

# The function of ant repellence by flowers: testing the “nectar protection” and “pollinator protection” hypotheses

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**Abstract** According to the “nectar protection” and “pollinator protection” hypotheses, ant repellents in flowers have evolved to prevent ants from exploiting floral nectar and chasing away pollinators, respectively. We used weaver ants, *Oecophylla smaragdina*, to determine the strength of ant repellence in 32 bee-pollinated plant species and used the comparative method to test whether nectar production, size of pollinating bees and plant growth form were related to floral repellence. Flowers were more likely to repel ants if they offered nectar as a reward and were pollinated by small bees than if they were nectarless and pollinated by large bees. Furthermore, tree flowers were more likely than shrub or vine flowers to repel ants. Our results validate the pollinator protection hypothesis and the nectar protection hypothesis. Depending on the ecological context, therefore, ant repellents can function as direct or indirect exploitation barriers: they can prevent ants from removing nectar without effecting pollination (direct barriers) and, when flowers are pollinated by large bees, the absence of ant repellents—or even the presence of ant attractants—can result in ants chasing small ineffective pollinators away (indirect barriers).

**Keywords** Ant repellents · Nectar · Bee size · Plant growth form · Pollination

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## Introduction

Mutualisms are reciprocally exploitative interactions that provide net benefits to both partner species (Thompson 1982). For example, in most pollination mutualisms plants provide pollinators with floral rewards and, in turns, pollinators offer pollination services to plants. Such systems, however, are subject to exploitation by cheaters that can obtain the benefits of mutualisms providing few or no goods in return. Cheaters, thus, may impose significant costs on the species they exploit which, in response, may develop mechanism to dissuade them: exploitation barriers (Rodríguez-Gironés and Santamaría, this issue).

Except for a few reported cases of ant pollination (Gómez and Zamora 1992; Gómez et al. 1996) there is evidence that ants can act as cheaters of plant–pollinator interactions. Ants can consume floral nectar without pollinating flowers and occasionally damaging reproductive structures (Galen 1999; Galen and Cuba 2001). They can also use flowers as hunting platforms, capturing potential pollinators (Rodríguez-Gironés et al. 2013). Many plants therefore use exploitation barriers to dissuade ants from visiting flowers, allowing the visit of effective pollinators.

A common filtering mechanism adopted by plants is the production of ant repellents during the fertile period of flowers (Willmer and Stone 1997; Ghazoul 2001; Willmer et al. 2009). Depending on how ants interfere with plant–pollinator mutualisms, two hypotheses, not mutually exclusive, have been proposed to explain the existence of ant repellents. According to the “nectar protection hypothesis” ant repellents have evolved to discourage ants from removing nectar without effecting pollination (Junker and Bluthgen 2008; Junker et al. 2011). According to the “pollinator protection hypothesis” ant repellents have evolved to discourage ants from scaring pollinators away (Willmer and Stone 1997; Nicklen and Wagner 2006; Junker et al. 2007). No study to date has demonstrated that ant repellents have evolved to prevent nectar theft and/or pollinator deterrence. However, the correlation between nectar production and ant deterrence supports the nectar protection hypothesis—since it is more likely that plants invest in protection when they produce larger volumes of nectar (Ballantyne and Willmer 2012).

To determine the selective pressures that triggered the evolution of ant repellents in flowers we reason that if flowers produce ant repellents to prevent nectar theft (pollen-eating ants are rare; Baroni Urbani and de Andrade 1997), nectarless flowers should produce no ant repellents; and, if competition for foral nectar is higher in the canopy [as suggested by Junker et al. (2007)], flowers from trees should show more repellence than flowers from plants occupying lower strata, such as shrubs or vines. Likewise, if the function of repellents is to discourage ants from chasing pollinators, repellents should only be present in flowers pollinated by animals that are susceptible to ant attacks—large *Xylocopa* bees, for instance, are not deterred by ants (González et al. 2013). In the context of the nectar protection hypothesis, ant repellents are exploitation barriers: they prevent nectar thieves from reaching the resource produced to attract pollinators. In the context of the pollinator protection hypothesis, they are not—ants interfere with pollination but do not consume the resources produced by flowers.

We predict that ant repellence should be smallest in nectarless flowers pollinated by large bees, intermediate in flowers producing nectar or pollinated by small bees and greatest in flowers producing nectar and pollinated by small bees. To test these predictions we did a comparative study across 32 plant species, determining for each one its growth form and whether its flowers repelled weaver ants, *Oecophylla smaragdina*, produced nectar, and were pollinated by bees susceptible to ant attacks. We did a phylogenetically

**Table 1** Plant species

Family	Species	Floral repellence	Main pollinators	Nectar presence	Growth form
Acanthaceae	<i>Thunbergia grandiflora</i> Robx.	0.51	<i>Xylocopa</i> sp. <sup>1, 20</sup>	Yes <sup>20</sup>	Vine <sup>24</sup>
Apocynaceae	<i>Anoloccalix yunnanensis</i> Pierre	0.49	<i>Xylocopa</i> sp. <sup>1</sup>	Yes <sup>1</sup>	Vine <sup>24</sup>
Apocynaceae	<i>Calotropis gigantea</i> (L.) W.T. Anton	0.48	<i>Xylocopa</i> sp. <sup>6,13</sup>	Yes <sup>13</sup>	Shrub <sup>24</sup>
Caesalpinaceae	<i>Bauhinia acuminata</i> L.	0.50	<i>Xylocopa</i> sp. <sup>1</sup>	Yes <sup>1</sup>	Shrub <sup>24</sup>
Caesalpinaceae	<i>Bauhinia purpurea</i> L.	0.48	<i>Xylocopa</i> sp. <sup>6</sup>	Yes <sup>13</sup>	Tree <sup>24</sup>
Caesalpinaceae	<i>Cassia auriculata</i> (L.) Roxb.	0.50	<i>Xylocopa</i> sp. <sup>1,11</sup>	No <sup>13</sup>	Tree <sup>24</sup>
Caesalpinaceae	<i>Cassia fistula</i> L.	0.50	<i>Xylocopa</i> sp. <sup>1,12, 13</sup>	No <sup>13</sup>	Tree <sup>24</sup>
Convolvulaceae	<i>Ipomoea pes-caprae</i> (L.) R. Br.	0.51	<i>Xylocopa</i> sp. <sup>18</sup>	Yes <sup>18</sup>	Vine <sup>18</sup>
Costaceae	<i>Costus speciosus</i> Koen ex. Retzsz.	0.50	<i>Xylocopa</i> sp. <sup>1,6</sup>	Yes <sup>6</sup>	Shrub <sup>24</sup>
Dilleniaceae	<i>Dillenia ovata</i> Wall ex Hook. f. & Thomson	0.50	<i>Xylocopa</i> sp. <sup>1,5</sup>	No <sup>1</sup>	Tree <sup>5</sup>
Dilleniaceae	<i>Dillenia suffruticosa</i> (Griff ex Hook. f. & Thomson) Martelli	0.52	<i>Xylocopa</i> sp. <sup>1,3,6</sup>	No <sup>3</sup>	Shrub <sup>1</sup>
Euphorbiaceae	<i>Mallotus japonicus</i> (L.f.) Müll. Arg.	0.42*	Small bees (Apidae, Halictidae) <sup>1,22</sup>	Yes <sup>22</sup>	Shrub <sup>24</sup>
Fabaceae	<i>Dalbergia</i> sp.	0.27***	Small bees ( <i>Apis cerana</i> , <i>A. florea</i> ) <sup>1,5</sup>	Yes <sup>1</sup>	Shrub <sup>1</sup>
Fagaceae	<i>Castanopsis indica</i> (Roxb.) A. DC.	0.18***	Small bees <sup>1,4</sup>	Yes <sup>23</sup>	Tree <sup>24</sup>
Hypericaceae	<i>Cratoxylon conchinchinesis</i> Bl.	0.42*	Small bees ( <i>Megachile</i> spp., <i>Nomia</i> spp.) <sup>5</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>
Lauraceae	<i>Lisea</i> sp.	0.29***	Small bees ( <i>Apis cerana</i> , <i>A. florea</i> ) <sup>1,2</sup>	Yes <sup>1</sup>	Tree <sup>1</sup>
Lythraceae	<i>Lagerstroemia speciosa</i> (L.) Pers.	0.50	<i>Xylocopa</i> sp. <sup>1,5</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>
Malvaceae	<i>Microcos paniculata</i> L.	0.21***	Small bees ( <i>Apis cerana</i> ) <sup>1,5</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>
Melastomataceae	<i>Melastoma malabathricum</i> L.	0.58**	<i>Xylocopa</i> sp. <sup>1,16</sup>	No <sup>16</sup>	Shrub <sup>24</sup>
Mimosaceae	<i>Mimosa pudica</i> L.	0.42**	Small bees ( <i>Trigona</i> sp.) <sup>1,5</sup>	No <sup>1</sup>	Shrub <sup>24</sup>
Myrsinaceae	<i>Ardisia elliptica</i> Thunb.	0.50	Small bees (halictid bees) <sup>1,9</sup>	No <sup>9</sup>	Shrub <sup>24</sup>
Myrtaceae	<i>Syzygium cumini</i> (L.) Skeels	0.40*	Small bees ( <i>Apis</i> sp.) <sup>1,14</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>
Myrtaceae	<i>Syzygium jambos</i> (L.) Alston	0.46**	Small bees ( <i>Apis cerana</i> ) <sup>1,15</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>
Oxalidaceae	<i>Averrhoa carambola</i> L.	0.29***	Small bees ( <i>Trigona</i> sp.) <sup>1,17</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>

Table 1 continued

Family	Species	Floral repellence	Main pollinators	Nectar presence	Growth form
Passifloraceae	<i>Passiflora edulis</i> Sims	0.45**	<i>Xylocopa</i> sp. <sup>1,7</sup>	Yes <sup>7</sup>	Vine <sup>24</sup>
Piperaceae	<i>Piper umbellatum</i> L.	0.46**	Small bees ( <i>Apis cerana</i> , <i>Apis florea</i> ) <sup>3</sup>	No <sup>3</sup>	Shrub <sup>24</sup>
Pitosporeaceae	<i>Pitosporum tobrira</i> (Thunb.) W. T. Aiton	0.25***	Small bees ( <i>Apis cerana</i> , <i>Tetralonia</i> sp.) <sup>10</sup>	Yes <sup>1</sup>	Shrub <sup>24</sup>
Rubiaceae	<i>Chassalia curviflora</i> (Wallich) Thwaites	0.47	Small bees ( <i>Trigona</i> sp. and diverse small insects) <sup>1,3,2,1</sup>	Yes <sup>3</sup>	Shrub <sup>24</sup>
Rutaceae	<i>Murraya paniculata</i> (L.) Jack	0.40***	Small bees ( <i>Apis cerana</i> ) <sup>1,14</sup>	Yes <sup>1</sup>	Tree <sup>24</sup>
Sapotaceae	<i>Chrysophyllum cainito</i> L.	0.14***	Small bees ( <i>Tetragonisca</i> spp.) <sup>8</sup>	Yes <sup>1</sup>	Tree <sup>8</sup>
Solanaceae	<i>Solanum trilobatum</i> L.	0.50	<i>Xylocopa</i> sp. <sup>1,13</sup>	No <sup>13</sup>	Shrub <sup>1</sup>
Verbenaceae	<i>Gmelina asiatica</i> L.	0.49	<i>Xylocopa</i> sp. <sup>19</sup>	Yes <sup>13</sup>	Shrub <sup>24</sup>

The table indicates, for each plant species, the proportion of time that ants spent on the treatment side of the Petri dish (floral repellence), Main pollinator, Nectar presence and Growth form. Asterisks in the “Floral repellence” column indicate significant results for repellence: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

References: 1 Field observations; 2 Devy and Davidar (2003); 3 Momose et al. (1998); 4 Singh (2004); 5 Kato et al. (2008); 6 Endress (1996); 7 Corbet and Willmer (1980); 8 Parker et al. (2010); 9 Pascarella (1997); 10 Kato (2000); 11 Dulberger (1981); 12 Murali (1993); 13 Raju and Rao (2006); 14 Thomas et al. (2009); 15 Siqueira de Castro (2002); 16 González et al. (2013); 17 Heard (1999); 18 Devall and Thien (1989); 19 Reddi et al. (1996); 20 Fiala et al. (1996); 21 Devy and Davidar (2003); 22 Yamasaki and Sakai (2013); 23 Bista and Shivakoti (2011); 24 eFloras (2008)

corrected analysis to test whether nectar production, pollinator susceptibility to ant attacks or growth form determined the expression of ant repellents in flowers.

## Materials and methods

### Study site and species

The study was carried out at Xishuangbanna Tropical Botanical Garden, Yunnan province, China, from May to August 2011 and at MacRitchie Reservoir, Singapore, from March to April 2012. We selected 32 species of flowering plants, belonging to 27 families, including trees, shrubs and vines (Table 1) and assessed whether young inflorescences of these species repelled weaver ants, *Oecophylla smaragdina*—it is usually young inflorescences, but not old ones, that repel ants during dehiscence (Willmer and Stone 1997; Ghazoul 2001; Raine et al. 2002). We included in our sample all the plant species pollinated by bees for which we found enough individuals in bloom to replicate the experiment. We selected weaver ants as model because they are abundant, mobile, aggressive and ecologically dominant canopy predators (Bluthgen and Fiedler 2004; Offenberg et al. 2004a; Crozier et al. 2010); they attack herbivores and parasites (Offenberg et al. 2004b; Van Mele et al. 2009) and ambush incoming pollinators at flowers (Rodríguez-Gironés et al. 2013); and they feed on nectar from extrafloral nectaries and on honeydew from ant-tended aphids (Bluthgen and Fiedler 2004).

### Characterizing flowers and pollinators

We grouped flowers along three axes: nectar production (yes vs. no), main pollinator (*Xylocopa* spp. vs. small bees) and plant growth form (trees, shrubs or vines; Table 1). We identified the main pollinators and determined the presence of nectar and growth form from field observations, complemented by published records.

Weaver ants attack *Xylocopa* bees without driving them away, while smaller pollinators, such as small honey bees (*A. cerana* and *A. florea*) and *Trigona*, are vulnerable to weaver ant attacks and decrease their visit rate in the presence of weaver ants (Tsuji et al. 2004; González et al. 2013; Rodríguez-Gironés et al. 2013). Romero et al. (2011) found similar results using crab spiders as ambush predators. In addition, Rodríguez-Gironés et al. (2013) reported weaver ants attacking small bees at 24 plant species, successfully capturing them at 16 species. Attacks on *Xylocopa* bees, on the other hand, were observed at eight plant species and never resulted in a successful capture. On the contrary, *Xylocopa* bees preferentially forage on ant-harboursing *Melastoma malabathricum* flowers, where resource competition is smaller (González et al. 2013) and they can even predate on weaver ants (Punekar et al. 2010).

For each plant species we tested, we also checked whether weaver ants were patrolling the plants and flowers. In addition, whenever there were pollinators visiting the flowers, we recorded their interaction with pollinators. We observed flower visits in the morning (9 a.m. to 12 p.m.), spending 15 min on 15 individual of each 32 plant species we tested for repellents. We considered pollinators floral visitors that touched the stigma of the flower while collecting floral resources.

## Floral repellence tests

Ant repellents were detected as reported in (Ghazoul 2001). We wiped one half of a 14-cm diameter Petri-dish with a newly opened inflorescence (treatment side) and the other half with a 3–4-day-old withering inflorescence (control side). We placed an ant in the centre of the Petri dish and, after a 20 s acclimatization period, we recorded the amount of time that the ant spent on each half over 300 s, rotating the Petri-dish 180° halfway. We replicated the assay 15 times for each species, using for each replicate flowers from different plants and ants from different colonies. To verify that weaver ants were repelled by cues in the new inflorescences, rather than attracted to the old ones, for a subset of species (*Melastoma malabathricum*, *Dalbergia* sp., *Ardisia elliptica*, *Mimosa pudica*, *Cassia fistula*, *Bauhinia acuminata*, *Mallotus japonicus* and *Piper umbellatum*) we conducted control trials by testing whether ants preferred old inflorescences to leaves. Ants were never tested on plants present within their foraging territory, defined as the area in which ants showed territorial behavior.

## Statistical analyses

Because related species may share the same traits due to shared ancestry, statistical analyses must incorporate phylogenetic correlations. The basic structure of our tree was built from published phylogenies (Bremer et al. 2003; Wojciechowski 2003; McDade et al. 2008; Wurdack and Davis 2009; Schaeferhoff et al. 2010), arbitrarily setting branch length equal to one throughout the tree and including polytomies when the available phylogeny was not completely resolved. We used Mesquite 2.75 (Maddison and Maddison 2009) to construct the phylogenetic correlation matrix and did a Phylogenetic Generalized Least Squares regression including the phylogenetic correlation structure as a random factor. We fitted a series of statistical models considering nectar presence, pollinator size and plant growth form as qualitative independent variables, and the proportion of time that ants spent on the treatment side of the Petri dish as dependent variable. For each statistical model we applied three evolutionary models—Brownian, Pagel and Ornstein–Uhlenbeck (Martins and Hansen 1997; Pagel 1999)—and used Akaike’s Information Criterion, corrected for small sample size, AICc, to select the most parsimonious evolutionary model (Akaike 1973). We report in detail those models that are within seven AICc units of the top-supported model (Burnham et al. 2011).

We analysed the data from the subset of species tested with leaves versus old inflorescences using an ANOVA with species as fixed factor and the proportion of time that ants spent on the “leaf” side of the Petri dish—0.5 as dependent variable. Significance test on the intercept determines whether the dependent variable is significantly higher or lower than zero. All statistical analyses were carried out using R version 3.0.2. (R Development Core Team 2010) except for the ANOVA testing whether ants preferred old inflorescences to leaves, which was conducted on STATISTICA version 10 (StatSoft, Inc 2011).

## Results

We hypothesised that ant repellence would be stronger in flowers with nectar than in nectarless flowers, in flowers pollinated by small bees than in flowers pollinated by large bees, in canopy flowers than in flowers from lower strata. To test these predictions, we measured ant repellence in 32 plant species and used phylogenetically corrected models to

test whether nectar production, size of bee pollinator and growth form affected ant repellence. Out of the 32 plant species we tested, 14 were trees, 14 shrubs and four vines; 23 produced nectar and nine did not; 16 were pollinated by the large *Xylocopa* while the main pollinators of the remaining 16 were smaller bees such as *Apis* (*A. cerana* and *A. florea*) or *Trigona* (Table 1). We never observed *Xylocopa* and smaller bees pollinating the same plant species.

The most parsimonious model included growth form as explanatory variable and assumed Pagel's evolutionary model. Two additional models, both of them assuming Pagel's evolutionary model, were within seven AICc units of this model (Table S1). These models included as explanatory variables nectar presence and growth form ( $\Delta\text{AICc} = 0.20$ ) and bee size ( $\Delta\text{AICc} = 5.23$ ).

We selected the three most parsimonious models for hypothesis testing (Table 2). Flowers from trees repelled weaver ants more frequently than flowers from shrubs, but the difference between shrubs and vines was not statistically significant (Table 2; Fig. 1a). When considering the most parsimonious model, flowers from trees did not show greater repellence than flowers from vines (Fig. 1a), however, in the next most parsimonious model, we did find significant differences between trees and vines (Table 2). Since we only introduced four vine species in the analysis, however, shrub-vine and tree-vines comparisons should be taken with caution. Furthermore, ant repellence was stronger in flowers producing nectar than in nectarless flowers and in flowers pollinated by small bees than in flowers pollinated by large bees (Figs. 1b, c, 2; Table 2). Because the data support all models within seven AICc units of the most parsimonious one (Burnham et al. 2011) we must conclude that nectar presence, growth form and pollinator size affected the strength of ant repellence.

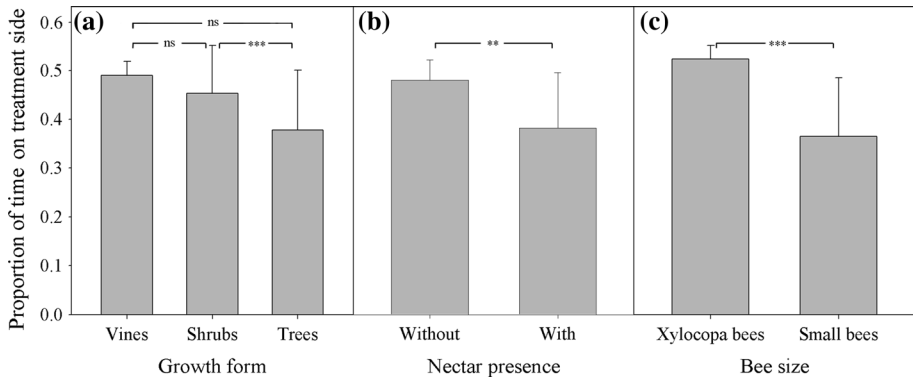
In the control tests, ants showed no preference for the “leaf” or the “old inflorescence” side of the Petri dish (intercept:  $F_{1,117} = 2.50$ ,  $p = 0.12$ ; species factor:  $F_{7,117} = 0.64$ ,  $p = 0.71$ ) confirming that differences between the proportion of time spent on the new or old inflorescence side of the Petri dish, when present, resulted from cues in new inflorescences.

In the field we observed weaver ants patrolling plants of 23 of the 32 plant species we tested in the lab (Table S2). Within these 23 plant species, 13 species did not produce ant repellents and 10 species produced them. Ants patrolled the flowers of all the species without repellents, but we never saw them on flowers with repellents, confirming the

**Table 2** Results of the different phylogenetic generalized least squares analyses for the three most parsimonious models

Model description	Evolutionary model	Factor	df	t	p
Growth form	Pagel	Shrubs-trees	30	>10	<0.0001
		Shrubs-vines	30	0.77	0.44
		Trees-vines	30	-1.60	0.43
Nectar + growth form	Pagel	With-without nectar	29	-2.98	<0.01
		Shrubs-trees	29	>10	<0.0001
		Shrubs-vines	29	1.39	0.17
		Trees-vines	30	-2.32	<0.05
Bee size	Pagel	Large-small bees	30	5.60	<0.0001

Models consider the proportion of time that ants spent on the treatment side of the petri dish as dependent variable



**Fig. 1** Proportion of time that ants spent on the treatment side versus **a** growth form, **b** nectar presence and **c** bee size. Asterisks denote significant differences between groups (\*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ) and ns indicates not significant. Error bars represent standard deviations

efficacy of ant repellents in nature (Table S2). We observed ants attacking *Xylocopa* bees on flowers of five species, but never a successful capture. Moreover, in two plant species with ant repellents (*Syzygium cumini* and *Castanopsis indica*) weaver ants managed to capture *Trigona* bees from branches. Finally, we only observed ants drinking nectar from the bracts at one species, *Costus speciosus*.

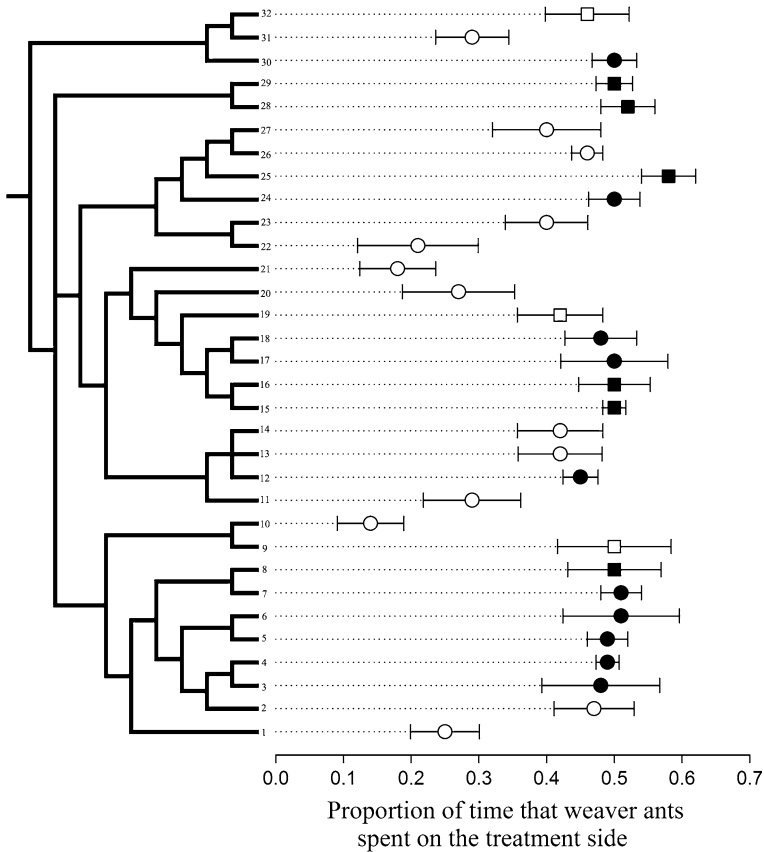
## Discussion

Our results show that flowers with nectar and flowers pollinated by small bees produced more ant repellents than nectarless flowers and flowers pollinated by large bees of the genus *Xylocopa*, respectively. In addition, flowers from trees showed more ant repellence than flowers from shrubs and vines. This study therefore confirms the pollinator protection and the nectar protection hypotheses and shows that the selective pressures promoting the evolution of ant repellence may be related to plant growth form. Although previous work supports the pollinator and nectar protection hypotheses (Junker and Bluthgen 2008; Ballantyne and Willmer 2012), this is the first study designed specifically to test them within the comparative framework, correcting for phylogenetic correlations between plant species.

By inflicting significant costs on plant fitness, ants may lead to selection for exploitation barriers to exclude them from flowers or, at least, to reduce their impact. Although plants have developed physical barriers such as sticky or waxy surfaces (Harley 1991), our findings confirm that ant repellents filter out undesired ants in order to allow the visit of effective pollinators (Willmer and Stone 1997; Nicklen and Wagner 2006).

The pollinator protection hypothesis was first formulated to explain ant repellence by *Acacia* flowers (Willmer and Stone 1997). Several *Acacia* species engage in tight mutualisms with aggressive ants: the trees provide ants with food and shelter, and the ants protect trees from herbivores (Janzen 1966). Willmer and Stone (1997) therefore suggested that flowers produce ant repellents in order that pollinators can visit flowers safely. Indeed, small bees are susceptible to predation by weaver ants and avoid visiting ant-patrolled flowers (Tsuji et al. 2004; González and Rodríguez-Gironés 2013).





**Fig. 2** Average proportion of time that weaver ants spent on the treatment side for each plant species, with the phylogeny represented on the side (the tip of each branch is connected through a dashed line to the corresponding point). Filled symbols represent flowers pollinated by *Xylocopa* bees, empty symbols flowers pollinated by small bees; circles represent flowers with nectar and squares nectarless flowers. Error bars are standard errors. Numbers represent plant species as follow: 1, *P. tobira*; 2, *C. curviflora*; 3, *C. gigantea*; 4, *A. yunnanensis*; 5, *G. asiatica*; 6, *T. grandiflora*; 7, *I. pes-caprae*; 8, *S. trilobatum*; 9, *A. elliptica*; 10, *C. cainito*; 11, *A. carambola*; 12, *P. edulis*; 13, *C. cochinchinense*; 14, *M. barbatus*; 15, *C. fistula*; 16, *C. auriculata*; 17, *B. acuminata*; 18, *B. purpurea*; 19, *M. pudica*; 20, *Dalbergia* sp.; 21, *C. indica*; 22 *Microcos paniculata*; 23, *Murraya paniculata*; 24, *L. speciosa*; 25, *M. malabathricum*; 26, *S. jambos*; 27, *S. cumini*; 28, *D. ovata*; 29, *D. suffruticosa*; 30, *C. speciosus*; 31, *Litsea* sp.; 32, *P. umbellatum*

An important insight of the present study is that, because large bees are not susceptible to predator attacks (Romero et al. 2011; González et al. 2013) the flowers they pollinate experience no selective pressure to evolve repellents. Large bees may even prefer to exploit ant-patrolled flowers, where inter-specific competition for resources is lowest, leading to a positive effect of ants on reproductive success (González et al. 2013). This asymmetry led us to predict that flowers pollinated by small bees should be more likely to produce ant repellents. This prediction was confirmed after correcting for phylogenetic correlations (Fig. 1c).

Weaver ants not only show territorial behaviour in their host plant, they also may act as ambush predators that wait on or under flowers of a widespread range of plant species for

the arrival of incoming visitors (Rodríguez-Gironés et al. 2013). In addition, the use of flowers as hunting platform also takes place in the secondary, hunting, territory of weaver ants (Rodríguez-Gironés et al. 2013). Therefore, and according to the pollinator protection hypothesis, we predict that the production of ant repellents is not limited to those plants that house weaver ants. Floral repellence may also evolve under selective pressures imposed by ants in plants without ant colonies—even if flowers are lacking nectar.

According to the nectar protection hypothesis, repellents are selected because they discourage ants from consuming nectar without effecting pollination. In support of this hypothesis, in Hawaii, where plants have not shared an evolutionary history with ants, nectar theft by introduced ants is more prevalent among native than alien species (Junker et al. 2011). Likewise, Ballantyne and Willmer (2012) found a positive correlation between ant repellence and volume of nectar per flower across 49 Costa Rican plant species.

In addition, ant repellence was more prevalent among tree than shrub or vine flowers. This agrees with the finding that canopy flowers have more repellents than understory flowers (Junker and Bluthgen 2008). These authors suggested that the need to prevent nectar theft is greater in the canopy, due to the higher ant abundance and greater nutritional requirements of canopy ants. The evolution of ant repellence in the canopy may also be favoured by the greater aggressiveness of canopy ants (Yanoviak and Kaspari 2000).

Ant repellence is a labile trait, which has evolved or disappeared repeatedly among the angiosperms (Ghazoul 2001). Within the 32 species we have included in the phylogenetic analyses, there have been at least eight transitions. We should therefore expect plants to produce ant repellence whenever it is advantageous, possibly leading to differences in the prevalence of ant repellence between habitats and biogeographic regions, paralleling the abundance of aggressive ants (Davidson et al. 2003) or their need for carbohydrates (Davidson 1997).

Although we have validated the nectar protection and the pollinator protection hypotheses, this study raises questions about the generality of our findings. For example, our results were based exclusively on the response of *Oecophylla smaragdina* to floral repellents. Due to the broad effect of floral repellents on several ant taxa (Ghazoul 2001), we expect that our results will apply to other ant species, although such extrapolation should be confirmed. In addition, plant traits other than pollinator size and nectar presence may also affect the evolution of ant repellents. Floral morphology, for example, may affect the efficacy of ant attacks, favoring the development of repellents in those flowers where ants successfully attack the incoming pollinators. In the same way, those flowers with accessible nectar may produce more frequently ant repellents than flowers where the nectar is hidden at the bottom of long corolla tubes. The effect of floral morphology on the evolution of ant repellents remains to be elucidated.

Within the tropical forests of SE Asia *Oecophylla smaragdina* is one of the most territorially-dominant ant species (Crozier et al. 2010), and their presence may reduce territories of neighboring colonies. This makes possible a greater influence of weaver ants on the development of ant repellents in flowers than other non-dominant ant species. In addition, comparable patterns of evolution of ant repellence is expected in flowers of tropical Africa, where a similar dominant species of the same genus, *O. longinoda*, occupies the same ecological niche. On the other hand, in the Neotropics, where co-dominance between arboreal ants is more frequent (Dejean et al. 2007), the evolutionary trajectory of ant-repellents may have been different due to the coexistence of dominant ant species with different behavioral traits. In these tropics, for example, we may predict that floral repellence may exhibit a weaker pattern of distribution within growth form and a more diffuse evolution when considering a specific plant species.

The pollinator and nectar protection hypotheses are not mutually exclusive. On the contrary, nectar-rich flowers pollinated by small bees obtain a double advantage from ant repellents. Within the context of the nectar protection hypothesis, ant repellents can be seen as a direct exploitation barrier (Rodríguez-Girones and Santamaría 2005, this issue): a trait that deters undesired visitors, preventing consumption of resources intended to reward pollinators. Within the context of the pollinator protection hypothesis, on the other hand, if flowers rely on large bees for pollination and small bees are parasites, ants may patrol repellent-free flowers keeping vulnerable undesired visitors at bay. Furthermore, in those cases where the presence of ants on flowers provides a net benefit on the reproductive success of the plant, flowers may develop ant attracting cues. For example, in *M. malabathricum* weaver ants are recruited on flowers and their presence deters less effective pollinators and attracts *Xylocopa* bees through an indirect effect on resource depletion. As result, there is an increase in the reproductive success of ant-harboring plants (González et al. 2013). In this case, ant attractants act as indirect exploitation barriers. We must point out, however, that *M. malabathricum* is the only species in our data set that produces ant attractants. As we have not measured the effect of weaver ants on the reproductive success of other species, we can only suggest that plants evolve ant attractants when the presence of ants at flowers increases their reproductive success. In the evolutionary history of ants and plants, it appears that the development of ant repelling/attracting cues is intimately related to the ecological and evolutionary trajectory of plant-pollinator mutualisms. We can therefore predict that flowers may develop repellents if, in the interaction between plants and pollinators, ants act as parasites (nectar thieves or pollinator predators) and attractants if they act as mutualists (pollinators or bodyguards preying on herbivores/parasites).

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