

Indirect mutualism: ants protect fig seeds and pollen dispersers from parasites

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Abstract. 1. Mutualisms are ubiquitous and ecologically important, but may be particularly vulnerable to exploitation by species outside of the mutualism owing to a combination of an attractive reward and potentially limited defence options. For some mutualisms, ants can offer dynamic and relatively selective protection against herbivores and parasites.

2. The mutualism between fig trees and their pollinating wasps, a keystone mutualism in tropical forests, is particularly well suited for ant protection because pollinators are protected inside hollow inflorescences but parasites are exposed on the outside.

3. In the present study, it was shown that the presence of ants provides a fitness benefit for both the pollinators and the hosting fig tree. The presence of ants (i) reduced abortions of developing figs, (ii) reduced herbivory of figs, and (iii) reduced parasitic wasp loads, resulting in more pollinators and more seeds in ant-protected figs. Even when taking costs such as ant predation on emerging pollinators into account, the total fitness increase of hosting ants was threefold for the tree and fivefold for the pollinators.

4. It was further shown that the seemingly most vulnerable parasitic wasps, of the genus *Idarnes*, have a specific behaviour that allows them to evade ant attack while continuing to oviposit.

5. Ants were present on 79% of surveyed Panamanian fig trees. Together with previous studies from the Old World, the results found here imply that ants are both powerful and common protectors of the fig mutualism worldwide.

Key words. Agaonidae, ant–plant, *Ficus*, species interaction, trophic relationship.

Introduction

Mutualisms are ubiquitous and essential for many ecosystems. Examples include the mycorrhizal fungi that provide nutrients to forest trees, pollinators that help flowering plants set fruit, and intestinal bacteria that help animals including humans take up nutrients (Herre *et al.*, 1999). In a mutualism, two species interact in such a way that both benefit. Mutualisms are, however, vulnerable to exploiters (cheaters, *aprovechados*, parasites), that could either be non-mutualistic individuals of the mutualistic partner species, or species external to the mutualism that reap the benefits without providing anything in return (Soberon & Martinez del Rio, 1985; Bronstein, 2001; Sachs & Simms, 2006). Species that are part of a mutualism may in fact be more vulnerable to external exploiters than species that are

not part of a mutualism, because in addition to the baseline risk of herbivory/predation they also offer an attractive reward to their mutualistic partner which non-mutualistic species might try to exploit (Bronstein, 2001). Ideal defences are those that specifically target external exploiters but spare mutualistic partners (Heil & McKey, 2003).

For some mutualisms, ants may provide exactly the selective and flexible protection needed. Ants frequently protect plants from herbivores and occasionally other competing plants (Janzen, 1966; Heil & McKey, 2003; Chamberlain & Holland, 2009). To entice ants to reside, plants may offer very specialised rewards. On the more obligate side of the spectrum, myrmecophytic plants such as some *Vachellia* (former *Acacia*; Seigler & Ebinger, 2005), *Cecropia*, *Piper*, and *Macaranga* offer both special nesting sites and lipid-rich food bodies (Janzen, 1966; Letourneau, 1998; Rico-Gray & Oliviera, 2007). More common is for plants to have a less obligate relationship with ants, but still benefit from their presence. Some plants (up to 30% of

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species in some locations Heil & McKey, 2003) have extrafloral nectaries to attract ants. In other cases, ants tend sap-sucking hemipterans or take advantage of a rich prey source such as wasps on fig trees (Rico-Gray & Oliviera, 2007; Schatz *et al.*, 2008). Although the outcome of individual relationships is not always clear, or may vary temporally or geographically, overall, plants seem to generally benefit from having ants present (Heil & McKey, 2003; Rico-Gray & Oliviera, 2007; Chamberlain & Holland, 2009). The flexible protection by ants may particularly benefit plants that have a nursery pollination system (where pollinator larvae develop in flowers, which may make general chemical defences less suitable) as long as parasites and pollinators are separated in time or space (Agrawal & Rutter, 1998). Defences that leave the pollinator larvae unharmed would be especially beneficial for plant species where the pollinators disperse pollen from their natal plant, such as in fig trees. In studies of fig trees and their pollinating wasps and yucca plants and their pollinating moths, the presence of ants reduced the number of parasites and increased the number of pollinators emerging from fruits (Compton & Robertson, 1988; Zachariades, 1994; Cushman *et al.*, 1998; Perry *et al.*, 2004; Schatz *et al.*, 2006; Harrison, 2014; Wang *et al.*, 2014). However, only one published study so far has also quantified the overall fitness effect that ants have on the plant host (Wang *et al.*, 2014).

In this study, I investigate the effect of the presence of ants on the fitness of both partners in the fig tree–fig wasp mutualism, a keystone mutualism in tropical forests with *c.* 750 fig species worldwide (Nason *et al.*, 1998; Herre *et al.*, 2008; Cruaud *et al.*, 2012). Fig trees are keystone species because they provide fruit for tropical frugivores year-round even when other trees do not (Shanahan *et al.*, 2001). Fig trees are solely pollinated by fig wasps, and fig wasp larvae develop only in fig flowers. Fig flowers and other fig structures can be exploited by larvae of pollen-free individuals of the pollinating wasp species ('cheaters', Jandér & Herre, 2010), of pollen-free wasp species that originate from pollinator lineages ('cuckoo wasps', Compton *et al.*, 1991; Peng *et al.*, 2008), of a variety of non-pollinating fig wasps ('NPFW') that are unrelated to pollinators *eg.* (West *et al.*, 1996), and of other herbivorous/predatory insects such as moths and beetles [hereafter 'herbivores' although they feed on both fig plant tissue, seeds, and wasp galls (Bronstein, 1988; Compton, 1993; Sugiura & Yamazaki, 2004)]. Because the NPFW and herbivores are accessing fig flowers from the outside of the fig (details below), they are perceivably more vulnerable to ant predation than are the pollinators that oviposit on the inside. Old World studies have shown ants to protect pollinators and seeds from NPFW (Compton & Robertson, 1988; Cushman *et al.*, 1998; Schatz *et al.*, 2006; Wang *et al.*, 2014).

I here investigate the effect ants have on a New World fig tree–fig wasp mutualism:

- 1 Does the presence of ants reduce the herbivore and parasite load of developing figs?
- 2 Does the presence of ants increase pollinator fitness? I uniquely take into account the estimated cost of ants' capturing emerging pollinators.
- 3 Does the presence of ants increase the fig tree's female and male fitness (seed numbers and pollen dispersing female pollinator wasps respectively)?

- 4 I observed an ant evasive behaviour by one of the NPFW genera (*Idarnes*) that seemed to allow continued oviposition. Was this behaviour effective at avoiding ant interference?
- 5 Using a field survey I estimate how frequently predatory ants are present on fig trees in the area, and thus how generalisable the results might be.

Methods

Study system

The studied fig trees and insects were part of natural populations at the Barro Colorado Natural Monument (BCNM) in Panama. All fig species here are monoecious, have synchronous crops with figs borne amongst the leaves, and are typically pollinated by one or two species-specific fig wasp species (Herre, 1989; Molbo *et al.*, 2003). *Ficus obtusifolia* Kunth (Moraceae) is here pollinated by the two cryptic fig wasp species *Pegoscapus hoffmeyer* A and *P. hoffmeyer* B Grandi (Agaonidae: Agaoninae) (Wiebes, 1995; Molbo *et al.*, 2003). Fig inflorescences (formally syconia; hereafter figs) are hollow structures lined with hundreds of uniovulate flowers. With few exceptions, pollinating fig wasps are the only insects able to enter through the narrow ostiole (Fig. 1a) to reach the fig interior where they oviposit in some of the flowers while also pollinating. A female pollinator fig wasp rarely leaves the first fig she enters; she lays her eggs and dies inside the fig. Her offspring develop in galls, one in each flower, and will emerge some weeks later (Fig. 1a). Male wasps emerge first from their galls, mate with the females, then dig an exit tunnel for the females. Females, carrying the host fig tree's pollen, fly off in search of a flowering fig tree where they can lay their eggs. Pollinators are thus protected from ant attacks except during the short time period when they locate and enter the ostiole (less than a minute; K. Charlotte Jandér, pers. obs.), and when they exit the fig.

There is also a variety of other fig-associated insects, of which I here will study a subset. The NPFW are unrelated to the pollinators and lay their eggs in fig structures from the outside of the fig and are therefore exposed to ants during the duration of their oviposition (Fig. 1b,c). At the study site, *F. obtusifolia* is most commonly associated with the following genera of NPFW, although others occur less commonly (West *et al.*, 1996; Marussich & Machado, 2007; J.-Y. Rasplus, pers. comm. for ID Mar. 2014; K. Charlotte Jandér, pers. obs.):

Idarnes Walker (Agaonidae: Sycophaginae; subgroups *carne* and *flavicollis*) (pollinator-sized wasps using the same flowers as the pollinators; *flavicollis* are likely competing early gallers and *carne* cleptoparasites; West & Herre, 1994; Elias, 2012). *Aepocerus* Mayr (Pteromalidae: Otitesellinae) and *Ficicola* Heydon (Pteromalidae) (large wasps that gall fig tissue; 'gallers'; Conchou *et al.*, 2014). *Physothorax* Mayr (Torymidae) (likely parasitoids of *Aepocerus* and *Ficicola*). *Eurytoma* Illiger (Eurytomidae) and *Heterandrium* Mayr (Pteromalidae: Otitesellinae) (unclear feeding habits, possibly parasitoids; Elias *et al.*, 2008). The NPFW of BCNM are not described to the species level. In this study, I will refer to wasps of the genus *Idarnes* (combined subgroups *carne* and *flavicollis*) as '*Idarnes*', but for simplicity combine all other NPFW (gallers, parasitoids, others)

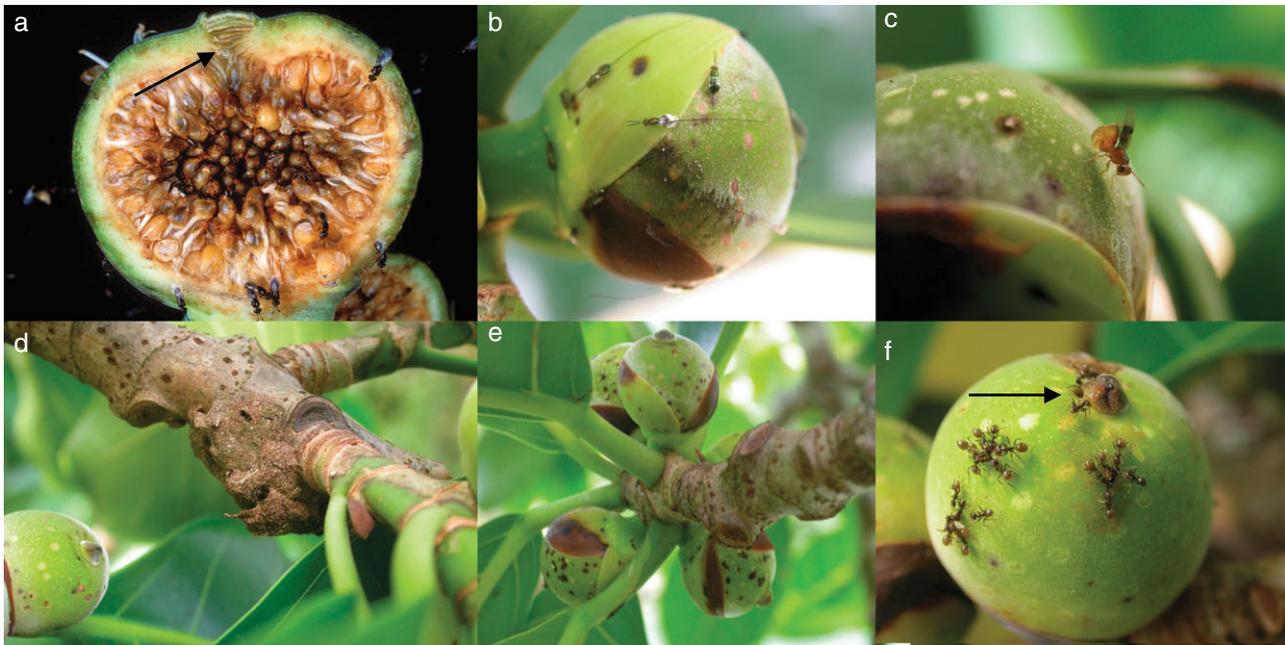


Fig. 1. Photographic illustration of the fig tree-wasp-ant interaction of *Ficus obtusifolia*. (a) Bisectioned mature fig fruit, showing yellow seeds along the outer edge and blackish galls from which pollinator wasps (*Pegoscapus hoffmeyeri*) emerge. The fig entrance covered in overlapping flaps (ostiole) is at the top of the photo (arrow). (b) Ovipositing *Idarnes* wasps in the flat position. (c) Ovipositing *Eurytomasp.* parasitic wasp. (d) *Azteca trigona* carton nest on an 'ant twig'. (e) A 'no-ant twig' lacking ant nest (note the numerous scars on figs from parasitic wasp ovipositions). (f) Ants surrounding captured emerging pollinator females; exit tunnel at top (arrow). See also Videos S1–S3. Photo (a) (©)Christian Ziegler/STRI.

into a category called 'parasitic wasps' as they take resources from the fig tree (and pollinators) without giving anything in return. There are also unidentified pyralid moths whose larvae consume developing seeds and wasp galls (Bronstein, 1988).

Ants are frequently present on BCNM fig trees. *Azteca* ants are the most noticeable, sometimes making a single large and/or multiple small satellite carton nests on twigs and branches. Large nests can hang from branches as thin as 4 cm in diameter; small nests are at the tips of the twigs and can be part of a colony that spans several trees (K. Charlotte Jandér, pers. obs.; B. Adams, pers. comm.). Although *Azteca* ants are the most aggressive, other ant genera are also present. In addition, spiders are often present on fig trees and I expect they consume fig wasps although I have not observed it. Nevertheless, ants are by far the most common, numerous, and aggressive predators present on fig trees at the study site. This study, therefore, focuses on their effect of the mutualism.

Field natural experiment

To investigate the effect of ants as a defence against parasites I made use of the large difference in ant abundance among the different twigs of a large *F. obtusifolia* tree. Many of the twigs (terminal branches with leaves) on this tree each hosted a small carton nest of *Azteca trigona* Emery (Formicidae: Dolichoderinae) ants (Fig. 1d), I will refer to these as 'ant twigs'. Whenever I observed these ant twigs, numerous ants were patrolling the leaves, stems, and figs, and aggressively and vigorously attacked anything that touched the tree structures.

Other twigs did not host any ant nests (Fig. 1e), and ants were absent or only rarely present; I refer to these as 'no-ant twigs'. I did not use any artificial means of excluding ants from the no-ant twigs. The *A. trigona* activity was confined to the twigs with the carton nests; I never observed *A. trigona* ants be present on 'no-ant twigs'. To evaluate the consistency of the ants' presence (ants vs. no ants) I counted the number of ants present on each twig (leaves, figs, and stem) on days 7, 8, 9, 10, 11, 12, 14, 20, and 26 after pollination. I monitored the tree more frequently days 7–14 after pollination because non-pollinating wasps were much more frequent during that period than later on. *Ficus obtusifolia* at this field site takes about 43 days (K. Charlotte Jandér, pers. obs.) from flowering (B phase figs) to mature wasp-releasing figs (D phase figs) (Galil & Eisikowitch, 1968). I compared the effect of the two states (ants vs. no ants) on the number of ants present with a repeated measures ANOVA.

Using a non-toxic permanent marker I numbered 63 figs on the ant twigs, and 53 figs on the no-ant twigs, and followed them until maturation. I noted if figs aborted (fell off the tree before maturation; typically a sign of herbivory or that they are not pollinated), which kills all developing wasp larvae and seeds inside (Jandér & Herre, 2010; Jandér *et al.*, 2012). I collected figs that had matured just before wasps emerged and allowed wasps from each fig to emerge in separate vials. For each fig, I also recorded if a herbivorous moth larva was present – such larvae filled the entire fig lumen and had consumed all the contents, seeds, and wasps alike. Wasps and figs were then frozen until they could be counted. The contents were counted of each remaining mature fig from the no ant twigs ($n = 20$) and

a random subset of figs from the ant twigs ($n = 21$): we counted male ('P_M') and female ('P_F') pollinators, *Idarnes* (combined subgroups *carne* and *flavicollis*) wasps ('I'), other parasitic wasps ('Par'), and seeds ('S'). As the original number of flowers in a fig can affect the number of seeds and wasps emerging from it (Herre & West, 1997), we also counted the total number of flowers in each fig so that we could control for that in the statistical tests. The mean number for each group (ants vs. no ants) was compared using Mann–Whitney *U*-tests, *t*-tests, or ANCOVAs with the number of flowers as a covariate. The pollinators' fitness measure in each fig was calculated as the combined number of male and female pollinators: $W_P = P_M + P_F$. The tree's fitness measure from a fig was calculated as the female pollinators (the pollen dispersers) plus seeds: $W_T = P_F + S$. To compare the tree's fitness measure across treatments when also taking the likelihood of abortion and herbivory in account I multiplied the tree's average fitness measure for a treatment with the proportion of figs that matured (i.e. did not abort or get destroyed by herbivory) in each treatment: $[W_T(\text{ants}) \times \text{prop matured (ants)}] / [W_T(\text{no ants}) \times \text{prop matured (no ants)}]$.

Observations of ant predation of pollinator wasps

When figs on *F. obtusifolia* matured, I observed the natural wasp emergence on three figs in the field. I counted how many pollinator and *Idarnes* females emerged and flew off, and how many were caught by ants. I also surveyed recently open figs (figs with an exit hole and emerging wasps) at three different times over two days and noted whether ants were present or not. When including the costs of ant-caught pollinators into the fitness estimates I first subtracted the estimated number of female pollinators that would be caught [$P_F(\text{ants}) \times 0.93 \times 0.065$] from the $P_F(\text{ants})$ detailed above (where 0.93 is the proportion of figs with ants, and 0.065 the proportion of female pollinators caught, see Results), and then proceeded with the calculations as detailed above. Ant predation of male pollinators, once they have exited the fig, does not negatively affect the fitness of either pollinators or the tree; the males' task is done when female pollinators are mated and an exit tunnel dug.

Field observations of ovipositing behaviour and *Idarnes* ant interactions

I observed the oviposition behaviour of different genera (identified to genera or other easily recognisable group by their morphological appearance in the field) of parasitic wasps arriving at a large *F. obtusifolia* tree with a recently pollinated crop. The oviposition behaviour of the parasitic wasps consists of a search phase when wasps walk around on the outer surface of the fig, an insertion phase when they insert their ovipositor into the fig, and then egg deposition (Bronstein, 1988; Elias, 2012). I quantified the duration of ovipositions, here defined as the time that the wasps were still on the fig (ovipositor insertion phase + egg deposition phase), for different wasp individuals of each genus using a stopwatch. In those cases where I timed more than one oviposition for an individual, I used the mean duration for that individual wasp. These observations took place in the

daytime (10.00–15.00 hours) on days 8–10 after pollination, when parasitic wasps were abundant. For comparison, I repeated this type of observation on a *Ficus dugandii* Standl. (days 10–22 after pollination) on wasps of the same genera as observed on *F. obtusifolia*. At BCNM, *F. dugandii* and *F. obtusifolia* share two galler species (genus *Aepocerus*), but at least some of the other parasitic wasp species (including *Idarnes*) are likely to be unique for each host species (Marussich & Machado, 2007). Nevertheless, these observations allowed some insight into whether some genera consistently had a longer duration of oviposition than others. Because *Idarnes* wasps seemed to exhibit a specialised ant-evasive behaviour, I also specifically observed 86 independent encounters between *Idarnes* wasps and *Azteca trigona* ants on *F. obtusifolia*.

Survey of ant prevalence

To roughly estimate the prevalence of ants on *Ficus* trees in this area (BCNM), I surveyed a total of 34 trees of 11 different species for presence or absence of ants' nests and ants. I recorded if there were any ant nests visible on branches or twigs and if there were any ants visible on leaves, twigs or figs (if present). To find out whether the presence of ants' nests and/or visible ants was a good indication of the predation pressure that arriving parasitic wasps and herbivores might experience, I placed bait on a subset of 18 trees of 7 different species (listed in Table S1, Appendix S1). I placed the bait [boiled ham, $1 \times 0.5 \times 0.2 \text{ cm}^3$; although chicken is typically more attractive to ants, ham is likely to give an acceptable estimate of the presence of dominant ant species (B. Adams, pers. comm.)] on five twigs per tree at the nodes by the petiolar bases, where figs would be. I then counted the number of ants that were present at each bait after 15 min and collected ants at the bait for later identification by J. Longino. I analysed the number of ants present per tree using a robust Welch test because variances were highly unequal across categories. I had to add 0.00001 ant to one of the 0 counts for the trees that did not have any ants at all recruited in order to make possible a variance calculation for the robust Welch test. I also analysed the data with a nested ANOVA (with ant category as the fixed treatment factor, and tree ID as a random factor nested within treatment). Because variances were highly unequal across categories, this test is not ideal, but is included for comparison.

Fisher's exact tests were performed using R version 3.1.0 (R Core Team, 2014), all other statistical analyses in the study were performed using SPSS 19.0 (IBM, Armonk, NY, USA).

Results

Field natural experiment

Consistency of ant presence. Throughout the study on *F. obtusifolia*, the twigs with the ants' nests had significantly (over 10 times) more ants present than did the no-ant twigs (repeated measures ANOVA on log-transformed data, $F_{1,21} = 586.5$, $P = 7.9\text{E}-17$, Fig. 2). *Azteca trigona* were only observed on the ant-twigs, never on the no-ant twigs, and were by far the most numerous ants present. Occasionally single ants

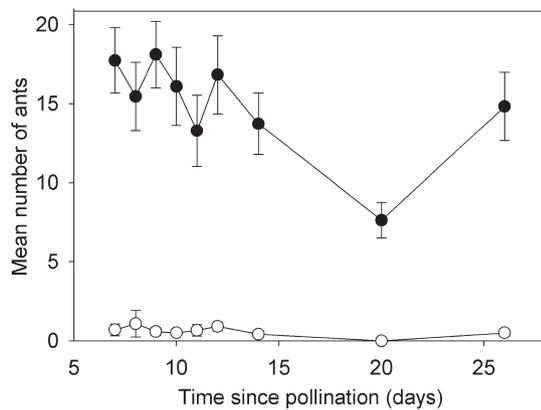


Fig. 2. Ants were much more numerous on ant twigs (filled circles) than on no-ant twigs (open circles) throughout the study. Error bars represent ± 1 SE.

(seven individuals in total; included in the ant counts) of the much larger *Camponotus sericeiventris* Guérin (Formicidae: Formicinae) would visit both the ant twigs and ant-free twigs. Although they are described to be generalists (Yamamoto & Del-Claro, 2008), I did not observe them attempting to prey upon either parasitic fig wasps or pollinators. On ant-twigs I did not observe any other type of ant, but on no-ant twigs there were occasional medium-sized ants, probably a smaller *Camponotus* species (unidentified); these were rare (two individuals in total) and are included in the ant-counts.

The presence of ants protected against abortion and herbivory of figs. Developing fig fruits experienced one of three fates: (i) they aborted before maturation (sensu Jandér & Herre, 2010), (ii) their contents were consumed by a moth larva (likely family Pyralidae; L. Palmieri, pers. comm. July 2014) or (iii) they matured and produced wasps and seeds. Both fig abortion and moth herbivory destroy all wasp larvae and seeds inside the fig. Fig abortion was 4.7 times more common when no ants were present (Fisher's exact test, $P = 0.041$; Fig. 3). Similarly, moth herbivory was 1.8 times more common when no ants were present (Fisher's exact test, $P = 0.013$; Fig. 3). As a result, figs on ant twigs were 1.7 times more likely to mature and thus produce wasps and seeds (Fisher's exact test, $P = 0.0021$; Fig. 3).

The presence of ants increased wasp offspring and seeds in mature figs. The number and type of wasp offspring that emerged from each fig fruit differed greatly between figs that developed on ant twigs and those that developed on no-ant twigs. Parasitic wasps (other than *Idarnes*) were 80 times less numerous in mature figs on ant twigs compared with figs on no-ant twigs (Mann–Whitney U -test, $n = 41$, test statistic -5.69 , $P < 0.001$; Fig. 4a). Ant figs also had fewer *Idarnes* wasps, 78% of that in no-ant figs, but the difference was not significant (t -test, unequal variances, $t_{34.7} = 1.63$, $P = 0.11$; Fig. 4b). There were 22% more fig flowers in ant figs than in no-ant figs (t -test, $t_{39} = -4.47$, $P = 6.5E-5$; Fig. 4c), so in the following analyses this is taken into account. There were three

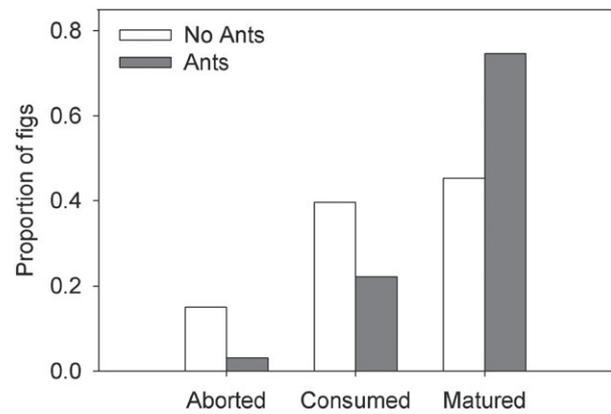


Fig. 3. Figs on ant-twigs were less likely to be aborted or consumed by herbivorous caterpillars, and more likely to mature and thus produce wasps and seeds than were figs on twigs where no ants were present.

times more pollinators emerging from ant figs than from no-ant figs (ANCOVA with flower number as covariate, $F_{2,38} = 12.56$, $P = 0.001$; Fig. 4d). There were 75% more seeds in ant figs than in no-ant figs (ANCOVA with flower number as covariate, $F_{2,38} = 32.45$, $P = 1.5E-6$; Fig. 4e). Consequently, the tree's fitness measure (female pollinators plus seeds) was more than doubled in ant figs compared with no-ant figs (ANCOVA with flower number as covariate, $F_{2,38} = 43.96$, $P = 7.8E-8$; Fig. 4f). If the likelihood of abortion and herbivory also is considered, the tree's fitness measure was 3.5 times higher in ant figs compared with no-ant figs, and the pollinators' fitness measure 5.1 times higher.

The cost of hosting ants: predation on emerging pollinators

If ants were present at the time, they caught a small proportion of emerging female wasps. Ants waited by the wasp-produced exit tunnel and caught wasps as they emerged from the mature fig (Fig. 1f; Video S3). I observed wasp emergence on two figs where ants were present: (i) ants caught 3 out of 50 female pollinators (6%), (ii) ants caught 9 out of 138 female pollinators (7%) and 18 of 87 female *Idarnes* (15%; caught by the long ovipositor in all cases). I also observed 69 pollinators and 18 *Idarnes* emerge from a fig without ants present; not surprisingly none of those wasps were caught. Not all figs with a recent exit hole and emerging wasps ('open figs') had ants present, although it varied with position on the tree. On the twigs with ant nests present ('ant-twigs' in the above study), ants were present on 93% of the open figs ($n = 63$ over two days). On the twigs labelled as 'no-ants' in the study, there were no ants even on open figs ($n = 4$; the rest were collected before opening as part of the study).

In an attempt to reach an overall fitness estimate of the effects of ants on the tree and pollinators, I combined these costs with the field-based benefit estimates. Based on my observations I assume that ants capture 6.5% of emerging pollinator females when present, and that ants are present on 93% of ant-figs. Despite the cost of captured pollinators, if including the protection from herbivory and abortion, the tree's fitness measure is still 3.4 times higher in ant figs than in no-ant

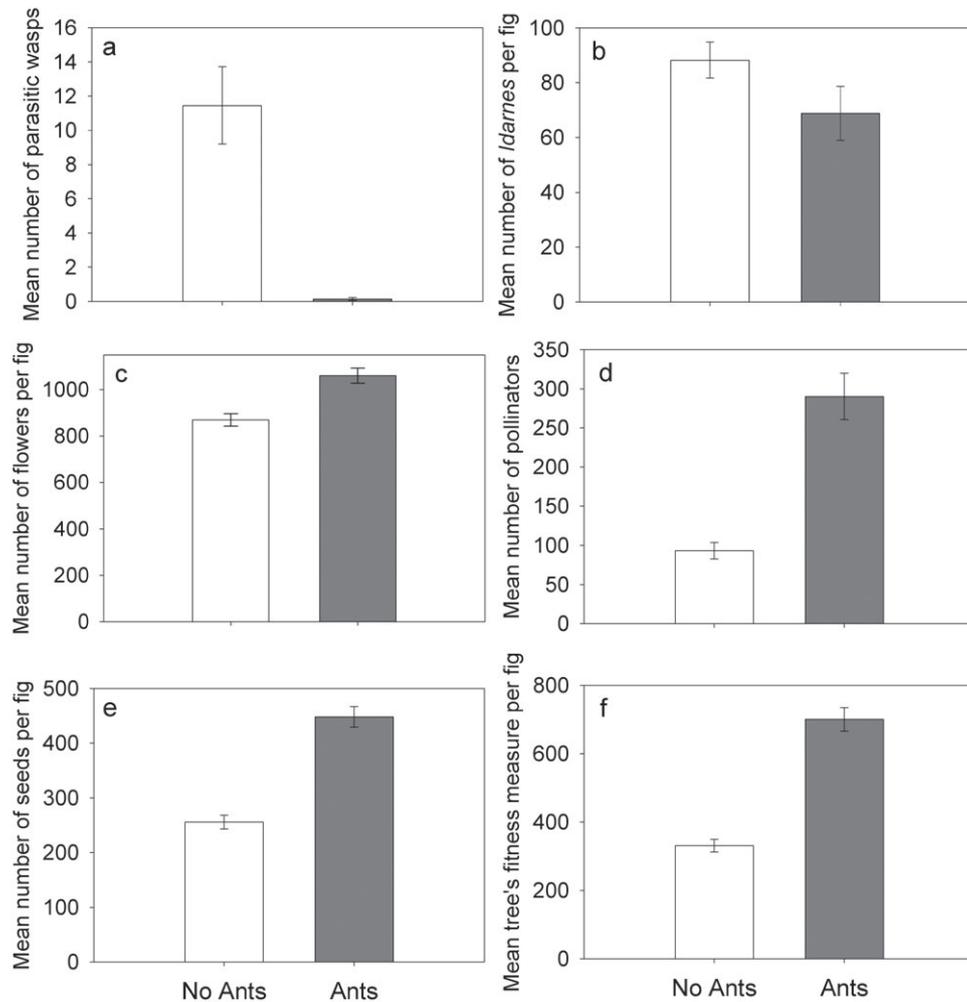


Fig. 4. Figs from ant vs. no-ant twigs differed greatly in the number of seeds and number and type of wasp offspring that developed within. (a) Parasitic wasps and (b) *Idarnes* were less numerous in ant figs. In contrast, (c) flower number, (d) pollinators, (e) seeds, and (f) overall tree fitness was higher in figs on ant twigs. Error bars represent ± 1 SE.

figs, and the pollinators' fitness measure 4.9 times higher in ant figs than in no-ant figs.

Field observations

Duration of oviposition varies across wasp genera. In *F. obtusifolia* the non-pollinating fig wasp genera differed greatly in the time that they were exposed to ant predation while ovipositing on the outside surface of the fig [ANOVA on log-transformed data, $F_{2,13} = 55.99$, $P = 1.7E-6$; Figure S1a, Appendix S1 (only species with >1 independent observation included in the ANOVA; more species are included in the figure)]. The *Idarnes* wasps sat motionless on the fig surface with their ovipositor inserted for 14–49 min, significantly longer than the other species (max 3 min) (Bonferroni's corrected Tukey HSD). Also on *F. dugandii*, *Idarnes* wasps had the overwhelmingly longest duration of oviposition compared with the other parasitic wasp genera (Figure S1b, Appendix S1) and therefore at

first seemed particularly vulnerable to ant attacks. However, on both fig species *Idarnes* wasps dealt with approaching ants differently than the other wasp species. Whereas all other NPFW wasp species I observed quickly pulled out their ovipositor and hopped off the fig when any danger such as an ant approached, *Idarnes* wasps pressed themselves flat against the fig surface, and thereby seemed to avoid ant attacks (Video S1 shows the *Idarnes* flattening behaviour).

Idarnes wasps flatten to avoid ants. To investigate the ant evasive behaviour of *Idarnes* further, I specifically observed 86 independent encounters between *Idarnes* females and *A. trigona* ants on *F. obtusifolia*; the *Idarnes* females flattened in 78 of these encounters, hopped off in 7, and remained standing in 1. Which behaviour the *Idarnes* wasps chose depended on what stage they were in the oviposition process (Fisher's exact test, $P = 5.6E-6$). Before having inserted their ovipositor into the fig surface, *Idarnes* wasps were equally likely to hop off or to

flatten ($n = 16$, 56% flattened). However, once they started the process of curling their body to insert their ovipositor into the fig, 96% of wasps flattened if an ant approached ($n = 25$), and once their ovipositor was fully inserted and they were in the flat oviposition position, 100% of wasps remained flat when an ant approached ($n = 45$). Video S1 shows *Idarnes* wasps in the three different ovipositing stages responding to an approaching ant by flattening or remaining flattened.

The flattening behaviour indeed seemed to prevent against ant attack (Fisher's exact test, $P = 0.051$, but note the very small sample size). Only two of the 78 flattened *Idarnes* females were attacked by the ants (one escaped, one did not), despite the ants investigating with their antennae all the flattened *Idarnes* females. None of the wasps that hopped off got attacked. The female that did not hop off or flatten but remained standing quickly got attacked and dragged away (Video S2).

Separate from these more systematic observations, I noticed several instances of *A. trigona* ants carrying *Idarnes* wasps that they had caught. I also observed a larger yellow and black unidentified vespid wasp grasp an ovipositing flattened *Idarnes*, cut her ovipositor to free her from the fig, and fly off with her.

Survey of ant prevalence

Of the 34 fig trees surveyed, 27 trees (79%) had visible ants on leaves or twigs, and 14 trees (41%) had also visible ants' nests. Of the 18 ham-baited trees, only 3 had neither ants nor ant nests visible, the remaining 15 trees (83%) had ants visible on the leaves and twigs before the baiting. Among the trees with visible ants, 8 trees had no visible ant nest, while 7 trees had externally visible ant nests like carton nests at the branch tips (*Azteca* nests), larger droop nests at the larger branches (*Azteca*), or ants living in hollow twigs (various ant genera).

The most common ant genus present on the ham-baited fig trees was *Azteca*, present on 14 (93%) of the 15 fig trees with ants. *Camponotus* ants were present on 9 (60%) of the trees. Less common genera were *Crematogaster*, *Cephalotes*, *Dolichoderus*, and *Pseudomyrmex* (complete genus/species list in Table S1, Appendix S1). The number of ants by the bait after 15 min varied widely across the three categories of pre-bait ant presence (no ants; ants only; ants plus external nests) (robust ANOVA on tree means, unequal variances, Welch's statistic_{2,8,6} = 9.78, $P = 0.006$). All three trees that lacked visible ants had no ants at all at the bait, which was significantly different from trees with visible ants (planned contrast unequal variances, $t_{10,3} = 4.56$, $P = 0.00097$; Fig. 5). Among trees with ants visible, those with externally visible ant nests had more ants at the bait than those without, but the difference was not significant (planned contrast unequal variances, $t_{10,3} = -1.48$, $P = 0.17$; Fig. 5). When analysing using a nested ANOVA, both the category of pre-bait ant presence ($F_{2,16} = 3.62$, $P = 0.05$) and the tree ID ($F_{16,77} = 11.8$, $P = 8.6E-15$) influenced the number of ants present at the bait. There were significantly more ants at the bait in the ants only category than in the no-ant category (planned contrast, $P = 1.22E-5$), and significantly more ants at the bait in the ants plus nests category than in the ants only category (planned contrast, $P = 8.7E-14$) (Fig. 5).

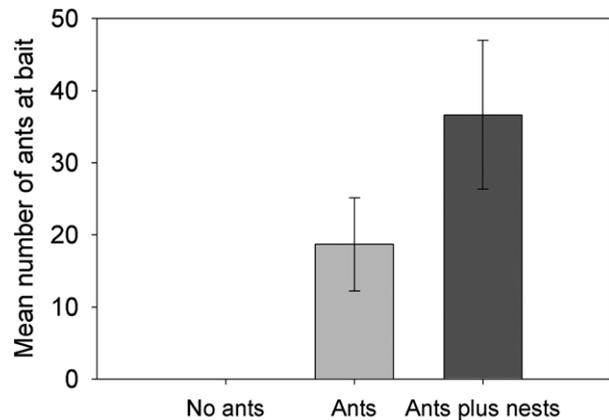


Fig. 5. There were more ants at the ham bait after 15 min on trees that had ants or ants plus ant nests visible than on trees where no ants were visible before the baiting. Error bars represent ± 1 SE.

Discussion

Here I have shown that the presence of ants dramatically increased the fitness of both the fig tree and the pollinators in a New World fig mutualism. Ants increased both fig maturation rates and the number of pollinators and seeds that developed, seemingly by preventing oviposition by herbivorous moths and parasitic wasps. The inferred relationships are summarised in Fig. 6. I here also give the first evidence of an ant-evasive behaviour that nevertheless allows continued oviposition of *Idarnes* parasitic wasps. Ants were present on 79% of surveyed Panamanian fig trees, implying that they are not only powerful, but also common protectors of the fig mutualism in the New World.

This study is the first to investigate the effect of ants on a fig tree–fig wasp mutualism in the New World. Ants efficiently protected figs from both herbivorous caterpillars and externally ovipositing parasitic wasps, leading to an over threefold increase in fitness (calculated as the number of seeds and female pollinators) for the tree, and nearly fivefold for the pollinators. Several mechanisms are responsible for this increased fitness: (i) ant-figs were less likely to abort, and hence more likely to mature, than no-ant figs. Although I was unable to collect the aborted figs and examine them to determine the cause of abortion I can hypothesize that ant-figs had lower rates of various types of herbivory (moth infestations of different genera can cause abortion of developing figs Jandér *et al.*, 2012). (ii) Ant-figs were less likely to be infested by herbivorous moth larvae that consume the entire fig content; presumably ants prevented moth oviposition. (iii) Ant-figs contained fewer large galls such as *Aepocerus* and *Ficicola* and other parasitic wasps; because they drain resources from the fig, fewer seeds and pollinators can be supported when parasitic wasps are present (West *et al.*, 1996; Conchou *et al.*, 2014). (iv) The large *Ficicola* galls oviposit very early in the fig development (K. Charlotte Jandér, pers. obs.) and may damage developing flower structures, thus reducing the total number of flowers available for pollinators and seeds (Conchou *et al.*, 2014). The lower flower number seen in the ant-free figs in this study may be as a result of such *Ficicola* damage.

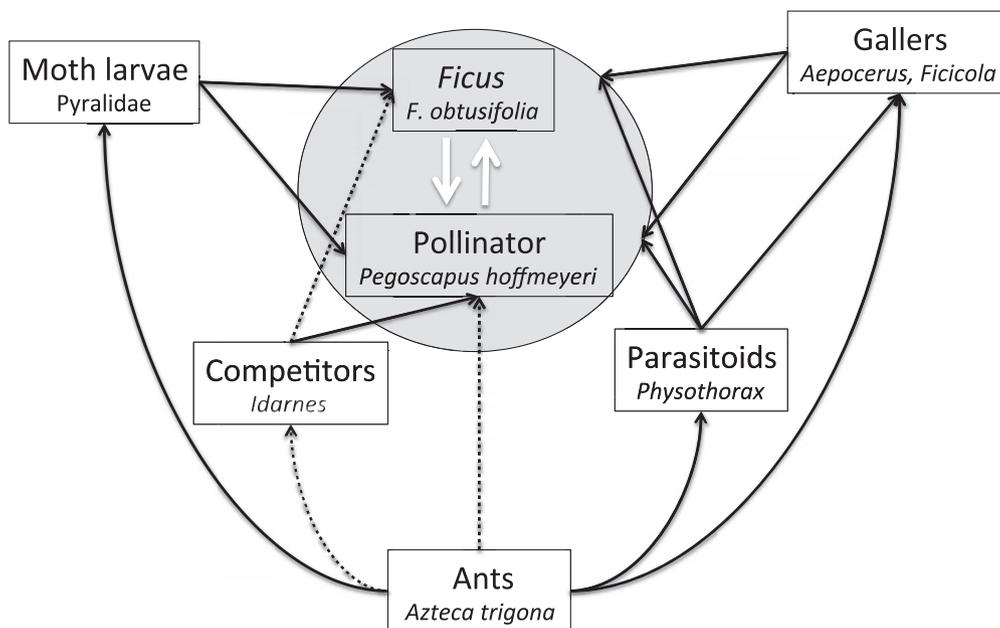


Fig. 6. Inferred interactions between the fig tree, pollinator wasps, non-pollinating fig wasps of different types, herbivorous moth larvae, and ants. Note that moth larvae and *Idarnes* affect pollinators and seeds directly, whereas gallers and parasitoids affect them more indirectly through their negative effect on the fig fruit. The grey circle represents the fig fruit. White arrows are positive interactions. Solid black arrows are negative interactions. Dashed black arrows are weak negative interactions.

(v) Ant-figs had somewhat fewer *Idarnes* wasps, and although *Idarnes* mostly compete with pollinators for flowers (West & Herre, 1994; Elias, 2012), they can also develop in seeds (Pereira *et al.*, 2007; BCNM: K. Charlotte Jandér, pers. obs.). Similarly, Old World studies of the effect of ants on the fig mutualism have shown reduced NPFW loads and/or increased pollinator and recently also seed numbers (Compton & Robertson, 1988; Cushman *et al.*, 1998; Schatz *et al.*, 2006; Zachariades *et al.*, 2010; Harrison, 2014; Wang *et al.*, 2014). Thus, although the dominant genera of NPFW and ants found on fig trees largely differ between the Old and New World (Segar & Cook, 2012; Bain *et al.*, 2014), the ecological niches of the interactors and the outcome is similar: that of predatory ants benefiting both partners of the fig tree–fig wasp mutualism by predominantly preying upon the externally ovipositing (thus easily accessible) NPFW.

Hosting ants may also incur costs to the tree. While ants may lower herbivory, they may also deter pollinators and/or bring other costs such as sap-sucking hemipterans (aphids, treehoppers etc) that ants tend for their honeydew (Compton & Robertson, 1988; Heil & McKey, 2003; Rico-Gray & Oliviera, 2007; Frederickson, 2012). I did not notice any hemipterans on either the ant twigs or no-ant twigs on the studied *F. obtusifolia*, but it is possible that sapsuckers were hosted inside the carton nests; I did not destroy any nests to look. However, the fitness effects calculated here are based on the number of seeds produced on twigs with ant nests present; hence any local fitness cost of potentially hosting hemiptera inside the ant nests is included in the overall effect.

While the presence of ants increased the number of pollinators, ants also preyed upon a small proportion of pollinator females as they exited the fig. Such ambush behaviour of ants has

previously been recorded in several fig mutualisms (Schatz & Hossaert-McKey, 2003; Schatz *et al.*, 2008; Zachariades *et al.*, 2010; Harrison, 2014). It is likely that the pollinators' behaviour of first gathering in the fig lumen then exiting the fig in quick succession is an adaptation to minimise the risk of being caught by ants (Zachariades *et al.*, 2010), as several wasps can escape while ants are busy handling the first wasps caught (Fig. 1f, Video S3). Although males of some fig wasp species will gather around the exit opening, thus occupying the ants and increasing the females' chance of escaping (Zachariades *et al.*, 2010; Harrison, 2014), I did not observe any evidence of this in *P. hoffmeyer*. Nevertheless, my estimate of 6.5% of emerging female pollinators caught corresponds well with previously published numbers of 1–9% depending on ant density (Zachariades *et al.*, 2010) and up to 10% (Harrison, 2014). Importantly, the proportion of females caught is small compared with the overall increase in pollinator numbers due to ants' deterring of parasites and herbivores, making the pollinators overwhelmingly benefit from the ants' presence. To my knowledge this is the first published attempt to estimate the overall fitness effect (including costs as well as benefits) for fig pollinators of the presence of ants. Pollinators are also vulnerable to ant predation when they are locating and entering the ostiole of receptive figs, although to a much lesser degree than externally ovipositing parasitic wasps as the pollinators quickly reach the sheltered fig interior (Bronstein, 1988; Schatz *et al.*, 2008). Although I did not specifically observe this aspect in the present study, the ants cannot have completely hindered the entering pollinators because abortion rates of figs on the ant twigs were very low (all *F. obtusifolia* figs unentered by pollinators abort; Jandér & Herre, 2010), and figs on the ant

twigs matured simultaneously with figs on the no-ant twigs, suggesting that pollination on ant twigs was not delayed.

There were dramatically fewer parasitic wasps developing in figs on the ant-twigs than on twigs with no ants. The exception was *Idarnes* wasps, discussed in a separate paragraph below. Although I occasionally observed ants catching a parasitic wasp, the main effect of the ants' presence is likely that they interrupt the parasites' ovipositions. Similar observations have been made on ants and wasps on Old World figs, and on *Helianthella* sunflowers where ants interrupt fly oviposition (Inouye & Taylor, 1979; Schatz *et al.*, 2006; Harrison, 2014). Because the 'natural experiment' part of this study was based on natural variation in ant density rather than randomised manipulative ant-exclusions, an alternative explanation of the results must be that parasites (especially non-*Idarnes* parasites) and ants do not interact but prefer slightly different microhabitats on the tree (Inouye & Taylor, 1979). I find this unlikely because (i) I observed non-*Idarnes* parasitic wasps constantly trying to oviposit on the figs on the ant-covered twigs but getting interrupted by ants, and (ii) non-*Idarnes* parasitic wasps are likely to share habitat requirements with the pollinators and the *Idarnes*, and both the last two were abundant in figs on both ant covered and ant free twigs. The most parsimonious explanation is that the ants interrupted the non-*Idarnes* parasitic wasps' oviposition, and therefore reduced the number of parasites developing in each fig. Previous randomised ant exclusion experiments have indeed shown that ants reduce the number of external parasitic wasps present on the surface of figs and/or developing from mature figs (Compton & Robertson, 1988; Zachariades, 1994; Cushman *et al.*, 1998; Schatz *et al.*, 2006; Harrison, 2014; Wang *et al.*, 2014).

This is the first study to show that one of the NPFW genera, *Idarnes* (subgroups *carne* and *flavicollis*), has a behaviour that seems specialised to avoid ant attack. In contrast with the other parasitic wasp genera that discontinued their oviposition and flew off when an ant approached, *Idarnes* wasps pressed themselves close to the fig surface when an ant appeared, and in doing so largely avoided ant attacks while being able to continue ovipositing. In addition to their flattening behaviour, it is possible that *Idarnes* wasps are chemically camouflaged (similar to the treehoppers in Silveira *et al.*, 2010) as the ants always touched the *Idarnes* with their antennae before ignoring them. It is also possible that the dorso-ventrally flattened body shape of *Idarnes* assists in their camouflage. Compared with other parasitic wasps at the study site, *Idarnes* wasps otherwise would be particularly vulnerable to ant attacks owing to their small body size and long ovipositing times. *Idarnes* wasps, especially of the *flavicollis* group, have an unusually convoluted way of depositing their eggs, first reaching their ovipositor into the fig lumen (from the outside), then inserting it through the stigma of a flower to deposit their eggs in the exact same location as pollinators do (Elias, 2012; Jansen-González *et al.*, 2012). Potentially they can use a single insertion through the fig wall to reach and deposit eggs in several individual flowers, all while sitting flat on the fig surface unharmed by ants. A similar ant-avoiding flattening behaviour has been observed in Old World *Sycophaga* wasps on *F. racemosa* although it is unknown how successful this is at preventing ant attacks (Bain

et al., 2014; F. Kjellberg and J.-Y. Rasplus, pers. comm.). In Africa, an encyrtid parasitic wasp of ant-tended hemipterans on *F. sur* also seems to escape ant attention through similar means (Zachariades *et al.*, 2009).

Compared with other plants, fig trees may be unusually benefited by hosting ants. While ants generally have a positive effect on their plant hosts, the three-fold fitness increase (3.5, including seed number, female pollinators and likelihood of maturation) for the tree that I documented here is definitely on the higher end of the plant performance effect typically documented for ants present on plants (Chamberlain & Holland, 2009). Even if I only look at the effect on seed numbers (1.8), or the effect on seed numbers combined with likelihood of fig maturation (2.4), the effect size of having ants vs. not having ants is for this study at the high end of the range. Although the experiment in this study was only on one tree, the strong protective fitness effect documented here is in line with what I typically observe at the study site when aggressive ants such as *Azteca* are present. Similarly, the effect of *Oecophylla* ants on the performance of *F. racemosa* in China was very strong, with over 5 times more pollinators and 2.5 times more seeds from figs on 13 trees where *Oecophylla* ants were present compared to 14 trees without (Wang *et al.*, 2014). The remarkably strong fitness effect seen for fig trees is likely because ants on figs specifically protect the seeds and the pollen dispersers rather than merely foliage. Fig trees also have a very special floral morphology with the flowers on the inside of a hollow inflorescence, which asymmetrically exposes parasites to ant predation while pollinators are protected [an exception are some Old World parasitic wasps that enter the interior of figs; while they are not affected by the presence of ants (Compton & Robertson, 1988; Cushman *et al.*, 1998), they may act as passive pollinators (Jousselin *et al.*, 2001)]. While *F. obtusifolia* and the other fig species in this study did not seem to present any rewards for their inhabiting ants other than intermittent food abundance when pollinators and parasites are arriving and emerging, this food reward may be sufficient to entice predatory ants to make their nests on fig trees rather than on other trees. Indeed, fig wasps alone may be a sufficient reward to attract ants: among dioecious fig trees on Borneo predatory ants and ant nests were 10 times more common on male trees (producing wasps) than on female trees (producing seeds only) (Schatz *et al.*, 2008). There are also a few reports of fig species that provide specific rewards to their inhabiting ants, such as hollow stems for nesting and/or extrafloral nectaries (Koptur, 1992; Maschwitz *et al.*, 1994; Blüthgen, 2003; Bain *et al.*, 2012, 2014; Harrison, 2014). Given the strong fitness consequences involved, if the results here and elsewhere are representative, I suspect that we will find more fig traits that encourage the presence of ants as we learn more about the many fig species around the world.

In conclusion, this study and earlier work show that not only does the presence of ants benefit fig pollinators, it also benefits the host tree. Ants are common inhabitants of fig trees, being present on 46% of surveyed fig trees in Africa (Cushman *et al.*, 1998), 100% in Costa Rica (Bronstein, 1988), and 79% in Panama. Aggressive ants protect seeds and pollinators against parasitic wasps and herbivores, and the presence of ants, therefore, increases the fitness of both pollinators and the fig tree.

Although trees may be paying their ant guards both a proportion of their phloem (through sapsuckers) and their pollen-dispersers, the price seems to be very low compared with the overall fitness benefit. Ants thus seem to be both common and powerful protectors of the fig mutualism, a keystone mutualism in tropical forests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:

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Appendix S1. Table S1 listing ant genera and species found in the ant survey; Figure S1 showing the duration of oviposition for different NPFW genera; Captions for videos 1–3.

Video S1. Video1_Idarnesflattening.

Video S2. Video2_Idarnescaught.

Video S3. Video3_emergingpollinators.

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Online supplement, Appendix S1

Indirect mutualism: ants protect fig seeds and pollen dispersers from parasites

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Table S1. Ant genera/species on the 16 trees in the survey where ants were present and collected (15 trees with ham bait and one without, *F. obtusifolia* Miller). J. Longino kindly identified the ants. Ants were further present but not collected on *F. citrifolia* (3 trees), *F. dugandii* (1), *F. maxima* (1), *F. nymphaeifolia* (1), *F. obtusifolia* (1), *F. popenoei* (1), *F. triangle* (2), *F. trigonata* (1). Ants were absent on *F. citrifolia* (1 tree), *F. costaricana* (1), *F. insipida* (2), *F. obtusifolia* (2), *F. perforata* (1).

Ficus species	Tree ID	Ant species
<i>F. insipida</i>	IT#1	<i>Azteca</i> sp. (instabilis group) <i>Camponotus</i> cf. <i>mucronatus</i> <i>Pseudomyrmex</i> sp. (pallidus group) <i>Cephalotes umbraculatus</i>
<i>F. insipida</i>	IT#2	<i>Azteca</i> sp. (instabilis group) <i>Camponotus</i> sp.
<i>F. maxima</i>	BS TC	<i>Azteca</i> sp. (trigona group?) <i>Camponotus novogranadensis</i>
<i>F. maxima</i>	BS#1	<i>Azteca</i> sp. (instabilis group)
<i>F. maxima</i>	BS#2	<i>Azteca</i> sp. (instabilis group) <i>Camponotus novogranadensis</i> <i>Camponotus</i> cf. <i>mucronatus</i>
<i>F. maxima</i>	BS#3	<i>Dolichoderus bispinosus</i>
<i>F. maxima</i>	BS#5	<i>Azteca trigona</i> <i>Camponotus</i> sp. (Myrmobrachus)
<i>F. dugandii</i>	BS#19	<i>Azteca</i> sp. (alfari group) <i>Camponotus novogranadensis</i> <i>Crematogaster curvispinosa</i>
<i>F. dugandii</i>	BS#20	<i>Azteca</i> sp. (alfari group) <i>Azteca</i> sp. (beltii-like)
<i>F. dugandii</i>	BS#25	<i>Azteca</i> sp. (instabilis group)
<i>F. nymphaeifolia</i>	BC#1	<i>Azteca</i> sp. (instabilis group) <i>Camponotus</i> sp.
<i>F. nymphaeifolia</i>	BC#2	<i>Azteca</i> sp. (instabilis group) <i>Crematogaster erecta</i>
<i>F. obtusifolia</i>	BS#23	<i>Azteca trigona</i> <i>Camponotus sericeiventris</i>
<i>F. obtusifolia</i>	IT#1	<i>Azteca</i> sp. (instabilis group) <i>Camponotus</i> sp.
<i>F. obtusifolia</i>	Miller (no ham bait)	<i>Camponotus</i> cf. <i>mucronatus</i> <i>Cephalotes minutus</i> <i>Crematogaster carinata</i>
<i>F. popenoei</i>	IL#1	<i>Azteca</i> sp. (instabilis group)

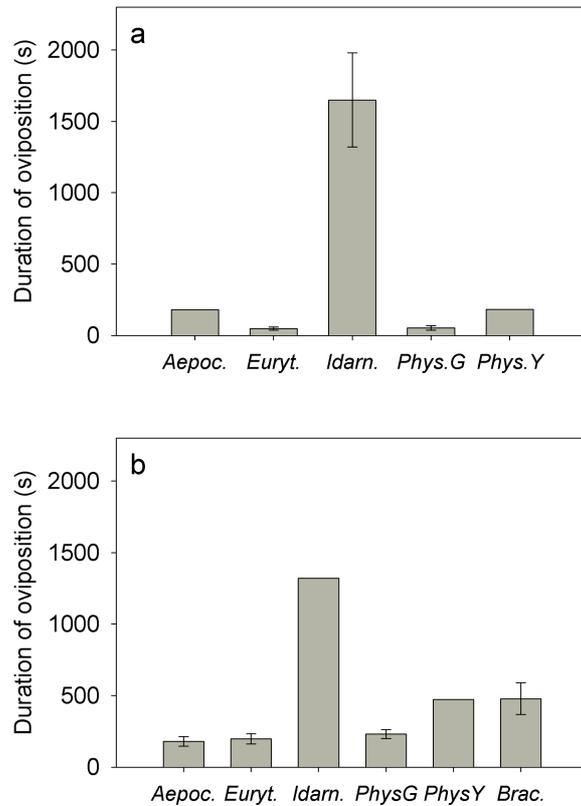


Figure S1. The duration of oviposition varied greatly across the different parasitic wasp genera/groups, with *Idarnes* spp. wasps taking overwhelmingly longer both on (a) *F. obtusifolia* and (b) *F. dugandii*. Error bars represent ± 1 SE. Abbreviations: *Aepoc.* = *Aepocerus* spp., *Euryt.* = *Eurytoma* spp., *Idarn.* = *Idarnes* spp. (*carne/flavicollis* groups), *Phys.G* = *Physothorax* spp. (Green), *Phys.Y* = *Physothorax* spp. (Yellow), *Brac.* = *Braconidae*.

Video captions

Video1_Idarnesflattening. Three *Idarnes* sp. wasps attempting to oviposit on a *Ficus obtusifolia* fig that is patrolled by *Azteca trigona* ants. Both the wasp that is inserting her ovipositor (upper left), and the wasp that is walking while searching for an oviposition site (middle) flatten and freeze when an ant approaches. The wasp that is ovipositing already (right) remains flat and still. None of the wasps are attacked by the ant despite being investigated.

Video2_Idarnescaught. Several *Azteca trigona* ants on *F. obtusifolia* are attacking an *Idarnes* sp. wasp that failed to flatten.

Video3_emergingpollinators. Female pollinators of *Ficus obtusifolia*, *Pegoscapus hoffmeyeri*, are emerging from the exit hole (top) one by one. Some have already been caught and are being surrounded by ants (*Azteca trigona*). Towards the end of the video a pollinator is caught by an ant waiting by the exit.