Nectar Theft and Floral Ant-Repellence: A Link between Nectar Volume and Ant-Repellent Traits?

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Abstract

As flower visitors, ants rarely benefit a plant. They are poor pollinators, and can also disrupt pollination by deterring other flower visitors, or by stealing nectar. Some plant species therefore possess floral ant-repelling traits. But why do particular species have such traits when others do not? In a dry forest in Costa Rica, of 49 plant species around a third were ant-repellent at very close proximity to a common generalist ant species, usually via repellent pollen. Repellence was positively correlated with the presence of large nectar volumes. Repellent traits affected ant species differently, some influencing the behaviour of just a few species and others producing more generalised ant-repellence. Our results suggest that ant-repellent floral traits may often not be pleiotropic, but instead could have been selected for as a defence against ant thieves in plant species that invest in large volumes of nectar. This conclusion highlights to the importance of research into the cost of nectar production in future studies into ant-flower interactions.

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Introduction

Ants are capable of disrupting pollination when visiting flowers (e.g., [1,2,3]). How often do plant traits prevent this from happening? When feeding on other plant surfaces ants can benefit a plant (e.g., preying upon herbivores, or disrupting their feeding and oviposition), whilst ants attracted by hemipteran honeydew may reduce levels of more damaging herbivores [4,5]. Many plant species produce extrafloral nectar (EFN), which actively attracts ants, and which may be sited to encourage ants to patrol vulnerable areas such as new growth [6] and inflorescences [7,8]. In tropical canopies plant exudates from hemipteran honeydew and EFN can support huge populations of potentially beneficial ants [9,10,11]. Some myrmecophytes also provide housing (domatia) for ant colonies, as hollow stems or thorns, and thereby acquire standing armies of specialized mutualists that they may feed with EFN, protein bodies, or indirectly with hemipteran honeydew [12,13,14]. These specialized ants may provide additional benefits by pruning encroaching vegetation (e.g., [15]), or supplying nutrients to their hosts through detritus within domatia (e.g., [16]).

However, there are some situations where attracting ant-guards may be detrimental, most obviously during flowering. Being flightless, ants are generally ineffective as pollinators themselves and may also reduce pollen viability due to the antibiotic secretions used in nest hygiene [17,18]. Additionally they may reduce visitation and disrupt pollination by stealing nectar or threatening incoming pollinators (e.g., [2,19,20]), resulting in decreased seed set [1]; this is especially damaging where plants are self-incompatible and rely on limited supplies of outcrossed pollen [21]. Ant attendance at flowers can therefore reduce plant fitness (e.g., [3,22,23]), and are only encouraged onto inflorescences by EFNs where their anti-herbivore benefits outweigh possible costs.

Perhaps for this reason, ants are repelled from certain flower species in response to contact with mechanical barriers such as trichomes, or through chemical deterrents including flower scent [24,25]. Thus, obligate potential flower visitors may be attracted to floral scents while facultative visitors such as ants may be repelled [26]. Most work on ant-floral conflict has focused on *Acacia*, both in Africa and the Neotropics, where repellence by floral scent has been demonstrated [27,28,29,30]; in these cases volatile organic chemicals (VOCs) released by young *Acacia* inflorescences repel the resident ant-guards for a few hours, allowing pollinators free access. Some VOCs from pollen may mimic ant alarm pheromones, and these VOCs peak in the myrmecophyte *A. seyal fistula* during dehiscence [30]. Other temperate and tropical plant species have also been found to possess VOC ant-repellence [30,31,32,33,34].

In most cases to date this repellence involves a pollen- or anther-derived scent, transferable though “pollen-wiping” (e.g., [27,35]). As yet, however, the range of plant species studied is small and the causes of repellence poorly understood. Where ant-repellence has been identified in non-ant-plants its function is less obvious, as the costs and benefits of ant attendance at flowers have only rarely been tested (e.g., [2,3,20]). Is ant repellence selected to reduce aggression towards pollinators, or to prevent nectar theft, or is it due to pleiotropic effects on other floral traits? This study aimed to identify patterns of occurrence of repellence in the following ways:

1. Which plant species possess ant-repellent traits and what form do those traits take: are they effective over a long range or do ants have to contact the flowers? 

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2. What plant and floral traits are correlated with floral ant-repellence? If repellence is adaptive it may be commoner in species investing most heavily in flowers (e.g., in advertisements such as copious pollen or large nectar volumes), and less common in plant species that limit access to rewards through morphological traits. If there is a relationship between rewards and/or accessibility then repellence may also relate to phylogeny and/or to pollination syndrome.

3. How effective are ant-repellent traits against different functional groups of ant species? If such traits function to reduce threats to pollinators, they might be selectively targeted at larger predatory ants.

Better understanding of the types of plants that restrict ant access to flowers will give insight into the potential selective pressures structuring interactions between ants and plants at this crucial stage in the plant life cycle.

Methods

a) Ethics Statement
In 2008 field work was carried out in Costa Rica at La Selva and Las Cruces, operated by the Organisation for Tropical Studies (permit issued by Javier Guevara, ref: 045-2008-SINAC). In 2009 and 2010 field work was carried out in Costa Rica at Santa Rosa National Park, operated by the Área de Conservación Guanacaste (permits issued by Roger Blanco, ref: ACG-PI-007-2009 and ACG-PI-003-2010). All fieldwork was carried out at these sites and during the periods covered by these permits.

b) Study sites
Fieldwork was carried out in Costa Rica initially in tropical wet forest at La Selva Biological Station (10°26'N, 83°59'W) and Wilson Botanical Garden at Las Cruces (8°47'N, 82°57'W) from February to May of 2008. Further data were gathered in tropical dry forest at Santa Rosa National Park in North-western Costa Rica (10°54'N, 85°39'W) from January to May of 2009 and January to February of 2010. These sites provided both logistical support and a broad diversity of plant species. The dry forest was structurally ideal for accessing and observing a wide variety of flowers and ants was especially common at Santa Rosa. Other ant species were common in plant species that limit access to rewards through floral scent. The number of times an ant changed direction (a turn of more than 90°) and/or crossed to the centre of the arena was recorded as a proxy for increased activity. Agitation or aggression were recorded as “charges”, “abdomen cocks”, “holding the head up” and “time spent grooming”. Time spent stationary was also recorded. If ants responded in any way to floral scent, trials were repeated with just vegetative plant parts to confirm that the VOCs responsible originated from floral tissue.

d) Detecting the presence and form of ant-repellent traits

i. Tactile response trials
Identified repellence when an ant contacted part of a flower. A single ant was placed in a Petri dish with three or four evenly spaced objects: a dehisced flower, an older pollen-depleted flower and/or a bud (as available from that species), and an appropriately-sized twig used as a control to rule out neophobic responses to unfamiliar objects. Various behaviours were recorded over four minutes: the number of antennations, the times an object was walked over or clearly avoided, and probing of the objects. Repellence was deemed to occur when an ant’s antennae contacted an object and the ant immediately jerked or turned away (often followed by grooming of the antennae). Recording began 10 s after an ant was placed in the arena, reducing the likelihood of recording purely neophobic responses. At least 12 trials were carried out for each species combination of ant and plant (unless fresh flowers became unavailable). We predicted negligible ant-repellence from buds and pollen-free older flowers but had no prior expectation of ant-repellence for specific non-ant plants. The upper petal surfaces of fresh flowers of Stachytarpheta jamaicensis (regardless of pollen availability) were strongly ant-repellent, so we tested these flowers in further tactile response trials using C. novograndensis. Fresh petals were rubbed against 1 cm² of filter paper with untouched paper for controls.

Tactile response data were analysed using the number of times that an ant walked over or were repelled by floral parts within a single trial. Although other behaviours were recorded, repellence from an object provided a clear, unambiguous response. Proportions repelled were calculated following Junker et al. [31]: ‘0’ = never repelled on contact, ‘1’ = always repelled on contact. An ant walking over just the base of a corolla was not included in the proportion data; and ants “repelled” by the twig “control” were discounted from the analysis entirely, though in practice this only excluded a very small number of trials. Floral parts for trials were picked with clean forceps and used immediately, and only once. Equipment was washed carefully with alcohol between trials.

ii. Scent response trials
Tested longer range (non-contact) repellence, arising from VOCs. An ant was placed in a Petri dish connected to two syringes, one empty and acting as a control (for air movement alone) and the second containing a flower or inoffuscence. (Although a larger number of flowers could have been used to concentrate the scent we decided to use scents at concentrations close to what an ant would experience when approaching a flower.) Ant behaviour was observed for 4 min alone inside the arena, 1 min after control air was gently blown through, and for another 1 min after this was repeated with the flower scent. The number of times an ant changed direction (a turn of more than 90°) and/or crossed to the centre of the arena was recorded as a proxy for increased activity. Agitation or aggression were recorded as “charges”, “abdomen cocks”, “holding the head up” and “time spent grooming”. Time spent stationary was also recorded. If ants responded in any way to floral scent, trials were repeated with just vegetative plant parts to confirm that the VOCs responsible originated from floral tissue.

e) Detecting plant and floral traits correlated with floral ant-repellence

Scores were assigned to each plant species for nectar volume and nectar accessibility. Mean nectar volume at dehiscence was scored categorically: 0 = no nectar detectable, 1 = volume too small to collect with a 1 µl microcap, 2 = <0.5 µl, 3 = >0.5 µl. Nectar accessibility to ants was scored by flow shape from 1...
(flowers with very limited access to nectar) to 4 (open access to nectar for ants of all sizes), taking into account width of the corolla and obstructing trichomes or anthers. Each plant species was defined as specialised or generalised (cf., [37,38,39] for detailed discussions of these terms) depending on the pollination syndrome to which the floral traits appeared to conform [39], and/or its known visitors.

f) Effectiveness of ant-repellent traits against different ant species

Plant species found to possess effective ant-repellent traits in tactile and/or scent trials with *C. novograndensis* were tested against other ant species. 14 species were tested against *Ectatomma ruidum* in tactile trials, and 11 species in scent trials (10 species from initial trials at La Selva and a single species, *Randia monantha*, at Santa Rosa). Five plant species with very different floral forms that were highly repellent to *C. novograndensis* were also tested against an additional 6–7 ant species with very different behavioral habits (detailed in Table 1). To test for repellence in another example from the genus *Stachytarpheta*, the ant species *Actromyrmex coronatus*, *Creontiades curvispinosa*, *Ectatomma ruidum*, *Megalomyrmex foreli* and *Pheidole fallax* were also tested in tactile trials against *Stachytarpheta frantzii*.

Table 1. Summary of behavioral trials carried out to address each experimental question.

| Experimental Questions | Method | Plant Species | Ant Species |
|------------------------|--------|---------------|-------------|
| 1 and 2                | Scent Trials | 33 plant species (see Table S1) | *Camponotus novograndensis* (Formicinae) [medium sized generalist] |
|                        | Tactile Trials | 49 plant species (see Figure 1 and Table S1) | *Camponotus novograndensis* (Formicinae) [medium sized generalist] |
| 3                      | Scent Trials | 11 plant species | *Ectatomma ruidum* (Ectatomminae) [large predator] |
|                        | Tactile Trials | 14 plant species (see Figure 3) | *Ectatomma ruidum* (Ectatomminae) [large predator] |

*Barleria ooeratheroides* (Acanthaceae) [under-canopy herb]; *Cardis alliadora* (Boraginaceae) [myrmecophytic tree]; *Malvaviscus arboresus* (Malvaceae) [shrub]; *Ruellia inudata* (Acanthaceae) [herb with defensive trichomes]; *Stachytarpheta jamesonii* (Verbenaceae) [EFN-bearing herb] | *Atta cephalotes* (Mymecinae) [leafcutter]; *Camponotus sericeiventris* (Formicinae) [large generalist]; *Cephalotes umbraculatus* (Mymecinae) [arboreal]; *Pachycondyla villosa* (Ponerinae) [large predator]; *Pheidole fallax* (Mymecinae) [small generalist]; *Pseudomyrmex gracilis* (Pseudomyrmecinae) [medium-sized generalist]; *Cephalotes setulifer* (Mymecinae) [C. alliadora plant-ant, only tested with *C. alliadora*]

Results

a) Which plant species possess ant-repellent traits and what form do they take?

We used *Camponotus novograndensis* in tactile trials with 49 plant species and observed a wide range of responses (Figure 1). The fresh flowers of 15 species (bold in Figure 1) repelled ants on more than half of the occasions that ants touched them. In 12 of these highly repellent species and in 4 that were less repellent (* in Figure 1), fresh dehiscing flowers were significantly more repellent than the control objects tested (older flowers with pollen-depleted anthers or unopened buds) (results of K-W tests shown in Table S1). In most cases repellence involved contact with pollen-bearing anthers, with the exception of *Rubolia inudata* and *Merremia agrippa*, where trichomes on the inflorescence were entirely or partly responsible respectively, and *Stachytarpheta jamesonii*, where the upper petal surface was repellent. Ants would occasionally walk over the bases of flowers with repellent pollen but would avoid the stamens.

Scent trials identified only the shrub *Randia monantha* as having a floral odour that influenced *C. novograndensis*, with a significant increase in agitation behaviour following injection of floral scent into the arena (*χ^2 = 5.3, df = 1, p = 0.021*). There was no increase in agitation with VOCs from a cut stem of the plant, i.e. the effect was specific to floral VOCs.

b) Which plant and floral traits are correlated with floral ant-repellence?

There was a significant positive relationship between the degree of floral ant-repellence and nectar volume (Figure 2a). Plants producing large nectar volumes were more likely to have protection against nectar theft. There were also significant differences between flowers with varying accessibility to nectar for ants (Figure 2b); however, there was no overall significant trend.

There was no significant difference in repellence between species with flowers exhibiting specialised pollination syndromes and more generalised species (*χ^2 = 0.702, df = 1, p = 0.402*). However, possibly due to the large nectar volumes required to attract endothermic vertebrates [39,40] flowers showing bird- and bat-pollination traits were significantly more ant-repellent than other flowers (*χ^2 = 36.654, df = 1, p < 0.001*). The proportions of ants repelled from flowers of species with different growth forms were significantly different (*χ^2 = 11.2, df = 3, p = 0.011*), but this was entirely due to the effect of climbers (no difference when excluded (*χ^2 = 2.6, df = 2, p = 0.28*).

The proportion of ants repelled varied significantly between plant families from which more than one species was tested.
\(\chi^2 = 30.236, \text{df} = 8, p<0.001\). However, the two best surveyed families, Acanthaceae (n = 6) and Fabaceae (n = 8), did not significantly differ in ant-repellence \(\chi^2 = 0.989, \text{df} = 1, p = 0.32\) suggesting that between-family variation in ant-repellence may to be an artefact of low sampling sizes within families.

c) How effective are ant-repellent traits against differing ant species?

Of the plant species that repelled *Camponotus novograndensis* in the tactile trials, 14 were also tested against *Ectatomma ruidum* (Figure 3), but only two had a significant repellent effect (the fresh anthers of *Malvaviscus arboreus*, and the upper petal surface of *Stachytarpheta jamaicensis*). *Stachytarpheta frantzii*, tested in 2008, had also provoked a repellent response from *E. ruidum*. In scent trials *Randia monantha* flowers had no effect on *E. ruidum* behaviour, and the scent trials carried out with *E. ruidum* at La Selva with 10 other plant species also found no repellence. Figure 4 (a–d) shows results with 4 plant species tested with multiple ant species.

a) As well as repelling *Camponotus novograndensis*, the fresh pollen of *Barleria oenotheroides* strongly repelled two other ants, *Cephalotes umbraculatus* and *Pseudomyrmex gracilis* (Figure 4a), both being species small enough to raid for nectar (although whether the normally arboreal *C. umbraclatus* forages on such low-lying vegetation is unknown). All other ant species tested were not repelled by *B. oenotheroides* and indeed often walked over pollen-laden anthers.

b) *Ruellia inudata* flowers were only repellent to *C. novograndensis* and *C. umbraculatus* (Figure 4b). This plant’s inflorescences below the flowers were covered in hairs, but these were only repellent to ant species with shorter legs.

c) *Cordia alliodora* pollen possessed a more generalised ant-repellence, affecting all ants tested except *E. ruidum* (Figure 4c) and the three ant species most strongly repelled were those most common on the tree at Santa Rosa.

d) *Malvaviscus arboreus* pollen provoked a strong repellent response from all the ant species tested (Figure 4d).

Figure 5 shows effects with both species of *Stachytarpheta* studied, which possessed strong, generalised ant-repellence elicited from the upper petal surface of fresh flowers. *S. jamaicensis* had the most potent general ant-repellence (Figure 5a). *Ectatomma ruidum* was repelled less by the petals of *S. frantzii* than by those of *S. jamaicensis* but there was no significant difference in the responses of *Pheidole florid*, (~80% repellent) (Figure 5b). The tactile response trials for *S. jamaicensis* wiped on filter paper showed no significant difference between the proportion of ants repelled by test and control pieces.
(average proportion repelled = 0.125; \( \chi^2 = 0.52, \) df = 1, \( p = 0.47 \)), so the repellence was not easily transferable to another surface.

Clearly ant species responded to flowers very differently. As the ant *Cephalotes umbraculatus* was moderately repelled by floral parts of all flower species tested, additional tactile trials were carried out using the flowers of *Cornutia grandiflora* (which had not proved repellent to *Camponotus novograndensis*), to ensure that it did not have a generic response to all flowers. *C. umbraculatus* was not repelled (average proportion repelled = 0.07; \( \chi^2 = 0.68, \) df = 1, \( p = 0.41 \)). At the other extreme, we found no particularly strong repellent effect of any flowers on *Atta cephalotes*. This species is not a nectar thief, and furthermore as these leafcutters can gain access to flower bases, it is unlikely that anther-based ant-repellence would provide any defence against them.

**Discussion**

**a) Ant-repellence - protecting an investment in nectar?**

In this study floral ant-repellence was observed most frequently in plant species producing high volumes of nectar per flower. Avoidance of nectar theft may therefore have influenced floral evolution, with selection for ant-repellling floral traits to protect against species (such as *Camponotus novograndensis*) that commonly visit flowers and steal nectar without providing any beneficial pollination services. In nearly all species tested here, individual flowers lasted for just a single day, and pollen was usually available for only a few hours after sunrise. With such a narrow window for male reproductive success, large numbers of ants thieving nectar from a flower could significantly reduce fitness. By repelling the initial forays of scouting workers, the plant prevents recruitment of large numbers of additional ants to valuable sources of sugar (and possibly, in an arid environment like Santa Rosa at the height of the dry season, of water [41]).

We suggest that when a plant invests in large nectar volumes it is more likely to protect that investment. Estimates for the costs of floral nectar production vary between species and depend upon other factors determining energy expenditure [42,43,44,45,46] but are probably high enough to "matter" to the plant. This view is reinforced as some plant species recoup part of the costs by reabsorbing nectar, which not only helps to maintain a stable concentration and volume of nectar [47] but also allows the recycling of unused resources after pollination, and their redirection towards seed production [48,49,50]. As demonstrated by Junker *et al.* [34] in Hawaii, when they are not needed floral defences against ant nectar-thieves are convergently lost by native plants. Future studies may link whole plant nectar costs with ant-repellence.

Pollen-based ant-repellence appears to be the commonest method used by angiosperms to repel ant-visitation, since most tests to date have localised the repellence to freshly dehiscing flowers, to anthers, to polyads, or to pollen grains [27,29,30]. This repellence is most effective at close range, in a similar way to the silk of some orb-web spiders being repellent to ants only upon contact [51]. As most of the species studied produced flowers that lasted less than a day, pollen-based repellence would be sufficient to dissuade ant-recruitment to flowers throughout the most crucial period of pollinator visitation, leaving only a short period of vulnerability in protandrous species where the stigma was receptive but no further self-pollen was present. In our tactile trials it was impossible to discern the exact nature of repellence from fresh flowers or anthers: whether it operates through VOCs in sufficiently high concentrations at close range, or through direct antennal contact. As Raguso [52] pointed out, insect interactions with flowers at close range "blur the distinction between olfaction, gustation and contact chemoreception as modes of action for chemical floral features". From a practical perspective, and from the perspective of the ants involved, there is little functional

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**Figure 2. Tactile ant-repellence, nectar volume and nectar accessibility.** a) Significant differences in ant-repellence occurred between plants with different volumes of nectar (\( \chi^2 = 42.9, \) df = 4, \( p < 0.001 \)) with a significant positive trend between nectar volume and the proportion of ants repelled (J-T = 5.9, df = 4, \( p < 0.001 \)). b) Ant-repellence differed significantly between plants with different levels of accessibility to nectar (\( \chi^2 = 26.6, \) df = 3, \( p < 0.001 \)), although the negative trend between accessibility and repellence was not significant (J-T = -1.5, df = 3, \( p = 0.13 \)). Significant differences between groups indicated with a/b/c. Error bars indicate 95% confidence intervals.

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difference between the two modes. However, understanding the mechanisms involved would be of interest in identifying selective actions on floral traits.

Where anthers were not involved, repellence came from trichomes on the inflorescences of *Ruellia inudata* and *Merremia aegyptia*, though only to smaller ant species, suggesting that they provide purely mechanical defence (no glandular trichomes were observed). *Stachytarpheta* species elicited repellence when ant antennae came into contact with the upper petal surfaces (cf., the ant-repellent petals of *Ferocactus wislizeni* [1]), and this triggered agitated antennal grooming without contact with anthers or pollen. At no time was repellent nectar observed. Instead, ants often fed on nectar that had leaked from the bases of repellent flowers, such as *Malvaviscus arboreus*.

Overall we found some form of floral ant-repellence in approximately a third of species tested in the dry forest environment. This is similar to the proportion reported by Willmer et al. [30] in the UK and by Junker et al. [31] in the wet forest of Borneo. However, there are some discrepancies between our results and those of Ghazoul [35], who also worked at Santa Rosa on some of the same plant species; e.g., some species that Ghazoul found to be ant-repellent (*Cochlospermum vitifolium, Ipomoea trifida* and *Gliricidia sepium*) provoked no responses from ants in our trials. The 2 acacia mutualist ant species used by Ghazoul were possible reacting to unfamiliar, non-host, scents. The responses of the more generalist *C. novograndensis*, may better reflect how common nectar thieving ants interact with flowers.

b) Relationship between other plant and floral traits and ant-repellence

While the likelihood of floral ant-repellence appears to be linked with nectar volume, accessibility to nectar may also be taken into account. In many cases accessibility will be more strongly influenced by other selection pressures on floral evolution, especially selection for the most efficient pollinator. Selection to prevent nectar theft by ants will either be complimentary to existing floral traits, as in the case of the narrow-flowered hawkmoth-pollinated *Randia monantha*, or will act separately from floral morphology, as in the open bat-pollinated *Crescentia cujete*. This contrasts with the consistent trade-off of VOC repellence and physical protection identified by Willmer et al. [30] and Junker et al. [34]. Out of the 18 species studied by Junker et al. [31] access to nectar was not a predictive factor for ant-repellence, but as nectar volume was not reported we cannot say if this was correlated with ant-repellence. The one species tested that did not produce nectar, *Diospyros durionoides* (Ebenaceae), was not significantly ant-repellent.

Whether or not a plant species develops ant-repellent traits may also depend on its degree of self-compatibility, and the likelihood...
and costs of geitonogamy. Nectar theft may often lead to reduced seed set, favouring ant-repellence; but lack of such repellence may be more favoured where visitation by ants promotes outcrossing by making pollinators move on more frequently, increasing pollen dispersion [53,54]. It is possible that other species cannot produce ant-repellence, either at the level of biochemical pathways or because repellence may have too great a disruptive influence on legitimate floral visitors. Conversely, the presence of certain floral traits, such as essential oil glands, may increase the likelihood of ant-repellence in some lineages.

While the proportion of ant-repellence appeared to differ significantly between plant families the effect was reduced as...
coverage within families increased. Related plant species were usually similarly repellent only when morphologically similar, indicating a stronger link between repellence and pollination syndrome than repellence and phylogeny. Thus, within the Malvaceae tested, the hummingbird-pollinated shrubs Malvaviscus arboreus and Helictores guazumafolia possess very similar floral structure and were both repellent to C. novograndensis (though different repellent components must be involved since Ectatomma ruidum was repelled only by M. arboreus); but the open-flowered Pavonia cancellata (also Malvaceae) had no such repellent traits. Similarly within the Fabales a clear contrast existed between the ant-repellent Mimosoideae (Acacia collinsii, Bauhinia ungulata, Enterolobium cyclocarpum), and the non-repellent Faboideae (Centrosema pulcherrimum, Desmodium sp., Gliricidia sepium, Haematoxylum brasiletto, Securidaca sylvestris). Ant-repellence is common within the American and African Acacia [28,30] but it may also be common in related genera within the mimosoid subfamily, which usually have highly exposed anthers giving easy access to pollen. Within the Faboideae tested (with the exception of Haematoxylum brasiletto) the pollen is protected behind keel petals, and anthers are often spring-loaded to cover the first visitor with pollen. None of the species with enclosed anthers had repellent pollen, which provides further (indirect) evidence that repellence may be adaptive and dependent on the nature of nectar and pollen rewards provided.

c) Ant-repellence effectiveness against different ant species
In some plant species ant-repellent traits had a broad effectiveness against a variety of ant species, e.g., Stachytarpheta jamaicensis, Malvaviscus arboreus, and Cordia alliodora flowers were all effectively repellent against multiple ant species, to varying degrees. In contrast, the effect of Barleria oenotheroides pollen and Ruellia inudata trichomes was restricted to a small number of ant species.

The large predatory ant Ectatomma ruidum was only influenced by the floral traits of two of the many species that were repellent to Camponotus novograndensis. Therefore there may be little selective pressure to protect potential pollinators from direct ant predation. Ants’ impact on pollinator populations is still poorly understood, but perhaps the threat they pose, in comparison to more efficient crab spiders [55] or competing bees [56], is too small to influence floral evolution. One exception may be Acacia collinsii, where repellence is likely to have evolved for pollinator protection. While A. collinsii’s ant-repellence is not effective against E. ruidum it does trigger a strong response from C. novograndensis, though this species rarely comes into contact with A. collinsii inflorescences and poses no threat to potential pollinators. This response in a non-mutualist is probably a by-product of selection to ensure that ant-guards do not interfere with pollination. No effect of A. collinsii VOCs was detected in our scent trials, suggesting that floral its VOCs are very limited in range. The other ant-plant used here, C. alliodora, is inhabited by ants with very low aggression posing no threat to

Figure 5. Floral ant-repellence in two species of Stachytarpheta tested against different ant species. (a) Stachytarpheta jamaicensis and (b) S. frantzii. * indicates significant difference between freshly dehisced flowers and old pollen-depleted flowers. Error bars indicate 95% confidence intervals. doi:10.1371/journal.pone.0043869.g005
pollinators, so its repellence probably arose once again to prevent nectar theft.

**d) Conclusions**

This study provides further groundwork for our understanding of how ants and flowers interact. It highlights the importance of potential constraints on floral evolution imposed by recruitable ants acting as nectar-thieves, and the complexity of floral characteristics that together attract potential mutualistic flower visitors and defend against exploitative visitors. While several different types of ant-repelling traits have been identified, by far the most common is ant-repellent pollen. Discovering the range of chemicals involved in this repellence will be crucial not just for understanding how it arose but also in understanding how ants interpret varied chemical signals from their environment, especially those similar to ant pheromones.

The positive correlation between floral ant-repellence and nectar volume suggests that to understand the role of ant nectar-thieves in floral evolution further information about the costs of nectar production is essential. Conversely, defence of pollinators against aggressive ants may be rather unimportant with no particular repellence of large predatory ant species. Given that interactions with other animals, such as pollinators and herbivores, are strongly selective on floral traits it is interesting that less recognised interactions with nectar-thieves could produce a significant trend in floral ant-repellence.

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**Supporting Information**

**Table S1** Plant species tested against Camponotus novograndensis in tactile trials. K-W tests were for differences among agitated responses in ants from fresh flowers and other floral part used (older, pollen and nectar depleted flowers in most cases) with significant differences shown in bold. Mode of pollinations was determined from visits observed while flowers were selected and peak dehiscence time determined combined with literature searches for each species. N = number of ants used in tactile trials. (DOCX)

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**Author Contributions**

Conceived and designed the experiments: GB PW. Performed the experiments: GB. Analyzed the data: GB. Contributed reagents/materials/analysis tools: GB PW. Wrote the paper: GB PW.
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