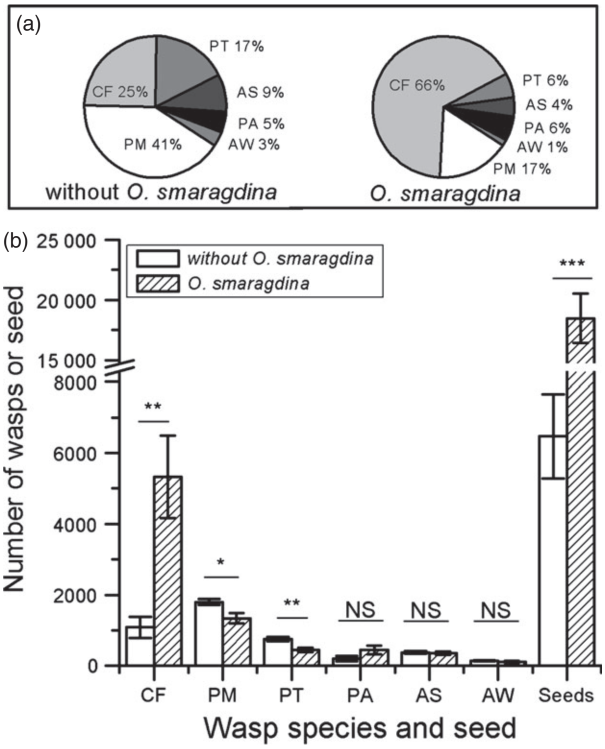

Fig. 4. The numbers of wasps from trees with weaver ant nests or inhabited by other ants: (a) species composition, and (b) comparisons of wasp and seed numbers between trees. Columns and bars show mean ± SE. Mixed-effects model, with ant (trees with or without *Oecophylla smaragdina*) as a fixed factor, and tree as a random factor, *($P < 0·05$), **($P < 0·01$), ***($P < 0·001$), NS = not significant. CF = *Ceratosolen fusciceps*; PM = *Platyneura mayri*; PT = *Platyneura testacea*; PA = *Platyneura agraensis*; AS = *Apocrypta* sp.; AW = *Apocrypta westwoodi*.

Discussion

There is typically one dominant ant species on each *F. racemosa* tree at Xishuangbanna. Ant diversity is low on each tree, which may be the result of ant territorial behaviour (Holldobler 1983), with aggressive species repelling others. Ants of the genera *Crematogaster*, *Oecophylla*, *Myrmicaria* and *Philidris* have been previously reported as fig wasp predators (Schatz & Hossaert-Mckey 2003; Harrison 2013). However, we found no *Myrmicaria* or *Philidris* species at XTBG. The most common *Crematogaster* species was present on six trees, but was only the dominant ant species numerically on two of these. By contrast, the weaver ant *O. smaragdina* was the most common and often the numerically dominant ant species on *F. racemosa* trees at XTBG during the study. Based on observations on many tree species in Asia and Australasia, Rodríguez-Gironés *et al.* (2013) have argued recently that weaver ants may play a substantial role in shaping plant/pollinator interactions throughout the Old World tropics.

Oecophylla smaragdina preyed mainly on *P. mayri* during the fig receptive phase (when figs are pollinated), which may reduce its oviposition rates. Each *P. mayri* offspring effectively removes a flower that could otherwise support development of a pollinator offspring or a fig seed (Wang & Zheng 2008). As a result, the community structure of wasps developing in figs was strongly associated with presence of *O. smaragdina*. Trees inhabited by *O. smaragdina* were dominated by the pollinating wasp, *C. fusciceps*, and produced more seeds than trees without weaver ants. By contrast, the non-pollinating galling wasp, *P. mayri*, was the numerically dominant species in figs from trees without weaver ants. The difference was large because pollinator progeny comprises 66% of all wasp progeny developing on trees with weaver ants, but only 25% on those without. Our results suggest that weaver ant predation may strongly shape fig wasp community structure. Weaver ant predation thus directly reduces the population density of common gall-making wasps, which may indirectly increase the fitness of both mutualists (plant and pollinator). Trees not occupied by weaver ants, but by other ant species (including species of the genera *Crematogaster*, *Dolichoderus*, and *Tetramorium*), may thus have figs dominated by *P. mayri* because these ants prey on, and/or disturb gall makers less.

Oecophylla smaragdina is an aggressive, tree-nesting predator (Holldobler 1983; Peng, Christian & Gibb 1995) that can indirectly protect host plants from attack by various phytophagous insects, and has been used as a bio-control agent (Peng & Christian 2005). Schatz *et al.* (2006) reported that *O. smaragdina* can reduce the ovipositing numbers of *Apocrypta* parasitic fig wasps on both *F. racemosa* and *F. condensa*, in Brunei and India. It was therefore suggested that ant predation on parasitic wasps may indirectly benefit both mutualists. *Apocrypta* sp. has low abundance in *F. racemosa* figs at XTBG. Few ants were present on the figs at the stage when *Apocrypta* sp. oviposits, resulting in *Apocrypta* sp. being unlikely to be a major prey item of *O. smaragdina* at XTBG. In contrast, *P. mayri* is the most abundant NPFW galler on *F. racemosa*. *O. smaragdina* appears to reduce oviposition of *P. mayri* by both direct predation and disturbance, which may indirectly benefit the figs and pollinators. Gall makers, such as *P. mayri* and *P. testacea*, lay eggs in the female fig flowers (Wang & Zheng 2008; Wang, Sun & Zheng 2010) and analogous galler species in other fig–fig wasp systems having also been reported to reduce pollinating wasp and/or fig seed production (Kerdelhue & Rasplus 1996; Cardona, Kattan & de Ulloa 2012).

We found that weaver ant numbers peaked during the fig receptive phase, when the ants captured more gall-making NPFWs than pollinating wasps. Fig odours, the bouquets of which vary according to developmental stage, have been shown to attract weaver ants (Schatz & Hossaert-McKey 2010). Ants may thus use chemical cues of figs to maximize their likelihood of capturing preferred prey (Ranganathan & Borges 2009; Schatz & Hossaert-McKey 2010). However, although weaver ants captured more *P. mayri* than pollinators, this may be simply because *P. mayri* are easier to catch. *P. mayri* females oviposit from the outer surface of figs, whereas pollinating wasps enter the fig cavity quite quickly to lay their eggs in predator-free space (Dunn *et al.* 2008).

When ants were experimentally excluded, we observed an increase in the numbers of ovipositing NPFWs on figs. Inspection and dissection of figs showed that this resulted in more NPFW oviposition scars on the fig surfaces and fewer pollinator foundresses inside figs than when ants were present. It is unclear why fewer pollinators entered figs without ants, but they may have been attracted less to figs with higher numbers of ovipositing NPFWs. A possible explanation is that *P. mayri* oviposition may damage figs and provoke a defence reaction from the plant and thus altering the bouquet of volatile emissions. Such plant volatile-mediated changes are common in other plant–herbivore–pollinator interactions (e.g. Strauss, Conner & Rush 1996; Krupnick, Weis & Campbell 1999; Suárez, Gonzáles & Gianoli 2009).

In summary, predatory ants affect lower trophic levels within the community of wasps supported by the figs of *F. racemosa* and may benefit both fig tree and pollinator mutualists. Through biased predation and disturbance (both DMII and TMII may be involved in this process),

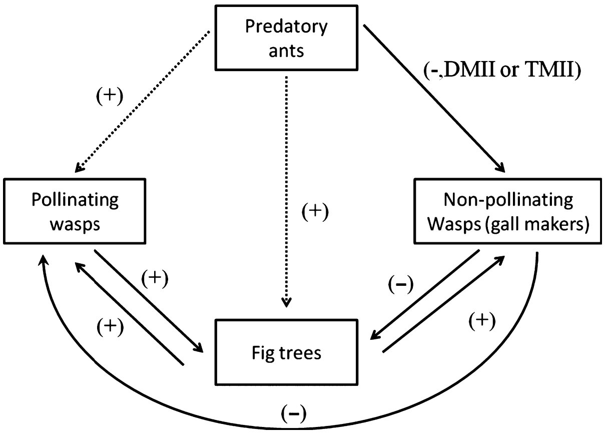

Fig. 5. Proposed interspecific relationships between ants, pollinating wasps and non-pollinating wasps associated with *Ficus racemosa*. Solid lines represent direct interactions, and dashed lines represent indirect interactions. Individual beneficial (+) and antagonistic (−) interactions are explained in the main text.

ants reduced the density of a key non-pollinating fig wasp that competes with the pollinator to lay eggs in fig flowers (Fig. 5). Predators can have strong effects on competition between prey species (Holt & Lawton 1993; Morris, Lewis & Godfray 2004) and thus change prey species abundance and diversity. Our finding that a predatory ant species changes the community structure of fig wasps, and indirectly benefits fig trees, appears novel. It may be a common phenomenon in fig–fig wasp systems, because ants are associated with many *Ficus* species. Previous work has mostly highlighted the capacity for complex interactions between bug-tending ants and the fig/fig wasp mutualism, but some predatory ants are also widespread in the tropics. In particular, *O. smaragdina* is common throughout much of Asia and Australasia (Rodríguez-Gironés *et al*. 2013).

Wang, Sun & Zheng (2010) and Wang, Yang & Wiggins (2014) reported that dominance of NPFWs may incur sanctions by fig trees through the abortion of overexploited figs during development, or restraining the offspring development of all wasps. Such sanctions by figs could reduce overall fig production and be detrimental for wasps within aborted figs. Because figs are a food source for many animals, including insects, birds and mammals, reduced fig production may have much wider ramifications within tropical and subtropical forest ecosystems, through bottom-up effects of primary productivity (Janzen 1979). We realize this scenario is hypothetical and the effects of long-term exclusion of predatory ants require further study.

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Data accessibility

Data deposited in the figshare repository: doi: 10.6084/m9.figshare.949682

References

Anderson, A.J. (1971) Ordination methods in ecology. *Journal of Ecology*, **59**, 713–726.

Bain, A., Harrison, R.D. & Schatz, B. (2013) How to be an ant on figs. *Acta Oecologica*, in press. Doi: http://dx.doi.org/10.1016/j.actao.2013.05.006

Bronstein, J.L. (1988) Predators of fig wasps. *Biotropica*, **20**, 215–219.

Cardona, W., Kattan, G. & de Ulloa, P.C. (2012) Non-pollinating fig wasps decrease pollinator and seed production in *Ficus andicola* (Moraceae). *Biotropica*, **45**, 1–6.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Compton, S.G. & Hawkins, B.A. (1992) Determinants of species richness in southern African fig wasp assemblages. *Oecologia*, **91**, 68–74.

Compton, S.G. & Robertson, H.G. (1988) Complex interactions between mutualisms: ants tending homopterans protect fig seeds and pollinators. *Ecology*, **69**, 1302–1305.

Dieckmann, U., Marrow, P. & Law, R. (1995) Evolutionary cycling in predator-prey interactions: population dynamics and the red queen. *Journal of Theoretical Biology*, **176**, 91–102.

Dukas, R. (2005) Bumble bee predators reduce pollinator density and plant fitness. *Ecology*, **86**, 1401–1406.

Dunn, D.W., Segar, S.T., Ridley, J., Chan, R., Crozier, R.H., Yu, D.W. *et al*. (2008) A role for parasites in stabilising the fig-pollinator mutualism. *Plos Biology*, **6**, e59.

Galil, J. & Eisikowitch, D. (1968) Flowering cycles and fruit types of *Ficus sycomorus* in Israel. *New Phytologist*, **67**, 745–758.

Gotelli, N.J., Ellison, A.M., Dunn, R.R. & Sanders, N.J. (2011) Counting ants (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. *Myrmecological News*, **15**, 13–19.

Griffin, C.A.M. & Thaler, J.S. (2006) Insect predators affect plant resistance via density- and trait-mediated indirect interactions. *Ecology Letters*, **9**, 338–346.

Harrison, R.D. (2013) Ecology of a fig ant–plant. *Acta Oecologica*, in press. Doi: 10.1016/j.actao.2013.05.008.

Heithaus, M.R., Frid, A., Wirsing, A.J. & Worm, B. (2008) Predicting ecological consequences of marine top predator declines. *Trends in Ecology & Evolution*, **23**, 202–210.

Holldobler, B. (1983) Territorial behavior in the green tree ant (*Oecophylla smaragdina*). *Biotropica*, **15**, 241–250.

Holt, R.D. & Lawton, J.H. (1993) Apparent competition and enemy-free space in insect host-parasitoid communities. *The American Naturalist*, **142**, 623–645.

Hossaert-McKey, M., Gibernau, M. & Frey, J.E. (1994) Chemosensory attraction of fig wasps to substances produced by receptive figs. *Entomologia Experimentalis Et Applicata*, **70**, 185–191.

Janzen, D.H. (1979) How to be a fig. *Annual Review of Ecology and Systematics*, **10**, 13–51.

Kenkel, N.C. & Orloci, L. (1986) Applying metric and nonmetric multidimensional scaling to ecological studies: some new results. *Ecology*, **67**, 919–928.

Kerdelhue, C. & Rasplus, J.Y. (1996) Non-pollinating Afrotropical fig wasps affect the fig-pollinator mutualism in *Ficus* within the subgenus Sycomorus. *Oikos*, **75**, 3–14.

Kerdelhue, C., Rossi, J.P. & Rasplus, J.Y. (2000) Comparative community ecology studies on old world figs and fig wasps. *Ecology*, **81**, 2832–2849.

Kobmoo, N., Hossaert-McKey, M., Rasplus, J. & Kjellberg, F. (2010) *Ficus racemosa* is pollinated by a single population of a single agaonid wasp species in continental South-East Asia. *Molecular Ecology*, **19**, 2700–2712.

Krebs, C.J. (1999) *Ecological Methodology*, 2nd edn. Addison-Welsey Educational Publishers Inc., Menlo Park, CA.

Krupnick, G.A., Weis, A.E. & Campbell, D.R. (1999) The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology*, **80**, 125–134.

Moran, M.D. & Hurd, L.E. (1997) A trophic cascade in a diverse arthropod community caused by a generalist arthropod predator. *Oecologia*, **113**, 126–132.

Morris, R.J., Lewis, O.T. & Godfray, H.C.J. (2004) Experimental evidence for apparent competition in a tropical forest food web. *Nature*, **428**, 310–313.

Mougi, A. (2012a) Predator–prey coevolution driven by size selective predation can cause anti-synchronized and cryptic population dynamics. *Theoretical Population Biology*, **81**, 113–118.

Mougi, A. (2012b) Unusual predator–prey dynamics under reciprocal phenotypic plasticity. *Journal of Theoretical Biology*, **305**, 96–102.

Muñoz, A. & Arroyo, M.K. (2004) Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub. *Oecologia*, **138**, 66–73.

Noguchi, K., Gel, Y.R., Brunner, E. & Konietschke, F. (2012) nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments. *Journal of Statistical Software*, **50**, 1–23.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B. *et al.* (2013) vegan: Community ecology package. R package version 2.0-9.

Pace, M.L., Cole, J.J., Carpenter, S.R. & Kitchell, J.F. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution*, **14**, 483–488.

Palkovacs, E.P. & Post, D.M. (2009) Experimental evidence that phenotypic divergence in predators drives community divergence in prey. *Ecology*, **90**, 300–305.

Peacor, S.D. & Werner, E.E. (2001) The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 3904–3908.

Peng, R.K. & Christian, K. (2005) Integrated pest management in mango orchards in the Northern Territory Australia, using the weaver ant, *Oecophylla smaragdina*, (Hymenoptera: Formicidae) as a key element. *International Journal of Pest Management*, **51**, 149–155.

Peng, R., Christian, K. & Gibb, K. (1995) The effect of the green ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae), on insect pests of cashew trees in Australia. *Bulletin of Entomological Research*, **85**, 279–284.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. & the R Development Core Team (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-111.

Polis, G.A. (1999) Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos*, **86**, 3–15.

Proffit, M., Chen, C., Soler, C., Bessiere, J.M., Schatz, B. & Hossaert-McKey, M. (2009) Can chemical signals, responsible for mutualistic partner encounter, promote the specific exploitation of nursery pollination mutualisms? The case of figs and fig wasps. *Entomologia Experimentalis Et Applicata*, **131**, 46–57.

R Development Core Team (2013) *R: A Language and Environment for Statistical Computing*. (ed. R.F.f.S. Computing). R Foundation for Statistical Computing, Vienna, Austria.

Ranganathan, Y. & Borges, R.M. (2009) Predatory and trophobiont-tending ants respond differently to fig and fig wasp volatiles. *Animal Behaviour*, **77**, 1539–1545.

Ranganathan, Y., Ghara, M. & Borges, R.M. (2010) Temporal associations in fig-wasp-ant interactions: diel and phenological patterns. *Entomologia Experimentalis Et Applicata*, **137**, 50–61.

Rodríguez-Gironés, M.A., Gonzálvez, F.G., Llandres, A.L., Corlett, R.T., Santamaría, L. & Aizen, M. (2013) Possible role of weaver ants, *Oecophylla smaragdina*, in shaping plant-pollinator interactions in South-East Asia. *Journal of Ecology*, **101**, 1000–1006.

Rosenheim, J., Wilhoit, L. & Armer, C. (1993) Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. *Oecologia*, **96**, 439–449.

Schatz, B. & Hossaert-Mckey, M. (2003) Interactions of the ant *Crematogaster scutellaris* with the fig/fig wasp mutualism. *Ecological Entomology*, **28**, 359–368.

Schatz, B. & Hossaert-McKey, M. (2010) Ants use odour cues to exploit fig-fig wasp interactions. *Acta Oecologica-International Journal of Ecology*, **36**, 107–113.

Schatz, B., Proffit, M., Rakhi, B.V., Borges, R.M. & Hossaert-McKey, M. (2006) Complex interactions on fig trees: ants capturing parasitic wasps as possible indirect mutualists of the fig-fig wasp interaction. *Oikos*, **113**, 344–352.

Schatz, B., Kjellberg, F., Nyawa, S. & Hossaert-McKey, M. (2008) Fig wasps: a staple food for ants on *Ficus*. *Biotropica*, **40**, 190–195.

Schmitz, O.J. (2003) Top predator control of plant biodiversity and productivity in an old-field ecosystem. *Ecology Letters*, **6**, 156–163.

Schmitz, O.J. & Suttle, K.B. (2001) Effects of top predator species on direct and indirect interactions in a food web. *Ecology*, **82**, 2072–2081.

Segar, S.T. & Cook, J.M. (2012) The dominant exploiters of the fig/pollinator mutualism vary across continents, but their costs fall consistently on the male reproductive function of figs. *Ecological Entomology*, **37**, 342–349.

Segar, S.T., Pereira, R.A.S., Compton, S.G. & Cook, J.M. (2013a) Convergent structure of multitrophic communities over three continents. *Ecology Letters*, **16**, 1436–1445.

Segar, S.T., Dunn, D.W., Darwell, C.T. & Cook, J.M. (2013b) How to be a fig wasp down under: the diversity and structure of an Australian fig wasp community. *Acta Oecologica*, in press. Doi: 10.1016/j.actao.2013.03.014.

Strauss, S., Conner, J. & Rush, S. (1996) Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist*, **147**, 1098–1107.

Suárez, L., Gonzáles, W. & Gianoli, E. (2009) Foliar damage modifies floral attractiveness to pollinators in *Alstroemeria exerens*. *Evolutionary Ecology*, **23**, 545–555.

Wang, R.W., Sun, B.F. & Zheng, Q. (2010) Diffusive coevolution and mutualism maintenance mechanisms in a fig–fig wasp system. *Ecology*, **91**, 1308–1316.

Wang, R.W., Yang, Y. & Wiggins, N.L. (2014) Asymmetric or diffusive co-evolution generate meta-populations in fig-fig wasp mutualisms. *Science China: Life Science*, in press. DOI: 10.1007/s11427-014-4653-y.

Wang, R.W. & Zheng, Q. (2008) Structure of a fig wasp community: temporal segregation of oviposition and larval diets. *Symbiosis*, **45**, 113–116.

Wang, R.W., Yang, C.Y., Zhao, G.F. & Yang, J.X. (2005) Fragmentation effects on diversity of wasp community and its impact on fig/fig wasp interaction in *Ficus racemosa* L. *Journal of Integrative Plant Biology*, **47**, 20–26.

Wang, R.W., Sun, B.F., Zheng, Q., Shi, L. & Zhu, L. (2011) Asymmetric interaction and indeterminate fitness correlation between cooperative partners in the fig–fig wasp mutualism. *Journal of The Royal Society Interface*, **8**, 1487–1496.

Wei, Z.D., Peng, Y.Q., Xu, L. & Yang, D.R. (2005) Impact of *Oecophylla smaragdina* on the percentage number of offspring of pollinator and non-pollinating wasps on *Ficus racemosa*. *Zoological Research*, **26**, 386–390.

Weiblen, G.D. (2002) How to be a fig wasp. *Annual Review of Entomology*, **47**, 299–330.

West, S.A. & Herre, E.A. (1994) The ecology of the New World fig-parasitizing wasps *Idarnes* and implications for the evolution of the fig-pollinator mutualism. *Proceedings of the Royal Society of London B. Biological Sciences*, **258**, 67–72.

West, S.A., Herre, E.A., Windsor, D.M. & Green, P.R.S. (1996) The ecology and evolution of the New World non-pollinating fig wasp communities. *Journal of Biogeography*, **23**, 447–458.

Yang, C.-Y., Wang, R.-W., Zhao, G.-F. & Yang, D.-R. (2005) Diet of non-pollinating wasps and their impact on the stability of fig-pollinator wasp mutualism. *Zoological Research*, **26**, 379–385.

Yoshie, H. & Yusa, Y. (2011) Indirect interactions in a rice ecosystem: density dependence and the interplay between consumptive and non-consumptive effects of predators. *Freshwater Biology*, **56**, 302–310.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Ant species sampled from *Ficus racemosa* in Xishuangbanna. Data of figures deposited in the figshare repository: doi: 10.6084/m9.figshare.949682.