Flowers attract weaver ants that deter less effective pollinators

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Summary

1. Many flowers produce ant-repellent substances that prevent ants from discouraging pollinator visits. When a flower's most effective pollinator is unaffected by predatory ants, however, flowers should benefit from the presence of ants that deter less effective pollinators from consuming resources.

2. Behavioural assays revealed that *Melastoma malabathricum* flowers, pollinated by large carpenter bees, *Xylocopa* spp., produce ant attractants that recruit weaver ants, *Oecophylla smaragdina*.

3. The presence of ants was associated with an increase in the reproductive success of *M. malabathricum* flowers. This outcome likely resulted from the filtering effect of ants on the community of flower visitors: ants deter less effective pollinators and attract *Xylocopa* bees through an indirect effect on resource depletion.

4. *Synthesis*. Although plant–pollinator interactions are classified as mutualisms, not all flower visitors are effective pollinators, and some can be parasites or conditional parasites. As a result, predators that deter flower visitors can have positive or negative effects on plant fitness, depending on whether they deter all visitors or a subset of them, and on the relative effectiveness of deterred and undeterred visitors.

Key-words: ambush predators, ant–plant interactions, ant repellents, mutualism, *Oecophylla smaragdina*, tritrophic interactions, reproductive ecology

Introduction

Arboreal ants can benefit their host plant through the consumption of herbivorous insects. Plants have developed an array of strategies, such as providing ants with food or shelter, to attract them (Janzen 1966) – leading to tight mutualistic relationships between both taxonomic groups (Davidson, Snelling & Longino 1989; Fiala *et al.* 1994; Pringle, Dirzo & Gordon 2011). Ants, however, are likely to attack all insects visiting the plant, and their presence risks deterring pollinators from visiting flowers (Willmer & Stone 1997; Tsuji *et al.* 2004; Willmer *et al.* 2009), potentially decreasing the plant's reproductive success. To counteract this negative effect, some plant species produce ant-repellent substances during the flower's fertile period (Willmer & Stone 1997; Ghazoul 2001; Raine, Willmer & Stone 2002; Junker, Chung & Bluthgen

2007; Willmer *et al.* 2009), ensuring pollination without losing the protection of ants. Ants are not alone in interfering between plants and their pollinators. Other predators, notably crab spiders, ambush visiting insects at flowers (Morse 2007), affecting the foraging choices of pollinators (Dukas 2001; Dukas & Morse 2003) and sometimes reducing plant reproductive success. It has been suggested, however, that plants may benefit from the presence of ants on flowers or other ambush predators. This would be the case if their negative effect (reduction in pollinator visits) is compensated by the protection they offer by removing florivorous insects and seed predators (Higginson, Ruxton & Skelhorn 2010).

We further predict that plants could benefit from ants ambushing at flowers if they selectively deter ineffective pollinators and that this benefit could promote floral traits that raise the probability of ant recruitment at flowers. To test whether flowers attract ants that deter ineffective pollinators, thus increasing the plant's reproductive success, we conducted a number of experiments and observations on the interaction between weaver ants, *Oecophylla smaragdina* Fabricius, the tropical shrub *Melastoma malabathricum* L. and its flower

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visitors. Our aim was accomplished by performing the following steps: (i) we first studied the association between plant quality and weaver ant presence, (ii) we then quantified bee visit rates at plants with and without weaver ant nests, (iii) we assessed the pollination effectiveness of the different flower visitors, (iv) we examined the association between weaver ant presence and fruit and seed set of the host plant, (v) we tested whether weaver ants were attracted to flowers and, finally, to study the mechanisms responsible for the bee foraging choices, (vi) we developed an optimal-foraging model and compared its predictions with observed patterns.

Materials and methods

The study was carried out at MacRitchie Reservoir in the Central Catchment Nature Reserve, Singapore, from April to June 2010. Weaver ants are extremely aggressive generalist predators that build their nests with the living leaves of a broad range of tree and shrub species. In our study site, they colonized about half of the *M. malabathricum* plants, where we often observed them patrolling flowers or tending aphids at their base. The nectarless flowers of *M. malabathricum* have an inner and outer whorl of stamens, apically poricidal, and attract bees able to extract pollen by sonication. This species is a self-compatible shrub, but pollen vectors are required to effect fruit set (Gross 1993). Flowers are visited by a wide array of insects, but due to the gap between anthers and stigma, only large bees seem likely to effect pollination. In our study population, the most common visitors to *M. malabathricum* flowers were two carpenter bees, *Xylocopa latipes* Drury and *X. confusa* Pérez, and a smaller solitary bee, *Nomia strigata* Fabricius: of the 436 visits to unmanipulated *M. malabathricum* flowers that we recorded (see Effect of weaver ant presence on bee visits), 432 (99.1%) were by *Xylocopa* and *Nomia* bees. We therefore restrict our study to *Xylocopa* and *Nomia* bees, ignoring in what follows the possible effect of infrequent visitors such as *Amegilla zonata*, *Ceratina* spp. or *Lasioglossum* spp.

COLONIZATION BY WEAVER ANTS AND PLANT QUALITY

We selected and tagged 25 *M. malabathricum* plants with weaver ant nests and 25 plants without nests. If a trait differed between colonized and uncolonized plants, the difference could result from the presence of ants at some plants, or from some other factor, which affected the trait under study and the probability of ant colonization. To estimate the possibility that ant-colonized plants had higher fitness because ants selected high-quality plants to build their nests, we assessed plant quality through height, number of flowers per plant and day (as most *M. malabathricum* flowers last one single day; Gross 1993) and pollen production per flower. Stamens from bagged, unvisited flowers were kept in 70% ethanol, and the number of pollen grains produced was determined by counting under a microscope (Olympus BHT-BH2) as detailed in Luo, Zhang & Renner (2008). We used *t*-tests to determine whether plants with and without weaver ant nests differed in height, number of flowers or pollen production.

ASSOCIATION BETWEEN WEAVER ANT PRESENCE AND BEE VISITS

We observed bee visit rates at the 50 tagged plants. Each plant was observed four times, in pseudorandom order (i.e. no plant was observed twice before all other plants had been observed at least once). At each observation, we selected four flowers and recorded the number of times that they were visited by *Xylocopa* and *Nomia* bees over a 10-min period. The number of *Xylocopa* and *Nomia* visits per plant was averaged over the four observation periods. Averages were log transformed to achieve homogeneity of variances and analysed with a repeated-measures ANOVA, having plant as subject, bee species as within-subject repeated measures and ant presence as a categorical factor. A preliminary analysis distinguished between *X. latipes* and *X. confusa* visits. We found no differences between the two *Xylocopa* species. To increase the power of the tests comparing *Xylocopa* and *Nomia*, which was the main purpose of the study, and given that we observed no obvious differences in their time of activity or behaviour, in this and subsequent analyses, we pooled data from the two *Xylocopa* species.

POLLINATION EFFECTIVENESS OF *NOMIA* AND *XYLOCOPA* BEES

We evaluated the pollination effectiveness of *Nomia* and *Xylocopa* bees by measuring pollen removal, as well as fruit and seed set of flowers with known visitation histories. To study pollen removal, we bagged flowers before opening. Upon unbagging the flowers, we removed an internal and an external stamen to assess pollen production, observed the flowers and, after a known number of bee visits, removed all remaining stamens to determine pollen removal rates. The number of pollen grains remaining after a known number of visits was determined as explained previously (Luo, Zhang & Renner 2008). The amount of pollen left in anthers was fitted to a generalized linear model (GLM) with normal distribution and log link function, having the number of *Xylocopa* and *Nomia* visits as continuous covariates, anther origin (internal or external stamen) as a categorical variable and the two-first-order interactions between number of visits and anther origin. Statistical significance was assessed through likelihood ratio tests for this and subsequent GLM analyses. In these analyses, we selected the combination of error distribution and link function that provided the best fit (lowest AIC; Akaike 1987) to our data.

We studied the effect of bee visits on fruit and per-fruit seed set by following the same set of bagged flowers until fruit ripening. For each mature fruit, we estimated seed production by counting the number of viable (white) seeds for one carpel and multiplying by five. The relationship between the probability of fruiting and the number of bee visits was analysed with a GLM with binomial error distribution and complementary log–log link function ($\log(-\log(1 - \pi))$). The relationship between seed production per fruit and the number of bee visits was analysed with a GLM with gamma distribution and power link function. In both cases, models included the number of *Xylocopa* and *Nomia* visits as separate, continuous covariates.

ASSOCIATION BETWEEN WEAVER ANT PRESENCE AND FRUIT AND SEED SET

We marked 12 flowers per plant on 25 plants with ant colonies and 25 without ant colonies to determine the relationship between ant presence and fruit set and both per-fruit and per-plant seed set, although 16 marked flowers were lost due to branches breaking during storms in plants with ant nests. We estimated fruit set by following flowers until fruit ripening. Per-fruit seed set was calculated by counting the number of viable seeds for one carpel and multiplying by five. Per-plant seed set was estimated by multiplying, for each plant, mean per-fruit seed set by the number of flowers produced by

the plant and the proportion of marked flowers that produced a fruit. This is an estimate of the number of seeds produced per plant each day. The relationship between the probability of fruiting and ant presence was analysed with a GLM with binomial distribution and logit link function. Estimated per-fruit and per-plant seed sets of plants with and without weaver ants were compared with GLMs, introducing plant as a random factor in the former analysis.

ATTRACTION TEST

To test whether *M. malabathricum* flowers produce ant-attracting cues, we wiped one half of a 14-cm-diameter Petri dish with a newly opened flower and the other half with a 2-day-old withering flower (Ghazoul 2001). We collected weaver ants from nests without *M. malabathricum* in their foraging territories, and we set an individual ant in the centre of the dish, recording over five minutes the amount of time spent on each half. The experiment was performed using 20 replicates (a different plant and ant colony was used for each replicate). To confirm that the preference for the 'new flower' side was due to an ant-attracting cue in new flowers, rather than to the presence of ant-repellent substances in withering flowers, we repeated the test using *M. malabathricum* leaves and twigs instead of new flowers. Because residuals were normally distributed for each data set, we used paired t-tests to check whether ants spent more time on one side of the Petri dish or the other.

All analyses were carried out using STATISTICA version 10 (StatSoft, Inc. 2011) except for those testing the relationship between seed set and ant presence, which were conducted on R version 2.8.0. (R Development Core Team. 2010).

EFFECTS OF TROPHIC COMPETITION ON BEE VISITS

The pattern of bee visit rates that we observed suggested that *Nomia* bees were visiting mainly flowers where predation risk was lowest and that *Xylocopa* bees were avoiding competition for resources. To assess the likelihood of this interpretation, we developed an optimality model to predict the frequency of *Xylocopa* visits to flowers at plants with and without weaver ant nests. The model was parameterized with data collected in this study and assumed that each *Xylocopa* bee foraged in such a way as to maximize her pollen intake rate, taking into account how other bees were behaving. Model predictions were then compared with the rates at which *Xylocopa* bees visited flowers at plants with and without ant nests.

Results

COLONIZATION BY WEAVER ANTS AND PLANT QUALITY

Height was similar for *M. malabathricum* plants with and without ant nests (159.56 ± 41.90, 163.44 ± 50.24 cm, respectively; $t_{48} = -0.29$, $P = 0.38$), showing that ants did not select large (or small) plants for their nests. The number of flowers opening per day was also similar in plants with and without ants (7 ± 2.56, 6.8 ± 2.98, respectively; $t_{48} = -0.25$, $P = 0.80$), indicating that ants did not select plants with more flowers either. We found no differences ($t_{48} = -0.54$, $P = 0.58$) in the number of pollen grains produced by external stamens of flowers in plants with ($119\,73$ $2.00 \pm 16\,507.57$) and without ($116\,251.55 \pm 21,264.92$) ant nests. Pollen production in internal stamens of plants with ($92\,004.00 \pm 11\,781.57$) and without ($89\,276.00 \pm 9408.34$)

ant nests was also similar ($t_{48} = -0.90$, $P = 0.37$). It follows that ants did not select to build their nests in the most (or least) productive plants.

ASSOCIATION BETWEEN WEAVER ANT PRESENCE AND BEE VISITS

While the average rate at which bees visited flowers was similar at plants with and without ant nests ($F_{1,48} = 0.15$, $P = 0.70$) (Fig. 1), there was a strong interaction ($F_{1,48} = 32.75$, $P < 0.0001$) between plant type and bee species: small *Nomia* bees concentrated their foraging effort on ant-free plants and large *Xylocopa* bees preferentially exploited flowers on ant-harbouring plants (Fig. 1). When exploiting ant-harbouring plants, *Nomia* bees spent several seconds inspecting flowers, seldom landing on ant-harbouring flowers and being readily captured or chased away by the ants whenever they did so (Appendix S1). *Xylocopa* bees, on the other hand, were unaffected by the presence of ants and ignored their attacks (Appendix S2), occasionally flying off to the next flower with an ant hanging from their legs.

POLLINATION EFFECTIVENESS OF *NOMIA* AND *XYLOCOPA* BEES

The amount of pollen left in anthers decreased exponentially with the number of *Xylocopa* ($\chi_1^2 = 250.39$, $P < 0.0001$) and *Nomia* ($\chi_1^2 = 287.62$, $P < 0.0001$) visits (Fig. 2). Pollen removal was also affected by anther type ($\chi_1^2 = 68.69$, $P < 0.0001$) but not by the interactions between anther type and the number of either *Xylocopa* ($\chi_1^2 = 3.07$, $P = 0.079$) or *Nomia* ($\chi_1^2 = 0.30$, $P = 0.584$) visits. Both species extracted comparable amounts of pollen per visit [confidence intervals of regression coefficients for *Xylocopa* and *Nomia* visits: $(-0.64, -0.46)$ and $(-0.78, -0.56)$, respectively].

Despite the efficiency of *Nomia* bees at collecting pollen, they only fertilized flowers when they accidentally landed on the stigmas. Of 185 flowers visited exclusively by *Nomia* bees, only 11 (5.9%) produced fruits, while 53.9% of the 206 flowers visited by *Xylocopa* bees set fruit (G-test for the dif-
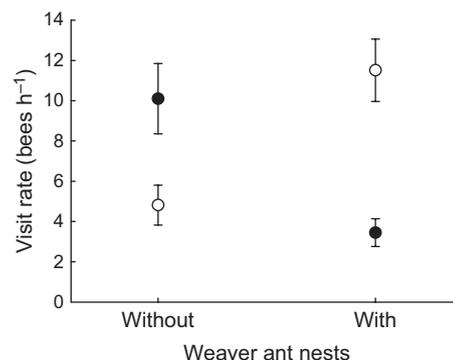
Fig 1. Visit rates by *Nomia* (black circles) and *Xylocopa* (empty circles) bees at plants with and without weaver ant nests. Error bars represent standard errors.
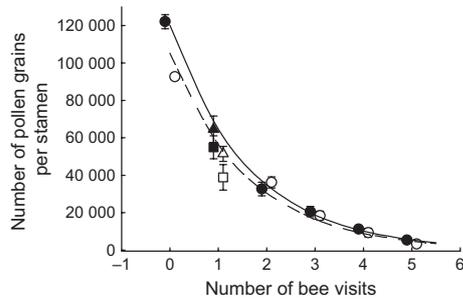
Fig 2. Pollen removal. Exponential decrease in the number of pollen grains left in outer (black symbols) and inner (empty symbols) anthers with increasing numbers of bee visits. For the case of a single bee visit, we distinguish between the number of pollen grains remaining after a *Nomia* (squares) or *Xylocopa* (triangles) visit. For more than one visit, different combinations are pooled for clarity. Error bars are standard errors. Lines represent the fitted regressions (solid line external anthers, dotted line internal anthers) for the most parsimonious model.
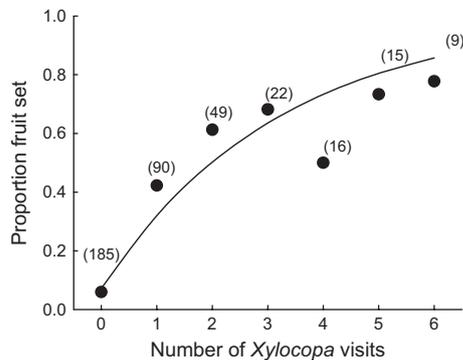
Fig 3. Increase in proportion of fruit set with the number of *Xylocopa* visits. Proportion of flowers producing mature fruits vs. the number of *Xylocopa* visits. Numbers in brackets are sample sizes. Line represents the fitted regression for the model with lowest AIC, log(1-fruit set) = −0.074 to 0.312 *Xylocopa* visits.

ference between flowers with and without *Xylocopa* visits: $G = 114.75$, $P < 0.0001$. When the analysis was restricted to flowers that received a single bee visit, 27 of 55 flowers visited only by a *Xylocopa* bee set fruit, while only 5 of 64 flowers visited by a single *Nomia* bee set fruit ($G = 24.87$, $P < 0.0001$). Moreover, for those flowers receiving several *Xylocopa* visits, the probability of setting fruit increased with the number of visits ($\chi_1^2 = 109.66$, $P < 0.0001$) (Fig. 3), while the effect of increasing numbers of *Nomia* visits was not statistically significant ($\chi_1^2 = 0.62$, $P = 0.431$).

When we concentrated on those flowers that had actually set fruit, however, the number of viable seeds produced per fruit (per-fruit seed set) was not affected significantly by the number of *Nomia* (slope: mean ± SE -1.27 ± 13.62; $\chi_1^2 = 0.007$, $P = 0.93$) or *Xylocopa* (slope: mean ± SE 28.33 ± 21.26; $\chi_1^2 = 2.34$, $P = 0.12$) visits, although there was a tendency for per-fruit seed set to increase in fruits with more than three *Xylocopa* visits: per-fruit seed set was 363.90 ± 35.25 when there were three or fewer *Xylocopa* visits and 469.22 ± 35.25 when there were more than three visits ($F_{1,67} = 2.23$, $P = 0.14$). As a

result, per-fruit seed set was similar for flowers that had been successfully fertilized by *Nomia* (mean ± SD: 457.12 ± 274.44; $N = 8$) and *Xylocopa* (398.30 ± 291.69; $N = 61$).

To recap, although *Nomia* and *Xylocopa* bees removed similar amounts of pollen per visit, *Xylocopa* bees were much more likely to fertilize flowers. Nevertheless, once a flower had been successfully fertilized, seed set did not depend on the number of visits it had received or the species of the bee that had fertilized the flower.

ASSOCIATION BETWEEN WEAVER ANT PRESENCE AND FRUIT AND SEED SET

Because ant-harbouring plants received more visits from the most effective pollinators, *Xylocopa* bees, ant-free plants were visited mainly by the least effective pollinators, *Nomia* bees. Fruit set, per-fruit seed set and per-plant seed set were higher at plants with ant nests. Only 40% ($N = 300$) of marked flowers in ant-free plants set fruit, while fruit set increased to 65% ($N = 284$) in plants with ant nests ($\chi_1^2 = 38.46$, $P < 0.0001$) (Fig. 4). In the same way, fruits at ant-free plants produced significantly fewer seeds than fruits at ant-harbouring plants ($F_{1,48} = 31.50$, $P < 0.0001$; mean ± SD: 731.2 ± 191.1 and 1194.0 ± 297.3, respectively) (Fig. 4). Note that per-fruit seed set in unbagged flowers was double that in bagged flowers, probably because unbagged flowers received more bee visits than bagged ones. This difference suggests that per-fruit seed set increases with the number of *Xylocopa* visits and that the trend detected with bagged flowers (per-fruit seed set was higher when flowers received more than three *Xylocopa* visits, although the difference was not statistically significant) would have become significant if we had left some flowers exposed for a longer time. Because plants with and without ant nests produced similar numbers of flowers, while fruit set and per-fruit seed set were higher at plants with ant nests, per-plant seed set was 2.7 times higher at plants with weaver ant nests (5281.70 ± 555.11) than at plants without nests (1941.13 ± 253.20; $F_{1,48} = 30.79$, $P < 0.0001$) (Fig. 4).

ATTRACTION TEST

Ants spent significantly more time on the 'new flower' than on the 'withering flower' half of the dish ($t_{19} = -3.53$, $P = 0.002$) (Fig. 5). When we repeated the test using *M. malabathricum* leaves and twigs instead of new flowers, ants spent similar amounts of time on both sides of the dish ($t_{19} = 0.30$, $P = 0.77$ and $t_{19} = -0.18$, $P = 0.86$, respectively) (Fig. 5), confirming that the preference for the 'new flower' side was due to an ant-attracting cue in new flowers, rather than to the presence of ant-repellent substances in withering flowers.

EFFECTS OF TROPHIC COMPETITION ON BEE VISITS

As we have seen, *Nomia* bees preferentially visited flowers at ant-free plants, and *Xylocopa* bees preferentially visited flowers at ant-harbouring plants. The behaviour of *Nomia* bees can be explained as an antipredator response. But how
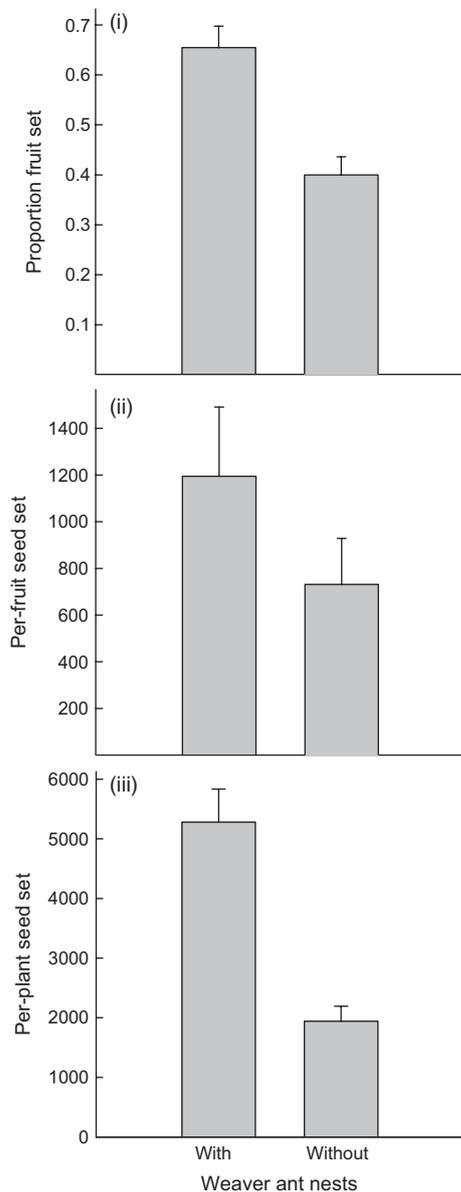
Fig 4. Effect of weaver ant presence on (i) proportion fruit set, (ii) per-fruit seed set and (iii) per-plant seed set. (i) Proportion of flowers producing mature fruits at plants with and without weaver ant nests. (ii) Number of viable seeds per fruit produced at plants with and without weaver ant nests. (iii) Estimated number of viable seeds per plant produced in a day at plants with and without weaver ant nests. Error bars are standard errors.

do we explain the preference of *Xylocopa* bees for ant-harbouring plants? Because weaver ants pose little threat to the large *Xylocopa* bees, which ignored the presence of ants at flowers and their attacks (see Results), we hypothesize that *Xylocopa* bees were simply avoiding interspecific competition, foraging at those plants where pollen depletion was less severe. To assess the viability of this hypothesis, we calculated the optimal-foraging strategy of *Xylocopa* bees and compared it with the strategy we observed.

The foraging strategy of a bee exposed to the risk of predation is affected by factors such as the proportion of flowers harbouring predators, the ability of bees to detect the presence
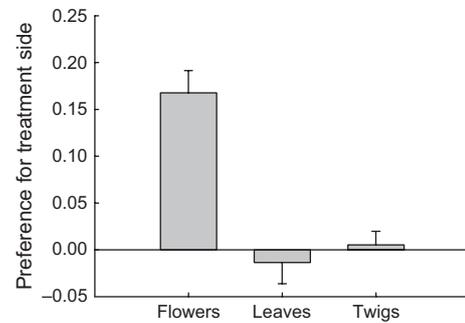
Fig 5. Attraction of weaver ants to *Melastoma* flowers. Ant preference for the half of the Petri dish wiped with new flowers, leaves or twigs. For simplicity, data are plotted as preference for the treatment side (i.e. newly open flower, leaf or twig) relative to the control side (withering flower). Preference = (time (in secs) spent on treatment side − 150)/150. This preference index ranges from −1 if ants spent all the time in the control side to +1 if they spent all the time in the treatment side. Error bars are standard errors.

of predators and their probability of being captured upon landing on a predator-harbouring flower (Clark & Dukas 1994 Rodríguez-Gironés & Bosch 2012). Because most of these parameters are unknown for the *Nomia*–weaver ant system, we cannot determine the extent to which *Nomia* bees were following their optimal strategy. For *Xylocopa* bees, on the other hand, we can assume that there was a negligible predation risk and calculate their optimal-foraging strategy, given how *Nomia* bees were behaving.

If *Xylocopa* bees were optimal foragers, the proportion of *Xylocopa* visits to flowers of plants with and without ant nests would be such that the expected pollen intake rate per flower of a *Xylocopa* bee would be independent of the plant type it exploited (Possingham 1992). If we assume that handling times are equal at flowers of plants with and without weaver ant nests (the validity of this assumption will be checked below), the equality of intake rates translates into equality of resources found per flower. In other words, if *Xylocopa* bees were optimal foragers, pollen removal would follow the same pattern in flowers of plants with and without ant nests. Because the amount of pollen removed per visit by *Nomia* and *Xylocopa* bees was similar, pollen depletion depended solely on the total rate at which flowers were visited, regardless of the bee species that was visiting the flowers. Thus, the condition for optimal foraging is simply that the rate at which flowers were visited, combining *Xylocopa* and *Nomia* visits, was the same for flowers of plants with and without ant nests.

Flowers of plants with and without ant nests received an average of 3.45 and 10.09 *Nomia* visits per hour, respectively (Fig. 1). If we denote by v_0 and v_1 the rate of *Xylocopa* visits to flowers at plants without and with ant nests, then the optimal-foraging condition becomes as follows:

$$10.09 + v_0 = 3.45 + v_1 \qquad \text{eqn 1}$$

Equation 1 does not allow us to predict optimal visit rates in absolute terms: the actual rate at which *Xylocopa* bees are expected to visit flowers depends on many factors, such as the number of bees and flowers, the distance between bee

nests and *M. malabathricum* plants, and the availability of alternative resources. Nevertheless, eqn (1) predicts the relationship between *Xylocopa* visit rates to flowers of plants with and without weaver ant nests:

$$v_1 - v_0 = 6.64. \qquad \text{eqn 2}$$

In our observations, we found (Fig. 1) $v_1 = 11.51 \pm 1.55$ and $v_0 = 4.81 \pm 0.99$ visits per hour, so that $v_1 - v_0 = 6.70$ – in perfect agreement with the predicted difference of 6.64 visits per hours.

Equations 1 and 2 rely on the assumption that *Xylocopa* handling times were similar for flowers at plants with and without weaver ant nests. To check this assumption, we videotaped 68 *Xylocopa* visits (34 at plants with ant nests and 34 at plants without ant nests) and counted the number of frames from landing on the flower to departure. *Xylocopa* handling times (mean $\pm$ SD) were 1.20 ± 0.33 s for flowers at plants with ant nests and 1.16 ± 0.17 s in plants without ant nests. This difference was not statistically significant ($t_{66} = 0.29$, $P = 0.77$), confirming the validity of the model's assumption.

Discussion

The presence of weaver ant nests was associated with an increase in the reproductive success of *M. malabathricum* shrubs: fruit set, per-fruit seed set and per-plant seed set were higher at plants with than without nests. This was most likely the indirect effect of changes in small-bee behaviour in response to predation risk by ants. Small bees were easily captured by ants and avoided plants with ant nests, quickly depleting resources at ant-free shrubs. Larger *Xylocopa* bees, safe from predation by weaver ants, responded to interspecific competition by concentrating their foraging effort at ant-harbouring plants. Because *Xylocopa* bees were by far the most effective pollinator of *M. malabathricum* flowers, the combination of the antipredator response of *Nomia* bees and competition avoidance of *Xylocopa* bees was associated with a higher reproductive success of ant-harbouring plants. Although we did not quantify pollen flow, given that both *Nomia* and *Xylocopa* bees removed similar amounts of pollen and that the probability of pollen transfer from bees to flower stigmas was much higher for *Xylocopa* bees (*Nomia* bees seldom fertilized flowers), male reproductive success must also have been higher for plants with weaver ant nests than for plants without nests.

In principle, the higher reproductive success of *M. malabathricum* plants with ant nests could be causally unrelated to the presence of ants. For instance, ants might be building their nests in more vigorous plants or on those growing on more fertile soil. This explanation, however, seems unlikely. Plant density was very high in our population, and ant-colonized plants were interspersed between the ant-free plants. If abiotic factors were affecting plant growth and ant colonization, the heterogeneity of such factors must take place in a very small spatial scale. Most important, plants with and without ant nests had similar sizes, numbers of flowers and

pollen production rates, making it unlikely that ants were selecting plants with traits correlated with higher fecundity.

BEE BEHAVIOUR

A number of studies have shown that social and solitary bees are able to avoid predator-harbouring flowers, plants and patches (Dukas 2001, 2005; Schmalhofer 2001; Dukas & Morse 2003). While it is not presently clear whether bees learn to associate some areas with predators and avoid foraging at them, or whether they detect and avoid predator-harbouring flowers, avoidance of ant-harbouring plants by *Nomia* bees fits well with existing literature. Why, however, were *Xylocopa* bees attracted to ant-harbouring plants? We suggest that *Xylocopa* bees were simply maximizing pollen intake rate and preferred plants where interspecific competition was lowest. The tight fit of optimal-foraging model predictions to the data supports this interpretation. As for the mechanism allowing *Xylocopa* bees to concentrate their foraging effort in plants with weaver ant nests, we know little about their foraging strategies, but comparison with other bee species may shed some light on the issue. Bees can track changes in the spatial distribution of resources (Keasar 2000; Cartar 2004; Westphal, Steffan-Dewenter & Tscharntke 2006; Lihoreau, Chittka & Raine 2010, 2011). Bumblebees, for instance, change their foraging territories when competitors exploiting neighbouring patches are experimentally removed (Thomson, Peterson & Harder 1987). If *Xylocopa* bees have similar cognitive abilities, it is not surprising that they concentrate their foraging effort where competition with *Nomia* bees is lowest. In this respect, it should be noted that resource partitioning is facilitated by the slow turnover rate of ant-harbouring plants: ant nests can remain in the same plants for months (personal observations).

EVOLUTION OF ANT ATTRACTION

Through its geographical range, *M. malabathricum* flowers are visited by a diverse array of small bees. However, due to the large gap between their anthers and stigma, *Xylocopa* bees seem to be their main pollinator. In contrast, small bees behave as less effective pollinators: they provide *M. malabathricum* with a weak pollination service in the absence of effective pollinators, but when *Xylocopa* bees are abundant, they decrease its pollination success. This happens because small bees, which seldom fertilize flowers, make them less attractive to the most effective pollinators, reducing the rate at which they visit flowers. Even worse: small bees sometimes scavenge pollen from stigmas in the late morning (Gross & Mackay 1998). Because weaver ants and *M. malabathricum* have almost identical distributional ranges and the two species have co-existed for at least 1 million years (Renner & Meyer 2001; Azuma *et al.* 2006), it seems likely that the same ecological play presented in this article, incorporating the interactions between flowers, ambush predators, pollinators and less effective pollinators, has been repeated generation after generation (with minor variations, perhaps, in the iden-

tity of the secondary characters, the less effective pollinators), applying a selective pressure on *M. malabathricum* to encourage weaver ants to patrol their flowers.

Possibly as a result of these selective pressures, *M. malabathricum* produces some unidentified cues (not necessarily volatile substances) that entice *O. smaragdina* ants to patrol flowers during anthesis. Although future work will be required to evaluate whether the production of these cues is a genuine adaptation, this possibility receives support from the fact that, of all flowers tested so far, *M. malabathricum* is only the second one reported to produce ant-attracting cues in fertile flowers. Of 64 plant species tested so far (Willmer & Stone 1997; Ghazoul 2001; Raine, Willmer & Stone 2002; Ness 2006; Junker, Chung & Bluthgen 2007; Agarwal & Rastogi 2008; Junker & Bluthgen 2008; Willmer *et al.* 2009; Schiestl & Glaser 2012), production of ant-repellent substances has been reported in 73% of the species studied. Attraction of ants to flower odours has only been reported twice. In the first case, volatiles produced by *Luffa cylindrica* flowers repel large ant species, but attract the tiny nectar-feeding *Tapinoma melanocephalum* (Agarwal & Rastogi 2008). Nevertheless, because the *T. melanocephalum* individuals used for the experiments regularly consumed nectar at *L. cylindrica* flowers, it is unclear whether flowers produce substances to attract them. It seems just as likely that *T. melanocephalum* ants had learnt to associate flower odours with the presence of nectar. In the second case reported, the alpine orchid *Chamorchis alpine* is pollinated by ants and uses floral scents to attract them (Schiestl & Glaser 2012). Here, however, we report for the first time that floral substances may play a new role by attracting predatory ants to flowers to deter ineffective floral visitors. Attraction of ambush predators to flowers can be seen as an indirect means of resource concealment, which may have evolved because predators deter ineffective pollinators, leading to a reduction in the rate of resource depletion that further attracts more effective pollinators (Rodríguez-Gironés & Santamaria 2005).

More importantly, our results stress the ecological lability of both plant–animal and animal–animal interactions – which may switch between mutualistic and antagonistic depending on their ecological context. When small bees are the only visitors of *M. malabathricum* flowers, they provide a certain pollination service (mutualistic interaction); however, in the presence of effective pollinators, small bees act as thieves, removing pollen and making flowers less attractive to the most effective pollinator (antagonist interaction) (Hargreaves, Harder & Johnson 2009). Conversely, ants that may reduce the reproductive success of plants by deterring small pollinators (antagonist interaction), enhance plants' fitness when more effective pollinators, unaffected by their predatory habits, are present (mutualistic interaction). Our system is, to our knowledge, the first one in which all actors in the play are broad generalists, yet their interaction may have resulted in the evolution of an unlikely floral trait – floral attractants for predatory ants.

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Supporting Information

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

Appendix S1. Video recording of a *Nomia* bee being chased away by a weaver ant on a *Melastoma malabathricum* flower.

Appendix S2. Video recording of a *Xylocopa* bee visiting *Melastoma malabathricum* flower patrolled by the weaver ant *Oecophylla smaragdina*.