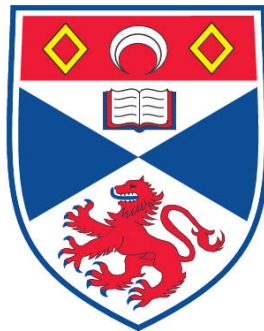


**ANTS AS FLOWER VISITORS: FLORAL ANT-REPELLENCE AND
THE IMPACT OF ANT SCENT-MARKS ON POLLINATOR
BEHAVIOUR**

Gavin Ballantyne

**A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews**



2011

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Ants as flower visitors: floral ant-repellence
and the impact of ant scent-marks on pollinator behaviour

Gavin Ballantyne

University of St Andrews

2011

Supervisor: Prof Pat Willmer

-

This thesis is dedicated to my grandparents,
the half that are here and the half that have gone,
and to taking photos of random things.

-

“Look in the mirror, and don't be tempted to equate transient domination
with either intrinsic superiority or prospects for extended survival.”

- Stephen Jay Gould

“I am comforted and consoled in finding it immeasurably remote in time,
gloriously lacking in any relevance for our day.”

- Umberto Eco

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I, Gavin Ballantyne, hereby certify that this thesis, which is approximately 59,600 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in June, 2007 and as a candidate for the degree of Ph.D. in Biology; the higher study for which this is a record was carried out in the University of St Andrews between 2007 and 2011.

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Acknowledgments

I am indebted to the staff and other researchers at La Selva, Las Cruces and Parque Nacional Santa Rosa for their help, taxonomic expertise and putting up with my terrible Spanish. Thanks to Arietta, Danielle, Martina and Erin for helping me to survive in the field (and at champagne receptions in German castles) and thanks to Nigel, Valerie, Elvin and the rest of the monkey people for chat at the Albergue and making me glad my study organisms hardly ever did anything in the midday sun.

The essential survival gear: nutella, barvaria beer, hammocks and headphones.

Thanks to David and Emma Illsey and everyone else I met in Mairena, who were always welcoming and an amazing help. At the Quinta de São Pedro, a big thanks to Armin, Ben and Helena.

Thanks to the Russell Trust Award for funding one of my field trips and Pat's class grant for funding the rest. Murray, for making and fixing so many bits of equipment. And in my aborted adventures into the world of chemistry thanks to Jette Knudsen for letting me borrow sampling tubes and Tom Shepherd for teaching me how to use a GC-MS.

Special thanks to Jane Wishart, for always making time to help and giving great advice. Steve Hubbard, for his valuable advice and comments. Will Creswell, for teaching me stats along with the third years. Jonathan, for being my first student and for all the ant chat. Thank you as well to Simon Potts and Alyson Tobin for their advice and comments on the thesis.

Thanks to Stu, Ashleigh and Dave for employing me in research after my undergrad and being the best bosses anyone could hope for.

Caroline "Carnage" King, Queen of Bees, for keeping me going for the last four years and introducing me to the world of rollerderby.

I would like to especially like to thank Pat Willmer for being an excellent supervisor and superb teacher. Thanks for giving me the freedom to make my own decisions and the guidance needed to not mess it up. Massive thanks to Graham Stone as well, for introducing me to Pat in the first place.

My friends and family, for putting up with me disappearing for months at a time and coming back with a tan. Thank you Mum and Dad, for the constant support and encouragement. Thank you Tay, for being an amazing sister and brilliant flatmate.

And Eyjafjallajökull, for giving me an extra week in Portugal.

Contents

• Abstract	
• Chapter 1: Ants, Plants and Pollination - a Review	
1.1 Introduction.....	3
1.2 Ant Exploitation of Sugar Sources.....	5
1.2a Extrafloral nectar.....	5
1.2b Herbivore-derived sugar sources.....	10
1.2c Competing sugar sources.....	14
1.3 Specialised Ant-Plants.....	17
1.3a Food and shelter.....	18
1.3b Balancing the costs of maintaining ant-guards.....	22
1.4 Exploitation of Mutualism.....	27
1.4a Defence against exploitation.....	27
1.4b Conflicts of interest.....	30
1.5 Ants as Pollinators.....	32
1.6 Disruption of Pollination.....	36
1.6a Pollinator predation.....	36
1.6b Floral larceny.....	40
1.6c Costs of nectar production.....	46
1.6d Visitation and floral scent-marking.....	48
1.7 Floral Ant-Repellence.....	52
1.7a Physical barriers.....	52
1.7b Repellent nectar.....	53
1.7c Chemical repellence.....	56
1.8 Summary and Chapter Plan.....	61
• Chapter 2: Nectar Theft and Floral Ant-Repellence	
2.1 Introduction.....	64
2.2 Methods	
2.2a Study sites.....	66
2.2b Study species.....	67
2.2c Detecting the presence and form of ant-repellent traits.....	68
2.2d Detecting plant and floral traits correlated with floral ant-repellence.....	70
2.2e Effectiveness of ant-repellent traits against a different ant species.....	70
2.2f Statistical methods.....	72
2.3 Results	
2.3a Which plant species possess ant-repellent traits and what form do they take ?.....	77
2.3b What plant and floral traits are correlated with floral ant-repellence	77
2.3c How effective are ant-repellent traits against a range of ant species?.....	81

2.4 Discussion	
2.4a Ant-repellence - protecting an investment in nectar.....	87
2.4b Ant-repellence, phylogeny and morphology.....	89
2.4c Ant-repellence as pollinator protection.....	92
2.4d Frequency of ant-repellence across different studies.....	93
2.4e Ant-repellence trigger – volatile or contact?	94
2.4f Conclusions.....	96
• Chapter 3: Floral Ant-Repellent Effects on a Native and an Invasive Ant	
3.1 Introduction.....	98
3.2 Methods	
3.2a Study site.....	102
3.2b Study species.....	102
3.2c Detection of floral ant-repellence.....	105
3.2d Statistical methods.....	106
3.3 Results	
3.3a <i>Linepithema</i> response.....	106
3.3b <i>Messor</i> response.....	109
3.3c <i>Arbutus unedo</i> meristems.....	110
3.4 Discussion	
3.4a Susceptibility of an invasive ant to floral ant-repellence.....	110
3.4b Comparison with other studies.....	112
3.4c Implications for ant invasions.....	113
3.4d Conclusions.....	114
• Chapter 4: Floral Visitors and Ant Scent-Marks	
4.1 Introduction.....	115
4.2 Methods	
4.2a Study sites.....	120
4.2b Study species.....	120
4.2c Experimental procedure.....	122
4.2d Insect Identification.....	123
4.2e Statistical methods.....	124
4.3 Results	
4.3a <i>Digitalis purpurea</i>	124
4.3b <i>Bupleurum fruticosum</i>	127
4.3c <i>Brassica juncea</i>	131
4.4 Discussion	
4.4a Responses of flower visitors to ant scent-marked flowers.....	133
4.4b Scent-marks deposited by ants.....	134
4.4c Why did scent-marks not influence flower visitor behaviour; and when might they do so?.....	135
4.4d Conclusions.....	137

- **Chapter 5: Floral Visitor and Ant Scent**

5.1 Contributions of this Thesis.....	138
5.2 Unknowns in the Role of Ants in Pollination Biology	
5.2a Floral ant-repellence.....	140
5.2b Ant scent-marks on flowers.....	143
5.3 Future Directions	
5.3a Identifying the chemical basis of ant-repellence.....	143
5.3b Identifying the role of coevolution in selection for ant-repellence.....	144
5.3c Identifying when and where ant-scent-marks are informative in bee foraging decisions.....	145
5.4 Summary.....	146

- **Bibliography**

Abstract

As flower visitors, ants rarely benefit a plant, commonly disrupting pollination by deterring other flower visitors, or stealing nectar. This thesis examines three aspects of ant-flower interactions, focusing on the occurrence of floral traits that prevent disruption of pollination and a novel means by which ants may influence pollinator behaviour.

To assess which types of plant species possess ant-repelling floral traits I carried out a survey of 49 Neotropical plant species. Around a third of these species were repellent to the common generalist ant *Camponotus novograndensis* (Formicinae). This repellence was positively correlated with large nectar volumes within individual flowers. It appears that there has been selection for floral ant-repellence as a defence against ant thieves in plant species that invest in large volumes of nectar. In some cases these repellent traits were effective against a wide range of ant species. However, in no plant species were predacious ants particularly repelled, indicating that there may be little selective pressure on non-ant-plants to defend potential pollinators from aggressive ants.

To investigate the importance of coevolution in determining the effectiveness of ant-repellents, a small but diverse range of Mediterranean plant species were tested with the invasive nectar thieving ant *Linepithema humile* (Dolichoderinae) and the native but non-nectar thieving ant *Messor bouvieri* (Myrmecinae). Responses of both ant species to floral traits were very similar. The ability of some plants to restrict access to ant species with which they have no evolutionary history may help to reduce the impact invasive species, as nectar thieves, have on plant-pollinator interactions.

It is reported that flowers recently visited by bees and hoverflies may be rejected for a period of time by subsequent bee visitors through the detection of scent-marks. Nectar-thieving ants could potentially influence the foraging decisions of bees in a similar way if they come to associate ant trail pheromones or footprint hydrocarbons with poor reward levels. However, my empirical work found no differences were found in bee visitation behaviour between flowers of *Digitalis pupurea* (Plantaginaceae), *Bupleurum fruticosum* (Apiaceae) or *Brassica juncea* (Brassicaceae) that had been in contact with ants and control flowers. Ant-attendance at flowers of these species may not reduce

reward levels sufficiently to make it worthwhile for bees to incorporate ant scent-marks into foraging decisions.

Investigations like these into the interactions between ants, flowers and other flower visitors are essential if we hope to understand the part ants play in pollination ecology, and determine how ants have helped shape floral evolution.

Chapter 1: Ants, Plants and Pollination – a Review

1.1 Introduction

Ants make up a massively important part of most terrestrial ecosystems and one of the reasons for this is the way that they interact with so many other species in beneficial or antagonistic relationships. All species interactions are best viewed as interactions between guilds of organisms, be they plants, pollinators, mutualistic ant-guards or exploiters (Stanton, 2003). This is especially true in any study of pollination ecology, as flowers have been shaped not just by the needs and sensory capabilities of their pollinators but also by interactions with herbivores, nectar thieves and other groups. Ants, being so ubiquitous, have the potential to influence pollination both beneficially and antagonistically.

Often interactions between ants and plants are beneficial to both parties, as ants can disrupt herbivore feeding and oviposition in ways that can be hard for generalist herbivores to circumvent (Stryksy & Eubanks, 2007). The most common way that plants gain the protective benefits provided by ants is through offering sugar-rich liquids produced by extra-floral nectaries (EFNs) or provided by hemipteran honeydew. EFNs can be found on a wide variety of plant parts and throughout a huge taxonomic range of species and act primarily to attract enemies of herbivores (Rico-Gray & Oliveira, 2007). In the tropics the consistently stable conditions leading to consistent herbivory pressure and therefore consistent benefits of ant attendance have allowed certain plant and ant lineages to become obligate partners in mutualistic relationships. These obligate ant-plants, known as myrmecophytes, house their ant-guards and often provide them with food, effectively investing in a standing army that defends against a range of threats (Janzen, 1977; Stanton *et al*, 2002).

Ant visitation to flowers is often very common, making up 58.5% of visitations in a Mediterranean grassland habitat for example (Bosch *et al*, 1997). However, the importance of floral nectar to ant communities has historically been overlooked (Blüthgen *et al*, 2004a), with greater emphasis on EFN-visitation. During visits to flowers ants may effectively pollinate flowers and ant-pollination has been demonstrated in a small but fascinating number of species (Hickman, 1974; Vega

et al, 2009). Most ants, being flightless, smooth-bodied and possessing antibiotic secretions that can kill pollen, are very poor pollinators (Beattie *et al*, 1986), and so ants usually act as nectar thieves. Floral larceny is a burden on flowers, inflicted by a taxonomically diverse range of flower visitors that steal nectar or pollen without providing any direct benefits as pollinators (reviewed by Inouye, 1980). As it is nectar alone that ants feed on at flowers it is useful to consider the costs involved in its production and those suffered when it is removed. Aggressive ants, like those employed as ant-guards, can also pose a threat to potential pollinators. Predation risks at flowers, due to ants or more specialised predators like crab spiders, alter the behaviour of potential pollinators in a variety of ways (Dukas, 2001; Ings & Chittka, 2008). Both nectar theft and predation risk can make flowers less attractive to potential pollinators, although this does not necessarily result in a loss of plant fitness as pollen flow may be altered to promote outcrossing, which can benefit populations that are not pollen-limited (Maloot & Inouye, 2000; Irwin *et al*, 2001). From the perspective of the pollinator, however, depleted flowers or those that carry with them a risk of increased predation are never beneficial and bees are often adapted to detect and avoid such flowers by scent alone, detecting marks left by previous visitors (Stout *et al*, 1998; Gawleta *et al*, 2005), possibly including ants.

As more attention is being focused on the implications of ant attendance at flowers it is becoming clear that plants are commonly adapted to limit ant access to flowers through the use of physical barriers and repellent chemicals (Kerner 1878/2008; Willmer *et al*, 2009). Such adaptations are especially obvious where they have been looked for in the ant-plants, reliant as they are on ants for protection but in need of mechanisms to prevent ant-disruption of pollination (Willmer & Stone, 1997; Willmer *et al*, 2009). Interactions between ants and flowers, however, temporally and spatially make up only part of the relationship between ants and plants as a whole. So, before discussing the interactions between ants, flowers and other flower visitors, it is important to first review the variety of relationships that exist between ants and plants and how other species, especially homopterans, can influence their outcomes.

1.2 Ant Exploitation of Sugar Sources

Ants that visit flowers to feed on nectar also acquire sugar from other sources, especially EFNs and homopterans. These interactions span multiple trophic levels, influencing, in a species-specific and density-dependent manner, not just the host plant but also the community of herbivores and predators that live on or visit it. Sophisticated deployment of EF-nectar by plants can focus ant activity on vulnerable areas and both plants and homopterans may compete for the attention of ant colonies (Figure 1 a-c). These multitrophic interactions are by far the most common impact ants have on plant fitness and also highlight the importance of nectar sources to ant populations.

1.2a Extrafloral nectar

EFNs are found in a wide range of species and are especially abundant in certain tropical habitats, such as rainforests and Brazil's cerrado savanna (Oliveira & Freitas, 2004). By 2007, the ant-plant relationship was studied in 67 species with EFNs (Rico-Gray & Oliveira, 2007) and EFNs themselves occur on species in at least 93 flowering plant and 5 fern families (Koptur, 1992), with new examples of EFNs regularly being reported on large numbers of species (Díaz-Castelazo *et al*, 2004). Ants attracted to EFNs provide protection primarily against direct herbivory, by attacking or disrupting herbivores feeding or egg laying (Letourneau, 1983; Strysky & Eubanks, 2007; Figure 1a). In a growing number of cases ant attendance at EFNs has also been linked to increases in plant fitness: for example, seed set in wild cotton plants is positively correlated with EFN frequency (Rudgers, 2004). This effect is especially pronounced in the tropics where herbivory pressure is generally higher (Rosumek *et al*, 2009).

Although fine details are extremely rare in fossils we know that EFNs were present on extinct poplars that lived around 30 million years ago, during the Oligocene (Pemberton, 1992), and ant-aphid interactions have been preserved in amber on ferns from the early Tertiary, around 60 million years ago (Wheeler, 1914). Protective ant-plant interactions are important not just ecologically but also economically in the time before pesticides were in widespread use. *Oecophylla smaragdina*, weaver ants, (Formicidae) have commonly been used in China to help protect citrus crops against large pest

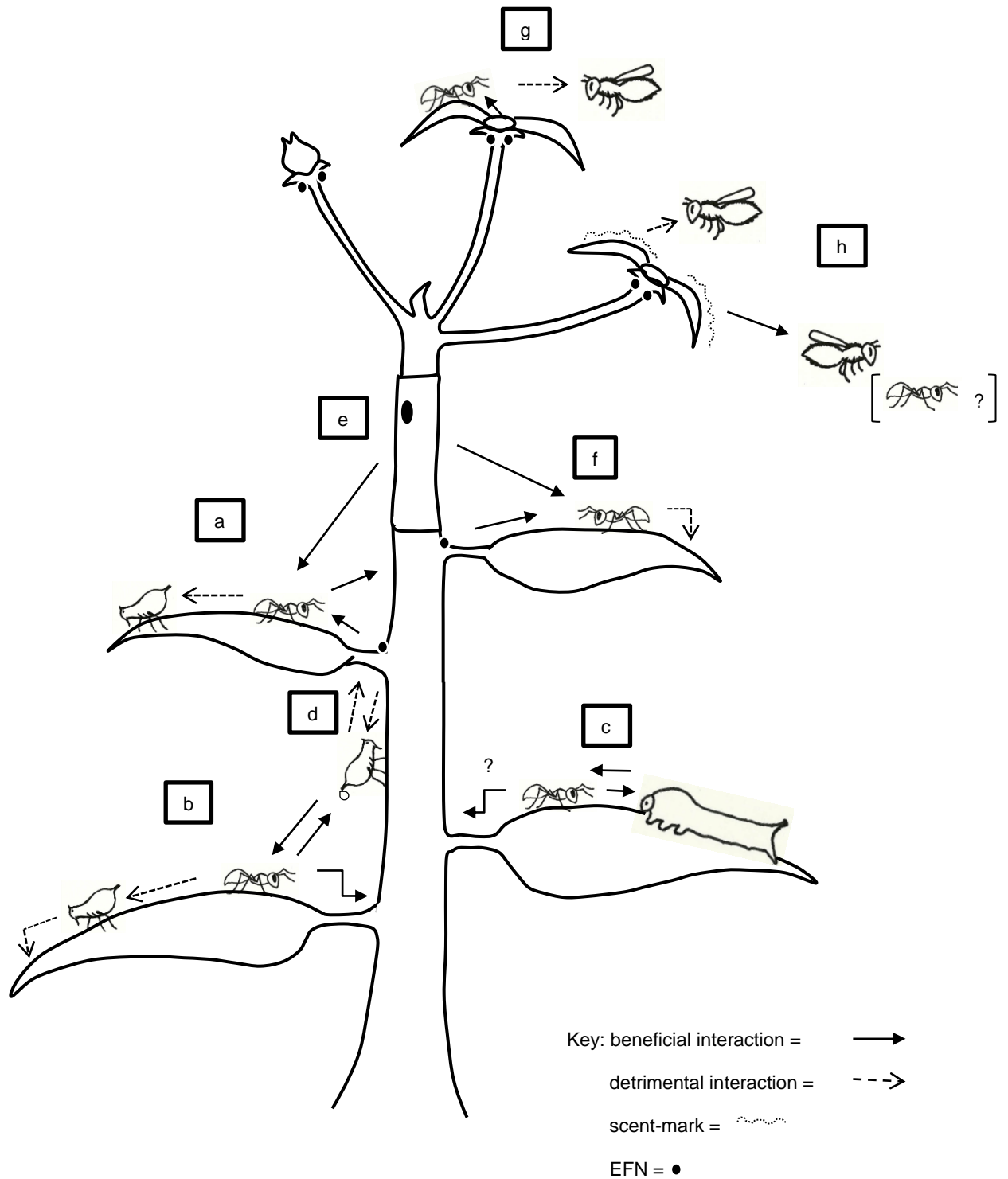


Figure 1: a) Ants visiting EFNs on new growth or inflorescences reduce costs imposed by herbivores. b) Ants tending homopterans reduce density of non-tended herbivores. c) Ants tending lycaenid caterpillars, no resulting benefit to host plants found. d) Competition between sugar sources for ant attendance. e) Ant-plant traits, such as domatia and food bodies, maintain ant-guards that reduce herbivore density. f) Parasitic species exploiting ant-plant traits without provide benefits to the plant, g) Ants stealing floral nectar and disrupting pollination. h) Scent-marks deposited by floral visitors (possibly including ants) repel potential pollinators by indicating low reward levels.

species for hundreds of years (Huang & Yang, 1987).

The threat of disruption by ants causes many herbivores to avoid ant-attended plants altogether. The beetle *Ryparida wallacei* (Chrysomelidae) preferentially feeds on leaves from plants outside of *Oecophylla smaragdina* territory, presumably due to detection of potentially long lasting ant trail pheromones (Offenberg *et al*, 2004). By removing potential vectors of plant disease, such as non-ant tended aphids, ants may also provide protection against pathogen damage (de la Fuente & Marquis, 1999), although this is complicated by ant-attendance of other species. The benefits provided by ant colonies to plants are generally identified through the use of exclusion experiments, the majority of which identify an increase in herbivory when ants are absent (Rosumek *et al*, 2009). However, these studies are complicated by the fact that they also exclude other small walking invertebrates. Future studies may also take advantage of techniques involving the addition of single ant nests to stems to isolate the effects of individual species (Byk & Del-Claro, 2010).

EFN placement is often crucial, promoting visitation to vulnerable areas such as meristems and young leaves, or inflorescences, where ants can protect against seed predators. Ants visiting EFNs on the inflorescences of the herb *Costus woodsonii* (Costaceae) disrupt the oviposition behaviour of *Euxesta* picture-wing flies (Ulidiidae) that would otherwise reduce seed set as their larvae develop on *Costus* seeds (Schemske, 1980). Ants attracted by EFNs to the inflorescences of the perennial vine *Canavalia cathartica* (Fabaceae) go on to nest in cavities produced in the fruit by moth larvae and reduce the likelihood of further moth damage being done (Yamashiro & Yamashiro, 2008). Placement of EFNs and quality of EF-nectar differs between *Passiflora* species (Passifloraceae) and as a consequence each species of vine attracts different ant species in different numbers, providing varying degrees of protection against herbivory (Apple & Feener, 2001).

The efficiency of ant-guards attracted by EFNs depends on a wide range of factors, such as the ant's diet, territoriality and size. While a variety of ant species provide protection for the EFN-bearing tree *Ouratea spectabilis* (Ochnaceae), when access is limited to *Cephalotes pusillus* ants (Myrmicinae) no benefits were detected (Byk & Del-Claro, 2010). *Eunica bechina* butterflies (Nymphalidae) have been shown to avoid ovipositing on host plants with dried ants of potentially

dangerous *Camponotus* (Formicinae) species pinned to them, but freely oviposit on plants with harmless *Cephalotes* ants are attached (Sendoya *et al*, 2009). This ability to detect predaceous ants, through visual (Freitas, & Oliveria, 1996; Sendoya *et al*, 2009) or olfactory means (Offenberg *et al*, 2004), may be common among specialist herbivores of EFN-bearing plants. The presence or absence of ant-tended homopterans, discussed in detail below, is also crucial (Heil & McKey, 2003).

EF-nectar can substantially increase worker ant longevity, especially in species with physiological adaptations to liquid feeding such as formicine ants (Lach *et al*, 2009). Most ants preferentially feed on solutions of sugar and amino acids (Lanza, 1988; Blüthgen & Fiedler, 2004). While worker survival in many species does not alter when they are provided with a sucrose solution without amino acids, the amino acids may well be essential in the rearing of larvae (Lach *et al*, 2009). It appears that the exact nature of the amino acids is more important to ants that specialise on a particular plant species (and therefore have less access to an off-plant protein source): for example, *Acacia hindsii* (Fabaceae) ant mutualists are able to discriminate and preferentially chose nectar containing the same combination of amino acids as that produced by their host (González-Teuber & Heil, 2009), whereas other ants choose nectars randomly.

EF-nectar production in many species is inducible in response to herbivory, directing ants to sites of damage while conserving resources when ants are not needed. Following two days of herbivory, cotton and castor oil plants both respond by increasing EF-nectar production in those nectaries closest to the attack, an increase that persists post-herbivory for two and four days respectively (Wäckers *et al*, 2001). The amino acid content of *Impatiens sultani* (Balsaminaceae) EF-nectar peaks dramatically 24 hours after defoliation, making it more attractive to specialist ants, and after 72 hours has returned to normal levels (Smith *et al*, 1990). In the senita cacti *Pachycereus schottii* (Cactaceae), while EF-nectar production is inducible on floral buds, when the tissue develops into more valuable fruits EF-nectar is produced constantly (Holland *et al*, 2009). Such species conform to theories of optimal defence (McKey, 1974) by actively expending more energy when at risk and where the greatest resource investment can be protected.

A wide range of other arthropods exploit EFNs, including stingless bees, flies, beetles and

mites, and not all provide benefits in return. The concentrated nature of most EF-nectar due to high evaporation rates (Josens *et al*, 1998) not only decreases intake rate, so the length of time ants spend at EFNs is increased, but also helps to exclude less suitable visitors, such as butterflies that are unable to ingest solutions of such high viscosity as their nectar intake rate decreases with increasing sugar concentration (May, 1985). Despite this, thievery by species, including ants, that provide no protective services may still be widespread (Rudgers & Gardener, 2004).

Some of these other visitors, such as parasitoid wasps, spiders and predatory mites, benefit the plant by reducing herbivory in the same way as ants. The presence of jumping spiders has been shown to enhance seed production on the EFN-bearing herb *Chamaecrista nictitans* (Fabaceae) (Ruhren & Handel, 1999), while certain species of wasp may do the same on the EFN-bearing herb *Turnera ulmifolia* (Passifloraceae) (Cuautle & Rico-Gray, 2003). When ants are excluded from *T. ulmifolia* predatory *Polistes* wasps have free access to EFNs and provide even greater protection than smaller ant species (Torres-Hernandez *et al*, 2000). Parasitoid wasps are also common EFN visitors, and are present in much greater numbers on *Solanum adherens* (Solanaceae) compared with congeners without EFNs (Gentry, 2003). Parasitoids act against a different set of herbivores than ants. While they are less of a deterrent to oviposition their own ovipositors can access hidden herbivores that ants do not come into contact with. Hespeneide (1985) speculated that the shrub *Byttneria aculeata* (Malvaceae) benefited as much from the large numbers of parasitoids attracted to its' EFNs as the attending ants. The EFNs of the trees *Inga densiflora* and *I. punctata* (Mimosoideae) in Costa Rica both receive higher visitation by wasp and fly parasitoids at higher altitudes where ant visitation is lower, resulting in a higher incidence of caterpillar parasitism (Koptur, 1985). *Phaseolus lunatus*, lima bean (Fabaceae), responds to volatile emissions from conspecifics that have been damaged by herbivores by increasing EF-nectar production, which is positively correlated with higher densities of predators on the plants (Kost & Heil, 2005, 2006). Such herbivory-induced EF-nectar production also slows the dispersal of predatory mites that feed from EFNs and provide indirect defence against herbivorous spider mites (Choh *et al*, 2006). In the future a range of “mute” mutant plants, that are unable to produce volatile organic signalling molecules such as jasmonate, and “deaf” mutants, that do not possess receptors for the signals, may help us to understand the extent of such plant-plant “communication” (Baldwin *et al*, 2006). EF-nectar also helps to maintain predaceous mite populations

on castor bean *Ricinus communis* (Euphorbiaceae), especially pre- and post-flowering (van Rijn & Tanigoshi, 1999) and promotes predatory activity of lacewing larvae by increasing their longevity in the first instar on cotton (Limburg & Rosenheim, 2001).

In theory floral nectaries are capable of attracting beneficial predators in the same way as EFNs. Yano (1994) speculated that floral nectaries might possess a secondary defensive role in the herbaceous crucifer *Rorippa indica* (Brassicaceae) by attracting ants. A stronger case for floral nectaries acting in a role analogous to EFNs can be seen in the euforb *Croton suberosus* (Euphorbiaceae). Despite being anemophilous its flowers still produce nectar that attracts wasps, especially the caterpillar predator *Polistes instabilis* (Vespidae) (Domínguez *et al*, 1989).

1.2b Herbivore-derived sugar sources

The other common way ants obtain plant-based carbohydrates is by tending homopterans feeding on a plant. A large number of homopteran species are adapted to be tended by ants. When tended such species increase their population sizes up to 8-fold, partly because the appearance of winged reproductives is delayed, and also because each individual homopteran may increase its phloem uptake and excretion rates (Banks & Nixon, 1958; Carroll & Janzen, 1973; Takeda *et al*, 1982). By removing other insects from a plant, ants protect their homopteran partners from predators, such as ladybirds, lacewings and syrphids, and competitors, as they kill non-ant-tended homopterans (Sakata & Hashimoto, 2000). Where there is a surplus of partner homopterans, however, ants will often cull the excess (Pontin, 1978).

Facultative ant-tended homopterans are smaller in size when tended, and produce fewer embryos, presumably due to the costs of the higher rate of honeydew excretion (Yao *et al*, 2000). When forced to compete with other sugar and amino acid sources to attract dominant ants, a high quality of honeydew production may be maintained, regardless of host plant nutritional value. *Tuberculatus quercicola* aphids (Aphididae) change the composition of their honeydew when ant-tended, and when the availability of amino acids in plant phloem falls they still maintain the same concentration in their honeydew to ensure they are not deserted by their ant-guards (Yao & Akimoto, 2002).

In return for this sometimes costly honeydew production, homopterans are protected from predation and parasitism, and benefit from better hygiene as honeydew is removed by the ants before fungal growth can build up (Way, 1963; Nielsen *et al*, 2010). When under threat from ladybird beetles, treehoppers use vibrational alarm-calls to increase the activity of their ant-guards (Morales *et al*, 2008) and ants have also been shown to transport homopterans to new hosts of better quality (Collins & Leather, 2002). The relationship can, however, be exploited by parasitoids, such as the Aphidiid wasp *Lysiphlebus* (Braconidae), that actively seek out ant-tended colonies and evade ant detection so their offspring will be protected from hyperparasitism inside aphid mummies (Völkl, 1992). Many ant-tended aphid species have become entirely dependent on ants and their population density decreases with increasing distance from suitable ant colonies (Wimp & Whitham, 2001).

The consequences of ant-homopteran interactions for host plants depend on the density of ants present (Oliver *et al*, 2007), on plant genetic traits (Mooney & Agrawal, 2008), and most importantly on the presence of other potential herbivores (Figure 1b). Even the weather can produce differences, as rainfall reduces the effectiveness of homopteran-tending ants on the vine *Passiflora coccinea* in French Guiana (Writh & Leal, 2001). By reducing the levels of non-tended herbivores, plants may indirectly benefit from the presence of the mutualism. 73% of studies looked at by Styrsky and Eubanks (2007) showed an overall benefit to the host plants from the interaction, where the costs imposed by the herbivores that the ants disrupted were higher than those imposed by the ant-tended homopterans themselves. In temperate areas the presence of ant-tended aphids is especially important in spring, attracting ants that then deal with other herbivores before they are mature enough to cause serious damage (Floate & Whitham, 1994).

One of the most important costs to a plant of homopteran populations is their transmission of plant viruses (Maramorosch, 1963), a problem exacerbated by ant attendance. Ant presence can also have other indirect costs through effects on the rest of the arthropod community. High ant densities, leading to high homopteran densities, have been shown to increase the number of non-ant-tended chewing herbivores on *Vicia faba* (Fabaceae), possibly due to aphid-mediated effects on plant susceptibility (Oliver *et al*, 2007). Tended homopterans are not the only herbivores to benefit; while ants reduce some herbivore numbers and the overall species richness of herbivores is lowered, other

species, such as some homopterans and leaf miners, benefit from the ants due to reduced predation and interspecific competition (Fowler & MacGarvin, 1985). It is also possible that attracting certain ant species with homopterans or EFN can just result in reduced predator numbers, especially spiders, with no reduction in herbivores at all (Mody & Linsenmair, 2004).

The presence or absence of ants is influential in many agro-ecosystems. *Iridomyrmex rufoniger* (Formicidae) has been linked to outbreaks of scale insects in citrus canopies in New South Wales, which in turn leads to problems with sooty mould. When the ants are excluded there is a 2.8 - 4-fold increase in the abundance of other beneficial species, like spiders (James *et al*, 1999). Such interactions may render attempts at biological control of specific pests useless if ants drive off biocontrol agents. Although ant-homopteran mutualisms undoubtedly have a negative effect on biodiversity on individual plants, there is some evidence that in habitats where only some trees are inhabited by ants and homopterans, the mutualism may have an overall effect of enhancing the area's biodiversity (Wimp & Whitham, 2001). This is probably less likely to be the case when invasive ant species are involved.

Invasive ants have dramatic disruptive effects on the habitats that they are introduced to, displacing native species and altering the structure and function of the ecosystem (Holway *et al*, 2002). They are by definition dominant species that monopolise sugar sources wherever they spread and displace native ant species, usually through interference competition (Human & Gordon, 1997). By defending these sugar sources, invasives ant-attendance leads to an increase in the population density of ant-tended homopterans, which has far-reaching consequences for the entire arthropod community structure (Way, 1963; Ness & Bronstein, 2004; Kaplan & Eubanks 2005; Grover *et al*, 2008). Argentine ants, *Linepithema humile* (Dolichoderinae), are preadapted to tend novel aphid species in newly colonised habitats as they respond to the alarm pheromones of native poplar aphids, *Chaitophorus populicola* (Aphididae) in California (Mondor & Addicott, 2007).

Where invasive ant ranges meet those of invasive homopterans the two species can facilitate each other's expansion. *Solenopsis invicta* (Myrmicinae) and the mealybug *Antonina graminis* (Pseudococcidae) appear to be doing just that in the United States, with mealybugs providing around

45% of the ants' daily energetic requirements (Helms & Vinson, 2002). The impact these invasive partnerships have on endemic plant fitness is still unclear. In addition, the picture is never completely one-sided. For example invasive Argentine ants act as efficient biocontrol agents in parts of Portugal where the moth *Thaumetopoea pityocampa* (Thaumetopoeidae) can be a serious pest species (Way *et al*, 1999). Invasive ants may be especially beneficial if they out-compete native homopteran-tending ants that are less aggressive than the invaders. Argentine ants are more aggressive than the native *Dorymyrmex bureni*, pyramid ants (Dolichoderinae), in the United States, so when tending aphids on cotton plants they increase cotton reproduction when caterpillar numbers are high (Styrsky & Eubanks, 2010).

A similar relationship exists between ants and lycaenid caterpillars (Figure 1c). The subfamily Lycaenidae contains around a third of all butterfly species and about 75% of these are associated with ants to varying degrees, from facultative to obligate and parasitic to mutualistic (Pierce *et al*, 2002). The larvae possess a dorsal nectary organ on the seventh abdominal segment that secretes a sugar and amino acid rich substance that is harvested by ants. Pore cupolas around the nectary organ release pheromones mimicking those of ant brood, which attract ants and promote tending (Thomas & Elmes, 1993), while tentacular organs on the eighth abdominal segment also attract ants and increase ant activity levels (Axén *et al*, 1996). Both larvae and pupae attract ants using substrate-borne vibrational signals (Travassos & Pierce, 2000). These ant-attracting specialisations may be more costly than the modified excretion traits utilized by homopterans (Stadler *et al*, 2001). Myrmecophytic lycaenids specifically oviposit on plants with ants that can tend their larvae (Pierce & Elgar, 1985; Fiedler & Maschwitz, 1989) and ant attendance may allow them to survive on less suitable novel plants, promoting diversification to new hosts (Forister *et al*, 2010). Even naïve workers of ant species such as *Camponotus japonicas*, that regularly tend certain lycaenid species, preferentially feed from solutions that mimic the lepidopteron secretions in sugar and amino acid composition (Hojo *et al*, 2008).

In a small number of cases lycaenid larvae feed on the eggs, larvae and pupae of their ant hosts, often overwintering in their nests (Pierce, 1995). Lycaenids may manipulate ants in other ways, as where facultative relationships occur, simulated predation attempts may induce secretion of ant-

attracting rewards (Agrawal & Fordyce, 2000) and individuals secrete less when aggregated together with other lycaenids (Axén & Pierce, 1998). By triggering tending using brood pheromone mimics the lycaenids can promote unusual behaviour from their hosts. For example, both major and minor *Oecophylla smaragdina* workers attempt to tend the larvae, and this can result in fighting between the two castes (Saarinen, 2006). They may also parasitise ant-plant relationships by exploiting EF-nectar (DeVries & Baker, 1989). One crucial difference between ant-lycaenid and ant-homopteran mutualisms is that the attraction of ants by lycaenids has not yet been shown to benefit host plants.

1.2c Competing sugar sources

The same ant species that tend homopterans may also visit EFNs, giving rise to potential competition between homopterans and plants for attention from ant-guards (Figure 1d). The real importance of nectar (both floral and extrafloral) and honeydew to most opportunistic ant species is still unclear. All three sources can potentially provide the ten essential amino acids required by insects (Blüthgen *et al*, 2004a). Where large volumes are habitually ingested, seasonal changes in EF-nectar secretion can even be tracked to the level of carbon isotopes in the bodies of visiting ants (Rico-Gray & Stenberg, 1991).

Although studies may test for the optimal preference of an ant species in terms of EF-nectar composition, in reality sugar resources will be monopolised by the most dominant species in the area. So selective preferences only translate into actual resource use in competitively dominant species (Blüthgen & Fiedler, 2004b). As honeydew is often the best quality resource on offer, homopteran colonies are monopolised by dominant species, while EFNs sustain the range of other sub-dominant ant species (Blüthgen *et al*, 2000; 2004b). 96% of trophobioses (interactions in which food is exchanged between partners) looked at in Borneo were monopolised by a single ant species competitively excluding all others (Blüthgen *et al*, 2006). This can be achieved either through aggression or exploitation, as in *Camponotus floridanus* on the weed *Urena lobata* (Malvaceae), where EFNs are systematically emptied creating a lower standing volume of nectar and therefore a less attractive resource to competitors (Dreisig, 2000). The influence of neonatal imprinting on host tree species also seems to play a role in the structuring of dominant ant communities (Djipto-Lordon & Dejean, 1999). This may be part of the reason why, although ants are facultative visitors of most EFN-

bearing plants, they may still show strong specificity within local areas (Thompson, 1988).

In contrast with obligate mutualisms, in which a plant will provide most of the nutritional needs of its ant-guards, in facultative relationships it is in the plant's interests to supply very little nitrogen to patrolling ants. Tropical canopy trees maintain a huge ant population by providing a rich supply of nitrogen-poor EF-nectar. This has led to the 'ant-biomass paradox' where there are more predatory ants than prey present in the canopy (Tobin, 1994). Workers of sub-dominant species were found to feed almost exclusively on EF-nectar in Australian forest, while dominant predatory ants had a more varied diet, including honeydew (Blüthgen *et al*, 2003). So the exudates fuel the prey-hunting activity of these huge ant populations, benefiting the canopy trees (Davidson, 1997; Tillberg, 2004).

The efficiency of an ant as a forager of EF-nectar or homopteran honeydew will be partly determined by its physiological adaptations to ingesting large nectar volumes and feed at a lower trophic level as an omnivore, activity that is detectable through the analysis of stable nitrogen isotope ratios within the ant's bodies (Tillberg, 2004). The highly modified proventriculi of many formicines and some dolichoderines enable rapid uptake of large volumes. These sclerotised valves separate the anterior stomach (also known as the colony stomach, as liquids are often regurgitated to feed larvae or other workers) from the hindgut and limit the movement of liquid, allowing large volumes to be stored and then gradually released into the hindgut without the need for muscular activity (Cook & Davidson, 2006). Such structures are predominantly found among solitary foraging species that can best locate and exploit sugar sources within the canopy (Davidson *et al*, 2004).

Interspecific competition and the availability of nectar and honeydew sources are two of the key factors that influence the structure of ant communities in rainforest canopies are structured around (Blüthgen & Fiedler, 2004a, 2004b; Blüthgen *et al*, 2004b). Thompson (1982) was the first to suggest that EFNs may by-pass the ant-homopteran mutualism and obtain the defensive benefits for a plant. In fact, the benefits of distracting the ants from homopterans alone, without receiving any protection, may favour the evolution of EFNs (Becerra & Venable, 1989). When *Lasius niger* (Formicinae) workers are supplied with honey solution, their predation on aphids increases 8-fold and tending decreases (Offenberg, 2001). This kind of evidence provides strong support for the idea that EFNs protect plants from potentially harmful ant-homopteran alliances. While *Vicia faba* EFNs do reduce numbers of non-

tended aphids there is no evidence it distracts *Lasius niger* from *Aphis fabae*, which produces a honeydew rich in the ant-attracting sugar melezitose (Engel *et al*, 2001). In the competition between sugar sources for ant attention homopterans may decrease the relative attractiveness of EF-nectar by reducing its quality, through a general loss of plant vigour (Sakata & Hashimoto, 2000). Where one sugar source fails, ants readily switch to the best alternative. For example, despite tending leafhoppers for much of the year *Brachymyrmex obscurior* (Formicinae) ants switch to nearby EFN-bearing plants near the end of the dry season when the leafhoppers' host *Tripsacum* spp. (gama grass, Poaceae) dries up (Moya-Raygoza & Larsen, 2000).

Offenberg (2000) identified a positive correlation between ant-tending of aphids and the number of EFNs on Scandinavian EFN-bearing plants. While on some species homopterans may act as stand-ins for EFNs as they do for many myrmecophytes, it seems likely that in many cases aphids are parasites of the ant-plant mutualism, being attracted to plants with protective ants present. Another possibility is that traits promoting ant-tending were selected for in aphid species specialised to feed in host plant species that happened to have EFNs, as a defence against predation by ants (Offenberg, 2000).

Assessing the potential costs and benefits of attendance by any ant species, however, is not simple. The invasive yellow crazy ant, *Anoplolepis gracilipes* (Formicinae), displaces more herbivores when present on the EFN-bearing *Acacia lamprocarpa* (although not the unrewarding *Eucalyptus tetrodonta*, Myrtaceae) than the native ant *Oecophylla smaragdina*, but herbivory is still greater on trees inhabited by the invasive species (Lach & Hoffmann, 2011). There is a growing consensus that experiments to determine the fitness consequences of potentially mutualistic relationships need to avoid being biased by tempero-spatial variations in relationships by being carried out for many years and in a range of locations (Heil *et al*, 2001a). To complicate matters further, under certain levels of herbivory, especially where apical dominance of meristems is disrupted, plants may overcompensate and actually perform better than if left untouched (Huhta *et al*, 2003). Despite significantly reducing the numbers of herbivores, but not the number of predators on the mangrove tree *Rhizophora mucronata* (Rhizophoraceae), plants with *Oecophylla smaragdina* ants grew very slightly less than control plants that the ants had been excluded from (Offenberg *et al*, 2005), an unexpected result considering that

no homopteran-tending occurred in this system and no energy was expended to attract the ants in the first place.

Even where a significant reduction in folivory is detected, this does not necessarily translate directly into a significant fitness benefit. Many plants can withstand a great deal of defoliation, so why should such plants expend energy maintaining ant colonies? In unpredictable environments it can be essential for plants to have insurance against changes in herbivore abundance, even if investment in such defences is sub-optimal in the short term. One key problem with the bulk of the studies done into the fitness costs of herbivory is that the short time periods they run for may easily miss periods of peak herbivory or development when the benefits of ant colonies are greatest (Heil *et al*, 2001a; Mody & Linsenmair, 2004; Tutin *et al*, 2004). The same is true where EFN-ant and ant-homopteran-plant relationships are being investigated. Any benefits from fern foliar nectaries, for example, have proved to be consistently elusive (Rashbrook *et al*, 1992). Ants on the EFN-bearing *Cassia fasciculata* (Fabaceae) have been shown to decrease herbivory and increase growth without translating into an increase in seed set. Other more important factors may determine seed set, such as water limitation (Kelly, 1986). Taking all this variation into account, a lack of benefits in facultative interactions for much of the time may be common (Ruhren, 2003). Dependency between partners may grow when benefits are more reliable, until an obligate mutualistic relationship is formed.

1.3 Specialised Ant-Plants

The massive spatiotemporal variation in ant-plant relationships is likely to limit the opportunity for taxon-specific coevolution. Even in the tropics consistent biotic selection pressures may still be rare (Horvitz & Schemske, 1990). However, in a phylogenetically diverse range of plant groups the benefits of ant-attendance have been great enough to select for myrmecophytic traits such as housing areas, known as domatia, and food bodies (Davidson & McKey, 1993; Figure 1e). As these may be costly to produce, plants need methods to direct a limited pool of workers to where they are needed and investments in myrmecophytic traits often trade-off against other more conventional defences.

1.3a Food and shelter

To maintain colonies of obligate ant partners, myrmecophytes must provide all the nutrients and nesting space needed. Domatia are discrete pockets of empty plant tissue that ant colonies use for brood rearing, shelter and storage. *Acacia* myrmecophytes possess swollen thorns (Janzen, 1977), *Macaranga* trees (Euphorbiaceae) (Fiala & Maschwitz, 1992) and *Piper* shrubs (Piperaceae) (Tepe *et al*, 2007) have hollow stems, while hollow rhizomes are found in *Lecanopteris* ferns (Polypodiaceae) (Walker, 1986). In return the defence that ant-guards provide is crucial to plant fitness. The form of defence varies greatly between partnerships, from passive defence against egg laying provided by the *Pheidole* ants (Myrmicinae) on *Piper* myrmecophytes (Letourneau, 1983) to the highly coordinated strategic defence against encroachment by herbivores or competing plants practiced by some inhabitants of *Acacia* ant-plants (Amador-Vargas, 2008).

The nomenclature of the genera *Acacia* is currently controversial. This polyphyletic grouping has recently been split into several genera including *Vachellia* and *Senegalia*, with the predominantly Australian monophyletic group retaining the genus name *Acacia* (Theile *et al*, 2011). All African and New World *Acacia* species discussed below are currently classified as *Vachellia*. However, as there is no consensus over the current state of affairs and the nomenclature could soon change again, for the purposes of this thesis the genus name *Acacia* has been retained for all species mentioned. *Acacia* myrmecophytes produce a constant supply of EF-nectar to their ant-guards, encouraging a persistent ant presence over the plant. As the EF-nectar is present and exposed at all times the *Acacia* protects it from microbial infestation using a wide range of chitinases, β -1,3-glucanases and peroxidases that inhibit fungal and bacterial growth (González-Teuber *et al*, 2009). Non-myrmecophytic *Acacia* species only secrete EF-nectar when under attack by herbivores and their EF-nectar has fewer defences, as it is only available for short periods of time (González-Teuber *et al*, 2009). While EF-nectar from myrmecophytic *Acacia* species inhibits the growth of certain phytopathogens, non-myrmecophytic *Acacia* EF-nectar has no such effect (González-Teuber *et al*, 2010).

So while in some facultative relationships ants are recruited to areas of leaf damage by EF-nectar secretion, in many mutualisms this may be done using as yet unidentified volatile chemicals. The ant *Allomerus octoarticulatus* (Myrmicinae) has been shown to recruit to damaged areas on

Hirtella myrmecophila (Rosaceae) (Romero & Izzo, 2004), *Crematogaster laevis* (Myrmicinae) and *Pheidole minutula* to damage on *Maieta guianensis* (Melastomataceae) (Lapola *et al*, 2003), *Azteca* sp. (Dolichoderinae) to damage on *Cecropia* (Urticaceae) (Agrawal, 1998) and *Crematogaster laevis* and *Azteca* sp. to damage on *Tococa bullifera* (Melastomataceae) (Bruna *et al*, 2004). According to optimal defence theory such induced biotic defences are most useful where herbivory is low, such that the costs of constitutive defensive mechanisms, in this case maintaining a large enough ant population to patrol all areas of the plant simultaneously, would outweigh the benefits (Zangerl & Rutledge, 1996).

Just as benefits from ants can at times be elusive when studying facultative relationships the same can be true for all ant-plant interactions. The benefits provided may not always be immediately obvious. *Pheidole bicornis* on *Piper* ant-plants act as 'passive defenders', removing herbivore eggs and fungal growth rather than attacking the adult herbivores themselves (Letourneau, 1983). Within the *Piper* genus obligate relationships with different *Pheidole* species have evolved at least three times. Despite developmental differences, the domatia produced by each lineage have converged on the same structure (Tepe *et al*, 2009).

Large host plants often mean more nesting space for plant-ants and therefore potentially higher plant-ant fitness. *Myrmelachista schumanni* ants (Formicinae), found throughout the Amazonian rainforest, have mutualistic relationships with a number of ant-plants including *Cordia nodosa* (Boraginaceae), *Duroia hirsuta* (Rubiaceae) and *Tococa guianensis*. They not only provide protection against herbivores (Frederickson, 2005) but also poison plant competitors by chewing holes in a plant and then injecting formic acid inside, creating patches of forest bare apart from the host plants known as “devil’s gardens” (Frederickson *et al*, 2005). Nesting space is a serious factor limiting fitness in this polygynous species (Frederickson, 2009) so, in addition to enhancing host size by eliminating the competition, *M. schumanni* also induce galls in plants neighbouring the gardens that provide further nesting space (Edwards *et al*, 2009). Put together these strategies ensure a single colony can live for more than 800 years (Frederickson *et al*, 2005).

Certain plant groups provide additional nutrients for their ant partners in the form of food bodies. In contrast with more facultative relationships it is in a myrmecophyte's interest to provide

nitrogen for growing plant-ant brood. The small egg-like structures found at the base of *Cecropia* leaves, known as Müllerian bodies, are rich in glycogen, rarely found in plant tissue, and so are specifically tailored to the nutritional needs of the plant's arthropod defenders (Rickson, 1971). Their production is also dependent on the intensity of mechanical removal so no excess is produced (Folgarait *et al*, 1994). *Macaranga* myrmecophytes produce more food bodies when inhabited by ants and also when provided with added nutrients (Heil *et al*, 2001b). For obligate plant-ants food bodies provide essential nutrients; an estimated 80% of the nitrogen intake of *Azteca* workers derives from their *Cecropia* host (Sagers *et al*, 2000). More aggressive plant-ants, like *Azteca*, behave like ecologically dominant non-plant-ants but instead of eating the prey they catch they often let them drop from the plant as their protein requirements are also supplemented by their host (Dejean *et al*, 2009). Sagers *et al* (2000) also estimated that the *Cecropia* plants receive 93% of their nitrogen from detritus deposited by their ant-guards within the stem domatia, possibly helping to give these pioneer plants a foothold in new areas.

Plant-ants that get food from their host plants through homopterans gain these intermediaries by either horizontal transmission, when homopterans are acquired as the ant colony grows, or vertical transmission, when founding queens beginning new colonies or budding factions of existing colonies may carry homopterans with them to a new host plant. In some cases it may depend on the nature of the host plant how aphids are transmitted. Partner coccoids significantly increase brood production for the ant *Cladomyrma* (Formicinae), but have to be horizontally transmitted to new host plants as new *Cladomyrma* queens cut open the host domatia so would be forced to first put down any homopteran loads they carry. The wound caused by the queen scars over but the bugs gain access to the brood chamber later through secondary slits (Moog *et al*, 2005). Other myrmecophytes that provide direct access to EFNs, such as *Acacia*, may still have to tolerate homopteran tending by their obligate ant-guards (Young *et al*, 1997).

A common trait among obligate ant-plant relationships appears to be their asymmetrical nature. Often an obligate ant species may only ever be found on its one host species. This high degree of specialisation is due to competition for the limited number of ant-plants available (Davidson & McKey, 1993; Fonseca & Ganade, 1996). That same host species, however, may interact with a

number of other ant species or even survive without any, albeit under increased herbivory pressure. Such mutualistic networks are nested, with a core of reciprocal generalists accompanied by specialists that interact almost exclusively with generalists (Guimarães *et al*, 2006). Due to a heavy reliance on a small number of species such nested networks are highly vulnerable to the extinction of those species with links to many others (Memmott *et al*, 2004). While coevolution is much less likely in networks of more facultative interactions, where it or other changes (such as extinction) do occur the consequences will affect far larger numbers of species (Guimarães *et al*, 2007). Identifying the precise nature of an interaction, however, can be difficult, especially when that interaction is rare. Detailed spatio-temporal data as well as detailed natural history information are needed to understand any mutualistic network (Vázquez *et al*, 2009).

In *Macaranga* ant-plants there are often one-to-one pairings of ant with plant species (Fiala *et al*, 1999). Most plant-ant species, however, have to compete with other plant-ant species for access to hosts. Multiple ant partners are maintained by environmental heterogeneity. *Acacia drepanolobium* is occupied by a range of ant species with varying competitive dominance and mutualistic benefits. Competitively superior dominant species, such as *Crematogaster sjostedti*, usually occupy the largest trees, while subordinate plant-ants, like *Crematogaster nigriceps* and *Tetraponera penzigi* (Pseudomyrmicinae), colonise younger hosts more quickly and competition between queens can be fierce (Palmer *et al*, 2000; Stanton *et al*, 2002). The competitively subordinate *C. nigriceps* prevents its colony being deposited by the dominant species by pruning apical meristems, restricting lateral growth and so limiting contact with other trees and their ant-guards (Stanton *et al*, 1999). *T. penzigi* persist in another way, by being resistant to invasion, through their destruction of EFNs on their own host plants and by making very small entrance holes into the swollen thorns that other species cannot fit through. These different mechanisms are all crucial factors that help to maintain biodiversity within an environment (Palmer *et al*, 2002). Competitively subordinate ant-guards can still be highly effective plant defenders and the resulting diversity may benefit the plants as a mosaic of different ant-guards within the same habitat may make it more difficult for herbivores to predict safe feeding times and places (Cronin, 1998).

Many plants are involved in seed mutualisms with ants, in which ants feed on special

elaiosome organs attached to seeds and in doing so disperse the seeds themselves to areas where they are most likely to germinate. Such mutualisms are dependent on volatile signalling. Obligate ant-garden ants, such as *Camponotus femoratus*, use their host's seeds as construction material in arboreal carton nests. When they germinate, the seeds turn the nests into small hanging gardens. A complex blend of chemicals, including methyl-6-methyl salicylate, attracts ants and triggers seed carrying behaviour, possibly by mimicking ant brood, as well as presumably performing other functions such as seed-predator repellence (Seidel *et al*, 1990; Youngsteadt *et al*, 2008).

1.3b Balancing the costs of maintaining ant-guards

While the defensive benefits of ant-guard colonies can be dramatic, the costs of structural domatia and regular food supply can be high. Under conditions of low herbivory, ant-plants have been shown to lose myrmecophytic traits. *Cecropia peltata* populations on many Caribbean islands do not produce food bodies, probably due to lower herbivory pressure and less interspecific competition with vines (Janzen, 1973). Although it is unknown how long it took *C. peltata* to lose their myrmecophytic traits in other species changes have been shown to occur very quickly. After a year without grazing, *Acacia drepanolobium* trees decrease their investment in EFNs and swollen thorns, making it clear that the trigger for their phenotypic expression is extensive damage (Huntzinger *et al*, 2004). This apparent saving in terms of resources can have unexpected consequences in the short term. In areas that have been isolated from large herbivores for a decade, trees actually suffer greater damage from arthropod herbivores as they are more likely to be occupied by the less efficient mutualist *Crematogaster sjostedti*. Conspecific trees inhabited by *C. nigriceps* do not lose myrmecophytic traits as the ant-pruning activity simulates herbivory (Palmer *et al*, 2008). In another example, *Tococa guianensis* populations have been found to persist without their ant mutualists at the edge of their species range. These plants have significantly tougher leaves and higher densities of trichomes than those plants with mutualists. The myrmecophytic traits of the plants in such populations are probably maintained by gene flow from populations with ant partners (Moraes & Vasconelos, 2009).

To benefit the most out of the colonies they have invested in, plants use a variety of chemical cues to direct ant behaviour. *Allomerus decemarticulatus* ants are induced to preferentially patrol foliar wounds of *Hirtella physophora*. The trigger appears to be non-volatile chemicals as the ants

intensively antennate the wounds before recruiting nestmates (Grangier *et al*, 2008). *Crematogaster laevis* responds to sites of physical damage on both its host plants *Tococa bullifera* and *Maieta guianensis* (Bruna *et al*, 2004). *Crematogaster* plant-ants respond to volatiles from their host *Macaranga* plants by swarming out of internodes and aggregating around the damaged area. The ants respond aggressively to damage on parts of other sympatric *Macaranga* myrmecophyte species but are most aggressive in response to their own host's volatiles (Inui & Itioka, 2007). *Azteca* ants also respond both to extracts from their host *Tococa bullifera* as well as non-host extracts, while *Pheidole minutula* only responds to volatiles from its host *Maitea guianensis*. The difference may possibly be due to the habit of the *Azteca* species of building satellite nests on nearby vegetation (Bruna *et al*, 2008).

Leonardoxa africana subsp. *africana* (Fabaceae), in which EF-nectaries only become active on mature leaves, also use volatiles to promote patrolling of young leaves by their ant-guards *Petalomyrmex phylax* (Formicinae) (Brouat *et al*, 2000). A comparison of the volatiles emitted by *L. a. africana* show that all are also emitted by the closely related non-myrmecophyte *L. a. gracilicaulis*; however, 'green leaf volatiles' and methyl salicylate are released in much larger quantities by *L. a. africana*. Methyl salicylate is of particular interest as it is similar to the compound methyl-6-methyl salicylate, which is an alarm pheromone in some ponerine species (Duffield & Blum, 1975). Schatz *et al* (2009) confirmed that methyl salicylate alone is responsible for recruiting *P. phylax* to damaged leaves. These volatiles are produced by intact leaves and promote ant attendance regardless of damage, so could have arisen from intact leaves mimicking damaged ones. The response is specific to the mutualistic partner, as the parasitic ant species *Cataulacus mckeyi* (Myrmicinae) was not induced to damaged areas and does not patrol young leaves (Brouat *et al*, 2000).

Obligate plant-ant queens are usually attracted to a new host by volatile signals. Both *Azteca* and *Allomerus* foundresses are attracted to *Cordia nodosa* plants in this way (Edwards *et al*, 2006). In the unusually species-specific *Crematogaster-Macaranga* relationship queens use compounds of low volatility on the stem surfaces to identify their specific *Macaranga* partner species (Inui *et al*, 2001). No discernible pattern can be found in the leaf volatile profiles of different *Macaranga* species, regardless of phylogeny or status as a myrmecophyte or non-myrmecophyte, and foundresses probably respond

to key components within the profiles (Jürgens *et al*, 2006). Similarly *Hirtella physophora* is almost entirely inhabited by the plant-ant *Allomerus decemarticulatus*, and in the absence of any other exclusion filters, either competitive or mechanical, it appears that it is efficient host searching mediated by volatiles that ensures *A. decemarticulatus* monopolisation of *H. physophora* (Grangier *et al*, 2009).

To fully understand the services provided by an ant colony they need to be placed in the context of the suite of defences utilized by the plant. As defensive mechanisms are often costly to produce, a lack of redundancy is expected (Agrawal, 2006). This has led researchers to attempt to identify trade-offs between chemical and biotic defences in myrmecophytes. In a number of African *Acacia* myrmecophytes anti-fungal chitinase activity is at least six times lower than that of four non-myrmecophytic species studied in a phylogenetically independent manner (Heil *et al*, 2000). Chitinases were also found to be lower in myrmecophytic than non-myrmecophytic *Macaranga* species (Heil *et al*, 1999) while tannin content was found to be lower in those *Macaranga* species developing myrmecophytic traits early in ontogeny (Eck *et al*, 2001). In a survey of ten *Macaranga* species, Nomura *et al* (2011) found that the intensity of ant-defence was negatively correlated with non-ant defences and that low leaf turnover and shade-tolerance corresponded with non-ant defences. Insecticidal amide content is slightly lower in ant-inhabited *Piper cenocladum* than uninhabited plants, regardless of herbivory (Dyer *et al*, 2001). *P. melanocladum*, which does not possess ant inhabitants or strong chemical defences, has instead very tough leaves (Fincher *et al*, 2008). Costa Rican *Inga* species have higher levels of phenolics in their leaves in the highlands, where ants are less abundant (Koptur, 1985). Heil *et al* (2002) identified another apparent trade-off in the myrmecophyte *Leonardoxa africana*, which has lower tannin content than non-myrmecophytic species. However, in the same study while a great deal of variation in the quantities of phenolic compounds was identified between different *Acacia* and *Macaranga* species, this variation does not correspond with the presence of myrmecophytic traits.

It has become clear that a simple chemical/biotic defence trade-off is an oversimplification. Trade-offs may only be common in the case of chemicals potentially dangerous to resident ants, such as chitinases and amides (Heil *et al*, 2002). Ant-guards need to be seen in the context of a synergistic network of plant defences (Agrawal, 2006). No single biotic or abiotic (chemical or physical) defence is

going to be capable of combating the whole array of enemies faced by a plant. Maximum defence is expected to be achieved by deploying different traits (often at different periods of development) against different threats, ideally with as little wasteful overlap as possible. *Pheidole bicornis* ants have been shown to be effective against specialist herbivore eggs laid on *Piper cencladum*, while the plant's secondary metabolites deter orthopterans and leaf cutting ants (Dyer *et al*, 2001). Young myrmecophytes, too small to house an ant colony, usually possess better chemical defences than older plants (Bryant *et al*, 1991). Trager and Bruna (2006) concluded that young *Cordia alliodora* myrmecophytes are no less palatable than older individuals but instead are fast growing and tolerant to herbivory. Llandres *et al* (2010), however, found that both *Cordia alliodora* and another myrmecophyte *Croton suberosus* were more palatable to caterpillars after they had developed their indirect defences (those that support ant-guards). Despite this, herbivory was higher in the related species *Cordia elaeagnoides* and *Croton pseudoniveus* that lack indirect defences, possibly because the indirect defences in *Cordia alliodora* and *Croton suberosus* effectively repel generalist herbivores (Llandres *et al*, 2010). The drawbacks of investing too much in specific defences are well illustrated by Itino and Itioka's (2001) work on *Macaranga*. While some threats are neutralised in the case of one plant species, that species is left relatively defenceless to others. For example, species well defended against insect herbivores by ants but possessing little mechanical defence fall victim to birds that prise open the stems to reach the ants within (Itino & Itioka, 2001).

All forms of resistance incur allocation, auto-toxicity, opportunity and ecological costs. Such induced resistance to threats may save energy and increase plant fitness in the long-term and a major challenge in ecology is to monitor plant fitness for long enough to fully appreciate the benefits provided (Cipollini and Heil, 2010). Interestingly, there is increasing evidence for the short-term costs of these traits that may impact on plant fitness (Heil, 2002; Heil & Baldwin, 2002). Allocation costs arise when resources are diverted away from reproduction and form part of the complex issue of how much of its resources a plant should allocate to growth and how much to defence (Herms & Mattson, 1992). The induction of photosynthesis-inhibiting nicotine production in *Nicotiana* spp. (Solanaceae) is a clear autotoxicity cost of short-term herbivore resistance (Baldwin & Callahan, 1993). Ant-guards can provide an ideal solution for some potential biochemical pathway problems in new leaves, as the traits needed to maintain a colony (such as food bodies) are located elsewhere (Heil *et al*, 2001a). However

plants attracting ant-guards incur numerous ecological costs, the most important being the risks of homopteran tending. It is becoming clear that there is a strong selective disadvantage to herbivore resistance in herbivory-free environments and understanding the costs involved is central to understanding selection pressures on plant defence (Strauss *et al*, 2002).

Domatia are normally unvarying characters of fully grown myrmecophytes. The African tree *Humboldtia brunonis* (Fabaceae), however, is polymorphic for domatia. When present these domatia may be inhabited by a range of invertebrates, from mutualistic ants to earthworms or pollinating bees (Shenoy & Borges, 2008). Domatia are also sometimes inducible by ants, as in the Amazonian tree *Vochysia viciniaefolia* (Vochysiaceae) (Blüthgen & Wesenberg, 2001). The inducible domatia present in semi-myrmecophytes may provide evidence for the selection pressures driving domatia evolution. In some plant species domatia have evolved to house predatory mites. They may even benefit the plant when housing phytophagous mites as this helps to stabilise predatory mites against population crashes (Romero & Benson, 2004). The domatia of *Piper urostachyum* by comparison are occupied by mirid bugs, which defend against herbivory in much the same way as ant defenders, following optimal defence theory by predominantly patrolling vulnerable, young, leaves (Hodson & Gastreich, 2006). Hybridization between myrmecophytes and closely related non-myrmecophytes may lead to F1 hybrids with intermediate phenotypes that rarely survive until reproduction. Hybrids between *Leonardoxa africana* subsp. *africana* and *L. africana* subsp. *gracilicaulis* have neither domatia that are habitable to ants nor adequate chemical defences. An obligate symbiosis with ants may therefore drive speciation as it contributes to the reproductive isolation of the species (Léotard *et al*, 2008).

The more details of the natural history of ant-plant mutualisms are uncovered the wider the web of interactions that is discovered. The relationship between *Leonardoxa africana* and its ant partner *Petalomyrmex phylax* not only involves homopterans but also a species of fungus. The fungus is only found within domatia inhabited by *P. phylax* and is absent in the presence of parasitic ant species. There is some evidence that the ant mutualists transport the fungus, which may well be involved in nutrient transfer between ant and plant mutualists (Defosse *et al*, 2009). Other potential players in ant-plant mutualisms have been found within the ant digestive tract. Several of the bacterial taxa found within the *Acacia* mutualist *Pseudomyrmex ferrugineus* (Pseudomyrmicinae) are potentially

nitrogen fixing, helping the ants survive on the low nitrogen diet provided by their host (Eilms & Heil, 2009). With so much to gain from such complex multitrophic interactions between plant, ants and other partners it is not surprising that the relationship is often parasitised.

1.4 Exploitation of Mutualisms

To prevent exploitation of the mutualism between plant and ant-guard, plants have evolved a variety of traits to sanction cheating behaviour by partners and filter mechanisms to exclude parasites. These traits are comparable to those discussed later that help to prevent the disruption of the relationship between plants and another guild of plant mutualists, the pollinators. Even mutualists may not always act in the best interests of their partners and such conflicts also have to be resolved if the mutualism is to persist.

1.4a Defence against exploitation

In any relationship where organisms mutually exploit each other there is a risk that third parties will impose costs on the relationship without providing benefits (Figure 1f). Cheaters are defined as species that have evolved from ancestors that had a mutualistic relationship with the host plant (Segraves *et al*, 2005), while parasites are those species whose ancestors were never mutualists (Bronstein, 2001). Even species of ant that provide some protection to a plant may be viewed as exploiters of a relationship between the host and a more efficient defender in the same habitat, as they impose an opportunity cost on the host plant (Yu & Pierce, 1998; Clement *et al*, 2008).

Parasites of ant-plant relationships can come in many different forms. Predatory *Phyllobaenus* beetles exploit the mutualism between *Piper obliquum* ant-plants and *Pheidole bicornis*. The beetle larvae occupy domatia and consume both food bodies and ant brood (Letourneau, 1990). There is some speculation that food bodies may require specialised adaptations for their consumption by larval ants, reducing the likelihood of exploitation by other ant species (Raine *et al*, 2004), but *Charterginus* wasps have been observed using cooperative behaviour to steal *Cecropia* food bodies (LaPierre *et al*, 2007). Even more fascinating is the jumping spider *Bagheera kiplingi* (Salticidae) that has become effectively herbivorous and feeds almost exclusively on the Beltian bodies of *Acacia collinsii* ant-plants

(Meehan *et al*, 2009).

In most ant-plant mutualisms looked at, however, other ant species act as parasites of the relationship, using up domatia space and consuming EF-nectar without providing benefits to the host plant. When inhabited by parasitic ant species *Cordia nodosa* places sanctions on those colonies by reducing domatia production and survival, thereby limiting colony growth (Edwards *et al*, 2006). The rattan ant-palm *Korthalsia furtadoana* (Arecaceae), however, appears to be incapable of such sanctioning in response to herbivory and so is regularly inhabited by parasitic ant species other than the mutualistic *Camponotus* species which does effectively protect (Edwards *et al*, 2010).

While most myrmecophytes are inhabited by one species of ant (mutualist or parasite) at any one time, the Neotropical tree *Cordia alliodora* is unusual in that individuals may accommodate a number of ant colonies in a mosaic within their canopies. Tillberg (2004) examined the common inhabitants and found a hierarchy of relationships. *Azteca pitteri* is an obligate mutualist and *Crematogaster curvispinosa* a facultative mutualist, while *Cephalotes setulifer* and *Cephalotes mutispinosus* are obligate and facultative parasites respectively. Those species feeding at a higher trophic level (i.e. more carnivorous) were shown to be the most efficient mutualists (Tillberg, 2004). As host plants are normally a limiting resource for plant-ants it is not yet clear why competitively inferior species are not territorially excluded from *C. alliodora*. In this case the costs imposed on the tree by the parasitic ants in its canopy may be negligible in the presence of more efficient defenders. Alternatively, *Cephalotes* colonies may be too difficult to outcompete once entrenched, as their major workers use their wedge-shaped heads to block the entrances to domatia (personal observation). However, where parasites cause a significant loss of fitness, selection is expected for the evolution of exclusion or filter mechanisms to protect the plant's resources from exploitation. These filters are usually imposed by the host plant and overcome by its mutualists alone.

Filters limiting exploitation may be structural or physiological in nature. A structural exclusion mechanism evolved independently in numerous genera, including *Piper*, *Macaranga* and *Leonardoxa*, is the prostoma. Only specialised ants can chew through this thin layer of tissue to gain access to the domatia beyond. Different species of the African trees *Leonardoxa* possess their own uniquely shaped

prostomas that correlate with the head shapes and sizes of their specific ant mutualists (Brouat *et al*, 2001). Many species of *Macaranga* also exclude non-specialist ants with wax blooms on their stems that only their sure-footed mutualists can scale, although how exactly the 'wax runner' species adhere to their hosts is still unclear as they perform even worse than non-specialists in their attachment to smooth cylinders (Federle *et al*, 2000). Federle *et al* (1997) hypothesised that it is less costly for the plants to maintain and protect less dominant, ant colonies requiring fewer resources than to sustain highly aggressive defenders. Those myrmecophytic but non-glaucous *Macaranga*, possessing mutualists unable to climb slippery stems, present food bodies in secluded closed stipules and have fewer EFNs providing less nectar (Federle & Rheindt, 2005).

In a range of myrmecophytes, including *Cordia nodosa*, *Maieta guianensis* and *Triplaris poeppigiana* (Polygonaceae), trichomes appear to play some part in limiting the size of the ant inhabitants, as a positive correlation can be seen between worker body length and spacing between trichomes (Davidson *et al*, 1989). Larger ants are forced to cut trails through the trichomes so this provides the plant's ant inhabitants with a small degree of defence against larger competitors (Davidson *et al*, 1989). The myrmecophyte *Vitex thyrsoflora* (Verbenaceae) is unusual in being a climber, as these plants are normally too prone to invasion by dominant ant species to evolve true myrmecophytism. This species appears to maintain its mutualistic colonies of *Tetraponera tessmanni* by possessing domatia formed from hollow stems that have no internodes, allowing the ants to travel through the plant in safety (Djiéto-Lordon *et al*, 2005).

Physiological filters are also common adaptations. Mexican *Acacia* myrmecophytes provide EF-nectar with a very low sucrose concentration due to high sucrose-cleaving invertase activity (resulting in higher glucose and fructose concentrations). Facultative ant species prefer sucrose-rich nectar, while *Pseudomyrmex* specialists of *Acacia* prefer sucrose-free nectar and workers have barely any invertase activity in their digestive tracts (Heil *et al*, 2005). This filtering mechanism has not prevented exploitation completely as the plants are still visited by three parasitic ant species, including *Pseudomyrmex nigropilosus* that is an obligate parasite. Unlike the mutualists, these species cannot induce EF-nectar production but they can forage and nest off of their host plant so are not completely dependent on it for resources. This obviously limits their investment in the vigour of their hosts. The

constraints placed on the behaviour of ant mutualists, however, do seem to prevent cheating as none of the exploiters are descended from mutualists (Kautz *et al*, 2009).

Mutualisms also appear to be reinforced by the investment strategies of myrmecophytic *Acacia* in Mexico, since those species with the highest investment in ant resources are also those that receive the best protection as secretion of EF-nectar is positively correlated with ant activity. Such reciprocal exchange between partners stabilizes the mutualism (Heil *et al*, 2009). In fact, despite the large amount of theoretical work that suggests mutualisms are prone to collapse into cheating, very few cases of a mutualist reverting to cheating have been found (Bronstein, 1994; Ferrière *et al*, 2007). Where it has been found, in fig-fig wasp and yucca-yucca moth systems, it appears to have occurred after a host shift to another plant species that already has a mutualist so the coexisting cheater-mutualist pairs are not sister taxa (Pellmyr *et al*, 1996; Machado *et al*, 2001). Examples of cheating are rare or at least short-lived, and more commonly partners simply abandon the mutualism, an outcome especially likely in facultative interactions (Sach & Simms, 2006). Thus more commonly mutualisms face exploitation from parasites, be they a non-defending ant species or a non-pollinating nectar thief. As the presence of exploiters selects for traits in both mutualistic partners that make them more resistant to exploiters, the loss of long established exploiter species may in fact put the mutualism at risk of extinction if new exploiters are encountered (Ferrière *et al*, 2007).

1.4b Conflicts of interest

Although outright cheating by mutualistic partners is not common, conflicts of interest between partner species may still strain the relationship. In most facultative situations plants are routinely exploited as the fitness of the ants visiting them, and offering varying degrees of protection, is usually not tied to the fitness of the plant. Myrmecophytes with obligate ant partners have a great deal more control over the relationship through their control of resources and nesting space (Edwards *et al*, 2006). However their ant partners are still horizontally transmitted. Even under ideal conditions, where both partners benefit overall, it is likely that some traits a partner possesses will have less than beneficial effects on the other mutualist (Young *et al*, 1997). One of the best examples of such a conflict can be seen in host castration behaviour. The conflict arises over how a plant should invest its resources. While the plant may benefit from sexually reproducing, the ant colony more directly benefits from further vegetative

growth and therefore more domatia and food production.

The pruning behaviour of *Crematogaster nigriceps* may effectively sterilise *Acacia drepanolobium*. Although they do not specifically focus on floral tissue, by chewing shoot tips these ants modify the canopy architecture and in the process castrate their host (Stanton *et al*, 1999). As another example, where large amounts of food bodies are removed from *Macaranga bancana*, *Crematogaster* plant-ants are more likely to attack host flowers, encouraging new growth (Heil & McKey, 2003). The most notorious host castrators, however, belong to the genus *Allomerus*. Workers of *A. octoarticulatus* and *A. demerarae* attack buds and flowers of *Cordia nodosa* and *Hirtella myrmecophila* respectively (plants that they otherwise defend). How the ants identify sexual reproduction-associated tissue has still to be determined. *C. nodosa* that are artificially castrated produce significantly more domatia (Frederickson, 2009). The mutualism appears to be maintained by *H. myrmecophila* through the imposition of a kind of host sanction. By dropping old domatia, *H. myrmecophila* not only limits the ant colony size but also keeps older branches free of ants, allowing mature flowers to develop (Izzo & Vasconcelos, 2002). In the case of *C. nodosa* a second ant mutualist appears to stabilize the mutualism, as the trees may also be inhabited by *Azteca* species that do not castrate (they do not need to, building carton nests away from the host tree instead). While *Allomerus* colonies are more fecund, *Azteca* queens disperse further. This competition/dispersal trade-off maintains fully reproductive plants in areas of low *C. nodosa* density (Yu *et al*, 2001). The importance of host castration, however, may be overestimated due to the short time periods of the studies. *C. nodosa* lives for around 77 years while an *Allomerus octoarticulatus* colony only lives for an average of 7.8. Throughout its life a plant can concentrate on vegetative growth when inhabited by *A. octoarticulatus*, and on sexual reproduction when inhabited by *Azteca* (Frederickson, 2009). Without any kind of host sanction or competing ant-plant, host castration is unlikely to drive the partnership to extinction as long as the castrator is dispersal-limited. Exactly this situation is found in the relationship between *Hirtella physophora* and *Allomerus decemarticulatus*, where the dispersal-limited *A. decemarticulatus* has evolved a lower level of castration virulence (Szilágyi *et al*, 2009).

Other conflicts of interest arise when very different mutualisms are taken into account. Many myrmecophytes are defended by highly aggressive ant colonies that attack anything they encounter. If

the plant species is even partly pollinated by insects however, contact with pollinators is obviously desirable. How do plants that may otherwise attract and benefit from ants achieve pollination if their aggressive ant-guards can chase away or kill their pollinators? Related to this, there is the risk that ants of any type may pose in making flowers less attractive by draining them of nectar.

1.5 Ants as Pollinators

It is generally accepted that ants are poor pollinators. They are small, smooth-bodied and (in the case of workers) flightless, so only capable of transporting small amounts of pollen over short distances (Faegri & Van der Pijl, 1979; Willmer, 2011), and one of the main arguments against effective ant-pollination is the effect that they have on pollen viability. Ants produce antimicrobial secretions from glands in their integument, most notably the metapleural glands. These secretions are used to keep the nest clean and help prevent disease and are present in all species unless secondarily lost, for example in parasitic species (Beattie *et al*, 1986). Exposure to the relatively harmless fungal pathogen *Metarhizium anisopliae* (Clavicipitaceae) becomes lethal when metapleural glands of the leafcutter *Acromyrmex octospinosus* (Myrmicinae) are blocked (Poulsen *et al*, 2002) and Fernández-Marin *et al* (2006) showed that *Atta columbica* (Myrmicinae) increased grooming from metapleural glands when exposed to fungal conidia (compared with control talcum powder). Such active use of antimicrobial secretions indicates that they are probably metabolically costly and reveals a degree of sophistication that limits the dangers of antibiotic resistance.

Beattie *et al* (1985) first examined the effect of exposure to the ant integument on pollen. They showed that *Myrmecia nigriscapa* (Myrmicinae) metapleural gland secretions destroyed plasma membranes and prevented normal hydration of pollen grains from *Brassica* and *Acacia*. Hull and Beattie (1988) found decreased pollen viability in 46 out of 50 bioassays tested against metapleural gland secretions of the leafcutter *Atta texana*. However, it is clear that not all harmful secretions come from the metapleural glands. The genus *Camponotus* has secondarily lost these glands but contact with their integument has still been shown to reduce pollen viability (Beattie *et al*, 1985; Vega *et al*, 2009). The effect is not consistent and depends on the combination of plant and ant species with varying degrees of vulnerability and potency respectively. Contact with the integument of other social

Hymenoptera appears to be less harmful to pollen, possibly because the larvae of these species are kept in clean brood cells instead of being groomed with antibiotics as ants do (Harriss & Beattie, 1991). However, the effects of cuticular secretions have only been tested for a very small range of species.

In some cases a reduction in pollen viability may have little impact on male fitness, for example *Paronychia pulvinata* (Caryophyllaceae) pollen seems to suffer no significantly adverse effects from contact with *Formica neorufibarbis* (Formicinae) workers that can self-pollinate hermaphrodite flower morphs (Puterbaugh, 1998). But when ants of the same species are enclosed in *Polemonium viscosum* (Polemoniaceae) flowers, pollen viability is significantly reduced (Galen & Butchart, 2003). *Blandfordia grandiflora* (Blandfordiaceae) pollen grain germination was reduced by 6% after contact with ants, but this did not translate into a significant difference in seed-set (Ramsey, 1995). Such small reductions in pollen viability, however, may be important in pollen-limited species. The influence of reduced pollen viability is most important where plants have a relationship with ants. *Acacia constricta* pollen viability is significantly reduced by contact with the plant's partner ant *Formica perpilosa*, potentially causing a trade-off between the benefits the plant receives through increased female reproduction function and the damage done to male reproductive function (Wagner, 2000).

In certain circumstances ants may occasionally be efficient pollinators. Hickman (1974) was the first to categorise plant traits common amongst the few species known to be pollinated by ants. They are likely to be low-lying plants in dense populations, usually in hot dry habitats, that bloom synchronously and possess small volumes of nectar with little in the way of visual displays, so limiting visitation by flying insects. Such a low energy system, where little nectar is needed for a walking pollinator, is exemplified by the small annual *Polygonum cascadenae* (Polygonaceae). *Formica argenta* workers carry pollen on their heads between flowers of this self-incompatible species and appear to be essential for normal seed set (Hickman, 1974). The annual succulent *Diamorpha smallii* (Crassulaceae) is also pollinated by *Formica* species and again is self-incompatible. Cross-pollination is promoted by providing very few open flowers at one time (Wyatt & Stoneburner, 1981). Ants can also sometimes effect cross-pollination of female flowers of the gynodioecious *Fragaria virginiana* (wild strawberry; Rosaceae) (Ashman & King, 2005). Those visited exclusively by ants had 90% of the seed

set of those visited only by flying insects. However, the flower pistils suffered some ant damage.

Heterostyly, a form of floral polymorphism, structures ant pollination in *Fagopyrum esculentum* (buckwheat; Polygonaceae). Short anthers and a long stigma are found in the “pin” flower morph and vice versa in the “thrum” morph, with pollination occurring when pollen is transferred between the two. *Formica* workers are the right size to carry pollen from the short anthers of “pin” flowers to the short stigmas of “thrum” flowers. Honeybees efficiently transfer pollen in between both morphs but even in their absence half of the crop yield could potentially be maintained by ant pollination (Taki *et al*, 2009). In areas with more abundant flying flower visitors the potential contribution ants can make to pollination is only realised when flying visitors are excluded. *Euphorbia cyparissa* (Euphorbiaceae) is cross-pollinated by ants but as the seed set is the same when only flying visitors are allowed access as when all visitors have access it appears that in practice ants contribute little (Schürch *et al*, 2000).

A number of orchid species are successfully pollinated by ants. *Leporella fimbriata* (Orchidaceae) is obligately pollinated by winged *Myrmecia urens* (Myrmeciinae) males. While attempting to copulate with the flower, pollinaria are attached to the ants via sticky stigmatic secretions (Peakall *et al*, 1990). Similar secretions attach *Epipactis thunbergii* (Orchidaceae) pollinia to *Camponotus* workers, where hoverflies may move pollen between plants, but ants facilitate self-pollination (Sugiura *et al*, 2006). Such ant-pollinated species often possess traits that prevent pollen coming into contact with ant integument, such as sticky secretions, pollinia stalks and the dilution effect of so many pollen grains being present within pollinia (Peakall & Beattie, 1989; Sugiura *et al*, 2006).

Ant-mediated self-pollination may be more common than appreciated, especially where autogamous selfing does not take place. *Iridomyrmex* sp. workers pick up *Blandfordia grandiflora* pollen on their hairy legs, moving it from anthers to stigma within flowers. Although this plant is normally bird-pollinated, ants may play a role when bird pollinators are scarce (Ramsey, 1995). In the case of the herb *Lobularia maritima* (Brassicaceae) the seed set is the same whether the plants are self-pollinated by ants or visited by winged insects. As each flower only has two ovules there is no risk of pollen limitation due to less efficient pollinators (Gómez, 2000). The rare umbellifer *Trinia glauca*

(Apiaceae) is successfully pollinated by ants that carry significantly less heterospecific pollen than flying visitors (Carvalho *et al*, 2008). Unusually for ant-pollinated species, the Mediterranean root holoparasite *Cytinus hypocistis* (Cytinaceae) is pollinated by as many as ten different ant species that spend a long time on individual flowers, moving pollen and facilitating self-pollination (Vega *et al*, 2009). Where pollen is moved between plants it is limited to the small area around an ant nest, leading to the restricted gene flow seen in populations of the European perennial herb *Anthericum liliago* (Asparagaceae) pollinated by the ant *Myrmica sabuleti* (Peterson *et al*, 2002). The real impact of self-pollination, short-pollen transfer distances and the resultant inbreeding only become clear in later generations (Gómez, 2000; Lennartsson, 2002). The costs may be reduced, however, because many ant-pollinated species can still outcross through rare visitation by flying insects; for example *Cytinus hypocistis* is also visited by the fly *Oplisa aterrina* (Rhinophoridae) (Vega *et al*, 2009).

Although individually ants may be poor pollinators themselves, there are often large numbers of them. Some plant species, like the mass flowering shrub *Hormethophylla spinosa* (Brassicaceae), can be successfully ant-pollinated due to ant's high frequency of visitation (Gómez & Zamora, 1992). Gómez *et al* (1996) also estimated that ant pollination may be especially common in high mountain and arid zones due to the high proportion of ant floral visitors compared to any others. Five out of seven species they looked at in the high Mediterranean could be pollinated by ants, with one species (*Sedum anglicum*; Crassulaceae) possibly exclusively ant-pollinated (Gómez *et al*, 1996). In a comparable Californian climate the San Fernando Valley Spineflower *Chorizanthe parryi* var. *fernandina* (Polygonaceae) fruit set is significantly reduced when native ants are excluded from flowers, a pollination mutualism that may be especially at risk of invasion by Argentine ants and subsequent displacement of native ant species (Jones *et al*, 2010).

Crucially, floral traits facilitating ant pollination do not appear to be specifically selected for. In most cases the relationship appears to be the result of “ecological fitting” (sensu Janzen, 1985). These species are pre-adapted and when ant pollination does occur it is probably the result of prevailing ecological conditions (Peakall *et al*, 1990; Gómez *et al*, 1996; Vega *et al*, 2009). Their effectiveness will always depend on the context of other potential pollinators in the area. Efficient pollinators may still become parasites in the presence of better pollinators if they are wasting pollen that the superior

pollinator could have been depositing (Ne'eman *et al*, 2010).

1.6 Disruption of Pollination

Mutualisms between plants and their pollinators may either be disrupted directly by physical threats to the pollinators themselves or indirectly through parasitism of floral resources (Figure 1g). Such disruption can impact on plants in a number of ways as patterns of pollen flow are altered and potentially costly nectar is wasted. From the perspective of flower visitors, reduced attractiveness due to plundered resources impacts on foraging decisions so bees have adapted to avoid recently depleted flowers by detecting scent-marks left by previous visitors (Figure 1h). The greatest disincentive to visit a flower, however, is direct predation from crab spiders, mantids, predatory bugs and, at times, ants.

1.6a Pollinator predation

Where larger and more aggressive ants visit flowers there is a risk that they could pose a direct threat to potential pollinators. Honeybees are quick to associate otherwise desirable tastes or scents with risk or negative consequences and such cues are soon avoided (Abramson, 1986; Smith *et al*, 1991). Probably the most common threat flower visitors face is from crab spiders (Thomisidae) that are often well camouflaged on a flower. Bees also do not land on flowers with dead spiders or dead bees placed on them, and when an attempted ambush is staged at certain food sources those are avoided in future (Dukas, 2001). On milkweed, crab spiders have been shown to decrease floral visitation by the plant's smaller bumblebee visitor *Bombus ternarius* (Apinae) (Dukas & Morse, 2003). Such a decrease in floral visitation caused by crab spiders leads to lower seed set in *Leucanthemum vulgare* (ox-eye daisy; Asteraceae) (Suttle, 2003). Due to avoidance alone, artificial spiders placed on *Rubus rosifolius* (Rosaceae) flowers decrease seed set through pollen limitation (Gonçalves-Souza *et al*, 2008). Specifically the shape of the crab spiders' widely spread limbs alone is enough to trigger avoidance by hymenopterans, although butterflies have no such predatory avoidance mechanism, possibly due to their inherent unpalatability (Gonçalves-Souza *et al*, 2008).

Such avoidance is not an innate behaviour, but a learned one. Ings and Chittka (2008) found

no evidence that naïve bees avoided spider-shaped models, either conspicuous or cryptic, until simulated predation attempts were made, after which bees that encountered cryptic spider models did spend longer inspecting flowers and rejected far more perfectly safe flower models. Lizards have been shown to reduce the seed set of *Chuquiraga oppositifolia* (Asteraceae) shrubs, almost entirely due to disruption by failed attempts to catch pollinators (Muñoz & Arroyo, 2004).

Threats may even come from other pollinators. *Trigona* (Apinae) stingless bees are highly territorial and may monopolise flower patches and kill other bees that transgress the boundaries of their territory (Nagamitsu & Inoue, 1997; Maloof & Inouye, 2000). *Pavonia dasypetala* (Malvaceae) seed set is reduced by *Trigona* robbers indirectly through their deterrence of bird pollinators (Roubik, 1982). However, these dominance hierarchies rarely lead to encounters at flowers and do not translate into resource partitioning, possibly due to a trade-off between resource discovery and territorial dominance (Dworschak & Blüthgen, 2010).

Predatory wasps, such as the bumblebee wolf *Philanthus bicinctus* (Crabronidae), have a direct impact on pollinator numbers, with a typical wasp aggregation catching thousands of bees every day. *Bombus* spp. density has been shown to be far lower around *P. bicinctus* colonies and this leads to an indirect effect on the fruit-set of *Aconitum* (monkshood; Ranunculaceae) within the area (Dukas, 2005). Although in the past some have argued that pollinator predation is of very little importance (Pkye, 1979; Morse, 1986), it is becoming clear that the extent of top-down predatory effects on pollinators and their interactions with plants cannot be ignored.

While not as efficient at catching prey as more specialised predators, ants may also threaten flower visitors and may be present in far greater numbers than crab spiders, wasps or mantids. The large ponerine ant *Ectatomma ruidum* (Ectatomminae) regularly hunts halictid bees at nest entrances and food sources (Schatz & Wcislo, 1999). Highly aggressive species, such as *Oecophylla longinoda* (Formicinae), provide the best protection against herbivores but also pose the most obvious threat to pollinators. It may be in the plant's best interests to be visited by a less aggressive species (Djiéto-Lordon *et al*, 2004).

Highly competitive, invasive species like the Argentine ant (*Linepithema humile*) and the big-headed ant (*Pheidole megacephala*) may have serious impacts on native plant-pollinator interactions. Both species can commonly be found on flowers throughout invaded areas of Australia (Lach & Thomas, 2008) and *P. megacephala* is a common predator on honeybees in Africa (Yang *et al*, 2009). Argentine ants commonly visit the flowers of the shrub *Euphorbia characias* where they have a significantly detrimental effect on fruit and seed set, possibly through a reduction in flower visitation in infested areas (Blancafort & Gómez, 2005). They also reduce visitation by native pollinators on the tree *Metrosideros polymorpha* (Myrtaceae) in Hawai'i, although not visitation by introduced honeybees (Lach, 2008a). Argentine ants, however, do reduce honeybee visitation to *Leucospermum conocarpodendron* (Proteaceae) flowers in South Africa, although this does not result in a reduction in seed set presumably because this species is not pollen-limited (Lach, 2008b). *Oecophylla smaragdina* ants repel *Trigona* (stingless bee) visitors to the flowers of *Nephelium lappaceum* (rambutan; Sapindaceae) trees in West Sumatra in the same way (Tsuji *et al*, 2004). In the clearest, as well as most extreme, case uncovered so far the invasive ant *Technomyrmex albipes* (Dolichoderinae) has a significantly detrimental effect on both the pollination and seed dispersal of the critically endangered Mauritian liana *Rousseau simplex* (Rousseaceae) by preventing its' unusual, but essential pollinator and seed disperser the blue-tailed day-gecko, *Phelsuma cepediana* (Gekkonidae), from achieving access to flowers and fruits (Hansen & Müller, 2009).

Bees are not entirely defenceless. A combination of kicks and wing fanning can dislodge at least smaller ant species from hive entrances and occasionally from nectar sources (Spangler & Taber, 1970; Yang *et al*, 2009). The resins collected by *Trigona* bees are not only useful in nest building, but may also repel ant predators. Dead bees washed in chloroform were found to be more palatable to ants than unwashed bees, possibly due to the repellent terpenes present in resins and found on the bee cuticle (Lehmberg *et al*, 2008). Although visual cues play a role in ant detection by bees, scent is also important, especially inside the nest, and common ant alarm pheromones, including formic acid and 2-heptanone, can elicit defensive behaviour in honeybees (Spangler & Taber, 1970). One of the reasons Argentine ants may pose such a threat to pollinators is that they possess very little scent themselves and so can infiltrate a hive or nest undetected (Spangler & Taber, 1970). The cost of pollinator disruption extends further than those bees directly attacked. Honeybees threatened with

simulated attacks or aggressive competitors signal to nestmates visiting the same flower patch to stop their waggle dance behaviour (Nieh, 2010). This negative feedback signal short-circuits recruitment to that area and increases the potential cost to a plant of predation attempts on social bees.

Junker *et al* (2007) found that *Camponotus* and *Polyrhachis* (Formicinae) ants reduced floral visitation rate to the shrub *Cassia alata* (Fabaceae), while *Tapinoma melanocephala* (Dolichoderinae) on flowers of the perennial *Wedelia trilobata* (Asteraceae) and *Dolichoderus thoracicus* (Dolichoderinae) on flowers of the tree *Diospyros durionoides* (Ebenaceae) both reduced duration of visitation. These results, however, may not necessarily be due to risk aversion in pollinators. One as yet untested hypothesis put forward by Tsuji *et al* (2004) is that ants may mark flowers with scent traces that repel potential pollinators.

The situation is never a simple one, as predators may also provide benefits to plants. *Oecophylla smaragdina* ants may repel pollinators, imposing costs on male reproductive success, but at other times have a facultative protective relationship with rambutan by reducing herbivory and increasing female reproductive success (Tsuji *et al*, 2004). The crab spider *Misumenops argenteus* (Thomisidae) on the shrub *Trichogoniopsis adenantha* (Asteraceae) lowers the density of some types of herbivorous insects (Romero & Vasconcellos-Neto, 2004). In this case all detectable spider effects are beneficial to the plant, partly because so few individual flower capitula are occupied and partly because the spiders do not prey on the plant's thrip pollinators.

The line between disruption of pollinator activity and disruption of plant enemies can be a very fine one. For example on figs, ants commonly predate both pollinating and non-pollinating fig wasps. Male figs of dioecious *Ficus* (Moraceae) species are often monopolised by dominant predatory ants that may actually benefit male reproductive output, as non-pollinating wasps that are more likely to spend time at risk on the outside of the fig are caught or disturbed by ants, leaving more flowers available within the fig for mutualistic wasps that enter through the ostiole, resulting in a greater number of pollen-carrying wasps in the next generation (Schatz *et al*, 2008). On *Yucca* (Asparagaceae) flowers *Formica* ants do kill some pollinating moths and damage buds but they ultimately increase fitness through disruption of non-pollinating moths (Perry *et al*, 2004). Although

they decrease pollinator visitation to *Turnera ulmifolia* flowers, attendance by a variety of ants also increases seed set, possibly because they reduce herbivory (Torres-Hernández *et al*, 2000). Leal *et al* (2006) hypothesised that the seed set increase experienced by *Passiflora coccinea* when EFNs on the inflorescence were visited by ants may have been due to disruption of nectar robbers. Finally, the large predatory ant *Ectatomma ruidum* actually increases seed set of the self-incompatible *Psychotria limenensis* (Rubiaceae) by causing pollinators to make shorter foraging trips to fewer flowers per plant, thereby increasing movement between plants. However, the ants also have a negative effect on fruit removal by birds (and hence seed dispersal) later in the plant's development (Altshuler 1999).

1.6b Floral larceny

While ants may disrupt pollination by physically threatening potential pollinators as predators, the most important way that ants influence pollination ecology could be in their role as floral nectar thieves. There are two main forms of floral larceny, robbery and theft (Inouye, 1980). Robbery occurs when a floral visitor pierces some part of a flower to gain access to nectar, possibly damaging reproductive organs or nectaries in the process. Thieves obtain nectar without damaging the flower but without efficiently pollinating it either, although the same individuals may be termed secondary robbers if they access the nectar through openings created by primary robbers (for example *Apis* in *Xylocopa* (Xylocopinae) -produced holes, Barrows, 1980).

The most commonly robbed flowers are those with morphologies that limit access to resources, especially long corollas or nectar spurs, that can be robbed by a wide range of bee and bird visitors. In practice this includes many bee-pollinated and most bird-pollinated species. In Costa Rica over 90% of *Aphelandra golfodulcensis* (Acanthaceae) and *Justicia aurea* (Acanthaceae) flowers studied were robbed by noon by *Trigona* bees: and as neither species replenishes its nectar after it is drained, robbed flowers were almost entirely avoided by hummingbird pollinators (McDade & Kinsman, 1980). As nectar robbery happens more frequently to such flowers the costs involved may impose limits on selection for longer corollas (Laras & Ornelas, 2001a). Many birds, such as finches, tanager flower-piercers and hummingbirds, may also rob flowers (Traveset *et al*, 1998; Lara & Ornelas, 2001a; Mauck & Burns, 2009). Often the beaks of such thieves are specially adapted for piercing long corollas, being serrated in the case of many short-billed humming birds (Ornelas, 1994) and bearing

long down-curving hooks in the case of tanagers (Mauck & Burns, 2009).

Species with enclosed flowers are also commonly targeted by robbers. Robbed keel flowers of *Polygala vayredae* (Polygalaceae) suffer from significantly reduced pollen dispersal and seed production (Castro *et al*, 2008). In some populations at least, robbers are more likely to target *P. vayredae* flowers with longer corollas, thus imposing indirect negative selection on floral traits (Castro *et al*, 2009). *Bombus occidentalis* robbing behaviour reduces both male and female function of the perennial herb *Ipomopsis aggregata* (Polemoniaceae) by decreasing the attractiveness of flowers to legitimate pollinators (Irwin & Brody, 1999). In this case seed set was reduced by 50% and through the use of genotyping it was estimated that seed siring was reduced by 56% (Irwin & Brody, 2000).

Nectar larcenists may also be legitimate pollinators, as they may visit or contact the anthers and stigma on other occasions while foraging for pollen (Irwin *et al*, 2001; Navarro, 2001). Bumblebees visit young pink Northern bluebells (*Mertensia paniculata*; Boraginaceae) legitimately, buzz-pollinating them, and then later, when the flowers turn blue and produce nectar, the same bee species rob the flowers for the nectar reward. The displays of pollinated blue flowers attract bumblebees to the inflorescence, where they then pollinate the young pink flowers, so nectar robbery appears to be integral to the mutualism (Morris, 1996).

A review by Maloof & Inouye (2000) of studies on nectar robbery found that incidences of positive, neutral and negative effects on the plant were roughly equal. Another meta-analysis carried out by Irwin *et al* (2001) concluded that floral larceny overall had a weak negative effect on female reproductive success. They found greater negative effects when the perpetrators were birds but found no significant difference between the effects of thieves and robbers. As nectar has no direct role in reproduction, unlike pollen, nectar thieves and robbers will only influence plant fitness indirectly. It is also less likely compared with pollen theft or robbery that nectar theft influences selective pressures on plant sexual systems (Hargreaves *et al*, 2009), as nectar is usually found in both male and female flowers. Newman and Thomson (2005) hypothesised that robbed flowers may act in an analogous way to EFNs, attracting secondarily nectar-robbing ants that then provide protection to the inflorescence, but while ants did provide some protection against beetle herbivore damage on the toadflax *Linaria*

vulgaris (Scrophulariaceae) they provided no more when flowers were pierced than when unpierced (Newman & Thomson, 2005).

Consequences of ant nectar theft are similarly complicated. When present in flowers of the autogamous perennial weed *Rorippa indica* ants reduce numbers of *Pieris* (Pieridae) butterfly larvae and appear to cause minimal negative effects (Yano, 1994). Flowers of the perennial herb *Frasera speciosa* (monument plant; Gentianaceae), although extensively visited by nectar-thieving ants that reduce standing nectar volumes and pollinator visitation rates, had no subsequently reduced level of seed set. It may be that nocturnal moth visits make up for the loss of diurnal visitation, or that very few visits are necessary for successful pollination (Norment, 1988). Nectar-thieving ants on *Asclepias syriaca* (Apocynaceae) did not affect pollinia removal but did affect female reproductive success, through a reduction in seed production (Fritz & Morse, 1981). Ant presence on *Asclepias curassavica* altered butterfly behaviour so they visited more flowers for less time, resulting in lower efficiency of pollinia insertion (Wyatt, 1980).

When acting as floral larcenists ants are usually thieves but there are cases of robbery when floral parts are damaged. Most flowers of the sphingid moth-pollinated epiphytic orchid *Aerangis verdickii* are robbed by weaver ants (*Polyrhachis* spp.), which make small slits at the base of the nectaries. This is possibly one reason why nectar is reabsorbed by this species following pollination (Koopowitz & Marchant, 1998). Floral resources other than nectar may also suffer from the attentions of ants. *Formica neorufibarbis* acts as a herbivore on flowers of the herb *Eritrichum aretiodes*, damaging the coronal ring by feeding for lipids and reducing seed set (Peterbaugh, 1998).

Damage caused by visiting ants has influenced floral morphology of the pollen-limited alpine skipper *Polemonium viscosum* (Galen, 1985). *Formica* ants plunder nectar and dislodge the style from the ovary, directly reducing seed set. Those flowers with the broadest corollas are most attractive to both bumblebee pollinators and ants, putting the larger floral morphs under conflicting selective pressures and presumably helping to maintain narrower floral corollas within ant-abundant habitats, as they suffer less from ant damage (Galen, 1999). Flowers are also found in two scent morphs, sweet and skunky. *Formica neorufibarbus gelida* steal nectar and bite through the styles of sweet-smelling

flowers twice as often as skunky ones. By eliciting a neophobic response the skunky flowers often repel ants on first contact, although when this fails the same amount of damage is done to skunky flowers as sweet ones. This defence appears to keep the density of skunky flowers high at low altitudes where ants are more common, despite being less attractive to bumblebee pollinators but more attractive to flies (Galen, 1983). The situation is even more interesting when the protective benefits the ants provide to the plant as a whole are taken into account. Galen and Cuba (2001) looked at the ant-attractive sweet-smelling flowers in two different habitats. Alpine tundra populations under low predation pressures had a lower proportion of flowers with long corollas than lower elevation (krummholtz) populations under high predation pressure. The fitness loss suffered by flowers with long corollas when attended by ants was larger where the ants benefited the plant less. Flower size is also correlated with scent as shorter, narrower flowers are more likely to smell unpleasant and skunky (Galen *et al*, 1987). The influence of different flower visitors, ants and bumblebees or flies, appears to be reinforcing divergence of the populations in scent and morphology. A similar trade-off between defence and pollinator attraction also occurs within sweet scent morphs, as higher rates of volatile 2-phenylethanol emission is correlated with both reduced bumblebee and reduced ant visitation (Galen *et al*, 2011).

Thieves as small as mites may also influence plant-pollinator relations. Hummingbird mites breed within inflorescences and travel between flowers in the nares of hummingbirds. Such mites are probably parasites on the shrub *Hamelia patens* (Rubiaceae), and an average population of mites consumed an estimated 40% of nectar and 16% of pollen from each flower (Colwell, 1995; Paciorek *et al*, 1995). Similarly, *Tropicoseius* (Ascidae) hummingbird mites reduced standing nectar of the shrub *Moussonia deppeana* (Gesneriaceae) by 50%. Here female fitness was not significantly reduced and mites may even act as secondary pollinators of this self-compatible species (Lara & Ornelas, 2001b). The lower nectar volumes in mite-infested flowers decreases the frequency and duration of hummingbird visits but also increases the number of times each flower is probed, an action apparently beneficial to seed set, although this conclusion was arrived at using stuffed hummingbirds (Lara & Ornelas, 2002). It is clear, however, that by altering the volume of nectar, thieves can influence pollinator behaviour, and their influence is not restricted to hummingbird-pollinated species. Mites within the bee-pollinated flowers of the bromeliad *Neoregelia johannis* (Bromeliaceae) can reduce the

amount of sugar available to flower visitors by up to 31% (Guerra *et al*, 2010).

Finally, another common group of nectar thieves may be microbes. Without altering the volume of nectar present microbial infestations that are capable of circumventing chemical defences may alter and decrease the sugar content of floral nectar significantly. Herrera *et al* (2008), who have dubbed nectar yeast communities the “dark matter” of plant-pollinator interactions, found that yeast infestations of nectar first reduce the concentration of sucrose by converting it into glucose and fructose, before then consuming almost all of the sugar available. Such yeast communities examined to date have a low species richness and are numerically dominated by a very small number of those species (Pozo *et al*, 2011). The importance of microbe activity in nectar has probably been underestimated.

By decreasing the amount of available nectar in flowers and increasing the variance in nectar volumes of flowers within a patch, floral larceny can have far-reaching consequences for plants and communities. The proportion of empty flowers present in large inflorescences determines the number of flowers within such an inflorescence that are visited by bumblebees (Ishii *et al*, 2008). If the absence of nectar alters the behaviour of legitimate pollinators in this way, then the pattern of pollen dispersal will be changed. When pollinators do not avoid the flowers completely they are likely to spend less time foraging at them, which can lead to a lower number or quality of ovules fertilised (Thomson & Plowright, 1980). On the other hand, as legitimate pollinators are forced to travel further to reach flowers with nectar, pollen dispersal distances may be increased. This will promote outcrossing and so reduce the cost of geitonogamy (Biernaskie *et al*, 2002). Variability in nectar volumes between flowers on the same plant was positively correlated with the number of flowers available on nine American herbaceous species (Biernaskie & Cartar, 2004), a strategy that hermaphroditic plants may use in concert with within-plant variation in nectar composition (Herrera *et al*, 2006). *Lapageria rosea* (Philesiaceae) flowers thieved by hummingbirds exported less pollen than non-thieved flowers, but what pollen was exported travelled further (Valdivia & González-Gómez, 2006).

Inbreeding can be costly even for self-compatible plant species (e.g. Charlesworth &

Charlesworth, 1987) and greater pollen dispersal can lead to an increase in multiple paternity of seeds. The genetic variability within the seed set is especially beneficial in a heterogeneous environment. By affecting a population's neighbourhood size in this way, through alteration of the pollen dispersal curve, floral larceny may also affect genetic drift and natural selection (Austerlitz *et al*, 2004). Irwin (2003) investigated the influence of robbery on pollen flow in *Ipomopsis aggregata* using dyed pollen to specifically identify such benefits. But robbery by *Bombus accidentalis* decreased visitation rate and reduced pollen flow between populations. So, although geitonogamy can be extremely costly to *I. aggregata*, there were still no detectable benefits from nectar robbery. As the costs of geitonogamy are only obvious when pollen competition is taking place populations that are pollen-limited experience no benefits from a reduction in geitonogamy, and populations with low visitation, such as heavily robbed ones, are typically pollen-limited (Gonçalves-Souza *et al*, 2008).

Reviews by Burd (1994) and Knight *et al* (2005) found pollen limitation to be common (62% and 63% respectively), with self-incompatible species and woody species most likely to be pollen-limited. The actual effect this had on populations was found to differ greatly between taxa and habitats. In areas of unpredictable pollen receipt, plants may possess a high number of ovules per flower if the cost of ovule production is outweighed by the potential benefits from occasional gluts in pollen receipt. Unrealistic aspects of the hand pollination techniques used, such as applying only outcrossed pollen, may also over-inflate estimates of pollen limitation (for an in-depth analysis of these issues see Ashman *et al*, 2004). However, it is becoming clear that the influence of pollen limitation is greater in fragmented populations where even self-pollinating species can suffer increased extinction risk due to pollen limitation (Lennartsson, 2002) and that pollen-limited species suffer higher costs when robbed of nectar (Burkle *et al*, 2007).

The effect of pollen limitation ultimately depends on the role of seed set in population dynamics. There is likely to be limited selection against pollinator disruption where seed limitation occurs and factors determining germination and establishment are more important. Short-lived species are especially sensitive to fluctuation in seed production so are also most likely to be influenced by pollen limitation at the population level (Ashman *et al*, 2004). Benefits of outcrossing will only occur when populations are spatially structured so that there is a direct link between pollen flow and realised

gene flow (Adler & Irwin, 2005). In addition, estimating the influence of pollinator disruption on male fitness is often difficult as realised male function is always going to be constrained by resource limitations on female fitness (Irwin & Brody, 2000).

1.6c Costs of nectar production

Nectar theft may indirectly affect plant fitness and pollen flow by emptying flowers and increasing the variance of nectar volumes (Biernaskie *et al*, 2002), but a more basic cost of nectar theft is the cost of the stolen nectar itself. Hummingbird-pollinated *Macleania bullata* (Ericaceae) flowers are commonly robbed by bees but, although they suffer no damage to reproductive organs or loss in pollinator visitation, robbery is positively correlated with a decrease in fruit set due the cost of additional nectar production (Navarro, 2001).

There has not been a great deal of work done yet on the costs of nectar production itself and most of what has been done has focused on the energy costs of the carbohydrates involved. Southwick (1984) estimated that between 4% and 37% of the daily photosynthate of milkweed (*Asclepias syriaca*) was used for nectar production. An estimated 30% of the energy devoted to flower production in *Asclepias quadrifolia* is devoted to nectar production. This accounts for 4% of above ground energy (Pleasants & Chaplin, 1983). Some of the cost of nectar production may be offset by the photosynthetic activity of the flower itself (Williams *et al*, 1985), but in many species, including the orchids *Aerengis verdickii* (Orchidaceae) (Koopowitz & Marchant, 1998) and *Platanthera chlorantha* (Orchidaceae) (Stpicyńska, 2003) and both male and female flowers of the squash vine *Cucurbita pepo* (Cucurbitaceae) (Nepi *et al*, 2001), surplus nectar is reabsorbed by the plant following pollination, implying that a plant can obtain significant benefits from the recuperation of the cost of nectar. The hawkmoth-pollinated orchid *Mystacidium venosum* (Orchidaceae) also reabsorbs nectar following pollination, and flowers where the nectar is removed after pollination set significantly smaller fruits (Luyt & Johnson, 2002). Experimental removal of nectar from the perennial herb *Blandfordia nobilis* increased subsequent nectar production and resulted in reduced seed set (Pyke, 1991). Nectar replenishment following visitation is significantly more likely for plants in wet habitats but even these species are, on average, conservative when it comes to secreting sugars (Ordano & Ornelas, 2004).

Other studies, however, have failed to find any cost associated with nectar production, either in terms of a reduction in vegetative growth (Golubov *et al*, 2004) or vegetative growth and reproductive output (Leiss *et al*, 2004). Such results are not surprising as the cost of nectar production can vary greatly between even closely-related species. The tank bromeliads *Tillandsia deppeana* and *T. multicaulis* (Bromeliaceae) do not reabsorb nectar and show no plasticity in nectar production following visits. This may be necessary to attract sufficient numbers of pollinators to fertilise all of the seeds in each fruit or benefit from extended pollen export (Ordano & Ornelas, 2004). Repeated nectar removal from *T. deppeana* flowers produces no significant effect on seed production but the same procedure carried out on *T. multicaulis* flowers reduces seed number by 50% when compared with bagged and hand-pollinated flowers. This reduced seed set, however, is not significantly different from that if normal visitation is allowed (Ordano & Ornelas, 2005). The costs of floral larceny will vary greatly between species and clearly be much greater in resource-limited populations.

Nectar reabsorption may have other physiological functions. By reabsorbing sugar from its nectar the Australian tree *Grevillea robusta* (Proteaceae) maintains a low nectar concentration despite evaporation (Nicolson, 1995). By reabsorbing nectar a plant may also discourage potentially destructive post-pollination visits (Búrquez & Corbet, 1991). However, these same plants also miss the chance to attract ants, which following pollination may be potentially beneficial for the protection of seeds, or to attract further pollinators to other flowers in the display that have yet to be pollinated. Reabsorption is more likely to be energy saving where nectar is protected against the external environment and only exposed to the risk of evaporation for a short period of time, but whatever the benefits are they will have to exceed the costs associated with nectar reabsorption itself (Nepi *et al*, 2001).

Costs of EF-nectar production have received even less attention but also appear to be considerable. EFN-related traits appear to respond to selection pressures very quickly. Urban populations of the annual *Chamaecrista fasciculata* (partridge pea; Fabaceae), that suffer less herbivory than rural populations, have smaller EFNs with reduced nectar production, implying that EF-nectar production is costly and only invested in when necessary (Rios *et al*, 2008). However, long-term experiments are still needed to determine the specific costs of EFN production (Rutter & Ruasher,

2004).

1.6d Visitation and floral scent-marking

The activity of nectar thieves can clearly influence pollinator behaviour by altering the cost-benefit foraging decisions made by those pollinators. When visiting flowers, bee behaviour is mediated by a large variety of chemical cues from both the flower itself as well as previous visitors to the flower. Both honeybees and bumblebees deposit scent-marks on flowers as they forage that may repel subsequent floral visitors. The odours are detected not only by conspecifics but also by other bee species (Goulson *et al*, 1998; Stout *et al*, 1998). Therefore, another way ants may interfere with pollinator-flower interactions could be via scent-marks left by ants on flowers or possibly the disruption of scent-marks deposited by the bees themselves.

Numerous experiments using artificial flowers have identified attractive scent-marks deposited by a range of bee species. Bumblebees are attracted to the discs of rewarding feeders up to 15 minutes after they have been swapped on to unrewarding feeders (Schmitt & Bertsch, 1990) and *Nannotrigona* (Apinae) bees are also attracted to previously-visited feeders (Schmidt *et al*, 2005). Both the Amazonian bees *Melipona rufiventris* (Apinae) and *Trigona spinipes* are attracted to feeders previously visited by their own species and the more dominant and aggressive *T. spinipes* is also attracted to those marked by *M. rufiventris* (Nieh *et al*, 2004). Scent-marks deposited from tarsal glands are progressively built up by visits from *Melipona seminigra* to feeders, so that after forty visits attractive scent-marks last for two hours (Hrncir *et al*, 2004).

However experiments carried out on real flowers reveal a different picture. A wide range of both social and solitary bee species have been shown to reject flowers recently visited by both con- and hetero-specifics (Stout *et al*, 1998; Stout & Goulson, 2001; Gawleta *et al*, 2005; Yokoi *et al*, 2007; Yokoi & Fujisaki, 2009a). This behaviour translates into more efficient foraging, as for example honeybees foraging on *Lotus corniculatus* (Fabaceae) are more likely to reject flowers with less than average nectar volumes (Weatherwax, 1986). *Bombus hortorum*, *B. prascuorum*, *B. pratorum* and *B. terrestris* reject the flowers of the perennial *Symphytum officinale* (Boraginaceae) for 20 minutes after the flowers are visited by a conspecific or any of the other three species. This matches well with the

time it takes for the flowers to replenish most of their nectar (Stout *et al*, 1998). The time that a flower remains repellent following a visit is often linked to nectar replenishment. Bumblebee revisitation rates to a number of plant species, varying from 10 minutes to 24 hours, correspond well with nectar secretion rates for those species (Stout & Goulson, 2002). The conflict between experimental results from artificial and real flowers arises as most artificial feeders are poor simulations of real flowers. Most provide an *ad libitum* sugar source and so more closely resemble a nest to be raided or rotten fruit, while real flowers are often emptied by bees in a single visit (Goulson *et al*, 2000). When more realistic artificial flowers are used bumblebees are repelled from recently-visited flowers (Witjes & Eltz, 2007).

Attractive cues have still been found on real flowers. Honeybees, although initially repelled by flowers of yellow sweet clover *Melilotus officinalis* (Fabaceae) for up to 40 minutes after a previous visit, are more attracted to flowers visited 24 hours previously (Stout & Goulson, 2001). The opposite has been observed in *Anthophora plumipes* (Apinae) bees that are attracted to flowers of the honeywort *Cerinth major* (Boraginaceae) they themselves have visited for 1-3 minutes but repelled after that from flowers visited by themselves or any conspecifics for up to 30 minutes (Gilbert *et al*, 2001), although in this case the initial attraction is perhaps more likely to be due to individual memory. Some researchers have speculated that different scent-marks may be used to mediate attraction and repellence (Stout *et al*, 1998); however, it is possible that the same scent-marks may be interpreted differently depending on the situation and the potential benefits available, either through a change in scent composition as the more volatile components are lost or through different learned responses to the same volatiles (Saleh & Chittka, 2006; Saleh *et al*, 2006).

Bumblebees are more likely to probe scent-marked artificial flowers in a high reward treatment if they have experience of that reward (Saleh & Chittka, 2006) and are also more likely to probe a marked flower if that flower is structurally simple, so that handling time is short (Saleh *et al*, 2006). Bees may also take into account the aggressiveness of the species leaving a scent-mark. *Bombus terrestris* shows the strongest avoidance to scent-marks left by the territorial *Anthidium mericatum* (Megachilidae) (Gawleta *et al*, 2005). Females of the solitary bee species *Andrena prostomias* (Andreninae) reject flowers marked by males even more strongly than those marked by other females

(Yokoi & Fujisaki, 2009a). Information from scent-marks is ignored completely when rewarding and unrewarding flowers are different colours (Giurfa *et al*, 1994). They may also be ignored more commonly when food is scarce (Saleh *et al*, 2006). So not surprisingly the influence of scent-marks on foraging decisions varies considerably between plant species. *Halictus aerarius* (Halictinae) sweat bees revisit visited and unvisited *Erigeron annuus* (Asteraceae) flowers at the same frequency 60 minutes after visitation, while visited *Justicia procumbens* flowers are avoided for longer (Yokoi & Fujisaki, 2007).

During these trials bees do not appear to take into account information obtained remotely from the nectar. Experimental manipulations with artificial flower corollas moved between flowers with different nectar volumes (Schmitt & Bertsch, 1990) and manipulations of nectar volumes within real flowers (Goulson *et al*, 1998; Yokoi *et al*, 2007) clearly show that what is detected are the scent-marks on the flower or artificial corolla. These results contrast with other studies that have found nectar scent used as a foraging cue. For example, *Osmia* bees were seven times more likely to visit previously unvisited flowers with nectar than unvisited and nectar-depleted flowers, and this discrimination ability vanished when bee antennae were coated in silica (Howell & Alcarón, 2007). Many nectars acquire scent through passive absorption from surrounding volatile-producing tissues, but certain species, including the succulent *Agave palmeri* (Asparagaceae) and the desert evening primrose *Oenothera primiveris* (Onagraceae), contain unique scent compounds (Raguso, 2004). How such subtleties in scent within a flower affects visitation by potential pollinators is not well understood.

The repellent chemical markers used as floral scent-marks differ between bee species. Bumblebee species use slightly different mixtures of long-chain hydrocarbons, secreted from tarsal glands (Goulson *et al*, 1998; Stout *et al*, 1998) and very similar in composition to cuticular hydrocarbons (Witjes & Eltz, 2009) while honeybees use the more volatile 2-heptanone, secreted from mandibular glands (Vallet *et al*, 1991). It is not yet clear whether the marks left on flowers are deposited actively by bees or if they are left passively during the course of a visit. Giurfa *et al* (1994) speculated that a single bee visits so many flowers in a day that they were unlikely to actively mark all of them. As sweat bee repellence to visited flowers was related to the size of the bee species depositing the marks, Yokoi *et al* (2007) hypothesised that sweat bees possessed a generalized

avoidance of flowers contaminated by hydrocarbons common on insect cuticles. It may be unavoidable volatile residues deposited during a visit, especially from tarsal secretions, that are being detected (Gawleta *et al* 2005; Witjes & Eltz, 2007). Whatever the trigger, it does appear that the ability to identify previous visitation to a flower is a general trait among bee species and may be a basal trait of the bee lineage (Yokoi & Fujisaki, 2007; 2009a).

This detection ability extends to visits by other insects as well. Honeybees and bumblebees have also been shown to avoid flowers recently visited by hoverflies (Reader *et al*, 2005), although hoverflies show no such discriminating behaviour themselves (Reader *et al*, 2005; Yokoi & Fujisaki, 2009b). While no-one has identified bee-rejection of flowers previously visited by insects that do not consume pollen, bumblebees use scent-marks more when foraging for nectar (Stout *et al*, 1998). The ability to identify and reject flowers that have been visited by nectar thieves such as ants could well increase foraging efficiency.

A wide range of scent-marks are deposited by ants at food sources, but also as simple territorial markers. Multiple different trail pheromones are also produced by a single species that are utilised by different castes, even in monomorphic species (Jackson *et al*, 2007). The longevity of these trails depends on the substratum, as Pharaoh ant (*Monomorium pharaonis*; Myrmicinae) trails decay three times as fast on newspaper as on plastic (Jeanson *et al*, 2003). This could translate into differing trail longevity on different plant species or plant parts depending on the nature of the epidermis. Ants also passively deposit footprints from tarsal glands in the same way as bees. These secretions may include a highly volatile hydrophilic component that will quickly disperse but mostly they consist of hydrophobic and highly persistent residues (Federle *et al*, 2002). The ability to detect either ant trail pheromones or these lipid footprints could give bees the ability to avoid flowers recently visited by ant nectar thieves. Such cues are already known to be detected by other species. Some ant species share trails either mutualistically when species share a common nest or due to the scouting activity of one species being exploited by another (Menzel *et al*, 2010); and as previously mentioned, the beetle *Rhyparida wallacei* preferentially feeds on leaves from plants outside of *Oecophylla smaragdina* territories (Offenberg *et al*, 2004).

1.7 Floral Ant-Repellence

In order to prevent the disruption of pollination by ants through floral larceny and other means, many plant species have evolved traits that block access or repel ants. Van der Pijl (1955) observed that petals of the myrmecophyte *Myrmecodia* (Rubiaceae) repelled ants, and was the first to hypothesise that a volatile substance may be responsible for the phenomenon he termed 'myrmecophoby'. Traits that trigger myrmecophoby in flowers can be physical features of the inflorescence or chemical ones specific to certain tissues. It should be remembered that many physical traits that function to prevent floral larceny are likely to be exaptations, the result of selection to prevent more generalised herbivory of the flower or fruit (Armbruster, 1997). Chemical means of defence are more likely to be effective against specific thieves, including specific ant species. As ant-flower conflicts are especially likely in species that use ants in defence against herbivores, investigations have focused primarily on myrmecophytes, especially *Acacia*; but as a greater range of species is being examined, adaptations to prevent the disruption of pollination by ants are being found in species without a close coevolutionary history with any particular ant species.

1.7a Physical barriers

The most basic defences against nectar theft by ants are incorporated into the structure of flowers and their positioning on the plant. Ants are most commonly found on bowl-shaped flowers with exposed nectar (Herrera *et al*, 1984). Long petioles, obstructing bracts or trichomes and narrow corollas all effectively exclude ants of appropriate size (Guerrant & Fieder, 1981; Kerner 1878/2008; Willmer, 2011). The secretory trichomes covering the corollas of various species of *Plumbago* (Plumbaginaceae) are perfectly situated to deter herbivores and thieves (Willmer, 2011), while the fine obstructing hairs found within the flowers of many species with long corollas limit access to a wide range of potential nectar thieves.

Another possible strategy is tolerance, which involves providing enough nectar for thieves and pollinators alike. However, supplying large quantities of floral rewards would encourage inbreeding as pollinators would not have to travel far to be satiated (Hargreaves *et al*, 2009).

Water in bracts may prevent ants from accessing flowers (Willmer, 2011). One of the best examples of a physical barrier against ant thieves can be seen in *Heliconia* (Heliconiaceae) species that possess large upturned bracts and actively secrete a sticky liquid into the bract (Bronstein, 1986). When the bract liquid of *Heliconia wagneriana* was removed the flowers suffered greater physical damage, nectar theft by ants and reduced hummingbird pollination (Wootton & Sun, 1990). Similarly, the nectar-abundant flowers of teasel, *Dipsacus laciniatus* (Dipsacaceae), are held high above a water moat formed by a pair of leaves at the base of the stem that fill with water (Kerner, 1878/2008). The water calyces of the herb *Chrysothemis friedrichsthaliana* (Gesneriaceae), however, do not reduce visitation by ants but do significantly reduce herbivory by the moth *Alucita* sp. cf. *flavicincta*, the most common folivore (Carlson & Harms, 2007). The amphibious *Polygonum amphibium* is known to have no floral defences when floating in water but to develop sticky trichomes on the floral stems when the habitat dries out, giving access to ants (Kerner, 1878/2008).

Although it is now accepted that the primary role of EFNs is that of herbivory reduction, Kerner (1878/2008) suggested that they may also distract, or bribe, ants away from floral nectaries. Wagner and Kay (2002) tested this hypothesis using artificial structures with primary and secondary “nectaries”. For the short time period tested, the presence of secondary nectaries significantly reduced ant visitation to primary ones. Flowers of the senita cactus, *Pachycereus schottii*, do not repel the plants’ occasional *Crematogaster* ant defenders but the ants are distracted from flowers by increased EFN supply (Chamberlain & Holland, 2008). The distracting capabilities of EFNs however, may often be very weak. *Leucospermum conocarpodendron* (green pincushion tree) flowers are regularly visited by ants and covering the EFNs does not affect ant foraging at floral nectaries (Lach, 2008a). EF-nectar secretion on the pedicels of the shrub *Bixa orellana* (Bixaceae) actually drops when flowers open, only to increase again two days after opening (Bentley, 1977). Whether this acts to reduce ant visitation to the whole plant (and subsequent disturbance of pollination) or is down to physiological constraints is unclear.

1.7b Repellent nectar

Janzen (1977) was the first to speculate that repellent nectar may be an efficient mechanism to keep unwanted visitors away from flowers, and/or reduce the costs of nectar theft, especially by ants.

Schubart and Anderson (1978), however, were quick to point out that ants are commonly found in flowers and, so far, few examples of ant-repellent nectar have been found. There are, however, some fascinating cases.

Toxic nectar, containing iridoid glycosides, is produced by the bee-pollinated *Catalpa speciosa* (Bignoniaceae) and has been shown to reduce levels of floral nectar theft by small ants and skipper butterflies by poisoning those that attempt to feed from it (Stephenson, 1981, 1982). Kessler & Baldwin (2006) discovered high concentrations of repellent nicotine in the nectar of *Nicotiana attenuata* that limits the length of time pollinators spend at a flower. This may promote outcrossing or reduce nectar theft but may also be an unavoidable physiological consequence of the high nicotine concentrations found in the leaves.

The highly alkaline nectar of the protocarnivorous Purple Toothwort, *Lathraea clandestine* (Orobanchaceae), may be associated with ant repellence (Prŷs-Jones & Willmer, 1992). As root parasites, these plants present their flowers at the soil surface where nectar thieves could be a serious problem. Bumblebees, *L. clandestina*'s primary pollinators, appear to be unaffected by the high nectar pH. No such pollinator specialisation is seen in the case of the perennial vine *Gelsemium sempervirens* (Gelsemiaceae) where alkaline nectar reduces the quality, although apparently not quantity, of floral visits (Adler & Irwin, 2005). Using artificial mimics of *G. sempervirens*, Gegear *et al* (2007) found the opposite as *Bombus impatiens* avoided alkaline nectars, but visited for just as long as controls when visits did take place. Repellence, however, was context-dependent as the repellent effect could be overcome by particularly high sugar concentrations or tolerated when no better options were available (Gegear *et al*, 2007). *Aloe vryheidensis* (Xanthorrhoeaceae) is unusual in having dark red phenolic-rich nectar that is distasteful to specialised nectarivores like bees and sunbirds. Instead it is pollinated by unspecialised bird visitors that, for *A. vryheidensis* at least, are more efficient (Johnson *et al*, 2006). *Nicotiana attenuata* emits 1-hexanol from its nectar that attracts ants but repels pollinators making it maladaptive in floral nectar, but potentially useful in EF-nectar (Kessler & Baldwin, 2006). The nectar of the African milkweed *Xysmalobium orbiculare* (Apocynaceae) is also distasteful to potential visitors, possibly including ants, but is accepted by the plants' pompilid wasp pollinators, which are probably attracted by very specific chemical scent cues (Shuttleworth & Johnson, 2009). So

manipulation of pollinator behaviour with repellent nectar may be effective in populations that are not pollen-limited, are commonly visited by naïve individuals and do not have more rewarding species to compete with for attention. Only these species will be able to afford to move pollinators on quickly. Where distasteful pollen provides no benefits and result from defensive traits against herbivory, plants may overcome this repellent effect with particularly rewarding nectar (Gegear *et al*, 2007).

These examples appear to be the exceptions. Very little evidence for specifically ant-repellent nectar has been published. Surveys carried out by Guerrant and Fiedler (1981) and Koptur and Truong (1998) found no nectars that were consistently unpalatable to ants, both finding only nectars that were particularly appealing to ants. Feinsinger and Swarm (1978) observed ants jerking back after initial contact with the bitter-tasting nectar of the perennial herb *Hippobroma longiflora* (Campanulaceae) but this nectar was found to be palatable in other studies (Guerrant & Fiedler, 1981; Haber *et al*, 1981), perhaps illustrating the risks of sap contamination when examining insect responses to nectar (Willmer, 1986). As well as observing ants commonly feeding from flowers in lowland Costa Rica, Haber *et al* (1981) found nectar from a wide range of species palatable, including a number of nectars known to contain phenolics or alkaloids. More recently, Blüthgen *et al* (2004a) identified 3 out of 18 Australian plant species with nectar that was unpalatable to ants. Although the sugar concentrations of the nectar of these species were lower than average, and so inherently less attractive to ants, they suspected that unidentified repellent chemicals were responsible. As all three species had open flowers with exposed nectar, it is also possible that repellent pollen falling into the nectar may have been responsible or contamination by microbes. Hummingbirds do preferentially feed on more dilute solutions and production of such nectar by bird-pollinated species may indirectly benefit plant fitness as it helps to dissuade bee nectar robbers (Irwin *et al*, 2004). Out of 72 species of native and non-native plants examined in Germany, Junker and Blüthgen (2008) found only a single species whose nectar was consumed significantly less by ants than a sugar solution of the same concentration and this was the evergreen shrub *Nerium oleander* (Apocynaceae).

Potentially repellent substances in nectars such as phenolics, alkaloids, chitinases and non-protein amino acids are often found in both floral nectar and EF-nectar, and probably act as antimicrobial compounds. In much the same way as herbivore deterrent chemical defences, such

antimicrobials are probably effective against generalist infestations of nectar but fail to eliminate more specialised yeasts or bacteria (Herrera *et al*, 2008). These defences are tolerated by ants and other visitors, (Baker and Baker, 1978; González-Teuber *et al*, 2009, 2010). Ornamental tobacco (*Nicotiana langsdorffii* x *N. sanderae*) manufactures enough hydrogen peroxide within its nectar to inhibit the growth of phytopathogens through the nectar redox cycle, a process that may be common in many plants (Carter & Thornburg, 2004).

In those species where ant-repellence from nectar has been observed there is still no evidence for fitness benefits to the plant, and the repellence may have arisen due to pleiotropic effects (Adler, 2000). Untangling the effects of harmful secondary compounds in nectar is challenging, as legitimate and illegitimate floral visitors, herbivores and microbes within the nectar all need to be taken into account. As already mentioned, a close association with ants may in fact increase the concentrations of compounds, which may at first glance appear repellent, as in the EFN of myrmecophytic *Acacia* (González-Teuber *et al*, 2009). As the presence of repellent nectar is so rare, the trade-offs taking place between pollinator attraction and defence with regard to nectar appear to be typically weighted toward pollinator attraction. Repellent chemicals may be more common within floral tissue itself. While these non-volatile compounds in floral tissues have no effect on nectar thieves, deterrent chemicals in the perianth may play a role in defence against robbers. Hubbell *et al* (1984) found ant-repellent chemicals in the leaves of a number of plant species in Costa Rica. Many of these compounds, however, may in fact be anti-fungal in nature, as it was the preferences of the leafcutter *Atta cephalotes* that were tested and any leafcutter species need to protect their fungal gardens.

1.7c Chemical ant repellence

More subtle alternatives to physical defences are volatile chemicals timed to repel ants when the flowers first open. The aggressive ant-guards employed by many species of *Acacia* have the potential to disrupt pollination by physically threatening visitors. Acacias are self-incompatible (Kenrick & Knox, 1982) and so there is no chance that ant-guards can act as pollinators. Ant-guard patrolling on *Acacia zanzibarica* myrmecophytes, however, is at its lowest during dehiscence and peak pollinator visitation. This temporal partitioning is caused by a volatile chemical released from the flowers during dehiscence that repels ants, an effect that is transferable for a period of time when young flowers are

wiped on to other surfaces (Willmer & Stone, 1997). This mechanism maintains ant-guards on buds and fertilised ovules, reducing herbivory and increasing female fitness, but allows pollinators to operate unmolested on the dehiscing flowers. By eliciting out-of-context aggressive behaviour this sensory trap (sensu Edwards, 2009) enforces a behavioural pattern on the ant-guards and ensures that ants will not linger on flowers to disrupt pollination, helping to maintain the mutualism.

Raine *et al* (2002) discovered a similar mechanism in the Mexican *Acacia hindsii*. In this species no temporal partitioning of ants and pollinators was observed, but they were still separated in space by a transferable volatile repellent produced by young flowers. *A. hindsii* possesses racemose inflorescences with flowering initiated at the base, allowing ants to patrol developing fruits as flowers are pollinated successively from base to tip. Ants and pollinators are also separated both temporally and spatially in the American species *A. constricta* (Nicklen & Wagner, 2006). The repellent, produced by new inflorescences, is effective against all four visiting ant species, as all avoid prolonged contact with fresh flowers and pollen. When the lycaenid caterpillar *Hemiargus isola* (Lycaenidae) forages for pollen, however, the attractiveness of lycaenid secretions is enough to override the ants' repulsion and they continue to tend the caterpillars (Nicklen & Wagner, 2006). Further evidence of ant-repellence comes from various African acacias including *A. seyal fistula*, *A. etbaica* and *A. drepanolobium*. While *A. seyal fistula* and *A. etbaica* flowers are strongly repellent to their resident ants *A. drepanolobium* flowers are only weakly repellent. The difference probably relates to phenology as *A. drepanolobium* flowers *en masse* and therefore has no need for highly focused repellence produced by single flowers; instead the massed effect of whole branches repels ant-guards (Willmer *et al*, 2009).

What is crucial is the timing of repellence to coincide with peak pollinator activity. In African and Mexican *Acacia* communities different species of *Acacia* release pollen at different times of day, so large pollinator communities can be maintained while interspecies pollen transfer is minimized (Stone *et al*, 1996, 1998; Raine *et al*, 2007). A precisely timed and short-lived mechanism, like repellence specifically from the pollen, is all that is needed. As theory predicts, there is usually stronger repellence in ant-guarded species, and a strong correspondence between ant species aggression and floral repellence hierarchies. In both *A. seyal fistula* (Willmer *et al*, 2009) and *A. hindsii* (Raine *et al*, 2002) the most efficient defenders (i.e. the biggest threats to pollinators) are the species

most strongly repelled from dehiscing flowers. Less effective, but still significant, ant-repellent properties are found in flowers of the non-myrmecophytic *A. macracantha* and *A. angustissima*, suggesting that some level of floral ant-repellence may be widespread within the genus (Raine *et al*, 2002). Such a trait could help to preadapt *Acacia* trees to mutualisms with aggressive ants. The repellence in *A. seyal fistula*, and the Neotropical ant-plant *Acacia collinsii*, may be mediated by the presence of E,E- α -farnesene, a common ant alarm pheromone, within the floral bouquet (Willmer *et al*, 2009; Raine & Edwards, unpublished data). This would provide the perfect volatile repellent, although it is still to be determined if this alone is influencing ant behaviour.

In acacias the source of the repellence appears to be the polyads (Nicklen & Wagner, 2006; Willmer *et al*, 2009), although determining the exact source, pollenkitt or fresh anther tissue, has not yet been achieved. Pollenkitt is the oily substance covering the surface of many plant taxa that promotes adherence to other pollen and the body of potential pollinators (Pacini & Hesse, 2005). Ecologically, the origin of repellence is not important as ants make no discrimination. In strongly protoandrous species, however, the retention of anthers following pollen removal throughout the female phase could continue to provide ant repellence and it would also be useful to know exactly which floral traits selection is acting on and whether or not it is the same trait in every species. Wagner (2000) speculated that the lack of nectar in many myrmecophytic acacias, which may help to prevent pollinator disturbance, was also an adaptation to discourage damaging ant contact with the pollen.

Other myrmecophytes are less likely to need mechanisms of floral ant-repellence. *Macaranga* species are pollinated by thrips (Moog *et al*, 2002), which are unlikely to be disturbed by ant activity, and their flowers are also fairly enclosed, limiting ant access. Patrols by diminutive *Pheidole* ant-guards are commonly observed on the nectarless inflorescences of *Piper* ant-plants (Karin Gastreich, pers comm), but as they pose no threat to pollinators, selection for ant-repelling floral traits is unlikely in this group.

In an attempt to identify the ubiquity of floral ant repellence using pollen wiping experiments Ghazoul (2001) reported apparent repellence by *Acacia collinsii* ant-guards (*Pseudomyrmex spinicola* and *P. flavicornis*) to the pollen of 12 out of 13 species tested, including *Acacia collinsii* itself and

another myrmecophyte *Cordia alliodora*, although the pollen-wiping methods used in these trials did not conclusively rule out neophobic responses. 18 out of 25 non-ant-guard species were also repelled from *Acacia collinsii* pollen (Ghazoul, 2001). The common ant-guard *Crematogaster opuntiae* is also repelled from the flowers of the barrel cactus *Ferocactus wislizeni*, although *F. wislizeni*'s most aggressive defender, *Solenopsis xyloni*, is not repelled (Ness, 2006). In Borneo, contact between floral parts and ants in arenas identified 8 out of 18 plant species, predominantly from canopy habitats, to be repellent to *Dolichoderus thoracicus* ants (Junker *et al*, 2007). In contrast Jaffe *et al* (2003) found no repellence in the canopy species they surveyed in Borneo. Junker and Blüthgen (2008) surveyed the responses of groups of *Camponotus floridanus* and *Lasius fuliginosus* workers to floral scents of a range of native and non-native plant species and found that *C. floridanus* was repelled by 20 out of 30 species and *L. fuliginosus* by 8 out of 26 species, suggesting that repellence from floral scents may be a widespread adaptation even among plants with no specialised relationship with ants. Henson (2003) found that floral volatiles in 13 out of 37 species of native and non-native plants in the UK elicited aggressive responses in 2 ant species. *Formica rufa* was especially responsive to *Iris stylosa* (Iridaceae) and *Viburnum bodnantense* (Adoxaceae), while *Lasius niger* responded strongly to *Lathraea clandestina*. Willmer *et al* (2009) incorporated these results along with others and identified a significant trade-off between volatile repellence and physical defences or EFN decoys, as might be expected if both traits were potentially costly to produce.

The sponge gourd, *Luffa aegyptiaca* (Cucurbitaceae), an EFN-bearing herb and Indian crop plant, is visited by a range of ant species. All medium to large ants appear to be repelled from the flowers, specifically the pollen. Only the tiny *Tapinoma melanocephalum* ants are unaffected and visit floral nectaries, possibly because they are out-competed at the EFNs (Agarwal & Rastogi, 2008). Few other crop plants have been specifically tested for floral ant-repellence but some, such as cocoa (Anikwe, 2007), are reported as being visited by ants.

The response of flower visitors in general to floral scent has been shown to be a good predictor of the strength of the interaction between those two species within the structure of a visitation network (Junker *et al*, 2010a). Although repellence is harder to detect in natural situations, understanding both attraction and repellence is essential for our understanding of such species

interactions. A meta-analysis by Junker and Blüthgen (2010) showed that obligate flower visitors are more likely to be attracted to floral scents than facultative visitors, such as ants. Floral odours may have originated as deterrents to herbivores and only once some of these herbivores took on the role of pollinator did they become attractants (Pellmyr & Thien, 1986). It was this floral odour that was probably the first insect attractant to evolve, preceding colour cues, even before the origin of the angiosperms (van der Pijl, 1960). Porsch (1950) hypothesised that pollen odour was the very first floral scent (referenced in Thien *et al*, 2000). So from the very beginning plants have had to balance attractive traits with defensive ones.

The use of volatiles is known to repel other unwanted visitors in a similar way. The flowers of the shrub *Osmanthus fragrans* (Oleaceae) are visited commonly by two species of hoverfly but avoided by other potential pollinators; γ -decalactone released by the flowers has a strongly repellent effect on small white cabbage butterflies, *Pieris rapae*, which appears to be reinforced by isomers of linalool oxide also present in the floral fragrance (Ōmura *et al*, 2000). Presumably butterflies act as poor pollinators or nectar thieves of this species and so are excluded from the flowers. The orchid *Ophrys sphegodes* (Orchidaceae) attract males of the solitary bee *Andrena nigroaenea* with a mix of volatiles that effectively mimic the scent of sexually receptive females, but following pollination by pseudocopulation emission of these compounds drops and farnesyl hexanoate (indicative of mated females) is produced, making the flowers much less attractive to the males and promoting visitation to unpollinated plants (Schiestl & Ayasse, 2001).

A minor change in scent, texture or any specific floral organs to aid in ant-repellence could trade-off against pollinator attraction or pollinator efficiency when on the flower. In pollen-limited species these changes could translate into a loss of fitness (Ashman *et al*, 2004). The presence of conical epidermal cells on certain areas of snapdragon petal surfaces alone helps bees to grip the flowers efficiently (Whitney *et al*, 2009). The lack of floral repellence in many plant species may be the result of such trade-offs balanced toward pollinator attraction. As previously discussed, in *Polemonium viscosum*, the skunky scented morph is less attractive to ants but also less attractive to bumblebee pollinators and so both sweet and skunky morphs persist within the population (Galen, 1983). Developmental and physiological constraints may also dictate the composition of floral scents, making

interpretation of their components difficult. In most cases of repellent nectar, however, there is a clear trend towards pollinator specialisation.

The influence of defensive chemicals on pollinator behaviour is still an area that has received very little attention, despite the possibility of a conflict of interest existing between chemical defence and pollinator attraction. For example, herbivory-induced changes in plant metabolism in tomato plants, *Solanum peruvianum*, are associated with a reduction in pollinator attraction (Kessler & Halitschke, 2009). Repellent traits in pollen also affect pollinator foraging as honeybees discriminate against species with high levels of herbivore-repelling phenolic compounds in their pollen (Liu *et al*, 2006). Pollen produced by a number of species, including the buttercup *Ranunculus acris* (Ranunculaceae), Viper's Bugloss *Echium vulgare* (Boraginaceae) and species of Asteraceae, fail to support the development of unspecialised bee larvae, not because of a lack of nutritional content but because those bee species lacked the necessary physiological adaptations to detoxify the contents (Praz *et al*, 2008). So where pollen is toxic to most pollinators when consumed, either due to pleiotropic effects associated with herbivore repellence or specifically as an anti-pollen theft device, pollinator foraging is severely limited (Pimentel de Carvalho & Message, 2004). Dobson and Bergström (2000) hypothesised that the insect repellent α -methyl ketones produced predominantly by the pollenkitt of anemophilous species may be an adaptation to discourage visits by pollen-foraging insects. Ant-repellent chemicals emitted from pollenkitt of entomophilous species may have to be very specific in their function so as not to influence bee visitation. The outcome of potentially conflicting selection pressures from pollinators and nectar thieves will be determined by their overall influence on plant reproductive success.

1.8 Summary and Chapter Plan

Understanding the relationship between two species is not nearly enough to understand the workings of ecosystems. The reality of the situation is a complex and changing mixture of mutualistic and exploitative guilds of plants, herbivores, pollinators, predators and thieves. Although the rest of this thesis will focus on the normally detrimental effects of ants when they visit flowers it should always be remembered that their presence may still benefit the plant in other ways, by visiting EFNs or

homopterans and providing valuable protection, or by promoting outcrossing through increasing inter-flower movement of pollinators. The interactions between ants and pollinators and between ants and the flowers themselves have been often overlooked in the field of pollination ecology and a basic understanding of when and where floral ant-repellence occurs will provide an essential framework in which to address the influence of ants on the evolution of floral traits. How commonly is the disruption caused by ants, through nectar theft and physical threat, significant enough to select for ant-repellent traits that may have to trade-off against pollinator attraction? Considering how ubiquitous ants are in many ecosystems their role as potential agents of pollination disruption is one that deserves to be looked at closely.

It is crucial that we understand the processes that structure pollination ecology and the roles of individual species. There is a growing alarm over the declines documented in pollinator species due to habitat loss, disease, pesticide use, the spread of invasive species and climate change (Biesmeijer *et al*, 2006; Potts *et al*, 2010). Only by working towards a more comprehensive perspective that takes into account all possible interacting partners will we be able to predict the outcomes of such declines.

While a number of studies have focused on floral ant-repellence, they have mostly focused on myrmecophytes, only a small number of species have ever been tested and few have attempted to associate repellence with other floral traits. So, the main focus of this thesis, reported in Chapter 2, is a survey of floral ant-repellence carried out in a tropical dry forest in Costa Rica at a national park with local support and a wide diversity of flowering species. Here the issue of when and where ant-repellence occurs is addressed by examining a wide range of plant species, taking into account the amount of nectar available and its accessibility to ants, as well as what form the repellent trait(s) takes. The specificity of ant-repellence of a number of species was also examined in more detail to determine which types of ants were being excluded from flowers and how broad the effectiveness of the traits was.

Invasive ants are ideal study organisms when examining interactions between species where no coevolution has taken place. It is still not clear how important coevolution is in the formation of floral ant-repellence in non-ant plant species. Chapter 3 looks in more detail at the type of ants that respond

to repellent traits. Are repellent traits only effective against native ants with which the plant commonly interacts, or are they often broadly effective against a species with which the plant has had no such relationship? To address this question, floral interactions with two very different ant species, the economically important invasive *Linepithema humile* and a non-flower-visiting native seed collector *Messor bouvieri* (Myrmicinae), were examined to identify any repellence that took place.

Chapter 4 looks at the issue of pollinator disruption by ants from the perspective of the pollinators themselves. A great deal of work has already been done to demonstrate the influence of scent-marks deposited by con- and hetero-specific bees on bee foraging behaviour, but in this chapter the effects of ant scent-marks are investigated for the first time. Do bees commonly utilise scent mark left by ants, either as indicators of low nectar reward or of the potential danger posed by an aggressive ant species?

Finally Chapter 5 concludes with a summary of how the work carried out in the thesis has improved our understanding of interactions between ants, plants and potential pollinators and what questions remain to be answered.

Chapter 2: Nectar Theft and Floral Ant-Repellence

Abstract

As flower visitors, ants rarely benefit a plant. They are poor pollinators, and can also disrupt pollination by deterring other flower visitors, or 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 range to a common generalist ant species, usually via ant-repellent pollen. This 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 ant species and others producing more generalised ant-repellence. It appears that there has been selection for floral ant-repellence as a defence against ant thieves in plant species that invest in large volumes of nectar.

2.1 Introduction

Foraging ants may benefit plant species in a variety of ways. Ants foraging on plant surfaces may prey upon herbivores, or disrupt their feeding and oviposition, whilst ants attracted by hemipteran honeydew may benefit the plants by reducing levels of more damaging herbivores (Strysky & Eubanks, 2007). Many plant species produce extrafloral nectar (EFN), which attracts ants, and which may be sited to encourage ants to patrol vulnerable areas such as new growth (Hodson & Gastreich, 2006) and inflorescences (Schemske, 1980). In tropical canopies plant exudates from hemipteran honeydew and/or EFN can support huge populations of potentially beneficial ants (Davidson, 1997; Tillberg, 2004). Some myrmecophytes also provide housing (domatia) for ant colonies, in the form of hollow stems or thorns, and acquire standing armies of specialized mutualists that they may feed with EF-nectar, protein bodies, or indirectly with hemipteran honeydew (Heil & McKey, 2003). These specialized ants may then provide additional benefits by pruning encroaching vegetation (e.g. Suarez *et al*, 1997), or supplying nutrients to their hosts through detritus within domatia (e.g. Sagers *et al*,

2000). Such relationships between ants and plants provide fascinating model systems for ecological and evolutionary phenomena, including conflict between mutualistic partners (Heil & McKey, 2003).

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 with the antibiotic secretions used in nest hygiene (Beattie *et al*, 1985; Vega *et al*, 2009). In addition, they may disrupt pollination and reduce visitation by stealing nectar or threatening incoming pollinators (e.g. Altshuler, 1999; Lach, 2008a; 2008b), resulting in decreased seed-set (e.g. Ness, 2006); this is especially damaging where plants are self-incompatible and rely on limited supplies of outcrossed pollen (Lennartsson, 2002).

It has long been recognized that ants are repelled from certain flower species (Van der Pijl, 1955). This may occur in response to contact with mechanical barriers such as trichomes, or through chemical deterrents including flower scent (Guerrant & Fiedler, 1981). Most work on ant-floral conflict has focused on *Acacia*, both in Africa and the Neotropics, where repellence by floral scent has been demonstrated. Volatiles released by young *Acacia* inflorescences repel the resident ant-guards for a few hours, allowing pollinators free access (Willmer & Stone, 1997; Raine *et al*, 2002; Nicklen & Wagner, 2006). A range of temperate and tropical plant species has also been identified as possessing some form of ant-repellence (Junker *et al*, 2007; Agarwal & Rastogi, 2008; Junker & Blüthgen, 2008; Willmer *et al*, 2009). In some cases this repellence operates as a close range tactile signal, while in others the repellence works effectively as a volatile scent. In general, obligate flower visitors are more likely to be attracted to floral scents than facultative visitors such as ants (Junker & Blüthgen, 2010). Junker and Blüthgen (2008) found that terpenoids, such as linalool, effectively repel a number of ant species and are common in the floral scent of species that repelled ants, possibly playing a dual function as pollinator attractant and ant-repellent. Floral traits that perform defensive functions are often co-opted from otherwise attractive roles (Armbruster, 1997).

In most cases studied so far, including the acacias, this repellence involves a pollen- or anther-derived scent, transferable though “pollen-wiping”, the wiping of pollen from freshly dehiscent anthers onto older flowers or other surfaces (Willmer & Stone, 1997; Ghazoul, 2001). 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 immediately obvious, as the costs and benefits of ant attendance at flowers have only rarely been tested (e.g. Lach 2008a; 2008b; Hansen and Müller 2009). Are ant-repellent traits selected for to reduce aggression towards pollinators, or to prevent nectar theft, or are they a product of pleiotropic effects on other floral traits? This study aimed to identify the pattern of occurrence of such 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 the ants have to come into contact with the flowers?
2. What plant and floral traits are correlated with floral ant-repellence? If such repellence has adaptive significance it may be more common in species that invest most heavily in their flowers, for example in advertisements such as copious pollen or large volumes of nectar. It may be 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 then also relate to both phylogeny and pollination syndrome.
3. How effective are ant-repellent traits against a range of ant species? If such traits function to reduce threats to potential pollinators it might be expected that they are most efficient against larger predatory ants.

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

2.2 Methods

2.2a Study sites

Fieldwork, summarised in Table 1, 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. The majority of data was 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, throughout the bulk of the dry season when many of the forests' common plant species flower, and January to February of 2010. This area, managed by the government of Costa Rica, covers the greatest expanse of tropical dry forest in Central America and provides facilities for researchers, tourists and school groups. The dry forest was structurally ideal for accessing and observing a wide variety of flowers and ants are commonly found on all plants species.

Table 1: Response trials carried out in each study period.

Year	Field Site	Experiment	Species
2008	Las Cruces	Tactile trials	5 ant species with <i>Stachytarpheta frantzii</i>
	La Selva	Scent trials	10 plant species with <i>Ectatomma ruidum</i> .
2009	Santa Rosa	Tactile trials	49 plant species with <i>Camponotus novograndensis</i> . 14 plant species with <i>Ectatomma ruidum</i>
		Scent trials	33 plant species with <i>Camponotus novograndensis</i>
2010	Santa Rosa	Tactile trials	6 plant species with 8-9 ant species

2.2b Study species

To test for evidence of ant-repelling traits plant species were chosen to represent a wide variety of floral forms and cover a range of taxonomic groups. Selection was only limited by which species were flowering and accessibility to the flowers. Plants were initially located during the afternoons and then watched from dawn to identify the period of peak dehiscence; some species had pollen available all morning but others only in the short window after dehiscence, before all pollen was lost to visiting bees. Flowers were thereafter collected at times of high pollen availability. Priority was given to those species that flowered quickly over a few days. Nectar samples were collected from twelve individual flowers during peak dehiscence using 1µl microcaps, and refractometers (Bellingham & Stanley) used to determine sucrose concentration. A wide range of plants with different growth forms and from different taxonomic groups was used, but selection was limited by availability. Table 2 shows a complete list of plant species used.

The generalist formicine *Camponotus novograndensis* was used for the majority of experiments. It is a regular visitor to EFNs and flowers and was especially common at Santa Rosa. To test the effectiveness of repellence against a wider range of ant species, species were chosen that were both commonly found and also represented a variety of feeding preferences and taxonomic groups. The most important of these secondary ant species was the large ectatomminine *Ectatomma ruidum*. While *C. novograndensis* poses no obvious threat to pollinators *E. ruidum* has been shown to hunt bees (Schatz and Wcislo 1999).

2.2c Detecting the presence and form of ant-repellent traits

This question was investigated using the following behavioural trials:

Tactile response trials identified repellence when an ant contacted part of a flower. A single ant was placed in a Petri dish arena with three or four evenly-spaced objects: a fresh flower that was dehiscing, an older pollen-depleted flower and/or a bud and a twig used as a further control to rule out neophobic responses to unfamiliar objects. Behaviours were recorded over four minutes, including the number of antennations, times an object was walked over or clearly avoided and occasions when objects were closely probed. Repellence was deemed to occur when an ant's antennae made contact with an object and the ant immediately jerked or turned away. Such activity was often followed by grooming of the antennae. Recording began ten seconds after an ant was placed in the arena, to reduce the likelihood of recording initial neophobic responses. Comparisons between fresh flowers with pollen and old pollen-depleted flowers were especially useful in determining the repellent effect of pollen. At least 12 trials were normally carried out for each species combination of ant and plant (unless fresh flowers became unavailable). It was not always possible to acquire both buds and older flowers for each plant species as in some cases the flowers quickly abscised and withered and in others buds were hidden within the inflorescence.

An additional tactile trial was used in the case of *Stachytarpheta jamaicensis*, as it was the only species to clearly possess repellent petal surfaces. To narrow down the site and nature of strong ant-repellence in, the upper petal surfaces of fresh *S. jamaicensis* flowers were rubbed against 1cm²

of filter paper and tactile response trials carried out using *Camponotus novograndensis* (with untouched squares of filter paper for controls).

Tactile response data were analysed in terms of the number of times that ants walked over or were repelled by floral parts. The proportion of ants repelled was calculated, following Junker *et al*, (2007), where '0' indicated no repellence and '1' complete repellence. Where an ant walked over just the base of a corolla this was not included in the proportion data; and where an ant was "repelled" by the twig "control" those trials were not included in the analysis, though in practice this only excluded 5% of trials.

Scent response trials tested longer range (non-contact) repellence. An ant was placed in a Petri dish arena connected to two syringes, one empty and acting as a control (for air movement alone) and the second containing a flower or inflorescence. Ant behaviour was observed for four minutes alone inside the arena, for one minute after the control air was gently blown over the ant and for another minute after this was repeated with the flower scent. Ants tended to run around the circumference of the arena. 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. Changes in levels of agitation or aggression were recorded as charges, abdomen cocks, holding the head up and time spent grooming. The length of time an ant remained stationary was also recorded. Preliminary testing showed that there was no significant difference between behaviour during the minutes immediately prior to and following control air insertion.

Workers of average size for each ant species were used in all experiments. Each ant was collected by allowing passive entry into a clear glass tube from the ground or vegetation, and was used once and then returned to the area where it was found (except for those collected for identification). Floral parts for trials were picked with clean forceps and used immediately, and only once. Where equipment was marked with visible ant excretions it was washed carefully with alcohol.

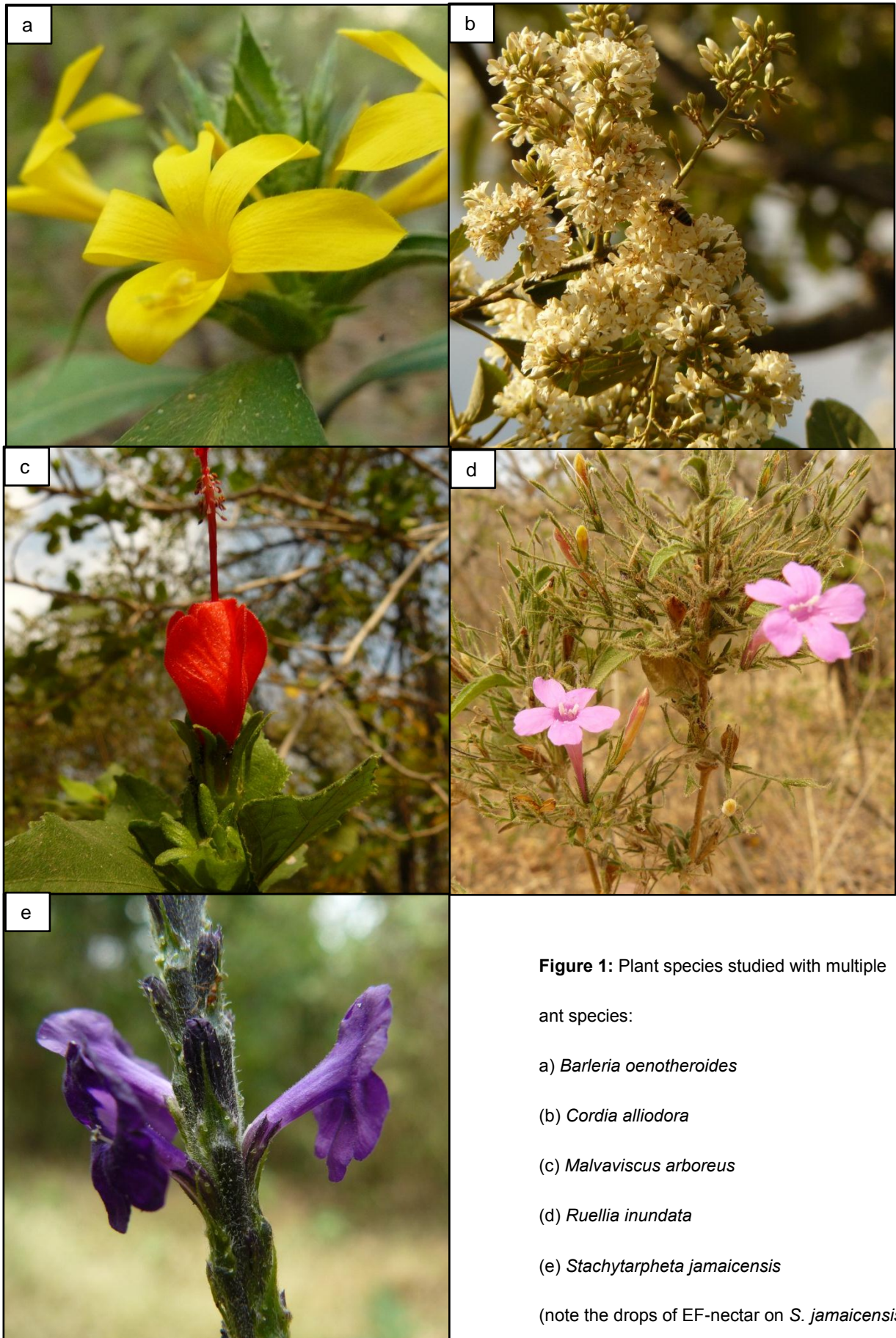
2.2d Detecting plant and floral traits correlated with floral ant-repellence

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 1µl microcap, 2 = <0.5µl, 3 = >0.5µl. Nectar accessibility to *Camponotus novgrandensis* was scored by flower 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 depending on the pollination syndrome the floral traits appeared to conform to (Willmer 2011), and/or its known visitors from observations and from the literature.

2.2e Effectiveness of ant-repellent traits against a different ant species

A number of plant species that were found to possess effective ant-repellent traits in tactile and/or scent trials with *C. novograndensis* were tested against other ant species to determine their effectiveness. 14 of these plant 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). In early 2010 the following five plant species with very different floral forms that were highly repellent to *C. novograndensis* were also tested against an addition 6-7 ant species with very different behavioral habits:

- ***Barleria oenotheroides* (Acanthaceae):** a small under-canopy herb, with large yellow flowers ~2.5cm long, with anthers and stigma extending out onto lower petal. Most commonly visited by small bees at Santa Rosa.
- ***Cordia alliodora* (Boraginaceae):** also known as laurel, this hardwood tree is a myrmecophyte notable for housing multiple ant species in the twig domatia of an individual plant (Tillberg, 2004). The species mass flowers for 6 weeks during the dry season. The simple white flowers, ~1cm long, possess a sickly sweet scent, produce small quantities of nectar and have a low degree of self-compatibility (Opler *et al*, 1975). The only flying visitors observed at Santa Rosa were honeybees.



- ***Malvaviscus arboreus* (Malvaceae)**: a hummingbird-pollinated shrub with large red flowers ~4.5cm long and copious nectar production. Hummingbirds are the plants' most efficient pollinator, but most pollen is removed by *Trigona* bees (personal observation).
- ***Ruellia inudata* (Acanthaceae)**: a perennial herb with purple flowers ~2.5cm long, often visited by butterflies, with a thick growth of trichomes around the inflorescence and meristems, but not on the flowers themselves.
- ***Stachytarpheta jamaicensis* (Verbenaceae)**: a perennial herb with a long EFN-bearing inflorescence from which two dark purple flowers open on average per day, each being ~1.3cm long. They are visited by a wide range of flying insects, especially butterflies. (*S. frantzii* is a widely cultivated shrub of very similar form).

These plant species were tested in tactile trails with the following commonly found ant species, which were chosen to represent a wide range of behaviours and diets: the leafcutter *Atta cephalotes* (Myrmecinae); the large generalist *Camponotus sericeiventris* (Formicinae); the common arboreal species *Cephalotes umbraculatus* (Myrmecinae); the large predatory *Pachycondyla villosa* (Ponerinae); the small generalist *Pheidole fallax* (Myrmecinae); and the medium-sized generalist *Pseudomyrmex gracilis* (Pseudomyrmecinae). *Cordia alliodora* is capable of housing multiple ant species in different twig domatia on the same plant (Tillberg, 2004) but the only inhabitant within reach at Santa Rosa was the obligate commensalist *Cephalotes setulifer*, so this ant was also tested specifically with this plant. The ant species *Acromyrmex coronatus*, *Crematogaster curvispinosa*, *Ectatomma ruidum*, *Megalomyrmex foreli* and *Pheidole fallax* were tested in tactile trials against *Stachytarpheta frantzii*, again being selected as they possess very different behaviours and diets.

2.2f Statistical methods

As data were not normally distributed Kruskal Wallis tests were used to compare the proportions of ants repelled from floral parts within species and to identify differences between proportion of ants repelled and floral traits of the various species used, while Jonckheere-Terpstra tests were used to test for correlations among these groups where appropriate. Behaviours recorded during the control and test minutes of the scent trials were compared using Kruskal Wallis tests. Where numerous statistical

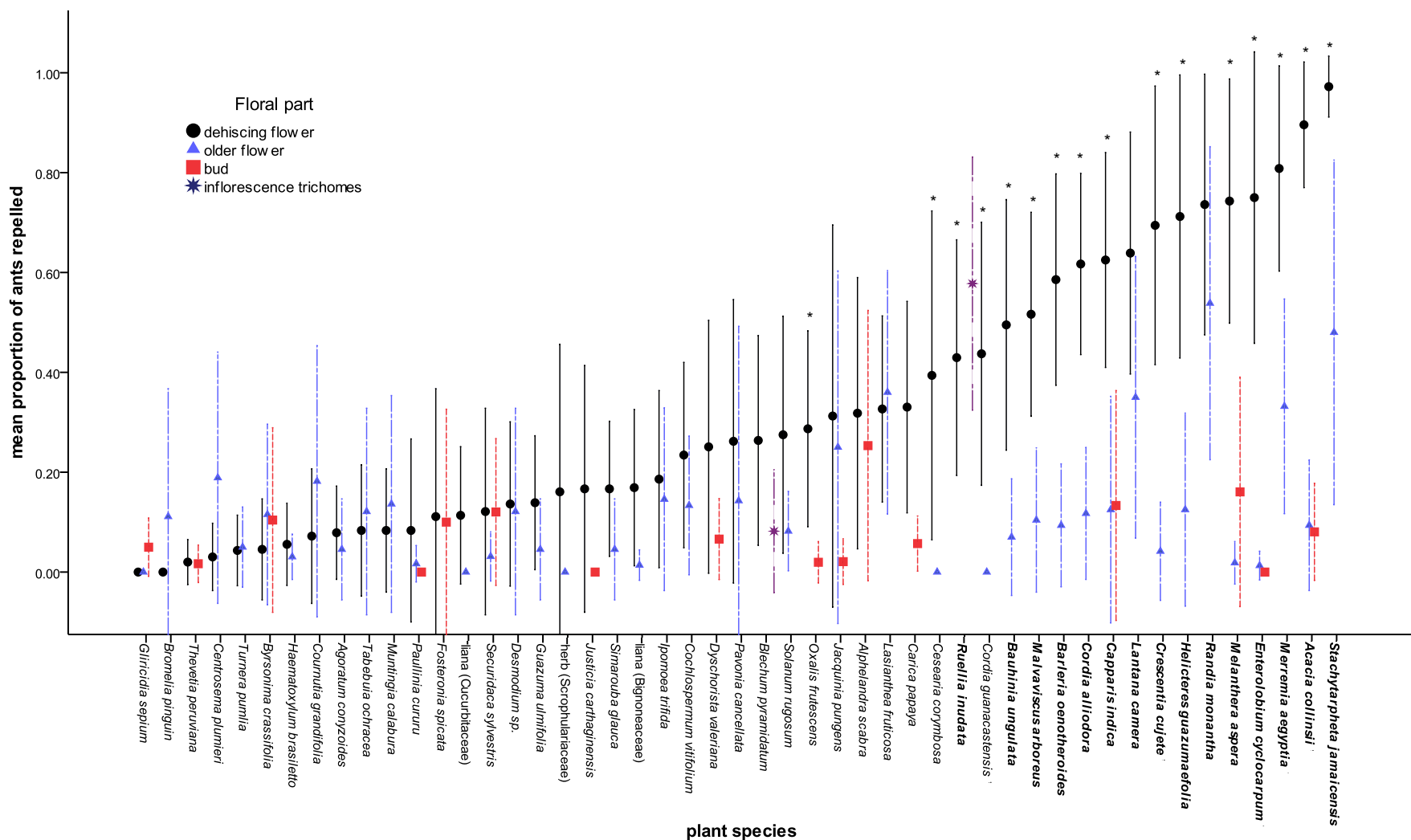


Figure 2: Tactile response results showing the mean proportion of *Camponotus novograndensis* workers repelled by the floral parts of each plant species, arranged by increasing repellence from fresh flowers. Floral parts of species in bold repelled ants on more than 50% of encounters and those marked * repelled ants significantly more from fresh flowers than other parts used. At least 12 replicates were carried out on each plant species.

Table 2: Plant species tested against *Camponotus novograndensis* in tactile trials. K-W tests were for differences between 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.

Family	Species	Growth Form	Nectar Volume	K-W χ^2	p-value	Mode of Pollination: Common Potential Pollinators	Tested in Scent Trials
Acanthaceae	<i>Aphelandra scabra</i>	shrub	3	0.198	0.656	Specialist: hummingbirds	y
	<i>Barleria oenotheroides</i>	herb	2	12.233	<0.001	Generalist: range of bees	y
	<i>Blechum pyramidatum</i>	herb	2	2.332	0.127	Generalist: range of bees	n
	<i>Dyschoriste valeriana</i>	herb	2	0.819	0.365	Generalist: range of bees	n
	<i>Justicia carthaginensis</i>	herb	2	0.495	0.482	Specialist: large bees	n
	<i>Ruellia inundata</i>	herb	2	0.791	0.374	Generalist: bees, butterflies	y
Apocynaceae	<i>Fosteronia spicata</i>	tree	1	0.006	0.939	Generalist: bees, butterflies	y
	<i>Thevetia peruviana</i>	tree	3	0.005	0.942	Generalist: bees, butterflies	y
Asteraceae	<i>Lasianthaea fruticosa</i>	herb	1	0.002	0.965	Generalist: bees, butterflies	y
	<i>Melanthera aspera</i>	herb	1	15.016	<0.001	Generalist: bees, butterflies	y
	<i>Ageratum conyzoides</i>	herb	1	0.86	0.354	Generalist: bees, butterflies	y
Bignoniaceae	<i>Tabebuia ochracea</i>	tree	2	2.692	0.260	Generalist: range of bees	n
	unidentified liana	climber	2	5.846	0.016	Generalist: range of bees	n
	<i>Crescentia cujete</i>	tree	3	16.091	<0.001	Specialist: bats	y
Boraginaceae	<i>Cordia alliodora</i>	tree	1	16.178	<0.001	Generalist: bees, butterflies	y
	<i>Cordia guanacastensis</i>	shrub	1	6.302	0.012	Generalist: bees, butterflies, muscids	y
Bromeliaceae	<i>Bromelia pinguin</i>	shrub	0	1.585	0.453	Generalist: range of bees	y

Table 2 cont.

Capparidaceae	<i>Capparis indica</i>	shrub	2	13.91	<0.001	Specialist: bat, hawkmoth	y
Caricaceae	<i>Carica papaya</i>	tree	3	5.241	0.022	Specialist: long-tongued bees, lepidopterans	y
Cochlospermaceae	<i>Cochlospermum vitifolium</i>	tree	0	1.182	0.277	Specialist: buzz pollinated by bees	y
Convolvulaceae	<i>Ipomoea trifida</i>	climber	2	1.426	0.232	Generalist: bees, butterflies, flies	y
	<i>Merremia aegyptia</i>	climber	2	8.121	0.004	Generalist: bees, butterflies, flies	n
Cucurbitaceae	unidentified dioecious liana (male)	climber	1	3.3	0.069	Generalist: bees, flies	n
Fabaceae	<i>Acacia collinsii</i>	tree	0	23.875	<0.001	Generalist: range of bees	y
	<i>Bauhinia unguolata</i>	shrub	3	7.43	<0.001	Specialist: bats	y
	<i>Centrosema plumieri</i>	climber	0	2.156	0.142	Specialist: large bees	n
	<i>Desmodium</i> sp	herb	0	0.2	0.655	Specialist: small bees	n
	<i>Enterolobium cyclocarpum</i>	tree	1	25.878	<0.001	Generalist: moths, beetles	y
	<i>Gliricidia sepium</i>	tree	0	5.84	0.054	Specialist: medium-large bees	y
	<i>Haematoxylum brasiletto</i>	tree	0	0.03	0.859	Generalist: range of bees	n
Fabaceae	<i>Securidaca sylvestris</i>	tree	0	1.138	0.566	Specialist: medium-large bees	n
Flacourtiaceae	<i>Casearia corymbosa</i>	shrub	1	6.111	<0.001	Generalist: bees, flies	y
Malpighiaceae	<i>Byrsonima crassifolia</i>	tree	0	0.328	0.849	Specialist: oil-collecting bees	y
Malvaceae	<i>Helictores guazumifolia</i>	shrub	3	7.5	<0.001	Specialist: hummingbirds	y
	<i>Malvaviscus arboreus</i>	shrub	3	10.66	<0.001	Specialist: hummingbirds	y
	<i>Pavonia cancellata</i>	climber	2	0.543	0.461	Generalist: bees, butterflies	n
Oxalidaceae	<i>Oxalis frutescens</i>	herb	1	6.374	<0.001	Generalist: small bees, flies	y
Rosaceae	<i>Muntingia calabura</i>	tree	1	0.035	0.852	Generalist: small bees, flies	y
Rubiaceae	<i>Randia monantha</i>	tree	3	1.007	0.316	Specialist: hawkmoths	y

Table 2 cont.

Scrophulariaceae	unidentified herb	herb	1	2.133	0.144	Generalist: small bees, flies	n
Simaroubaceae	<i>Simorouba glauca</i>	tree	1	2.56	0.110	Generalist: bees, flies	n
Sapindaceae	<i>Paullinia cururu</i>	climber	1	0.946	0.623	Generalist: bees, flies	y
Solanaceae	<i>Solanum rugosum</i>	shrub	0	1.839	0.175	Specialist: buzz pollinated by bees	y
Sterculiaceae	<i>Guazuma ulmifolia</i>	tree	1	1.643	0.200	Generalist: bees, flies	y
Theophrastaceae	<i>Jacquinia pungens</i>	shrub	1	3.866	0.145	Specialist: hummingbirds?	y
Turneraceae	<i>Turnera pumila</i>	herb	1	0.024	0.877	Generalist: bees, butterflies, flies	n
Verbenaceae	<i>Cornutia grandiflora</i>	shrub	0	0.022	0.882	Specialist: buzz pollinated by bees	y
	<i>Lantana camara</i>	shrub	1	2.89	0.089	Specialist: hummingbirds, butterflies	y
	<i>Stachytarpheta jamaicensis</i>	herb	3	7.411	<0.001	Generalist: bees, butterflies	n

tests were carried out it was decided that a Bonferroni correction was unnecessary as most significant values were $p < 0.001$. Error bars in all figures are 95% confidence intervals. All statistical tests were carried out using SPSS 18.0.

2.3 Results

2.3a Which plant species possess ant-repellent traits and what form do they take?

I tested 49 plant species (Table 2) and a wide range of responses was observed, with 15 species repelling ants when they came into contact with flowers on more than half of occasions. 14 of these highly repellent species and 2 that were less repellent possessed fresh flowers that were significantly more repellent than control parts tested (older flowers with pollen-depleted anthers or unopened buds) (Table 2). In most cases repellence was observed after contact with pollen-bearing anthers. Ants would occasionally walk over the bases of flowers with repellent pollen but would avoid the stamens. In repellent species there was a significant difference between the proportion of ants repelled by freshly dehiscent flowers and other floral parts, with some exceptions where both floral parts tested had a similar degree of ant-repellence.

Scent trials identified the shrub *Randia monantha* as the only plant species tested with floral scent that influenced *C. novograndensis* behaviour, with a significant increase in the number of agitated behaviours following injection of floral scent into the arena ($\chi^2=5.3$, $df=1$, $p=0.021$). There was no increase in agitation when volatiles from a cut stem of the plant were tested, indicating that the behaviour was specific to floral volatiles.

2.3b What plant and floral traits are correlated with floral ant-repellence?

There was a significant difference in ant-repellence between plants with different mean volumes of nectar ($\chi^2=46.4$, $df=3$, $p < 0.001$; Figure 3a) with a significant positive trend between nectar volume and the proportion of ants repelled (J-T=6.6, $df=3$, $p < 0.001$). Plants producing large nectar volumes were more likely to have protection against nectar theft. Ant-repellence differed significantly between plants with different levels of accessibility to nectar ($\chi^2=26.6$, $df=3$, $p < 0.001$; Figure 3b), and while there was

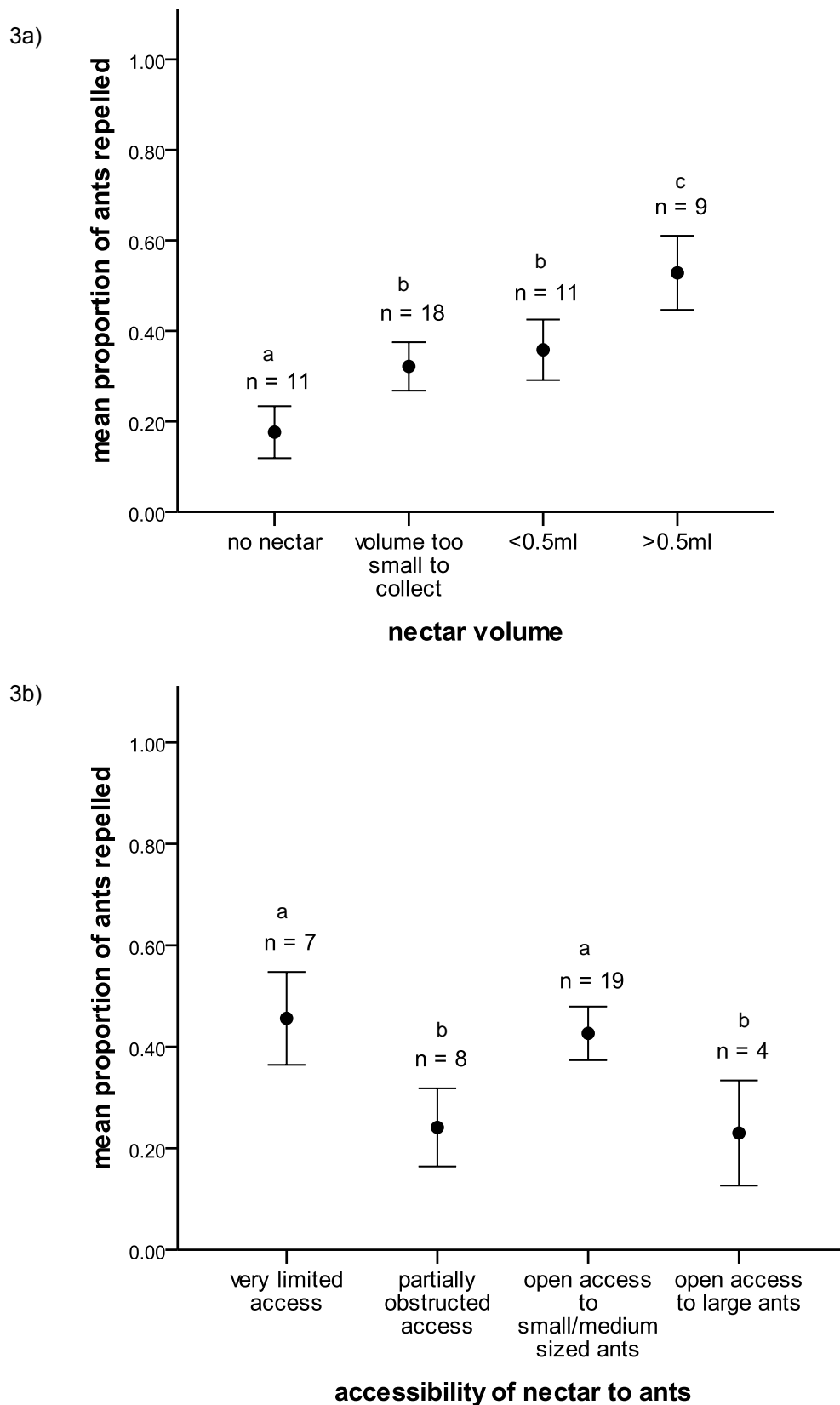


Figure 3: (a) Mean repellence to *C. novograndensis* of flowers with different nectar volumes.

(b) Mean repellence to *C. novograndensis* of flowers with different ant access to nectar.

Significant differences between groups indicated with a/b/c.

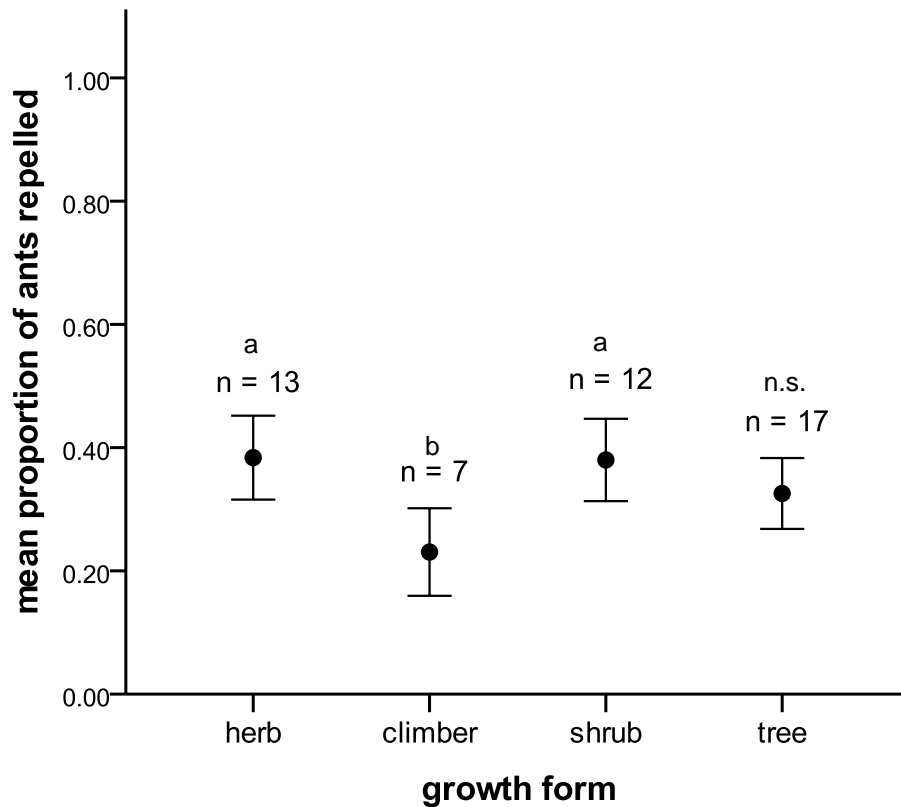


Figure 4: Mean proportion of *C. novograndensis* repelled from plant species with different growth forms. Significant differences between groups indicated with a/b/c.

a tendency for those species with the most limited accessibility to nectar to also possess the strongest ant-repellence, the overall trend was not significant (J-T=-1.5, df=3, p=0.127).

To determine which plant traits were correlated with floral ant-repellence the species were placed in a number of categories (summarised in Table 3). No significant difference in repellence was found between species with flowers of specialised pollination syndromes and more generalised species ($\chi^2=0.14$, df=1, p=0.708). However, bird- and bat-pollinated flowers were more likely to be ant-repellent ($\chi^2=36.654$, df=1, p<0.001). This is likely to be an artefact due to the high nectar volumes produced by such species. There was a significant difference between the proportion of ants repelled from flowers of species with different growth forms ($\chi^2=11.2$, df=3, p=0.011; Figure 3), but this was entirely due to the effect of climbers (no significant difference when excluded ($\chi^2=2.6$, df=2, p=0.279)).

Table 3: Subdivisions of the dataset with mean proportion repelled from fresh flowers: split by growth form, pollination syndrome (by specialist/generalist and by non-bird- or bat-pollinated/bird- or bat-pollinated), nectar volume and access to nectar. Means marked in bold where ants were repelled more than half of the time.

Subdivision		Number of species	Number of trials	Mean proportion of ant repelled from fresh flowers \pm St Dev
Nectar volume	None detectable	11	130	0.18 \pm 0.33
	Volume too small to collect	18	219	0.32 \pm 0.40
	<0.5 μ l	11	151	0.36 \pm 0.42
	>0.5 μ l	9	109	0.53 \pm 0.43
Access to nectar	Very limited	7	88	0.46 \pm 0.43
	Partially obstructed	8	87	0.24 \pm 0.36
	Open to small/medium sized ants	19	260	0.43 \pm 0.43
	Open to large ants	4	44	0.23 \pm 0.34
Growth form	Herb	13	152	0.38 \pm 0.43
	Climber	7	108	0.23 \pm 0.37
	Shrub	12	150	0.38 \pm 0.41
	Tree	17	199	0.33 \pm 0.41
Pollination syndrome	Generalists	30	396	0.33 \pm 0.41
	Specialists	19	213	0.35 \pm 0.41
	Non bird and bat pollinated	42	490	0.29 \pm 0.40
	Bird and bat pollinated	7	119	0.53 \pm 0.41

However, the sample size for climbers was lower and only one repellent example was found (*Merremia aegyptia*).

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

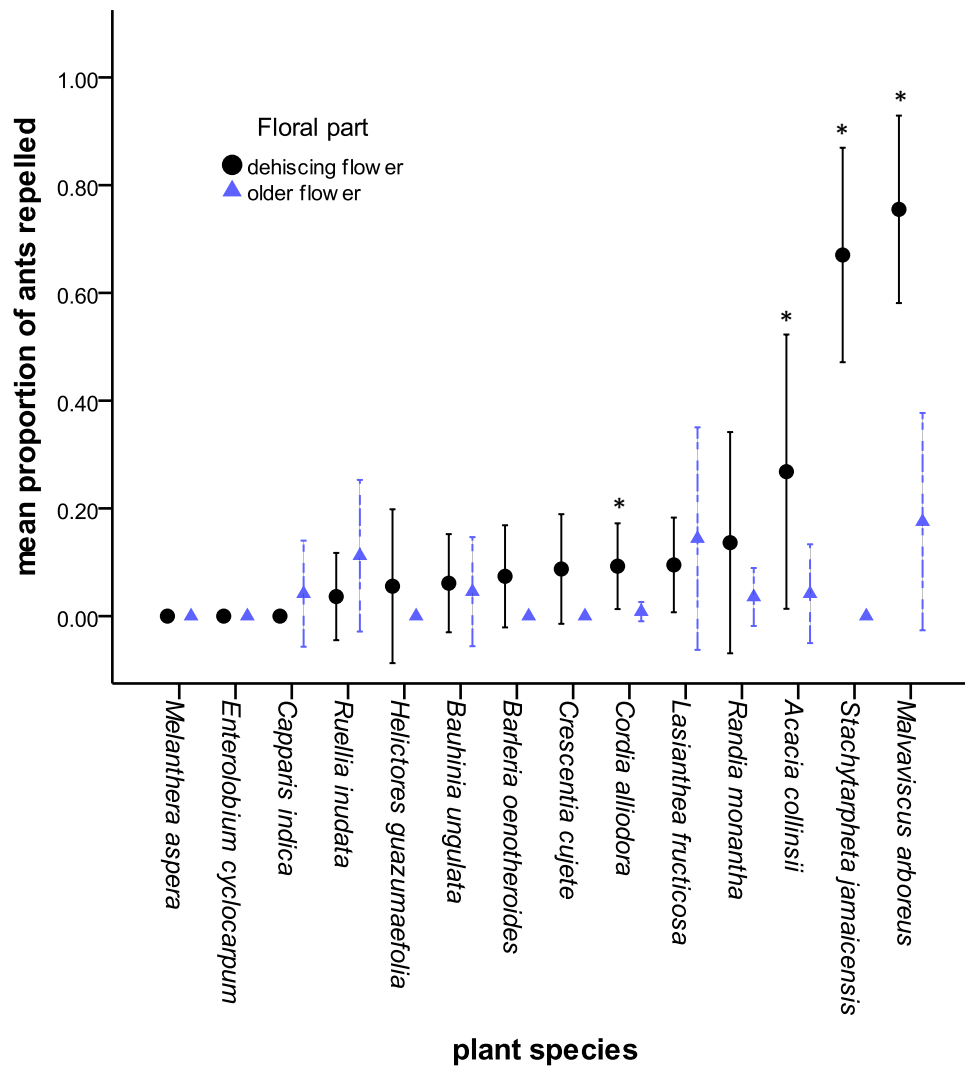


Figure 5: Plant species tested for tactile ant repellence with *Ectatomma ruidum*, where * indicates significant difference between freshly dehiscent flowers and old pollen-depleted flowers.

2.3c How effective are ant-repellent traits against a range of ant species?

Of the plant species that repelled *C. novograndensis* in the tactile trials, 14 were also tested against *E. ruidum* (Figure 5). Only two had a significant repellent effect on *E. ruidum*: 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 were found to have no effect on *E. ruidum* behaviour, and the scent trials carried out with *E. ruidum* at La Selva also found no evidence of floral scent influencing behaviour when tested against 10 other plant species (Table 4).

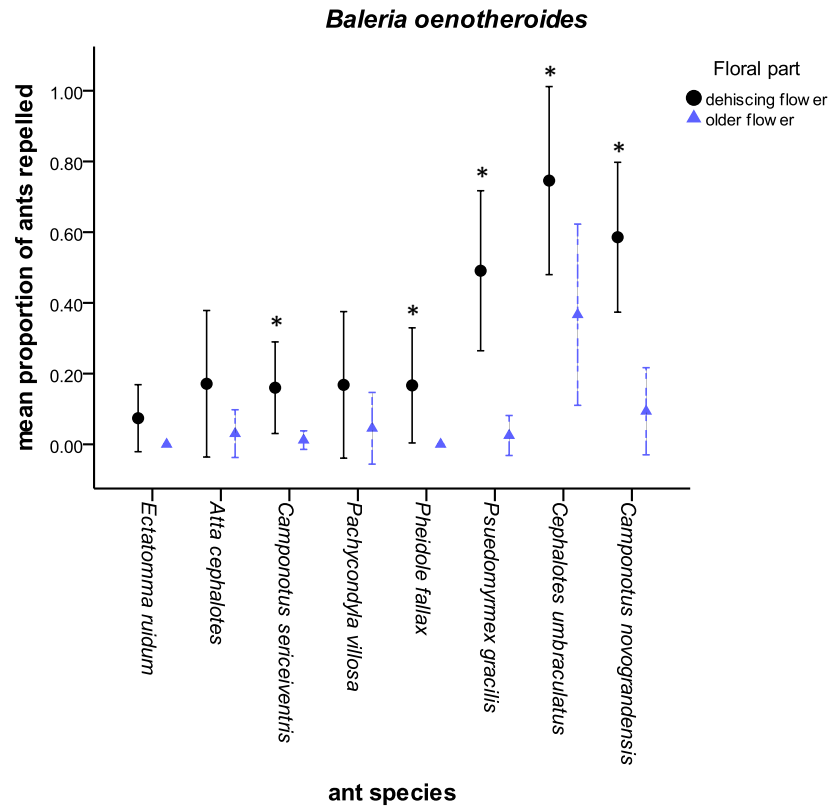
Table 4: Plant species tested in scent trials with *Ectatomma ruidum*. K-W tests for differences between agitated responses in ants during exposure to control air and floral scent. Eight trails were carried out for each species.

Family	Species	Growth Form	K-W χ^2	p-value
Commelinaceae	<i>Cochliostema odoratissimum</i>	shrub	1.519	0.218
Cucurbitaceae	<i>Gurania makoyana</i> (intrasexual protandry - male)	climber	1.496	0.221
Lamiaceae	<i>Plectranthus scutellarioides</i>	herb	0.003	0.956
Melastomataceae	<i>Miconia affinis</i>	tree	2.888	0.089
Piperaceae	<i>Piper silvianum</i>	shrub	0.048	0.826
Phytolaccaceae	<i>Phytolacca rivinoides</i>	shrub	0.071	0.790
Rubiaceae	<i>Hamelia patens</i>	shrub	0.208	0.648
	<i>Psychotria</i> sp.	shrub	0.205	0.651
	<i>Randia monantha</i>	tree	0.273	0.602
	<i>Warszewiczia coccinea</i>	shrub	0.185	0.667
Verbenaceae	<i>Stachytarpheta frantzii</i>	shrub	0.105	0.745

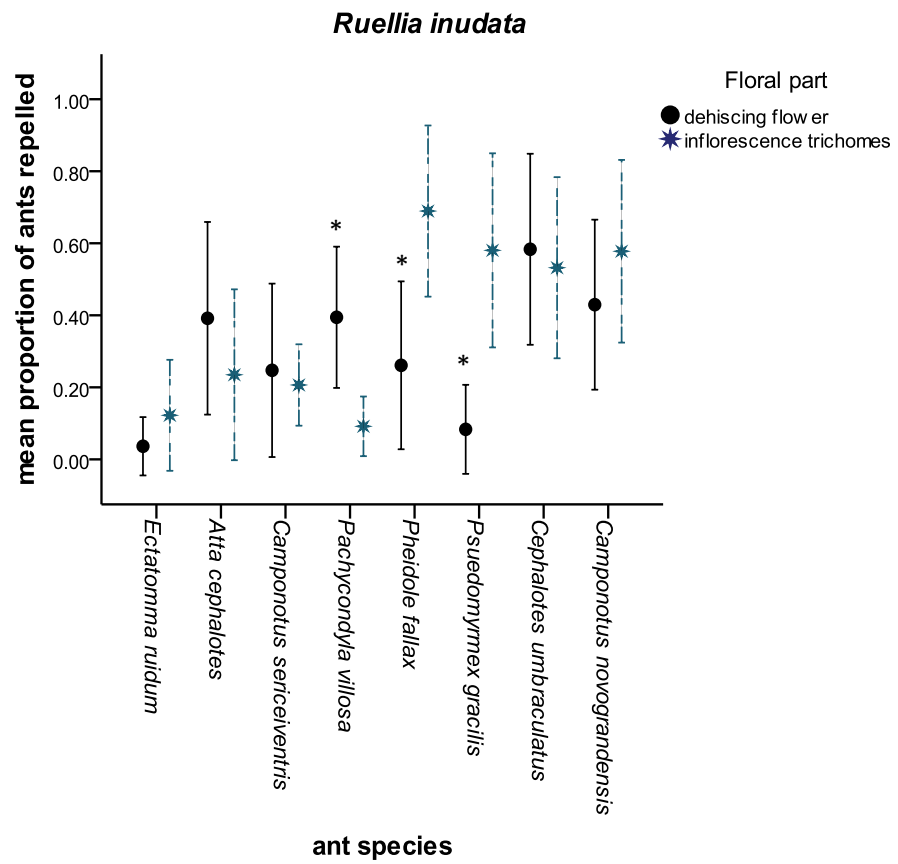
The plant species *Barleria oenotheroides*, *Cordia alliodora*, *Malvaviscus arboreus*, *Ruellia inundata* and *Stachytarpheta jamaicensis* were selected for tactile trials with six additional ant species (see Methods). *Cordia alliodora* was also tested with its obligate commensalist ant species *Cephalotes setulifer*. At least twelve trails were carried out with each ant species. Figure 6 (a-d) shows results with 4 plant species tested with multiple ant species:

- As well as repelling *Camponotus novograndensis*, the fresh pollen of *Barleria oenotheroides* strongly repelled two other ant species, *Cephalotes umbraculatus* and *Pseudomyrmex gracilis* (Figure 6a). These species are all small enough to raid *B. oenotheroides* for nectar. All other ant species tested were not repelled and often walked over pollen-laden anthers.
- *Ruellia inundata*'s inflorescence was covered in hairs and these were only repellent to smaller ants: larger species readily clambered over them (N.B. *P. gracilis* is only slightly smaller than *E. ruidum* but its legs are distinctly shorter). *R. inundata* flowers were only repellent to *C. novograndensis* and *C. umbraculatus* (Figure 6b).

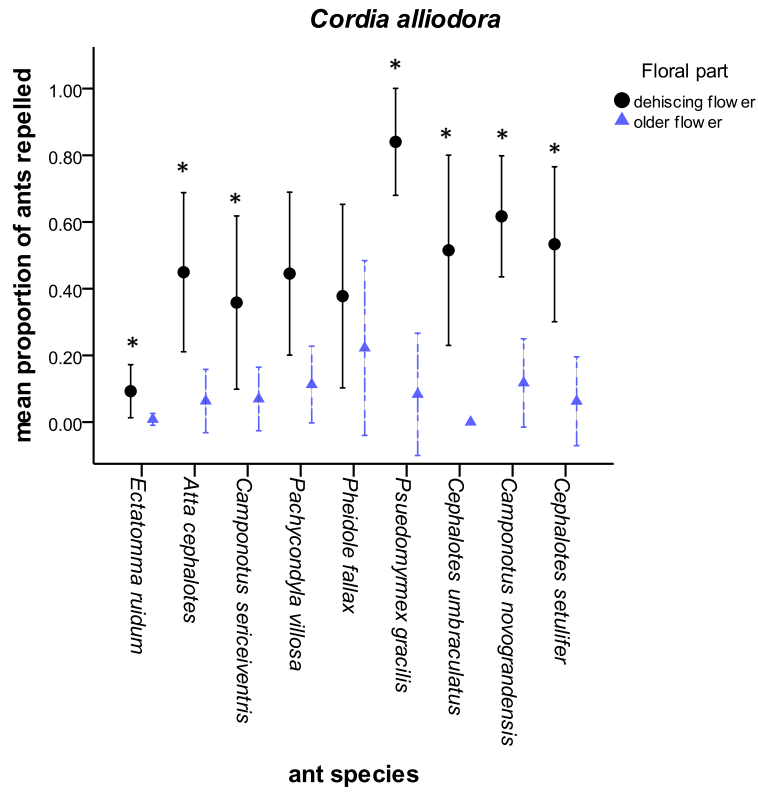
6a)



6b)



6c)



6d)

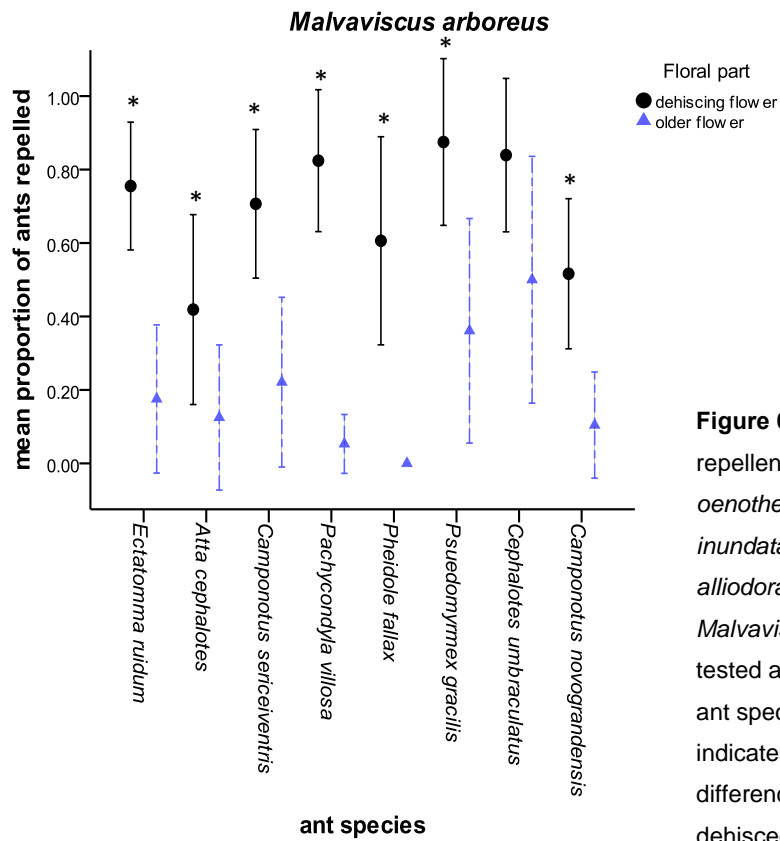


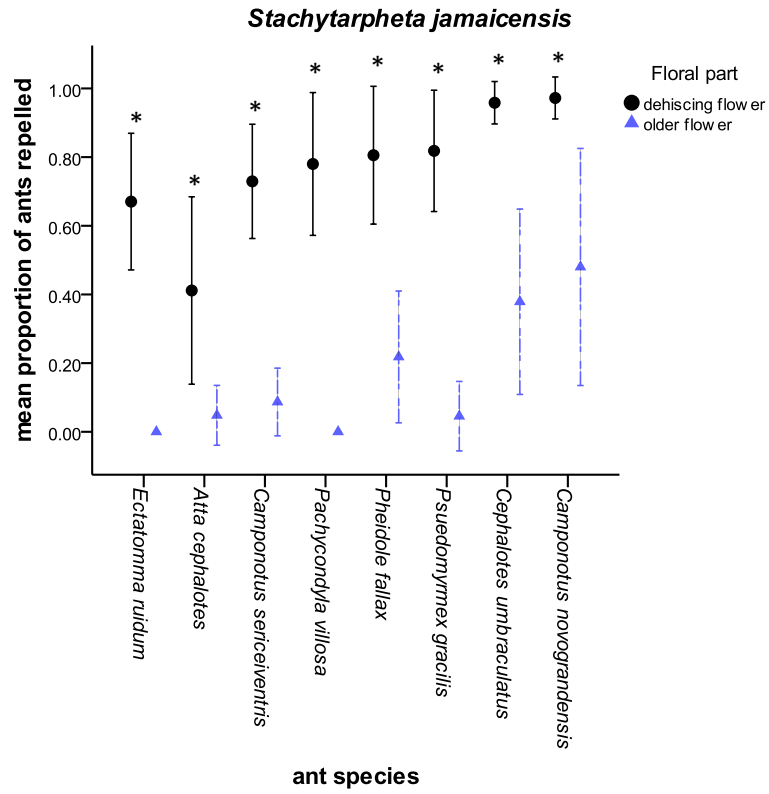
Figure 6: Floral ant-repellence in (a) *Barleria oenotheroides*, (b) *Ruellia inundata*, (c) *Cordia alliodora* and (d) *Malvaviscus arboreus* tested against a range of ant species, where * indicates significant difference between freshly dehiscid flowers and old pollen-depleted flowers or inflorescence trichomes.

- *Cordia alliodora* pollen possessed a more generalised ant-repellence, affecting all ants tested except *E. ruidum* (Figure 6c). The three ant species most strongly repelled from *C. alliodora* pollen were those species most common on the trees at Santa Rosa.
- *Malvaviscus arboreus*'s bright red pollen provoked a strong repellent response from all the ant species tested (Figure 6d).

Two of the ant species responded very differently. Firstly, it is clear that *Cephalotes umbraculatus* was moderately repelled by floral parts of all flower species tested, so additional tactile trials were carried out using the flowers of *Cornutia grandiflora*, which had not proved repellent to *Camponotus novograndensis* and in these *C. umbraculatus* was not repelled (average proportion repelled = 0.07; $\chi^2=0.68$, df=1, p=0.411). Secondly, in no case was there a particularly strong repellent effect of flowers on *Atta cephalotes*. As these leafcutters can gain access to the flower bases, it is unlikely that anther-based floral ant-repellence would provide any defence against castration and this species is not a nectar thief anyway.

Figure 7 shows effects of 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-repellent (Figure 7a). For *S. frantzii*, tested in 2008, with a different range of ants, *E. 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 fallax*, (~80% repelled) (Figure 7b). The tactile response trials on *Stachytarpheta jamaicensis* with 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 repellent trait was not easily transferable to another surface.

7a)



7b)

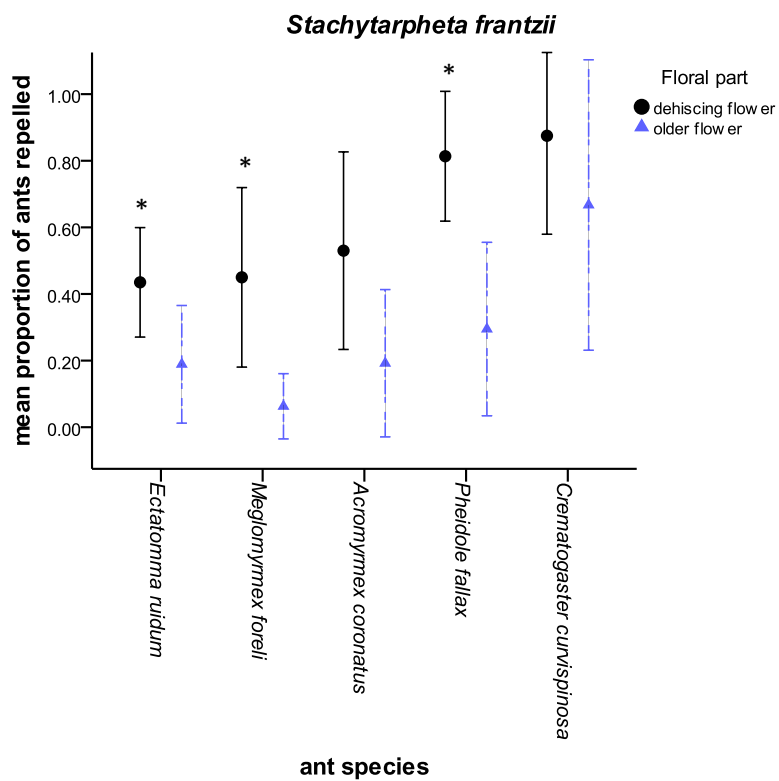


Figure 7: Floral ant-repellence in (a) *Stachytarpheta jamaicensis* and (b) *S. frantzii*, tested against different ant species, where * indicates significant difference between freshly dehiscing flowers and old pollen-depleted flowers.

2.4 Discussion

2.4a 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-repelling floral traits to protect against species like *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 collection 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 make a significant difference to fitness. By repelling the initial forays of scouting workers, the plant prevents recruitment of large numbers of ants to valuable sources of sugar and possibly, in an arid environment like Santa Rosa at the height of the dry season, of water (Willmer, 1983).

This study suggests that when a plant invests in a large volume of nectar 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 (Southwick, 1984; Pyke, 1991; Golubov *et al*, 2004; Leiss *et al*, 2004; Ordano & Ornelas, 2005) but are probably high enough to influence selection on floral traits. 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 (Nicolson, 1995) but also allows the recycling of unused resources after pollination, and their redirection towards seed production (Koopowitz & Marchant, 1998; Luyt & Johnson, 2002; Nepi & Stpiczyńska, 2008). Unfortunately data was not available to compare repellence between nectar absorbers and non-nectar absorbers within this study. Nectar production may in fact be relatively less costly for those species where large nectar volumes are produced but not defended, such as the entirely non-repellent tree *Tabebuia ochracea*. Alternatively, selection to protect nectar in these species may be outcompeted by conflicting selection pressures to attract pollinators. With the exception of *Acacia collinsii*, none of the species tested that produced no detectable nectar possessed any ant-repellent traits. While they may not need to be repellent, as nectar-thieves do not threaten

these species, another possibility is that species that provide pollen as the sole reward for pollinators are less likely to protect that pollen with secondary metabolites as the risk of interference in pollination is greater than for species that also provide nectar rewards (Kessler & Halitschke, 2009).

While the likelihood of floral ant-repellence appears to be linked to nectar volume, the picture becomes more complicated when accessibility to nectar is taken into account. It is possible that ant species evolve to recognise certain species that limit access to nectar and therefore provide no rewards, however, if that were the case then nectarless flowers would also be more likely to be repellent. In many cases accessibility will be more strongly influenced by the 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 hawkmoth-pollinated *Randia monantha*, that has a narrow corolla (around 5cm long) lined with obstructing hairs, or will act separately from floral morphology, as in *Crescentia cujete*, whose open bell-shaped flowers (around 4cm wide) are essential to accommodate the head of a bat (Figure 8). This contrasts with the consistent trade-off of volatile repellence and physical protection identified by Willmer *et al* (2009) between floral ant-repellence and morphological defences in temperate floral species.



Figure 8: Two ant-repellent flowers with very different morphologies (a) bat-pollinated *Crescentia cujete* (b) hawkmoth-pollinated *Randia monantha*.

Whether or not a plant species develops ant-repellent traits will also depend on its degree of self-compatibility, and the likelihood and costs of geitonogamy. Floral larceny may often lead to reduced seed set, favouring ant-repellence, but lack of such repellence may be more favoured where ant-attendance at flowers promotes outcrossing as pollinators move on more frequently, increasing the distance pollen is dispersed (Maloof & Inouye, 2000; Irwin *et al*, 2001). Pollinators are often risk-averse foragers and so are more likely to depart from a flower patch if the reward levels are variable (Biernaskie *et al*, 2002). Nectar thieves may increase the variance in nectar volumes of flowers within a patch. This will lead to transfer of pollen over longer distances and so reduce the cost of geitonogamy (Biernaskie *et al*, 2002). However, this will only be beneficial where the plant is not pollen-limited (Gonçalves-Souza *et al*, 2008) and pollen-limited species typically suffer higher costs from floral larceny (Burkle *et al*, 2007). It is possible that other species that 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.

Protection against nectar theft may also explain why all five night-flowering species tested here (from four different families and attracting different pollinators), were repellent to *C. novograndensis* in the tactile trials. While this sample size is small it would be interesting to test for a stronger ant-repellence in night-pollinated flowers: could ant-repellence be easier to incorporate into the floral scent of night-flowering species due to the preferences of their nocturnal pollinators? It is also possible that some ants such as *C. novograndensis* are more sensitive to such chemical stimuli after dark. However, there has been little work on ant responses to chemicals at different times of day.

2.4b Ant-repellence, phylogeny and morphology

Related species were usually all repellent only when morphologically similar, especially within the Malvaceae and Fabales, indicating a stronger link between repellence and pollination syndrome than repellence and phylogeny. While the proportion of ant-repellence appeared to differ significantly between plant families the effect was reduced as coverage within families increased. The lack of phylogenetic pattern at higher levels is not surprising as floral traits may vary dramatically between very closely related species and even phenotypes within the same species, like the scent morphs of

Polemonium viscosum (Galen, 1983). When night flowering tobacco, *Nicotiana attenuata*, is attacked by hawkmoth larvae the flowers produce less hawkmoth-attracting benzyl acetone and open flowers later to benefit from visits by hummingbirds (Kessler *et al*, 2010). The scent compounds produced by mistletoe (*Tristorix verticillatus*) flowers depend on the species that plant is parasitizing and this polymorphism results in differences in insect visitation (Troncoso *et al*, 2010). Very subtle structural differences can also alter the behaviour of insects interacting with flowers, such as the conical epidermal cells on the petals of snapdragons that improve bumblebee purchase on the flowers and therefore foraging efficiency (Whitney *et al*, 2009). An extensive survey of chemical components of floral scent by Knudsen *et al* (2006) concluded that floral scent chemistry was of little use for phylogenetic estimates above the genus level. Variation in the efficiency of floral ant repellence has now been found within the genera *Cordia* (this study), *Fordia* and *Ixora* (Junker *et al*, 2007). Small changes in scent or morphology may be all that is needed to alter the behaviour of floral visitors.

As nectar levels within flowers are higher in species with bird- or bat-pollination syndromes (Willmer, 2011) such species were more likely to possess ant-repellent traits. Within the Malvaceae tested, the hummingbird-pollinated shrubs *Malvaviscus arboreus* and *Helictores guazumifolia* possess very similar floral structure and were both repellent to *C. novograndensis*. Different repellent components must be involved, however, as *Ectatomma ruidum* was repelled only by *M. arboreus*. The open-flowered bee-pollinated *Pavonia cancellata* (also Malvaceae) had no such repellent traits.

Within the Fabales a clear contrast existed between the ant-repellent Mimosoideae (*Acacia collinsii*, *Bauhinia unguolata*, *Enterolobium cyclocarpum*), and the non-repellent Faboideae (*Centrosema pulmeri*, *Desmodium* sp., *Gliricidia sepium*, *Haemotoxylum brasiletto*, *Securidaca sylvestris*). Ant-repellence is common within the African and American *Acacia* (Raine *et al*, 2007; Willmer *et al*, 2009) but it may also be common in related genera within the mimosoid subfamily, which commonly have highly exposed anthers. Within the Faboideae tested (with the exception of *Haemotoxylum 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.

This argument can be extended to other families; for example the hummingbird-pollinated shrub *Aphelandra scabra*, in the Acanthaceae, protects its nectar and (non-repellent) pollen behind a petal lip that releases pollen down onto the head of a visiting bird. Although repellence is not involved it is another example of a bird-pollinated species with large nectar volumes protecting its investment.

The herbaceous *Barleria oenotheroides* (Acanthaceae) provoked repellence from only a subset of ants tested. *Camponotus novograndensis*, *Pseudomyrmex gracilis* and *Cephalotes umbraculatus* are all small enough to potentially raid *B. oenotheroides* for nectar (although whether or not the normally arboreal *C. umbraculatus* forages on such low-lying vegetation is unknown). The closely related *Blechum pyramidalis* and *Dyschorista valeriana* are both similar in floral morphology to *Barleria oenotheroides* but much smaller in size, and they did not provoke any repellent response from *C. novograndensis*.

In some cases, however, morphology gives no hint as to why a particular species should benefit from ant-repellent traits. *Ipomoea trifida* and *Merremia aegyptia* are both vines in the Convolvulaceae with very similar growth forms and flower shapes but, while *I. trifida* has no repellent traits, *M. aegyptia* has both trichomes and pollen that repel *C. novograndensis*. One key difference between these two species is their sexual system as *I. trifida* is self-incompatible while *M. aegyptia* is self-compatible (Macado *et al*, 2006). It is possible that the extreme ant-repellence employed by *M. aegyptia* prevents inbreeding caused by movement of pollen between flowers on the same vine by ants.

Junker & Blüthgen (2008) found that plants flowering at canopy level were more likely to possess ant-repellence. Having limited access to canopy-level flowers in this study, I was unable to make the same comparison but vines and lianas studied were less likely to possess ant-repellence (though this did include ground-level vines). It might also have been expected that short-lived herbaceous species would be more likely to protect their flowers (possibly their only chance to sexually reproduce) from interference by ants than would be the case for long-lived tree species.

However, no difference in the likelihood of repellence was found between herbaceous and tree species.

2.4c Ant-repellence as pollinator protection

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*. This suggests that there may be little selective pressure to protect potential pollinators from ant predation. Ants' impact on pollinator populations is still poorly understood, but it is possible that the threat they pose, in comparison to more efficient crab spiders (Dukas & Morse, 2003) or competing bees (Dworshak & Blüthgen, 2010), is too small to influence floral evolution. Although predatory ants pose less of a risk to pollinators than groups such as crab spiders, selection to override a repellent signal from a flower will be much weaker on predatory ants as they are not dependent on food acquired at flowers.

The floral ant-repelling traits found in *Cordia alliodora*, *Malvaviscus arboreus* and both *Stachytarpheta* species affected a range of ant species. The repellence acted at different intensities in each case, from the fairly weak repellence found in *C. alliodora* anthers, which were most effective against the three species observed on the tree at Santa Rosa (*Cephalotes setulifer*, *Camponotus novograndensis* and *Pseudomyrmex gracilis*) to the highly effective repellence of *Stachytarpheta jamaicensis* petals. These species demonstrate that generalised chemical ant-repellence is unlikely to differentiate between ants regardless of size or behavioural dominance.

Only the floral ant-repellence of *Acacia collinsii* is likely to have evolved as a means of pollinator protection due to the presence of their aggressive ant-guards and lack of nectar. Floral ant-repellents have been found in all *Acacia* ant-plants studied. While *A. collinsii*'s ant-repellence is not effective against *E. ruidum* it does trigger a strong response from *C. novograndensis*, despite the fact that this species rarely comes into contact with *A. collinsii* inflorescences and poses no threat to potential pollinators. Ghazoul (2001) also found that while *E. ruidum* was not repelled from *A. collinsii* a number of other species were. These responses in such non-mutualists are probably a by-product of

selection to ensure that ant-guards do not interfere in pollination and possibly mediated by ant-alarm pheromone mimicking E,E- α -farnesene produced by the flowers (Raine & Edwards, unpublished data). No effect of *A. collinsii* volatiles was detected in my scent trials, suggesting that the effect of floral volatiles is very limited in range. Although *Pseudomyrmex* ant guards were not tested in the trials (as they usually remained stationary when placed in an arena), it is also likely that the repellence has only a short range effectiveness against them as, while they were never observed on fresh inflorescences, they often ran right up to their base. The other ant-plant used here, *Cordia alliodora*, is inhabited by ants with very low aggression that would not pose a threat to pollinators so in this case the repellence probably arose to prevent nectar theft. The Cephalotini are the only tribe of ants known to feed on pollen, having a specialised proventriculus that allows them to regurgitate the undigestible cellulose coatings (Urbani & de Andrade, 1997) and *Cephalotes pusillus* was observed feeding on the pollen of the EFN-bearing plant *Ouatea spectabilis* in Brazil (Byk & Del-Claro, 2010). Although it is unclear how common this behaviour is within the genus, ant-repellence may also serve to prevent predation of *C. alliodora* pollen by *Cephalotes setulifer*.

2.4d Frequency of ant-repellence across different studies

Some form of floral ant-repellence was found in approximately a third of species tested in the dry forest environment. This is similar to the proportion reported by Willmer *et al* (2009) in the UK and Junker *et al* (2007) in the wet forest of Borneo. Out of the 18 species studied by Junker *et al* (2007) access to nectar was not a predictive factor for ant-repellence, but as nectar volume was not reported it is impossible to 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. Junker and Blüthgen (2008) found that the scents of 8 out of 26 plants were repellent to *Lasius fuliginosus* and 20 out of 30 to *Camponotus floridanus*. However, this study was complicated by the use of multiple ant workers within the test arena, where interactions between individuals (e.g. trail-following) may exaggerate responses.

There are some discrepancies between my results and those of Ghazoul (2001), who also worked at Santa Rosa on some of the same plant species. The responses of *C. novograndensis* and

E. ruidum to *A. collinsii* flowers were similar and both studies also identified repellent traits in *Cordia alliodora* and *Lantana camara*. Other species, however, which Ghazoul found to be ant-repellent (including *Cochlospermum vitifolium*, *Ipomoea trifida* and *Gliricidia sepium*) provoked no repellent responses from ants in my trials. Ghazoul's experiment involved 'wiping' flowers onto Petri dishes and recording the time spent in wiped and non-wiped areas by 2 *Acacia* mutualists (*Pseudomyrmex spinicola* and *P. flavicornis*). His results may reflect ants responding neophobically to non-host scents; my work explains better how ant species interact directly with flowers, and how less specialised ants are affected.

2.4e Ant-repellence trigger – volatile or contact?

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. It is possible that scented pollen is performing an ancestral function: floral odours probably originated as defence against floral herbivores (Pellmyr & Thien, 1986) so that as soon as nectaries were present to attract potential pollinators, conflict would have arisen with nectar-thieving ants and the scent-producing pollen could have provided a solution (as discussed in Chapter 1).

No repellent nectar was detected in my study, and it appears to be rare (Guerrant & Fiedler, 1981; Koptur and Truong, 1998; Blüthgen *et al*, 2004a; Junker & Blüthgen, 2008). When it does occur the plant concerned often has a specialised pollinator (Prŷs-Jones & Willmer, 1992; Johnson *et al*, 2006). It may also be used to maximise the number of flower visitors attracted to a small volume of nectar, as individuals do not feed for long (Kessler & Baldwin, 2006). Trichomes on the inflorescences of *Ruellia inundata* and *Merremia aegyptia* were also a source of repellence, though only to smaller ant species, suggesting that they provide purely mechanical defence (no glandular trichomes were observed).

Here I largely found only tactile responses to flowers. The lack of response from ants to floral scents in the tropical plant species tested contrasts with results obtained in temperate studies (Willmer *et al*, 2009; Junker & Blüthgen, 2010). The high temperatures in the tropics may cause volatile chemicals released by fresh flowers to disperse more quickly, and thus only be detectable at sufficient concentrations very close to the flowers, so that the response becomes indistinguishable from a tactile response. Additionally, plants in the tropics face significantly higher herbivory (Rosumek *et al*, 2009) so the benefits of attending ants are greater, especially on inflorescences where ants can potentially reduce seed predation (Schemske, 1980). Thus close-range repellent traits may be preferable to scents that keep larger areas of the plant clear of ants and may also be cheaper to produce than longer-range scent signals. (Note that although the scents of some tropical plant species were repellent to ants in Junker and Blüthgen's study (2008), this response was from temperate ant species.)

In my tactile trials it was impossible to discern the exact nature of repellence from fresh flowers or anthers: whether it operates through volatiles in sufficient concentrations at close range, or through direct antennal contact. As Raguso (2008) 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 difference between the two modes. However, understanding the mechanisms involved would be of interest in identifying selective actions on floral traits. No doubt close-range floral ant repellence arises from slightly different sources in different species. To determine the chemical components triggering ant repellence in any specific case, analysis of the floral volatiles and chemical composition of the oily outer pollenkitt layer would be necessary. Pollenkitt is a likely source for such close range repellence as the pollen odour is released at a very low rate compared with other floral parts (Dobson & Bergström, 2000). This would involve carrying out dynamic headspace collection of floral volatiles or, alternatively, solid phase microextraction. Pollenkitt composition could be determined by washing pollen with an appropriate solvent such as hexane, followed by GC-MS and experienced analysis of the output against an established library. However, such costly procedures fall outside the scope of this study.

The floral ant-repellence detected in the hummingbird-pollinated shrubs *Malvaviscus arboreus* and *Helicteres guazumifolia* probably originated from volatiles. In both cases the anthers are sited high above the corolla entrance, yet ants were never recorded entering or residing in a flower. Ants do gather around the base of the flowers and feed on nectar that has seeped out between the petals. Similarly, ants readily feed on nectar that has dripped out of *Crescentia cujete* flowers onto the outer edge of the corolla, but although the wide open bells of the flowers would allow ants to walk around the repellent anthers to reach nectar, ants were never observed within flowers on the tree.

In contrast, one case clearly involved primarily tactile repellence, but not from pollen: *Stachytarpheta* species elicited repellence when ant antennae came into contact with the upper petal surfaces, and this repellence triggered agitated grooming of the antennae. Here no contact with the anthers or pollen was required to repel ants. The repellent effect of petal surfaces was not transferable by wiping onto filter paper, so excluding superficial volatile chemicals as the source. However, cuticular waxes of plants may affect the behaviour of herbivores such as aphids (Alfaro-Tapia *et al*, 2007), and non-wipable waxes could be triggering ant-repellence in this case. This form of ant-repellence works elegantly with other traits so that ants are free to walk under the canopy of flowers and access the EFNs along the tall inflorescence. When given the chance ants readily feed on *Stachytarpheta* floral nectar, as when *S. frantzii* flowers were robbed by bananaquits (Aves; Thraupidae) at La Selva the holes made at the base of the corollas were secondarily robbed by ants.

2.4f Conclusions

This study forms the groundwork for a wider understanding of how ants and flowers interact. It highlights the importance of the potential costs that recruitable ants impose as nectar-thieves on floral evolution, and the complexity of floral characteristics that together attract potential mutualistic pollinators and defend against exploitative species. While several different types of ant-repelling traits have been identified, by far the most common is ant-repelling 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 that are similar to ant pheromones.

This study also provides the first indirect evidence that floral ant-repellence in non-ant plant species may be selected for mainly due to costs imposed by nectar theft. This has implications for the costs of nectar production and the consequences of nectar theft on floral attractiveness to potential pollinators. To fully understand this link between nectar volumes and ant-repellence it will be necessary to measure the costs of nectar production in species with similar nectar volumes and breeding systems but varying degrees of ant repellence. Plants do not, however, appear to have been strongly selected to defend their pollinators against aggressive ants, as there was clearly 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.

Chapter 3: Floral Ant-Repellent Effects on a Native and an Invasive Ant

Abstract

Ants are common nectar-thieves that can reduce pollinator visitation to the point where plant fitness is reduced. Some plant species possess ant-repellent floral traits, but do these traits function less effectively when faced with a novel invasive ant with which the plant species shares no evolutionary history and which has been shown to disrupt pollination interactions? A small but diverse range of plant species were tested in Portugal with the invasive ant *Linepithema humile* and the native, but non-nectar thieving, *Messor bouvieri*. Responses to floral traits were very similar. Pollen of *Acacia saligna* and glandular trichomes of *Erodium chium* were highly effective deterrents to both species, while the pollen of *Viburnum tinus* was more effective against *M. bouvieri*. The ability of certain plant species to restrict *L. humile* access to nectar may help to reduce the impact this invasive species has on plant-pollinator interactions.

3.1 Introduction

Floral nectar is an abundant resource for ants, but ants are often unwelcome visitors for many plant species. While some ants can provide valuable defensive benefits against herbivores (Heil & McKey, 2003; Stryksy & Eubanks, 2007), as flower visitors they are poor pollinators that are capable of damaging pollen with the antibiotic secretions they use to clean their nest and larvae (Beattie *et al*, 1985; Vega *et al*, 2009). They may also disrupt pollination by reducing nectar volumes and threatening potential pollinators, resulting in decreased seed-set (Altshuler, 1999; Ness, 2006; Lach, 2008a; 2008b).

Floral ant-repellence was first investigated in depth between the flowers of *Acacia* ant-plants and their ant-guards (Willmer & Stone, 1997; Raine *et al*, 2002; Willmer *et al*, 2009). By preventing access to flowers with repellent pollen scent, the plants ensure that potential pollinators can access the flowers without being harassed by the aggressive ant-guards. As more plant species are being

examined it is becoming clear that floral ant-repellence is common, not just among ant-plants, but in a wide variety of species (Junker *et al*, 2007; Junker & Blüthgen, 2008; Willmer *et al*, 2009; this thesis Chapter 2).

The ecological factors that select for repellence are still poorly understood. Although ant-repellence was positively correlated with nectar volume in a dry forest in Costa Rica (Chapter 2), some temperate studies have failed to find similar patterns (Willmer *et al*, 2009) implying variation in selective pressures both between species and between habitats. Variation in the costs of nectar theft by ants is probably an important factor.

The effectiveness of floral ant-repelling traits also varies with different ant species. How susceptible an ant species is to repellent floral traits will depend on the nature of the trait, the sensory abilities of the ant, and in some cases the coevolutionary history between both species. Physically repellent traits, such as trichomes and narrow access points, will only be effective against ants that are of appropriate size; larger species will step over trichomes and smaller species will manoeuvre along very narrow stems or down narrow corollas. Coevolutionary history is clearly influential in the case of ant-plants and their guards but history may also be important for species that succeed in blocking access by common nectar thieves within any environment. Alternatively, it may be common to evolve repellent traits that are effective against a wide range of ant species, regardless of coevolutionary history. Junker & Blüthgen (2008) and Willmer *et al* (2009) have reported the repellence of ants from flowers of invasive or imported species with which they had no evolutionary history.

While floral ant-repellence may be effective against native ant species, such defences could be less effective against invasive ant species. When invasive ant species enter a new environment are they repelled from flowers of the same species as native ants? The ability to exploit nectar resources that are usually “off-limits” to many of the native ant fauna could aid in colony establishment for invasives.

The Argentine ant, *Linepithema humile*, is one of the most successful invasive ant species. Native to South America, it has invaded Mediterranean-type ecosystems all over the world, forming

huge supercolonies with, due to the genetic bottlenecks the populations have gone through, little or no variation across vast areas in the composition of cuticle hydrocarbons that provide colony recognition systems (Giraud *et al*, 2002). In invaded areas *L. humile* has serious impacts on arthropod populations, reducing numbers of herbivores, predators and parasites and completely displacing many native ant species (Cole *et al*, 1992; Human & Gordon, 1997; Holway, 1998). Other invasive species, such as ant-tended hemipterans, may benefit from the disruption caused by invasive ants and in turn provide more resources to the growing ant population (Helms & Vinson, 2002).

The impact of invasive ants on plant-animal interactions is only starting to be understood. Most work has so far focused on plants whose seeds are dispersed by ants (myrmecochorous plants). Ants are attracted by elaisome food bodies on the seeds and in the process of elaisome removal disperse the seeds themselves over small distances. Due to the impact of myrmecochory on seed dispersal it is estimated that its evolution is directly related to increased speciation (Lengyel *et al*, 2009). In Australia, the invasive yellow crazy ant, *Anoplolepis gracilipes*, displaces more herbivores when present on the EFN-bearing *Acacia lamprocarpa* (although not the unrewarding *Eucalyptus tetrodonta*) than the native ant *Oecophylla smaragdina*. But herbivory is still greater on trees inhabited by the invasive species (Lach & Hoffmann, 2011), possibly due to the fact that *O. smaragdina* territorial pheromones are detected and avoided by some herbivores (Offenberg *et al*, 2004). *Anoplolepis gracilipes* ants on Christmas Island not only cause significant changes in seedling recruitment (by killing the native red land crabs that limit seedling growth and promote leaf litter breakdown) but also lead to canopy dieback due to their association with scale insects (O'Dowd *et al*, 2003).

Areas invaded by Argentine ants contain 92% fewer native seed dispersers and the invasive ants remove 47% fewer seeds (Rodriguez-Cabal *et al*, 2009). In the fynbos habitat in South Africa Argentine ants have brought about a shift in the composition of plant species. By displacing the native ant dispersers of large seeds and providing no dispersal services themselves, Argentine ants ensure large ant-dispersed seeds are predated by rodents or killed by fire (Christian, 2009). In South Africa *L. humile* has been shown to dangerously reduce seedling emergence of the shrub *Mimetes cucullatus*, by outcompeting more efficient ant mutualists, an effect that could extend to dozens of other Proteaceae with large ant-dispersed seeds (Bond & Slingsley, 1984). In southeastern Australia

Argentine ants also remove the most efficient seed dispersers but do some seed dispersal themselves. However, the quality of dispersal is much poorer. By replacing the native seed disperser *Rhytidoponera victoriae* in southeastern Australia, *L. humile* has promoted the spread of the invasive weed *Polygala myrtifolia* since it preferentially carries the plants' small seeds rather than the larger seeds of native species (Rowles & O'Dowd, 2009).

Myrmecochorous plant communities in temperate regions may also be susceptible to serious disruption by invasive ant species due to the specialised nature of the ant-plant relationships. *Aphaenogaster* species are keystone seed dispersers in temperate North America. These common ants are subordinate, and have flexible foraging territories, so ants from multiple colonies may disperse a single plant's seeds (Ness *et al*, 2009). In disturbed areas where larger seed dispersers, including *Aphaenogaster*, are absent the population density of the shrub *Sanguinaria canadensis* is significantly higher. Seeds are not dispersed away from the site so plants lose a major advantage from reproducing sexually (Pudlo, 1980). Until adequate network structures for ant-plant seed dispersal interactions are put together the extent of invasion or habitat change in most environments is still unclear. Such studies would highlight both the risks invasive ants pose to biodiversity and also the importance of these ant-plant mutualisms in maintaining plant and animal communities.

Invasive ants also displace native arthropods from flowers. In Mauritius the invasive ant *Technomyrmex albipes* infests both the flowers and fruits of the critically endangered endemic liana *Rousseau simplex*, reduce seed-set and seed-dispersal by excluding pollinating and frugivorous geckos (Hansen & Müller, 2009). In areas of Spain invaded by *L. humile* the ants reduced visitation rate to *Euphorbia characias* inflorescences, resulting in decreased fruit- and seed-set (Blancafort & Gómez, 2005). In South Africa *L. humile* colonies displace a wide variety of different arthropods from inflorescences of the tree *Leucospermum conocarpodendron* and reduce the length of time native honeybees spend at flowers by 75%, although this does not result in a decrease in seed-set as *L. conocarpodendron* is not pollen-limited (Lach, 2008a). In Hawai'i, *L. humile* and big-headed ants, *Pheidole megacephala*, reduce visitation by native bees and are negatively associated with nectar volumes of the tree *Metrosideros polymorpha* (Lach, 2008b). Although no studies have yet been

carried out, a shift towards clumped populations and vegetative reproduction might be expected where invasive ants disrupt pollination.

To assess the vulnerability of plant species to ants with which they have no history of coevolution I have examined the responses of *L. humile* and native seed-collecting *Messor bouvieri* ants to flowers of seven plant species in Portugal. Are any of these plants capable of preventing *L. humile* workers from disrupting pollination, and how do the invasives' responses differ from those of a native but non-nectar thieving ant species? If ant-repellence is typically very species-specific, due to coevolution, then it is unlikely to have any effect on invasives or even native non-nectar thieves. Where present, however, traits with broad effectiveness against a range of ant species could help to defend against ant-disruption even during changes in the ant community.

3.2 Methods

3.2a Study site

All fieldwork was carried out in April 2010 at the Quinta de São Pedro, a private nature reserve 10km south of Lisbon (38°65'N, 9°11'W). This small, well-managed reserve contains a wide diversity of plant species, including wildflowers, cultivated plants, and native and introduced species. Ants were also collected from large areas of abandoned ground near the site.

3.2b Study species

Ants

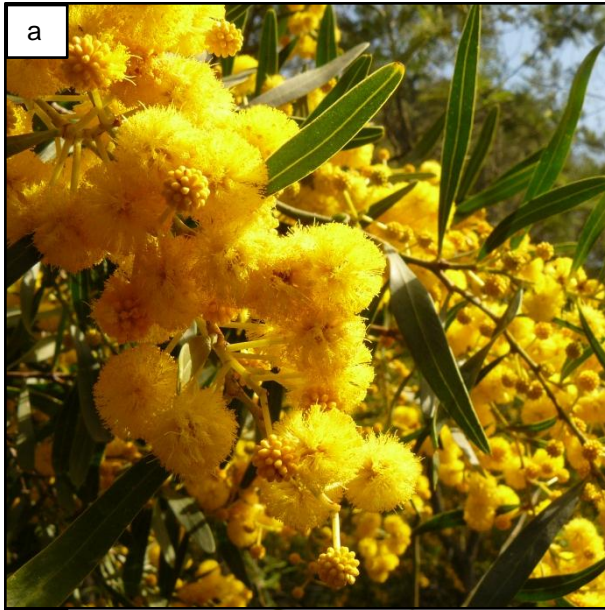
- ***Linepithema humile* (Dolichoderinae):** Present in Portugal since around 1900 (Way *et al*, 1997), *L. humile* has only become numerically dominant at the Quinta de São Pedro within the past ten years, as undergraduate ant-based projects from 2000 describe the behaviour of other ant species no longer present.
- ***Messor bouvieri* (Myrmicinae):** These seed harvesters are the most common ant species, besides *L. humile*, in the area; they have large conspicuous colonies common in fields and disturbed areas. Unlike seed-mutualist species *Messor* ants are usually post-dispersal seed predators (Willott *et al*, 2000). Smaller workers, close to the size of *L. humile*, were used in

experiments.

Plants

A small but florally diverse range of plant species were selected (Figure 1). None of these species are known to have specific associations with ant species in their home ranges. To increase the likelihood of identifying species with repellent traits, only plant species with no *L. humile* present within the flowers were chosen.

- ***Acacia saligna* (Fabaceae):** Formerly known as *A. cyanophylla* this invasive tree, native to Australia, produces spherical inflorescences ~2cm in diameter of tiny nectarless flowers. The species has a mixed mating system with preferential outcrossing in Australia (George *et al*, 2008).
- ***Cistus salviifolius* (Cistaceae):** A species of rockrose, this shrub produces large numbers of self-incompatible, open, white and yellow flowers each ~3cm wide. Nectar volume was too small to measure as individual nectaries produce very small quantities and were spaced far apart.
- ***Echium plantagineum* (Boraginaceae):** Purple viper's bugloss is an annual herb. The self-compatible flowers each ~3.5cm long contain 0-0.3µl of 13-26% sucrose nectar at peak dehiscence and are borne on a branched spike with densely haired bracts at the base.
- ***Erodium chium* (Geraniaceae):** Mediterranean storksbill is an annual that produces small, simple, purple outcrossing flowers ~1cm long with nectar volume too small to measure. Small sticky, glandular trichomes cover much of the plants' surface, including the bracts.
- ***Oxalis pes-caprae* (Oxalidaceae):** Indigenous to South Africa, this herbaceous plant is an invasive weed in many parts of the world. The large yellow flowers ~4cm long produce 0.05-0.2µl of 53-68% sucrose nectar at peak dehiscence. Only the short-styled form of this heterostylous species is common in Europe, and intramorph self-compatibility is very low so spread is usually vegetative (Castro *et al*, 2007).
- ***Pyrus amygdaliformis* (Rosaceae):** The almond-leaved pear produces large, open, white, outcrossing flowers ~2.5cm wide, with 0.05-0.35µl of 16-46% sucrose nectar in each at peak dehiscence.



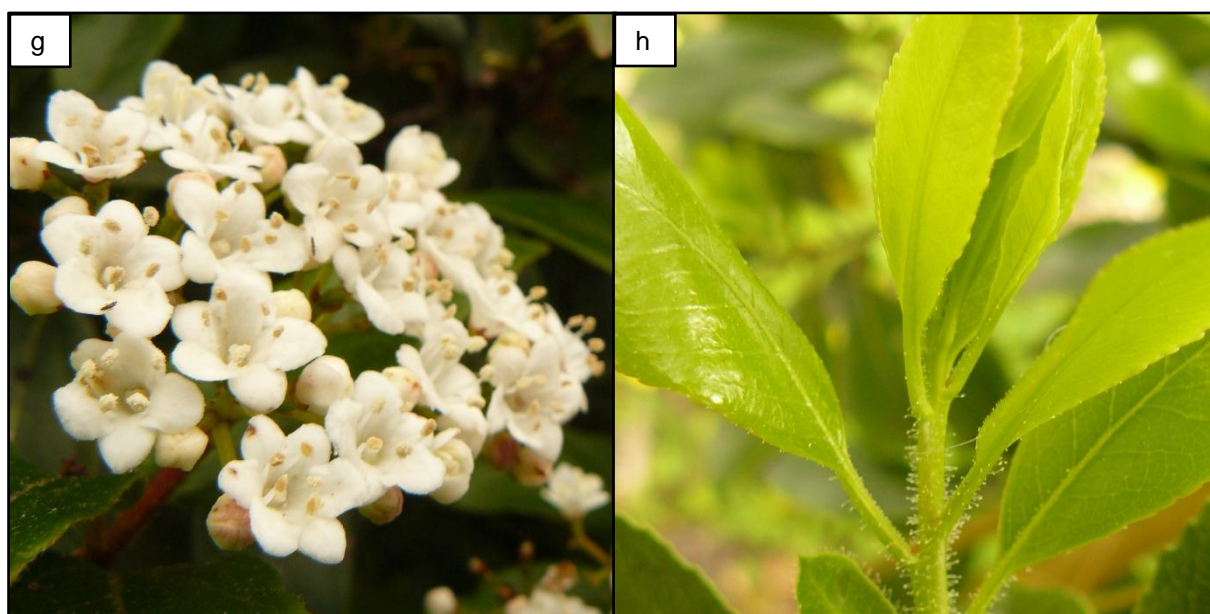


Figure 1: (a) *Acacia saligna* (b) *Cistus salviifolius* (c) *Echium plantagineum* (d) *Erodium chium* (e) *Oxalis pes-caprae* (f) *Pyrus amygdaliformis* (g) *Viburnum tinus* (h) *Arbutus unedo*.

- ***Viburnum tinus* (Adoxaceae):** Native to the Mediterranean, this is an evergreen shrub that flowers biannually, producing dense cymes of small, white, self-incompatible flowers ~0.7cm wide holding between 0-0.2µl of 6-13% sucrose nectar each at peak dehiscence. It has previously been found to possess scent repellent to an ant species it had not coevolved with, the Northern European and Northern Asian species *Formica aquilonia* (Willmer *et al*, 2009).
- ***Arbutus unedo* (Ericaceae):** The strawberry tree was also included in the study when dead *L. humile* workers were discovered attached to the glandular hairs around meristems that supported colonies of aphids. Tactile trials were carried out with sections of stem with trichomes from new growth and sections without trichomes from old growth.

3.2c Detection of floral ant-repellence

Each ant was collected by allowing passive entry into a clear glass tube from the ground or vegetation; it was only used once and then returned to the area it was found (except for a few collected for identification). Floral parts for trials were picked with clean forceps and used immediately, once only. Equipment was washed carefully with alcohol between trials. Tactile response trials and scent

response trials were carried out using the same methodology as outlined in Chapter 2. Around 12 trials of each type were carried out with each ant species on each plant species. Significant repellence was defined as being observed when ants were repelled by floral parts on nearly half of encounters (a proportion of 0.45, a definition that was relaxed slightly from the 0.5 used in Chapter 2). Fresh flowers with fresh pollen present were compared with older floral parts that were also used in the trials.

3.2d Statistical methods

Attempts were made to obtain normalised residuals from the tactile response data but the high proportion of zeros in the data prevented this. Kruskal-Wallis tests were carried out to compare repellence from fresh flowers with other floral parts in the tactile trials. As there were insufficient data from antagonistic behaviours observed in the scent trials, Kruskal-Wallis tests were carried out on the number of turnings made by ants to detect any increase in activity levels. Error bars in all figures are 95% confidence intervals. All statistical tests were carried out using SPSS 18.0.

3.3 Results

3.3a *Linepithema* responses

Out of the flowers of seven species tested in tactile trials, *Acacia saligna* and *Erodium chium* were the most repellent to *L. humile* workers (Figure 1; Table 1). Fresh inflorescences with pollen-laden anthers from *A. saligna* were significantly more repellent than older depleted inflorescences ($\chi^2=12.057$, $df=1$, $p=0.001$). The glandular trichomes covering the outer surface of the bracts of *E. chium* were marginally more repellent on old flowers than fresh flower ($\chi^2=3.652$, $df=1$, $p=0.056$). Trichomes from both old and fresh anthers elicited a repellent response in almost half of encounters. *E. chium* pollen, however, was freely walked over and never repellent. The pollen from fresh flowers of both *V. tinus* and *P. amygdaliformis* was only repellent in around 25% of encounters, but this still made fresh flowers significantly more repellent than old flowers ($\chi^2=5.506$, $df=1$, $p=0.019$ and $\chi^2=4.039$, $df=1$, $p=0.044$).

Table 1: Tactile repellent trails with *L. humile* and *M. bouvieri*. Means \pm st dev. shown for fresh flowers and Kruskal-Wallis test results for comparisons between repellence from fresh and old floral parts. Mean repellence in bold when greater than or close to 50%.

Species	<i>Linepithema humile</i>				<i>Messor bouvieri</i>			
	N	mean repellence from fresh flowers \pm st dev	K-W χ^2	p- value	N	mean repellence from fresh flowers \pm st dev	K-W χ^2	p-value
<i>Acacia saligna</i>	12	0.57 \pm 0.41	12.06	0.001	10	0.48 \pm 0.24	11.18	0.001
<i>Cistus salviifolius</i>	12	0.10 \pm 0.20	0.39	0.535	12	0.13 \pm 0.22	0.78	0.376
<i>Echium plantagineum</i>	12	0.05 \pm 0.13	0.11	0.740	12	0.31 \pm 0.28	3.28	0.070
<i>Erodium chium</i>	12	0.47 \pm 0.33	3.65	0.056	11	0.53 \pm 0.37	0.89	0.345
<i>Oxalis pres-caprae</i>	11	0.16 \pm 0.32	0.57	0.451	12	0.30 \pm 0.403	5.22	0.022
<i>Pyrus amygdaliformis</i>	11	0.24 \pm 0.33	4.04	0.044	12	0.25 \pm 0.31	0.01	0.920
<i>Viburnum tinus</i>	12	0.27 \pm 0.39	5.51	0.019	12	0.65 \pm 0.39	7.46	0.006
<i>Arbutus unedo</i>	12	0.70 \pm 0.17	18.01	<0.001				

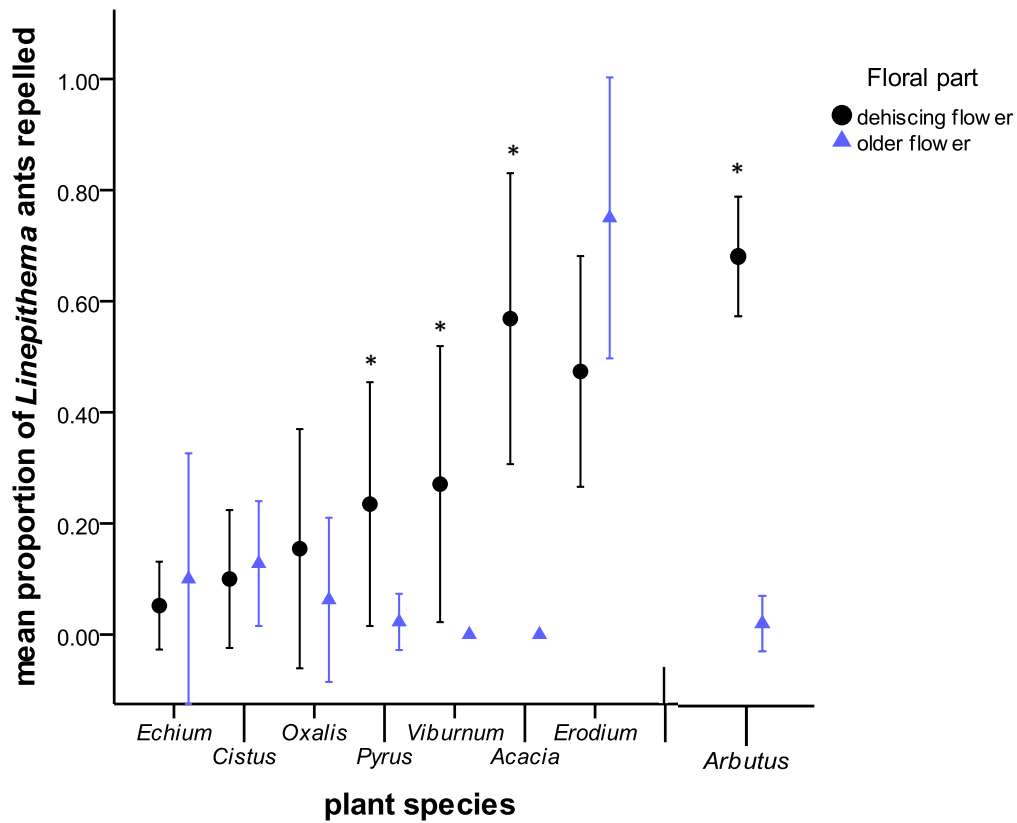


Figure 2: Proportion of *L. humile* workers repelled by flowers of seven plant species in tactile trials (and by the defensive hairs of *A. unedo*), where * indicates significant difference between freshly dehiscid flowers and old pollen-depleted flowers.

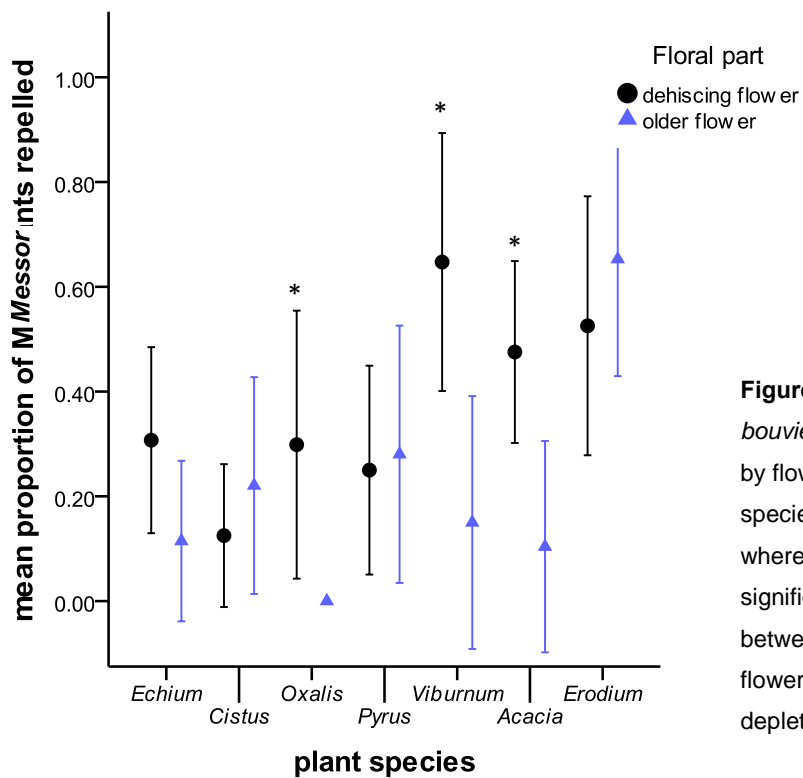


Figure 3: Proportion of *M. bouvieri* workers repelled by flowers of seven plant species in tactile trials, where * indicates significant difference between freshly dehiscid flowers and old pollen-depleted flowers.

Table 2: Kruskal-Wallis tests comparing directional changes when exposed to clean air and floral scents.

Species	N	<i>Linepithema humile</i>		<i>Messor bouvieri</i>	
		K-W χ^2	p-value	K-W χ^2	p-value
<i>Acacia saligna</i>	12	0.870	0.351	1.822	0.177
<i>Cistus salviifolius</i>	12	1.917	0.166	0.378	0.539
<i>Echium plantagineum</i>	12	0.632	0.427	1.113	0.291
<i>Erodium chium</i>	12	0.014	0.907	2.868	0.090
<i>Oxalis pres-caprae</i>	12	0.419	0.517	0.144	0.704
<i>Pyrus amygdaliformis</i>	12	0.245	0.621	0.123	0.726
<i>Viburnum tinus</i>	12	0.462	0.539	0.349	0.555

None of the seven species possessed floral scents that influenced *L. humile* behaviour at longer range in any way (Table 2).

3.3b *Messor* responses

Out of seven plant species tested in the tactile trials, *M. bouvieri* minor workers were significantly repelled from *Acacia saligna*, *Erodium chium* and *Viburnum tinus* (Figure 2; Table 1). Anthers of fresh *V. tinus* flowers were significantly more repellent than anthers of older, depleted flowers ($\chi^2=7.46$, df=1, p=0.006). Similarly, fresh inflorescences with pollen-laden anthers from *A. saligna* were significantly more repellent from than older depleted inflorescences ($\chi^2=11.18$, df=1, p=0.001). Glandular trichomes on the bracts of both fresh and old *E. chium* flowers elicited a repellent response in more than 50% of encounters, with no significant difference between the effectiveness of repellence from old and fresh flower bases ($\chi^2=0.892$, df=1, p=0.345). Again, *E. chium* pollen was freely walked over and never repellent. Fresh pollen of *O. pres-caprae* flowers was occasionally repellent, making fresh flowers significantly more repellent than older flowers ($\chi^2=5.217$, df=1, p=0.022). None of the seven species possessed floral scents that influenced *M. bouvieri* behaviour (Table 2).

3.3c *Arbutus unedo* meristems

Glandular hairs near the meristems of *A. unedo* were highly repellent to *L. humile* workers when compared to old growth without sticky hairs ($\chi^2=18.013$, $df=1$, $p<0.001$; Figure 1). Upon coming in contact with the hairs ants became trapped unless they immediately pulled away. This effectively cut them off from the aphids feeding among the hairs at the point of new growth. The sticky secretions, which had no repellent effect before contact, were effective enough to trap and kill ants. Dead ants were regularly found near aphid colonies and a quarter of the ants used were killed during the trials when they became ensnared.

These secretions were not toxic to the ants, as individuals that came into contact with the hairs but escaped were still alive and active the following day. Only those that were fully immobilised by the hairs were killed, succumbing to immobility within a few minutes.

3.4 Discussion

3.4a Susceptibility of an invasive ant to floral ant-repellence

Both ant species responded to the flowers of the different plant species in surprisingly similar ways. The repellent chemicals present on the pollen of *Acacia saligna* were highly repellent to *L. humile* and *M. bouvieri*, as are the glandular trichomes of *Erodium chium*. The ant species only differed in their response to *Viburnum tinus* pollen, as *M. bouvieri* was more effectively repelled. None of the other floral parts provoked a significant response from either ant species. Where the repellent trait was pollen, as in *A. saligna* and *V. tinus*, fresh flowers were significantly more repellent than older flowers. Where the repellent trait is trichomes, as in *E. chium*, the fresh flowers are not significantly more repellent than older flowers as they still retain some trichomes.

Flowers were tested against two ant species with which they shared little evolutionary history. *M. bouvieri* workers do climb onto some low lying plants but only to collect seeds. They were never found within flowers, so are unlikely to have had any influence on floral evolution (although they may affect the evolution of flower or fruit structure post-pollination). Ideally I would have liked to compare all plant species with a third ant species, a native ant that also visited flowers. However, with the

exception of small numbers of *Tetramorium* sp. and large numbers of tiny *Tapinoma* sp., *L. humile* had out-competed all other ant species in the Quinta.

The effectiveness of physical defences, such as the glandular trichomes of *E. chium* stems and bracts, is fairly predictable as these are probably effective against all ants of appropriate size. *L. humile* and the *M. bouvieri* workers used in the experiments were too small to walk over the trichomes and not strong enough to push through the sticky secretions easily, but larger ant species would readily be able to reach *E. chium* flowers.

Those plant species with no effective repellence against ant nectar-thieves may not have suffered significant costs in the past and some, especially self-compatible species such as *E. plantagineum*, may even have benefited from ant-attendance of flowers, as their presence can promote movement of pollinators and therefore reduce geitonogamy (Maloof & Inouye, 2000; Irwin *et al.*, 2001). The same situation may not be true under the novel conditions of the invaded ant community. Although the overall ant biomass remains the same, *L. humile* ants are often present in far larger numbers than the pre-existing ant species (Holway, 1998). With so many individuals recruiting in large numbers to flowers, *L. humile* may take higher volumes of nectar. It is also possible that their presence could benefit plant species in novel ways, for example by excluding harmful herbivores that are not threatened by native ant species.

The defensive hairs present around *A. unedo* meristems are probably of primary use as a defence against herbivores. The hemipterans commonly found feeding between the hairs are apparently immune to the sticky secretions. While some dead ensnared individuals were observed, they were far outnumbered by the living. The effect of the exclusion of ants from the hemipteran colonies is unclear and will probably depend on the species of hemipteran present. Infestations of ant-tended hemipterans will be limited by the trichomes as they are denied their ant-guards, while non-tended species may be protected from ant predation by the trichomes. However, trichomes play no part in limiting ant access to *A. unedo* flowers as they were not present on the inflorescence stems of those late-flowering plants present at the site.

3.4b Comparison with other studies

The ant-repellent properties of *Acacia* pollen have already been widely demonstrated. The strongest effects have been observed in myrmecophytic species, which possess potent chemical repellence that peaks in production during periods of initial dehiscence and high pollinator visitation (Willmer & Stone, 1997; Nicklen & Wagner, 2006). Willmer *et al* (2009) identified E,E- α -farnesene, a close relative to an ant alarm pheromone, as a component in the floral scent of *A. seyal fistula* and this has also been reported in *A. collinsii* (Raine & Edwards, unpublished data). The inflorescences of the non-myrmecophytic species *A. macracantha* and *A. angustissima* in Mexico also repelled ants more often than controls, although to a lesser extent than the myrmecophytic *A. hindsii* (Raine *et al*, 2002). Synthetic farnesol is also an effective *L. humile* repellent (Shorey *et al*, 1996). The present study is the first demonstration of floral ant-repellence in *A. saligna* and in the Australian acacia monophyletic group. Taken with the evidence of ant-repellent pollen in other Mimosoidae species presented in Chapter 2, this strengthens the hypothesis that ant-repellence is a basal trait not only within the African and American *Vachellia* monophyletic group but also throughout the Mimosoidae as a whole, preadapting them for myrmecophytic relationships. Indeed, such relationships have evolved independently in the *Acacia* of Africa and the Neotropics (Keeler, 1989).

Variation in ant species' responses to the same plant species has been observed a number of times. In ant-plants this variation may correspond with the aggressiveness of the ant species so that the most dangerous ants are mostly effectively excluded (Raine *et al*, 2002; Willmer *et al*, 2009). Junker *et al* (2008) found that *Camponotus floridanus* was significantly more likely than *Lasius fuliginosus* to be repelled from the flowers of a range of species in Germany. In Costa Rica *Camponotus novograndensis* was more likely to be repelled by floral traits than the larger *Ectatomma ruidum* (this thesis, Chapter 2). When additional ant species were also tested with five of these plant species the floral traits of *Stachytarpheta jameicensis*, *Malvaliscus arboreus* and *Cordia alliodora* were shown to be consistently effective against a range of ant species (with the exception of leafcutters) (this thesis, Chapter 2).

One big difference between this study and others carried out using temperate ant and plant species is the lack of response to scent compounds. The ants had to make physical contact with the

flowers to be repelled. This was especially surprising for *V. tinus* as the scent is an effective repellent against *Formica aquilonia*, inciting highly aggressive behaviour (Willmer *et al*, 2009). There are a number of possible explanations for this. In previous studies larger ants in the genera *Formica*, *Camponotus* and *Lasius* were used in the experiments (Junker & Blüthgen, 2008; Willmer *et al*, 2009). *L. humile* and the *M. bouvieri* workers used in this study were smaller and, in the case of *L. humile*, faster than those species and so it is possible that, while there were clearly no overtly aggressive displays, more subtle behavioural responses could have been overlooked. It is also possible that such behaviours do occur in response to the flower volatiles of other species. Trials with a wider range of plant species with repellent traits would be necessary to exclude this possibility.

3.4c Implications for ant invasions

The findings that *L. humile* responses to floral traits are not very different from those of native *M. bouvieri* ants indicates that they will not inevitably exploit plant species that are otherwise protected from native nectar-thieving ants. It seems that many plant species possess ant-repellent traits that have a broad application against very different ant species. Prolonged evolutionary exposure to one ant community may well pre-adapt plant species to changes in the composition of that community.

A greater difference would be expected when invasive species, such as *L. humile*, invade areas poor in ant numbers or diversity. The extreme case of this situation is seen in Hawai'i, where there are no native ants present. Floral nectar of native plants in Hawai'i is more heavily exploited by ants than that of introduced plant species, as the natives are more likely to lack ant-repellent traits (Junker *et al*, 2010b). Such traits are presumably more common in introduced species as they have shared an evolutionary past with ants. In some cases this new ant-attendance has actually benefited native species, when the ants reduce herbivore numbers leading to increased fruit set (Junker *et al*, 2010b).

Certain invasive ants are more likely to exploit floral nectar than others. Formicines, dolichoderines and some mrymecines are adapted for trophylactic exchange of liquid (Davidson *et al*, 2004). These species are the most common visitors to EFNs and tenders of hemipterans (Rico-Gray, 1993; Blüthgen *et al*, 2000) and so are also best adapted to consume large quantities of nectar. The

invasive formicine *Anoplolepis gracilipes* is especially well adapted to exploit nectar, while the invasive myrmecine *Pheidole megacephala* is less efficient (Lach, 2005). Additionally, the dolichoderines such as *L. humile* are well suited to the role of nectar thief as they are so numerous and recruit *en masse* to food sources.

3.4d Conclusions

The responses of the invasive *L. humile* and the native *M. bouvieri* to the floral traits of a small but diverse range of plant species were remarkably similar. The lack of responses to scent alone is surprising, but may reflect experimental techniques and/or the small number of plant species tested. A further comparison between native nectar thieves and *L. humile* would be useful in determining whether or not repellence is more effective against those species with which the plant has the longest evolutionary history. Floral ant-repellent traits with a broad range of effectiveness against different ant species should ensure these species have robust defences against changes in the ant community.

Chapter 4: Floral Visitors and Ant Scent-Marks

Abstract

Bee behaviour when visiting flowers is mediated by a large variety of chemical cues from both the flower itself and also previous visitors to the flower. Flowers that have been recently visited by bees and hoverflies may be rejected for a period of time by subsequent bee visitors. Nectar-thieving ants also commonly visit flowers and could potentially influence the foraging decisions of bees in the same way, through the detection of ant trail pheromones or footprint hydrocarbons. Visitor behaviour to the flowers of three plant species was compared between flowers that had been in contact with ants and those that had not; *Digitalis purpurea* and *Bupleurum fruticosum* with the nectar thieving ant *Lasius niger*, and *Brassica juncea* with predaceous *Formica seylesi*. No differences were found between the two treatments. Perhaps due to the context-dependent usage of chemical foraging cues, bee behaviour under these conditions was not influenced by chemical cues deposited by ants.

4.1 Introduction

A wide range of both social and solitary bee species have been shown to reject flowers recently visited by other bees (Kato, 1988; Stout & Goulson 2001; Yokoi *et al*, 2007). It is easy to see how such behaviour translates into more efficient foraging as recently depleted flowers are avoided. Honeybees foraging on *Lotus corniculatus*, for example, are more likely to reject flowers with less than average nectar volumes (Weatherwax, 1986).

Rejection of recently visited flowers occurs between both con- and hetero-specifics. Bumblebee species reject flowers recently visited by other bumblebee species (Stout *et al*, 1998) and bumblebees and honeybees each detect and reject flowers recently visited by the other (Stout & Goulson, 2001). Flowers visited by *Bombus terrestris*, or by solitary wood-carder bees *Anthidium manicatum*, are rejected by individuals of the other species (Gawleta *et al*, 2005). Other solitary bees, including *Halictus aerarius*, *Andrena prostomias*, *Colletes patellatus*, *Osmia orientalis* and *Tetralonia*

mitsukurii, all reject flowers recently visited by other bee species, although the intensity of repulsion depends on the identity of the previous visitor (Yokoi *et al*, 2007; Yokoi & Fujisaki, 2009).

In all cases repulsion from previously visited flowers appears to be context dependent. Bumblebees are more likely to probe marked artificial flowers in a high reward treatment if they have experience of that reward (Saleh & Chittka, 2006) and are also more likely to probe a marked flower if that flower is structurally simple so floral handling time is short (Saleh *et al*, 2006). Bees may also take into account the aggressiveness of the species leaving a scent-mark. *Bombus terrestris* shows the strongest avoidance to scent-marks left by the male *Anthidium manicatum*, which aggressively defends its territory (Gawleta *et al*, 2005). Females of the solitary species *Andrena prostomias* reject flowers marked by males even more strongly than those marked by other females (Yokoi & Fujisaki, 2009). Information from scent-marks is ignored completely when rewarding and unrewarding flowers are different colours (Giurfa *et al*, 1994). Scent-marks may also be ignored more commonly when food is scarce (Saleh *et al*, 2006).

The influence of scent-marks on foraging decisions varies considerably between plant species. *Halictus aerarius* sweat bees revisit flowers of Eastern daisy fleabane, *Erigeron annuus*, at the same frequency as untouched flowers 60 minutes after visitation by conspecifics, while around 40% of trailing water willow flowers, *Justicia procumbens*, are still avoided after 60 minutes (Yokoi & Fujisaki, 2007). The time that a flower remains repellent following a visit may be linked to nectar replenishment. *Bombus hortorum*, *B. prascuorum*, *B. pratorum* and *B. terrestris* reject the flowers of the perennial herb *Symphytum officinale* for 20 minutes after the flowers are visited by a conspecific or any of the other three species. This matches well with the time it takes for the flowers to replenish most of their nectar (Stout *et al*, 1998). This same correspondence between nectar secretion rates and revisitation time, varying from 10 minutes to 24 hours, was also found when bumblebees were tested with four different plant species (Stout & Goulson, 2002).

It is possible that in some of these situations bees detect the presence of nectar using scent. Solitary *Osmia* bees collecting nectar from the herb *Penstemon caesius* avoided previously unvisited flowers from which the nectar had been removed and/or replaced with water. This discrimination ability

was lost when the bees' antennae were coated with silicone (Howell & Alarcón, 2007). In the context of scent-marking experiments however, bees do not take into account information obtained remotely from nectar (Yokoi *et al*, 2007). Experimental manipulations with artificial flower corollas moved between flowers with different nectar volumes and manipulations of nectar volumes within real flowers clearly show that it is the scent-marks on the flower or artificial corolla that are detected (Schmitt & Bertsch, 1990; Yokoi *et al*, 2007). Thus, when nectar is removed from rewarding *Symphytum officinale* flowers bumblebees still visit them at the same rate as unadulterated flowers (Goulson *et al*, 1998).

The repellent chemical markers that trigger avoidance of previously visited flowers are volatile signals as they are usually detected before landing and are less effective when air is blown over flowers during trials (Giurfa & Núñez, 1992). The repellent signal produced by honeybees, at least from the perspective of conspecifics, is volatile 2-heptanone, secreted from mandibular glands (Vallet *et al*, 1991). The nature and source of the signal, however, varies between bee species. Different bumblebee species use slightly different mixtures of long-chain hydrocarbons, secreted from tarsal glands (Goulson *et al*, 1998; Stout *et al*, 1998), and very similar in composition to the cuticular hydrocarbons covering the rest of the bees' body (Witjes & Eltz, 2009). However, the tarsal glands of the stingless bee *Melipona seminigra* do not connect to the outer surface of the leg and instead probably serve to inflate the arolium. In this and other bee species footprints may originate from the tendon glands which do secrete onto the outer surface (Jarau *et al*, 2005). By analysing the footprint hydrocarbons (FHCs) deposited on flowers it is possible to identify, at least for bumblebees, the visitor species, and even to differentiate between workers and drones of different species (Witjes *et al*, 2010). In pollen-limited species, like *Symphytum officinale*, FHC deposition can even predict seed set as the total amount of FHCs was positively correlated with bumblebee visitation (Witjes *et al*, 2010).

It is not yet clear whether the marks left on flowers are deposited actively by bees or if they are left passively during the course of a visit. Giurfa *et al* (1994) speculated that a single bee visited so many flowers in a day that they were unlikely to actively mark all of them. It may be unavoidable volatile residues deposited during a visit that are detected, especially those from tarsal secretions (Gawleta *et al* 2005; Witjes & Eltz, 2007). Repellence of the sweat bee, *Halictus aerarius*, by previously visited flowers was related to the size of the bee species depositing the marks, and Yokoi *et*

al (2007) hypothesised that sweat bees possessed a generalized avoidance of flowers contaminated by hydrocarbons common on insect cuticles. Deposition of bumblebee cuticular hydrocarbons on deadnettle flowers, *Lamium maculatum*, increases linearly regardless of reward levels within the flowers, implying passive accretion (Eltz, 2006). Active deposition is more likely when the scent-mark originates from oral glands where some form of feeding or specific marking behaviour is needed, like the honeybee example and deposition of attractive octyl octanoate pheromone trails from the labial glands of *Trigona spinipes* (Schorkopf *et al*, 2007).

Whatever the trigger, it does appear that the ability to identify previous visitation to a flower is a general trait among bee species and may have been a basal trait of the bee lineage (Yokoi & Fujisaki, 2007; 2009). Especially among solitary bees, these cues act primarily as a signal to the bees that deposit them to avoid revisitation, and are then eavesdropped on by con- and hetero-specifics.

This ability to detect prior visitation may extend to visits by other pollinating insects as well. Honeybees and bumblebees have been shown to avoid flowers recently visited by hoverflies, although hoverflies show no such discriminating behaviour themselves (Reader *et al*, 2005; Yokoi & Fujisaki, 2009b). While bee-rejection of flowers previously visited by insects that do not consume pollen has not been reported, bumblebees use scent-marks more when foraging for nectar (Stout *et al*, 1998).

It is unclear as yet whether chemical marks deposited by other flower-visiting insects are taken into account by bees. Ants are common visitors to flowers and while their presence on an inflorescence may provide some defence against herbivores (Heil & McKey, 2003) they are poor pollinators and reduce pollen viability with the antibiotic secretions used in nest hygiene (reviewed in Chapter 1). As predators and nectar thieves, they also make flowers less attractive to other visitors by influencing the behaviour of potential pollinators (Altshuler, 1999; Ness, 2006; Lach, 2008). While a wide range of plant species possess physical traits that block ant access to flowers or chemical deterrents usually originating from pollen (Raine *et al*, 2002; Nicklen & Wagner, 2006; Junker *et al*, 2007; Willmer *et al*, 2009; this thesis Chapter 2), many do not and some even benefit from the increased pollination movement between flowers caused by ants (Maloof & Inouye, 2000; Irwin *et al*, 2001). From the pollinators' perspective, however, disruption of foraging activity by ants can only ever

be detrimental. Ants exploit nectar resources and larger predatory species can pose potential threats to the pollinators themselves (Schatz & Wcislo, 1999). While bees may defend themselves against smaller ants with a combination of kicks and wing fanning (Spangler & Taber, 1970; Yang *et al*, 2009), when present on flowers even harmless species may still reduce the length of visits by potential pollinators (Junker *et al*, 2007).

Some groups of ants are most likely to feed from flowers and influence pollination through interference competition. Formicine and dolichoderine ant species are the most common tenders of hemipterans and extrafloral nectar (Rico-Gray, 1993; Blüthgen *et al*, 2000) as their proventriculus is adapted for trophalactic exchange of liquid (Davidson *et al*, 2004). The proventriculus of formicine species is especially well adapted for the fast uptake of large nectar volumes (Davidson *et al*, 2004). However, in large numbers non-formicines may still significantly influence nectar volumes. For example, the nectar volumes of the Hawaiian tree *Metrosideros polymorpha* were negatively associated with the densities of the invasive ant species *Linepithema humile* (Dolichoderinae) and *Pheidole megacephala* (Myrmecinae) and visits by native pollinators were reduced (Lach, 2008).

Ant territorial scent-marks are already known to repel certain species of herbivores; for example *Rhyparida wallacei* beetles preferentially feed on leaves sampled from outside the territories of the aggressive weaver ant, *Oecophylla smaragdina* (Offenberg *et al*, 2004). While foraging in ant territory may not be as great a threat for potential pollinators, the ability of bees to identify and reject flowers that have been visited by nectar-thieving ants could increase foraging efficiency. The aim of this study was to determine if the behaviour of floral visitors was affected by scent-marks left by ants on three different wild flower species. If ant-deposited scent-marks influence visitor behaviour it is expected that control flowers will be visited more commonly. When they are visited it is expected that visits to ant scent-marked flowers will be shorter and that visits by bees are more likely to be influenced than visits by other potential pollinators like hoverflies as hoverfly visitation has never previously been shown to be influenced by prior bee or hoverfly visitation (Reader *et al*, 2005; Yokoi & Fujisaki, 2009b).

4.2 Methods

4.2a Study sites

Field work was carried out in disused areas of an organic farm near Mairena in Andalusia (37°0'N, 3°04'W) in August of 2009 and July/August of 2010 on *Bupleurum fruticosum* and *Brassica juncea*. Work was also carried out in a disused field (55°94'N, 3°16'W) in Edinburgh, near Arthur's Seat, on *Digitalis purpurea* in July 2010.

4.2b Study species

- ***Digitalis purpurea*** (Plantaginaceae) – The perennial herb, foxglove, produces elongated clusters of tubular, pendant flowers ~4cm long, which each last for 2/3 days holding at peak dehiscence 0.05 – 2.2µl of on average 22% sucrose nectar. They are visited primarily by a variety of bumblebee species but occasionally by smaller bees such as *Apis* as well.
- ***Bupleurum fruticosum*** (Apiaceae) – Also known as shrubby hare's ear, this evergreen shrub has open shallow flowers and contains small volumes of highly concentrated nectar on the flower surface throughout the day that is fed on by ants throughout the day. Individual flowers are ~0.4cm wide, last for 2/3 days are visited by a very wide range of bees, hoverflies, wasps, muscids, hemipterans and ants.
- ***Brassica juncea*** (Brassicaceae) – An annual weed, Indian mustard produces numerous small open flowers ~1.5cm long arranged in racemes, with 1-3 flowers opening each morning, each lasting for a single day and holding at peak dehiscence 0 – 0.07µl of on average 24% sucrose nectar. They are visited by a wide range of bee species and also by smaller hoverflies and muscids.

These species (see Figure 1) were chosen as they possess very different floral forms and were all very frequently visited by potential pollinators at the chosen sites. None possess any kind of physical or chemical floral ant-repelling traits to the ant species used and *Lasius niger* workers commonly tend homopterans on *D. purpurea* and forage for nectar on both *D. purpurea* and *B. fruticosum*.

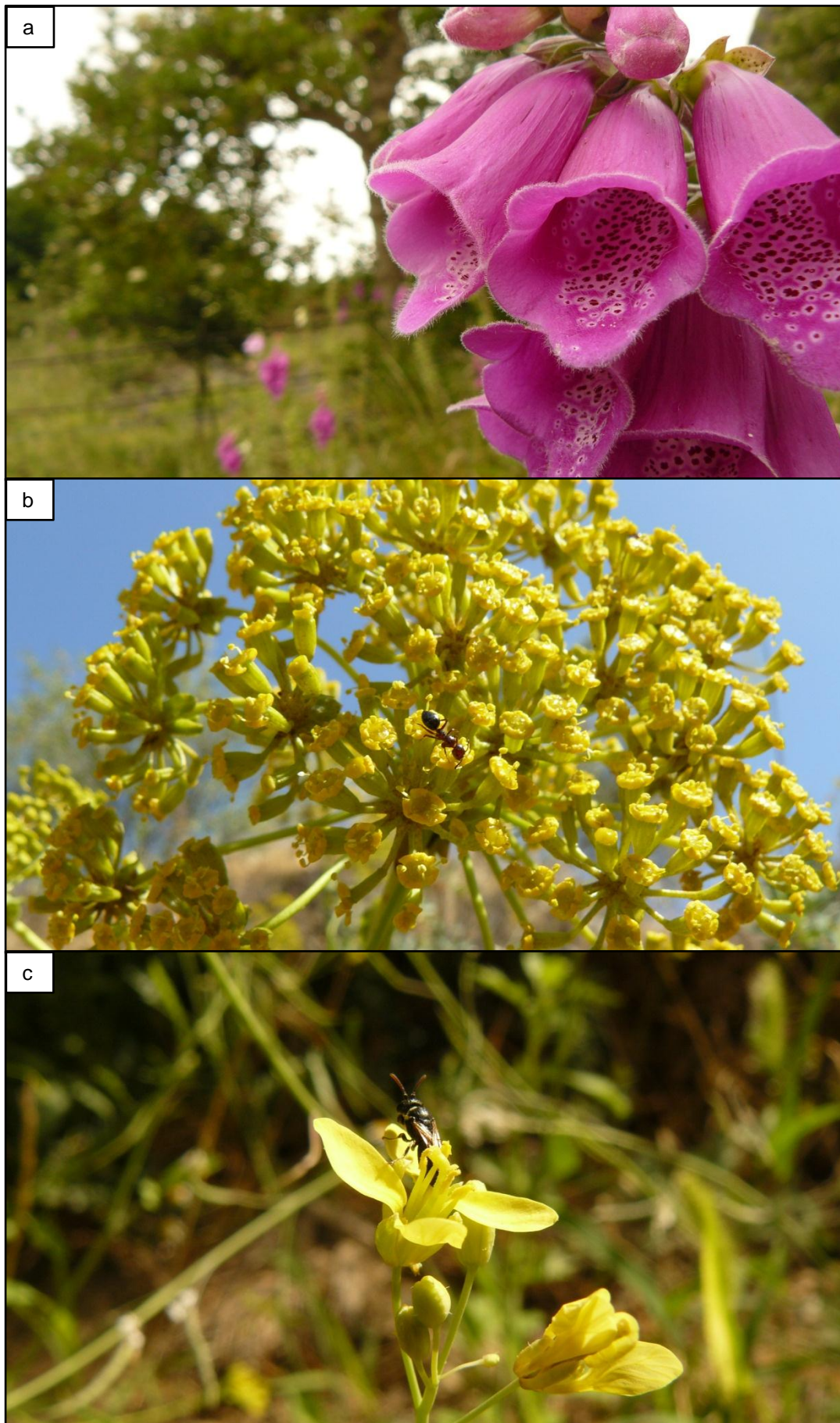


Figure 1: (a) *Digitalis purpurea* (b) *Bupleurum fruticosum* (c) *Brassica juncea*

4.2c Experimental procedure

Experiments were tailored to individual plant species and depended on the availability of ants at the site. Details of manipulated “ant-visitation” are given below for each plant species. In all trials, experimental and control flowers were bagged the night before observations were made, ensuring that the visits observed were the first of that day. Plants that did not already have ants foraging on them were selected. Flowers with freshly dehiscent anthers were chosen the next day for the experiments as these were more likely to have nectar (this could not have been ascertained beforehand without reducing the nectar volume and in doing so reducing the attractiveness of flowers to potential visitors). Ants were left in contact with experimental flowers for 5 to 10 minutes, depending on the species, while Control flowers were left untreated. Both ant-treated and control flowers were then watched until they were visited by a potential pollinator or until a 20 minute period elapsed. Time until visitation, length of time spent within a flower and visitor identity were all recorded. Aborted visits (inspections), when a flower was approached and then moved away from, were also recorded. It was hypothesised that if ant scent-marks dissuaded visitors from landing then there would be a greater proportion of aborted visits to experimental (ant-visited) flowers, and/or they would be visited later and/or visitation times would be shorter. If the effect of ant scent-marks is very short lasting it might be expected that they influence visitation only for a very short time (e.g. the first minute of the experimental trial).

1) *Digitalis purpurea*

Lasius niger workers were collected from nearby plants by carefully allowing them to walk into a vial. A single ant was held in each vial. Flowers with freshly dehiscent anthers were selected. One flower was held upright at the base with forceps and a single ant was dropped inside the bell of the flower and left there for 5 minutes. If the ant attempted to leave the flower after feeding on the nectar they were kept inside by gently tapping the side of the bell with a paintbrush. For each experimental flower a second control flower was similarly manipulated with no ant inside.

2) *Bupleurum fruticosum*

As the individual flowers of *B. fruticosum* are very small, whole inflorescences were bagged. *Lasius niger* workers foraging on *B. fruticosum* nectar were collected from plants some distance from the experimental plants by letting them walk into vials. Twelve ants were released onto an

inflorescence and allowed to forage on it for ten minutes. After this time remaining ants were carefully removed from the inflorescence with a brush. Both the experimental inflorescence and a control inflorescence were then observed. Attempts were made to observe visitation to smaller sub-umbels of inflorescences (each containing around 20 flowers), so that the number of individual flowers visited could be counted, but visitation rate to these smaller clusters was too low to acquire a sufficiently large data set for analysis.

An additional experiment was carried out to assess the effect of ants on visitor behaviour while the ants were still present on the flowers. Flowers were bagged in the same way as above. The bases of both experimental and control inflorescences were coated with Tanglefoot® pest barrier to prevent the ants from leaving the inflorescence. Ten ants were then placed on the inflorescence and allowed to forage on the nectar. Observations were carried out until the first visit or until a number of ants had escaped.

3) *Brassica juncea*

The medium sized ant *Formica selysi* was used in this experiment. Five ants were collected in vials and transferred to a single jar. Experimental inflorescences were encased within the jars with ants and control inflorescences were placed in empty jars. By modifying the lids of the jars with a slit the flowers were still attached to the plant and the stem was undamaged. The ants rarely fed on the nectar within the flowers but did run repeatedly over them as they sought to escape from the jar. After a period of 5 minutes the flowers were released from the jars and observations began.

4.2d Insect identification

Flower visitors were collected for later identification and photographs were taken of visitors on flowers. However, as a certain amount of distance is needed between the observer and the flowers so as not to disturb visitation, identifications in the field could not be done with complete accuracy. In Scotland *Bombus pratorum* is easily identified by the red/ginger tip of the abdomen and a yellow stripe but while bumblebees with a white abdomen were identified as *Bombus lucorum* it is also possible that some or all may have been large *Bombus terrestris*.

In Spain identifications were complicated by the diversity of species present. Many groups could only be identified to genus, including *Melitta*, *Andrena* and *Ceratina*. The smallest bee visitors consisted of a range of halictid species, most commonly from the genera *Halictus* and *Lasioglossum*. These have all been defined as halictids for the purposes of this analysis. Similarly all wasps similar to large *Vespa* sp. were considered as the single group “wasps”. Hoverfly visitors have been considered as a single group (“small hoverflies”) in the case of *B. juncea* as only small hoverflies of similar size visited the flowers, while hoverfly visits to *B. fruticosum* were divided into large and small hoverfly groupings.

4.2e Statistical methods

Pearson’s Chi-squares were used to test for differences between number of visits by different species in each treatment. Where necessary the time spent on flowers was transformed to obtain normally distributed residuals. Univariate ANOVAs were carried out on the visitation time, taking treatment and species as fixed factors, day of data collection as a random factor and time until visitation as a covariate. Statistics reported are from minimal models after all non-significant interactions and random factors were discounted. Tukey post hoc tests were used to identify the cause of significant differences where a number of visitor species were involved. Where normal distributions for the time spent on flowers could not be obtained Kruskal Wallis tests were carried out between time on flowers and factors and a Spearman’s Rho was carried out to determine if there was a significant correlation between time spent on flowers and time until visitation. Error bars in all figures are 95% confidence intervals. All statistical tests were carried out using SPSS 18.

Results

4.3a *Digitalis purpurea*

Two types of bumblebee accounted for 99% of visits to *D. purpurea* at the site: *Bombus pratorum* and *B. lucorum/terrestris* (Figure 2; data summarised in Table 1). Honeybees were also observed visiting flowers nearby but not in the study field, where the only other visitor was a single wood-carder bee *Anthidium manicatum*. *B. pratorum* workers were the most common visitors to *D. purpurea* (64.4% of visits). No significant difference was found between the visitation rates of bumblebees to flowers in

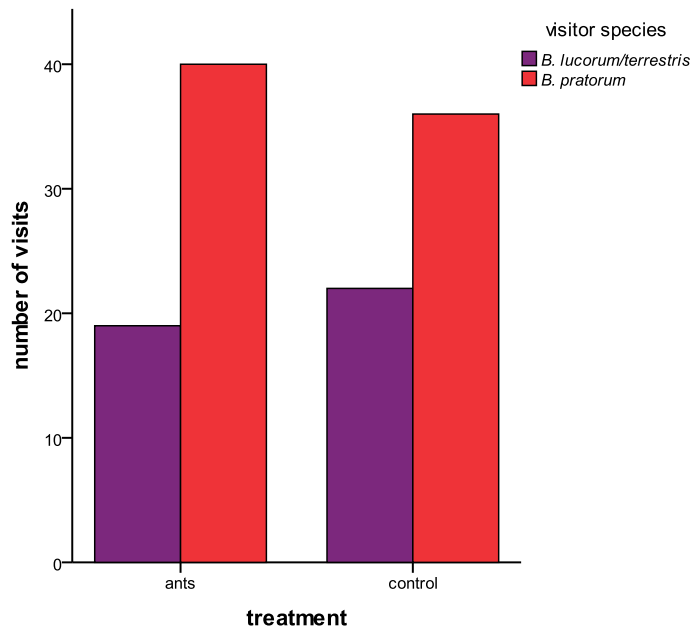


Figure 2: Number of visits to *D. purpurea* flowers in ant and control treatments by different groups of species.

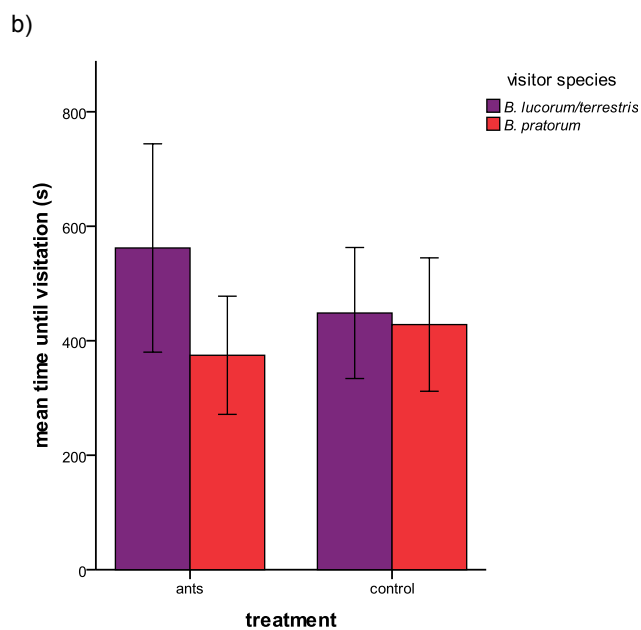
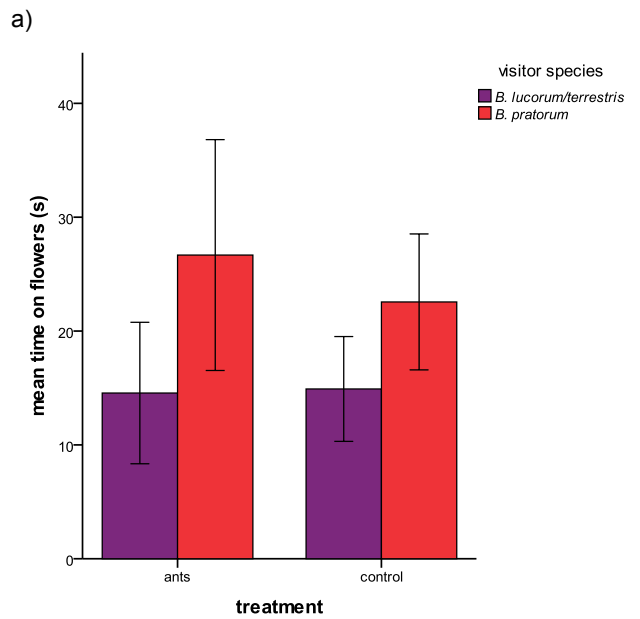


Figure 3: (a) Mean time spent on *D. purpurea* flowers by different visitor species in each treatment. (b) Mean time until visitation to *D. purpurea* flowers by different visitor species in each treatment.

Table 1: Sample sizes of different types of visitor to *D. purpurea* flowers in different treatments with mean \pm st dev. for time until visitation and time spent on flowers.

	Ant scent-marked		Control	
	<i>B. pratorum</i>	<i>B. lucorum/terrestris</i>	<i>B. pratorum</i>	<i>B. lucorum/terrestris</i>
Number of flowers visited	40	18	36	23
Time until visitation (s)	374.6 \pm 322.8	562.1 \pm 365.9	428.3 \pm 344.3	448.4 \pm 264.7
Time spent on flowers (s)	26.7 \pm 31.7	14.6 \pm 12.5	22.6 \pm 17.7	14.9 \pm 10.7

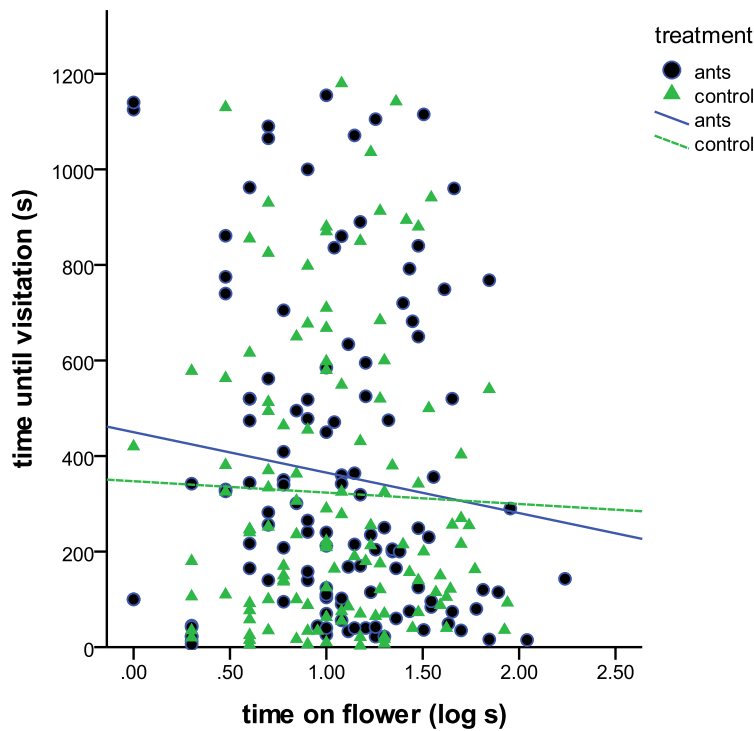


Figure 4: Time until visitation by bumble bees to *D. purpurea* flowers plotted against time spent on flowers were not significantly correlated. Shown here differentiated by treatment type. (Similarly, time until visitation did not predict time spent on flowers in *B. fruticosum* and *B. juncea* either).

different treatments ($\chi^2=0.422$, $df=1$, $p=0.516$). The proportion of flowers avoided by the first visitor did not differ between treatments (12 out of 69 ant-treated flowers, 13 out of 70 controls; $\chi^2=0.033$, $df=1$, $p=0.856$).

Treatment ($\chi^2=0.039$, $df=1$, $p=0.844$) and day of data collection ($\chi^2=5.226$, $df=10$, $p=0.265$) were not found to significantly influence the time bumblebees spent at flowers. Although *B. pratorum*

visits lasted for a shorter period of time, compared with *B. lucorum/terrestris* this difference was not significant ($\chi^2=3.156$, $df=1$, $p=0.076$). Time until visitation was not significantly correlated with time spent at flowers ($\rho=-0.170$, $p=0.067$; Figure 5). There were insufficient data from the first minute alone to carry out analysis of very early visits.

4.3b *Bupleurum fruticosum*

Honeybees were the most common visitor to *B. fruticosum* inflorescences (Figure 5; data summarised in Table 2), which were also visited by *Andrena*, halictids, and a wide range of hoverflies, wasps and muscids. No significant difference was found between the number of visits by different species to inflorescences in the different treatments ($\chi^2=2.979$, $df=1$, $p=0.811$). Very few flowers were obviously avoided by visitors and there was no significant difference between treatments (7 out of 85 ant-treated flower, 5 out of 86 controls; $\chi^2=0.385$, $df=1$, $p=0.535$).

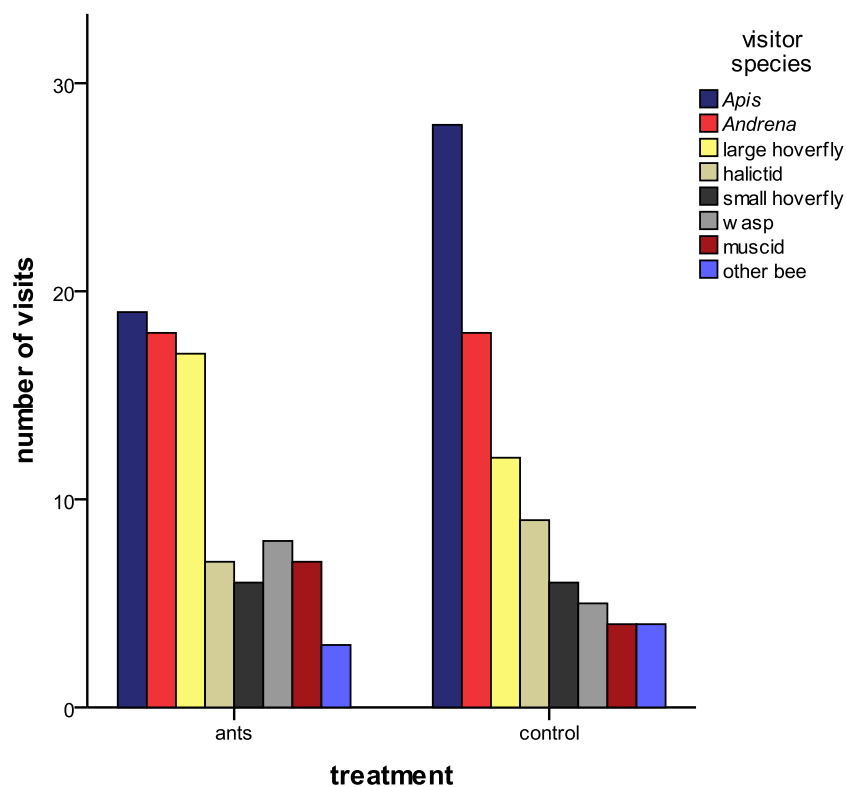
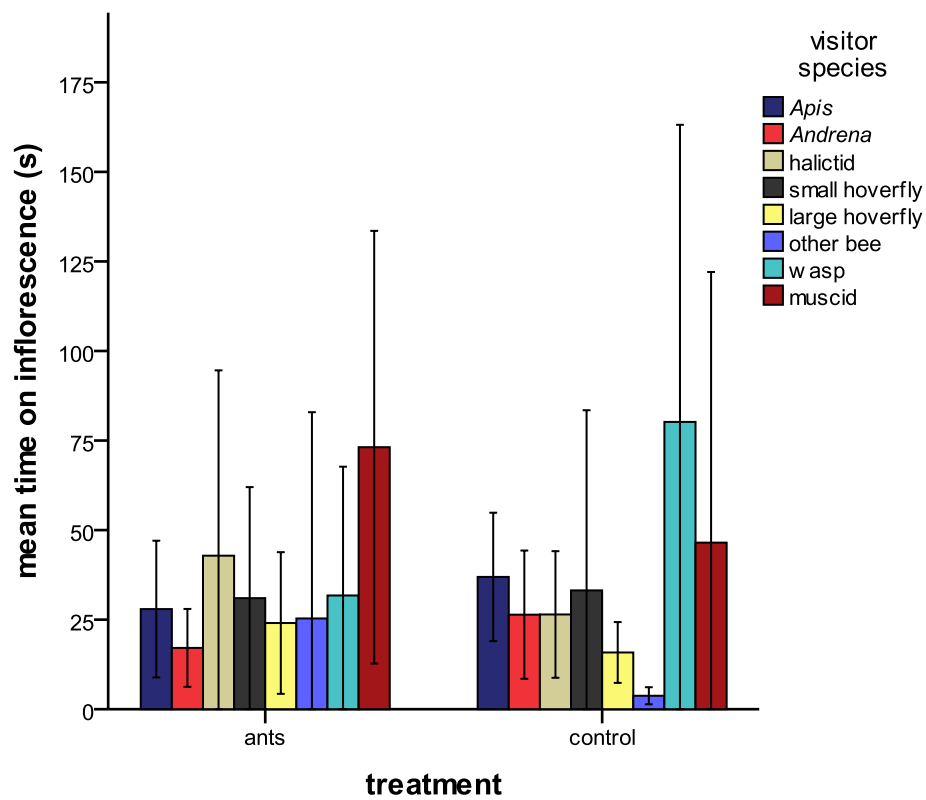


Figure 5: Number of visits to *B. fruticosum* inflorescences in ant and control treatments by different groups of species.

a)



b)

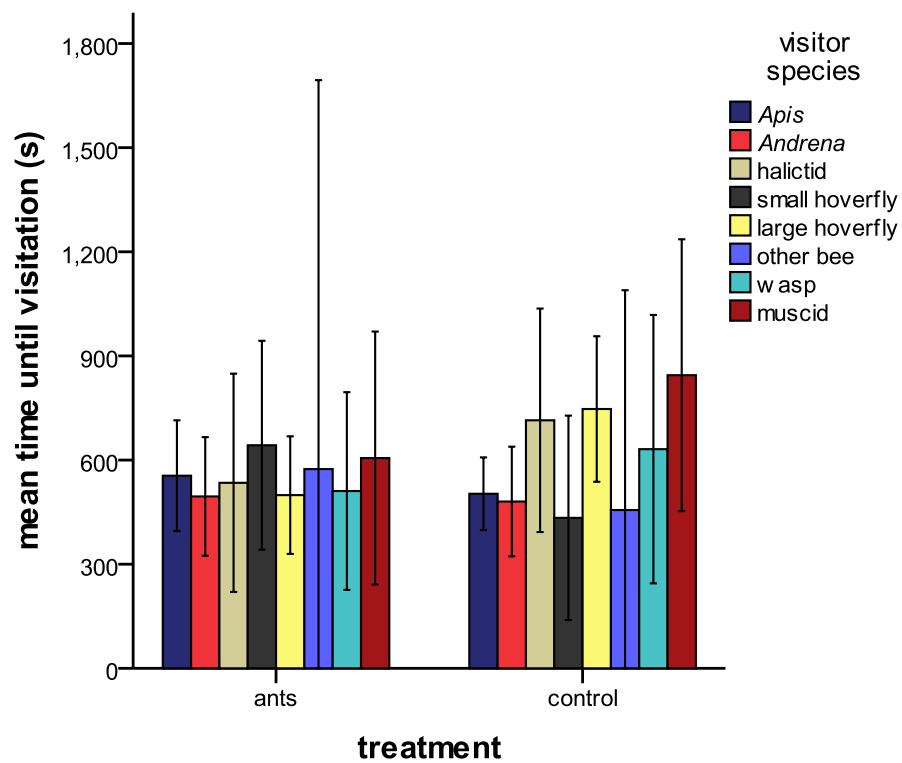


Figure 6: a) Mean time spent on *B. fruticosum* flowers by different visitor species in each treatment. b) Mean time until visitation to *B. fruticosum* flowers by different visitor species in each treatment.

Table 2: Sample sizes of different types of visitor to *B. fruticosum* inflorescences in different treatments with mean \pm st dev. for time until visitation and time spent on inflorescences.

Visitor species	<i>Apis</i>	<i>Andrena</i>	large hoverfly	halictid	small hoverfly	wasp	muscid	other bee
Ant scent-marked								
Number of flowers visited	19	18	17	7	6	8	7	3
Time until visitation (s)	555.0 \pm 331.2	495.2 \pm 343.7	499.0 \pm 329.5	534.4 \pm 340.0	642.7 \pm 286.8	510.8 \pm 340.7	605.7 \pm 394.1	574.0 \pm 451.1
Time spent on flowers (s)	28.0 \pm 39.6	17.1 \pm 21.9	24.1 \pm 38.5	42.9 \pm 56.0	31.0 \pm 29.5	31.8 \pm 43.0	73.1 \pm 65.3	25.3 \pm 23.2
Control								
Number of flowers visited	28	18	12	9	6	5	4	4
Time until visitation (s)	502.9 \pm 270.2	480.6 \pm 317.7	747.1 \pm 330.0	714.6 \pm 418.8	433.5 \pm 280.7	631.4 \pm 311.4	844.5 \pm 246.1	456.0 \pm 398.0
Time spent on flowers (s)	36.9 \pm 46.2	26.4 \pm 36.0	15.8 \pm 13.4	26.4 \pm 23.0	33.2 \pm 47.9	80.2 \pm 66.8	46.5 \pm 47.5	3.8 \pm 1.5

Table 3: Sample sizes of different types of visitor to *B. juncea* flowers in different treatments with mean \pm st dev. for time until visitation and time spent on flowers.

Visitor species	<i>Apis</i>	halictid	<i>Melitta</i>	<i>Ceratina</i>	other bee	small hoverfly
Ant scent-marked						
Number of flowers visited	48	28	19	12	9	3
Time until visitation (s)	366.5 \pm 326.9	385.5 \pm 304.4	248.7 \pm 234.7	396.8 \pm 433.3	479.2 \pm 447.5	242.3 \pm 279.4
Time spent on flowers (s)	8.9 \pm 6.1	30.9 \pm 26.9	15.3 \pm 11.8	41.3 \pm 46.3	16.3 \pm 15.0	24.0 \pm 35.5
Control						
Number of flowers visited	43	34	20	9	7	7
Time until visitation (s)	309.3 \pm 284.5	255.2 \pm 248.5	402.6 \pm 326.8	462.4 \pm 437.3	250.4 \pm 187.6	354.0 \pm 380.3
Time spent on flowers (s)	7.5 \pm 4.1	28.3 \pm 20.0	12.6 \pm 7.1	22.9 \pm 9.5	8.9 \pm 11.8	31.3 \pm 30.8

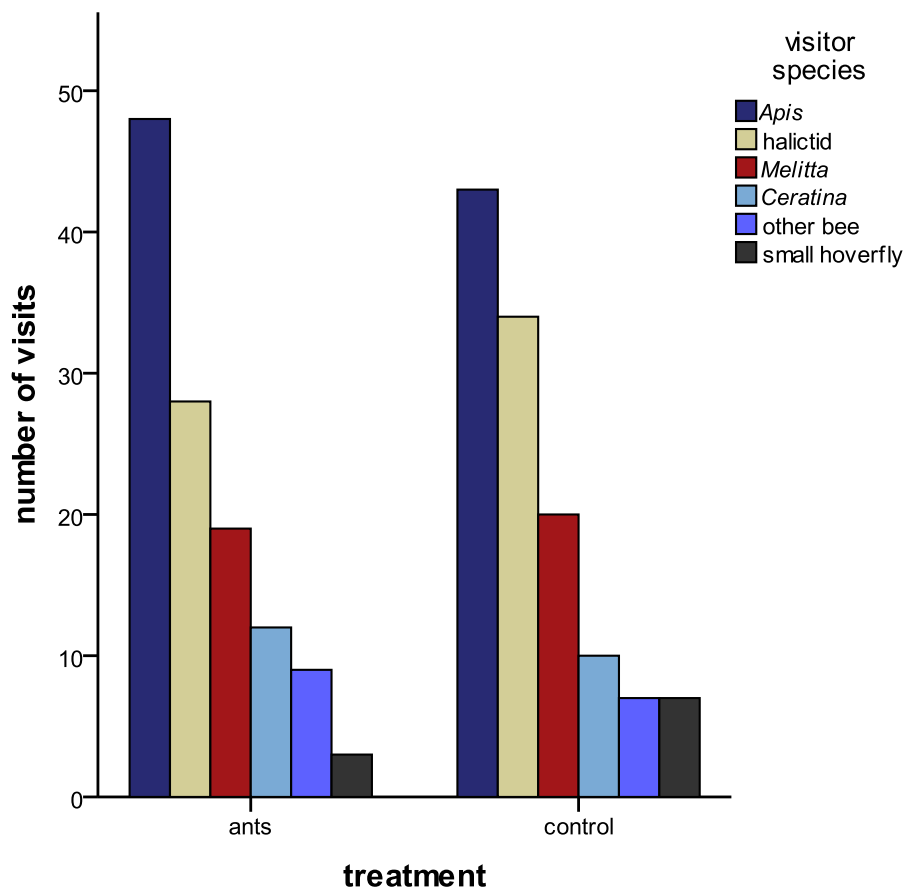


Figure 7: Number of visits to *B. juncea* flowers in ant and control treatments by different groups of species.

Treatment had no significant effect on length of visits ($F_{1,1}=0.204$, $p=0.726$), time until visitation had no significant effect on length of visits ($F_{1,1}<0.001$, $p=0.982$) and length of time spent at an inflorescence did not vary significantly between species ($F_{1,7}=1.596$, $p=0.140$; Figure 6). While more honeybees visited controls and more hoverflies visited treated inflorescences this did not result in a significant interaction between species and treatment in the model ($F_{1,7}=1.466$, $p=0.184$). There were insufficient data from the first minute alone to carry out analysis of very early visits.

When ants were left on the inflorescences throughout the trials, however, ant-infested inflorescences were visited for a significantly shorter period of time than control inflorescences (10.6 ± 12.5 seconds, $n=53$, on ant-infested inflorescences compared with 27.7 ± 38.4 seconds, $n=67$, on control inflorescences; $\chi^2=16.993$, $df=1$, $p<0.001$).

4.3c *Brassica juncea*

B. juncea flowers were visited by a range of bee species including *Apis mellifera* (the most common visitor), *Melitta*, *Ceratina* and halictids, as well as small hoverflies (Figure 7; data summarised in Table 3). No significant difference was found between the number of visits by different species to flowers in different treatments ($\chi^2=2.896$, $df=1$, $p=0.716$). Again, very few flowers were obviously avoided by visitors and there was no significant difference in avoidance between treatments (10 out of 130 ant-treated flowers, 8 out of 130 controls; $\chi^2=0.239$, $df=1$, $p=0.625$).

Time spent at a flower was dependent on visitor species and this resulted in a significant interaction with day of experiment due to variation in types of visitor on different days ($F_{1,36}=2.347$, $p<0.001$). Tukey post hoc tests revealed that there was a significant difference between time spent on flowers depending on the size of the bee (Figure 8). Large bees (*Apis mellifera*, *Melitta*, other

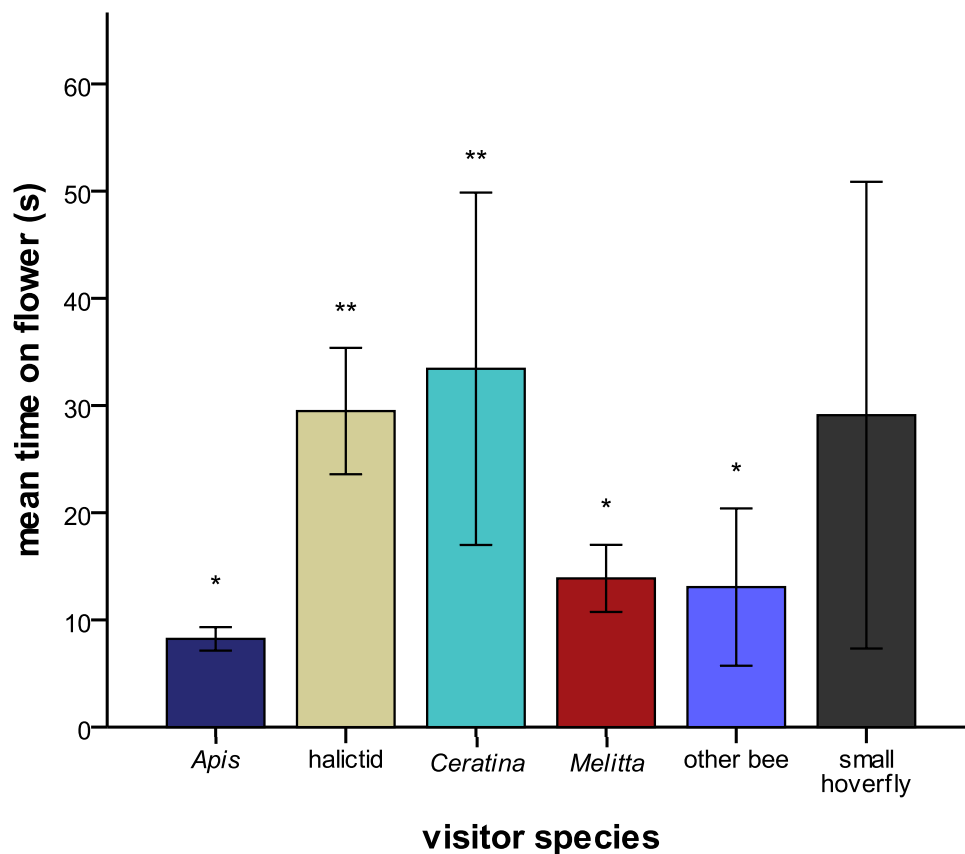


Figure 8: Time until visitation to *B. juncea* flowers data pooled across treatments. Significant difference in visitation time between the large *Apis*, *Melitta* and other bees (*) compared with the small halictid and *Ceratina* bees (**).

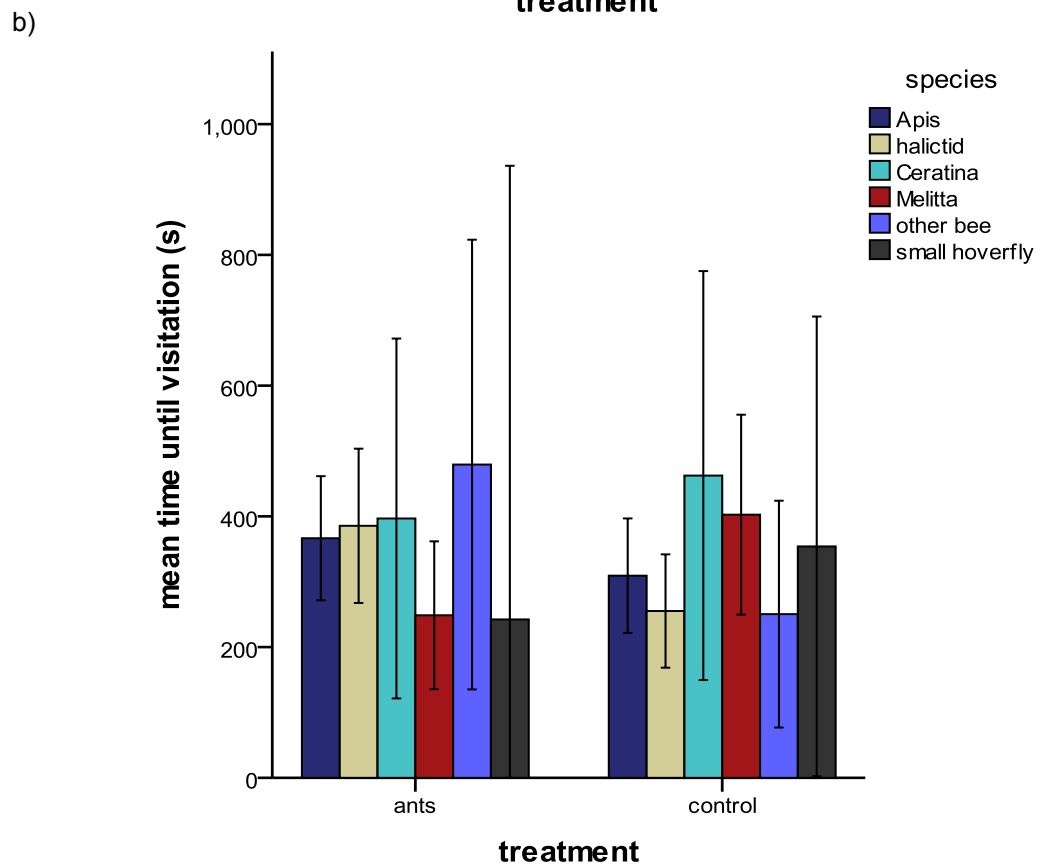
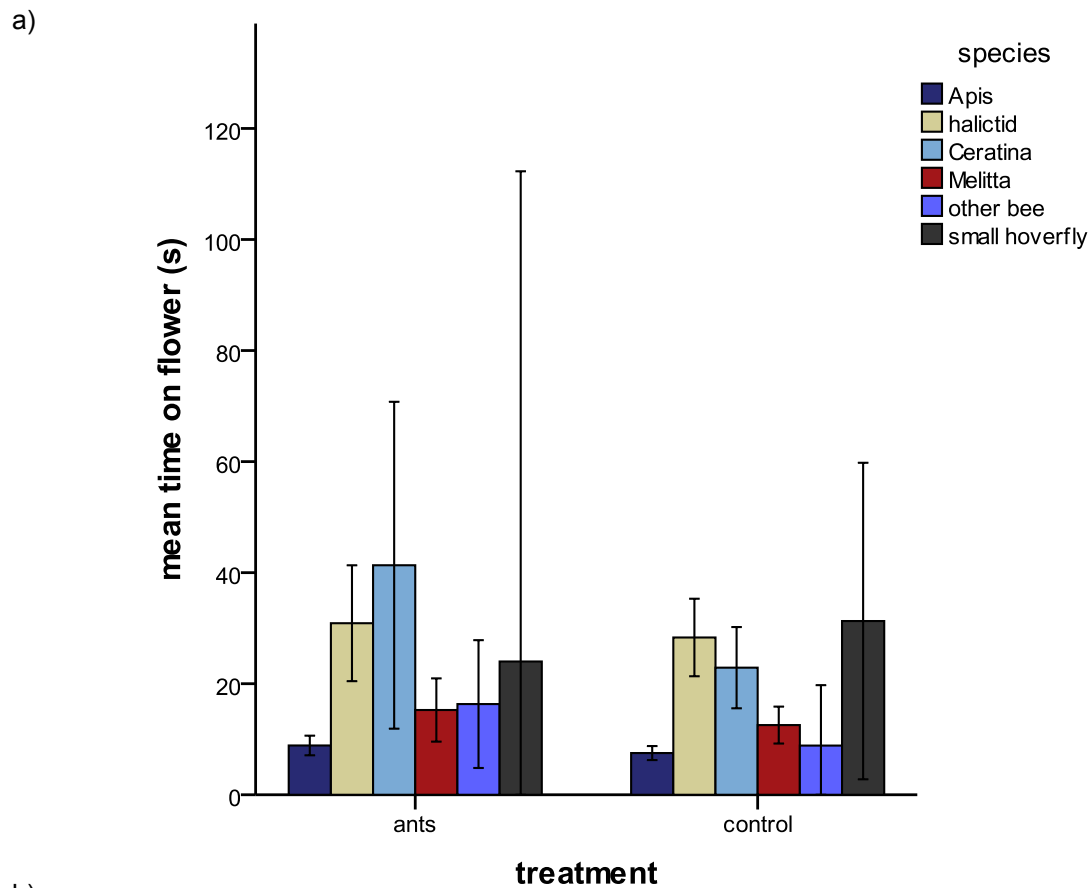


Figure 9: (a) Mean time spent on *B. juncea* inflorescences by different visitor species in each treatment. (b) Mean time until visitation to *B. juncea* inflorescences by different visitor species in each treatment.

bees) spent significantly less time on *B. juncea* flowers than small bees (halictids, *Ceratina*). Time spent by small hoverflies on the flowers did not differ significantly from either small or large bees. Time spent by visitors on *B. juncea* flowers was not, however, influenced by treatment ($F_{1,1}=0.015$, $p=0.902$; Figure 9) or by time until visitation ($F_{1,1}=1.308$, $p=0.254$). When data from the first minute alone were analysed to rule out scent-marks with very short half-lives (22 visits to ant-treated flowers, 21 to control flowers), the conclusions were the same: no significant effect of time until visit ($F_{1,1}=0.028$, $p=0.868$) or of treatment ($F_{1,1}=0.855$, $p=0.361$).

4.4 Discussion

4.4a Responses of flower visitors to ant scent-marked flowers

In all three study plants, potential pollinators did not discriminate between flowers ants had recently been present on and those that had remained ant-free. None of the pollinator species spent less time on previously ant-visited flowers. Additionally, visits did not become significantly more likely with increasing time since contact with ants. Visits to *B. fruticosum* inflorescences were likely to be shorter when the ants were still present foraging on the flowers. Bee foraging activity on *B. fruticosum*, however, is probably more disrupted by the large wasps that visited the inflorescences as they regularly chased off honeybees, sometimes stinging them to death.

It is possible that visitors were repelled from treated flowers more commonly than identified. Although flower avoidance was easy to identify as a bumblebee moved up an *D. purpurea* inflorescence, it was less simple for *B. fruticosum* and *B. juncea*, as in an environment in which insects are flying between and around flowers at all times repellence may be mistaken for avoiding a flower in an insect's flight path. However, when flower avoidance was observed it was not more common from flowers that had been in contact with ants.

Repellence from ant-deposited scent-marks may still have taken place for a very short time after an ant was present. Honeybees rejected 80% of *Borago officinalis* flowers 20s after they were visited by conspecifics but the scent-mark only had a half-life of 37s (Williams, 1998). A similarly short window of repellence is possible from ant scent-marks. However, no such short-term repellence was

found from *B. juncea* flowers, where there were sufficient data to carry out this analysis. As ants take longer to leave a flower than flying visitors, other ant workers often return soon after, it seems more likely that if a bee benefits from avoiding ant-attended flowers they will avoid them for a longer period of time.

Length of visits to *B. juncea* did depend on visitor species as honeybees, *Melitta* sp. and other medium to large bees spent less time on each flower than the smaller bees such as *Ceratina* sp. and halictids. This was possibly because the larger bees, with comparably longer tongues and larger pollen combs, harvested the resources from the small *B. juncea* flowers more quickly.

Hypotheses regarding potential disruptive effects of ant scent-marks on responses to bee scent-marks were not tested in these experiments. The experimental design precluded the possibility of pollinator visitation to the flowers before ant scent-marks were made. So even though ant scent-marks alone did not influence visitor behaviour it is still possible that ant scent-marks on these species may influence how bee scent-marks are interpreted by bee visitors.

4.4b Scent-marks deposited by ants

It is clear that ant-deposited scent-marks do not influence bee behaviour under the circumstances of the three trials. However, even without more sophisticated methods it is clear that scent-marks are being deposited and may be utilised by flower visitors under other conditions. Both *L. niger* and *F. seylesi* deposit FHCs from their tarsal glands that are primarily used by ants to aid adhesion of the tarsi (Federle *et al*, 2002). FHCs are also involved in colony-specific scent-marking in *Lasius niger* and *Lasius japonicus*, as more aggression is observed between ants of different colonies in areas previously conditioned (walked over) by ants but not marked with trail pheromones from the hind-gut (Devigne & Detrain, 2002; Akino & Yamaoka, 2005). The FHCs of *Lasius nipponensis* are very similar to their colony-specific cuticular hydrocarbons, lacking only n-alkanes, and so appear to act as a territorial pheromone in this genus (Akino *et al*, 2005). FHCs from the ants were presumably deposited on the flowers by both ant species, but the information provided by these volatiles was not taken into account by flower visitors when making foraging decisions.

Ants may also deposit pheromone trails. All primary formicine trail pheromones originate in the hind-gut, although the active trail-following compounds have only been identified for a small number of species (Morgan, 2009). *L. niger* workers deposit pheromone trails that may last for up to 24 hours as they move from a newly discovered food source back to the nest and continue to lay trails until they acquire a spatial memory of the food source (Evison *et al*, 2008). So it is likely that trail pheromones were also deposited on the flowers of *D. purpurea* as in all cases *L. niger* workers fed from the nectar within the flower before attempting to exit the corolla. Pheromone trails may have also been laid on the inflorescences of *B. fruticosum*, where ants were allowed to feed on the inflorescence and then leave, making their way through the inflorescence and down the stem. Many individuals that had left also returned to the inflorescence during the ten-minute period and commenced feeding again.

As *F. seylesi* workers rarely fed on the small volume of nectar available within the flowers of *B. juncea* it is unlikely that any equivalent foraging trails were left in this case. However, in both this experiment and with *L. niger* on *B. fruticosum* workers from the same colony interacted together on the flowers, antennating each other and possibly agitating each other with alarm pheromones.

D. purpurea and *B. fruticosum* flowers last for more than a day and so it is possible that flowers used in the experiment retained scents of visitors from the previous day. Scent-marks of low volatility attract *Bombus terrestris* individuals to artificial flowers that had previously proved to be rewarding 20 hours ago (Schmitt & Bertsch, 1990). Although initially repelled by previously visited flowers of the yellow sweet clover, *Melilotus officinalis*, honeybees were more attracted to flowers that were visited 24 hours previously (Stout & Goulson, 2001). In some cases such long-lived attractants could counteract other short-lived repellents.

4.4c Why did scent-marks not influence flower visitor behaviour; and when might they do so?

Potentially repellent scent-marks may have been ignored by flower visitors that were primarily foraging for pollen, as pollen-collecting bumblebees reject fewer scent-marked *Symphytum officinale* flowers than nectar-gatherers (Stout *et al*, 1998). Repellence from ant scent-marks may be more likely in undehisced or pollen-depleted flowers that are only attractive to nectar foraging bees. In practice

however pollen-foraging bees still visited such flowers but spent very little time on them. By using freshly dehiscent flowers, it was more likely that nectar would also be present.

Repellence from ant scent-marks would require not just a negative incentive for visiting bees but also experience of that cost by individual bees themselves. Bees visiting *D. purpurea* and *B. fruticosum* would have been familiar with potential *L. niger* scent-marks as ants regularly forage for nectar on *B. fruticosum* and nectar and homopteran honeydew on *D. purpurea* inflorescences. In the case of *B. juncea*, *F. selysi* do not normally forage on the flowers and so *F. selysi* scent-marks would be a novel signal in this context. Even if these aggressive ants depleted resources or posed a threat to flower visitors under other conditions, bees may not necessarily associate *F. selysi* scent-marks with danger or with low resource levels at *B. juncea* flowers.

Considering the versatility and well-developed learning capacity of social bees it would be surprising if they did not learn to use ant scent-marks as foraging cues under certain conditions. Early work with bees and artificial flowers identified scent-marks as attractive signals that promoted visitation. A number of species were even attracted to landing discs after they were exchanged with unrewarding feeders (Schmitt & Bertsch, 1990; Schmidt *et al*, 2005). However this was due to rewards being presented in the feeders *ad libitum*, so that such feeders more resembled food stores within a hive than natural flowers. In such situations bees learned to interpret conspecific scent-marks as attractants (Goulson *et al*, 2000). When more realistic volumes were used bees interpreted these same scent-marks as signals of unrewarding flowers (Giurfa & Núñez, 1992; Saleh & Chittka, 2006).

There are also other reasons why ant-deposited scent-marks could have been ignored. Due to the extra time it takes to make accurate decisions it may benefit bees to be fast and often inaccurate. For example, Burns (2005) analysis of Chittka *et al*'s (2003) experiment, in which bumblebees visited artificial flowers, discovered that fast, inaccurate bees performed better than slow, accurate ones. Slower, accurate bees would be expected to perform better where longer handling times were involved. Individual bees appear to have consistent behavioural patterns that benefit the colony as a whole, in much the same way as different castes fulfil different roles, ensuring efficient foraging on a variety of resources (Burns, 2005; Chittka *et al*, 2009).

Further experiments with artificial flowers could identify conditions under which repellence from ant-marked flowers does occur. Bees would be expected to be repelled from ant-visited artificial flowers if only small quantities of nectar were available in each, so that visiting ants made a significant impact on the reward levels of these “flowers”. Bees would then quickly learn to avoid ant-scents and so optimize their foraging by only visiting those flowers with nectar. The effect of such scent-marks could then be proven by moving the “corollas” of the artificial flowers to fully stocked “flowers” and seeing if these were still avoided. However, although attempts to carry out this experiment were made in the field, it quickly became clear that a captive bee population trained to visit such unnatural flowers was required. Such a colony could then be reared on a pollen-rich, nectar-poor diet and so encouraged to forage for nectar (Raine & Chittka, 2008).

4.4d Conclusions

While this study does not rule out avoidance of ant scent-marks on flowers by bees, it does start to narrow down when these cues may influence bee behaviour. Ant-attendance at flowers of these species might not reduce reward levels enough to make it worthwhile incorporating ant scent-marks into foraging decisions. Additionally, such an impact on reward levels will need to happen commonly enough within the locality that bees learn to associate the scent-marks with poor rewards. In the case of *B. juncea* the presence of *F. seylesi* scent-marks may not have had any prior associations with either low reward levels or danger, so they were ignored due to their novelty in that context.

Due to the sophisticated nature of bee decision-making, responses to stimuli such as ant scent-marks depend not only on innate ability to detect the information but also the previous experience of individual bees. It is likely that bees can easily detect ant pheromone trails and FHCs but these chemical cues had no negative associations for bees foraging on *D. purpurea*, *B. fruticosum* or *B. juncea*. Future research with captive bee populations may be able to identify any conditions under which ant scent-marks provide useful information to foraging bees. By identifying corresponding conditions in the field it will then be possible to determine if, when and where ant attendance of flowers impacts on pollinator behaviour through scent-marks.

Chapter 5: Conclusions and Future Directions

5.1 Contributions of this Thesis

The interactions between species visiting flowers can be complex and their precise nature not always obvious. Determining which players are benefiting or hampering each other under which conditions is essential for a comprehensive understanding of pollination ecology. Ants provide a good example of a group of species that regularly interact with flowers but whose influences on plants and other flower visitors are not readily apparent. They may act as pollinators, nectar thieves, predators of other visitors, indirect agents of outcrossing or unbidden guests that are more or less excluded with physical and chemical defences. Being able to predict the role of ants in pollination processes will involve experiments with a wide range of species under a wide range of conditions.

The work carried out in this thesis has broadened our understanding of the ubiquity and nature of floral ant-repellence and explored new avenues of research into interactions between species visiting flowers. While there are obvious selective benefits to producing floral ant-repelling traits in ant-plants such as *Acacia*, where aggressive ant-guards pose a clear danger to potential pollinators, until recently there has been little evidence that floral ant-repellence in non-myrmecophytes has resulted from specific selective pressures. The significant positive correlation between the intensity of ant-repellence and the volume of nectar found within individual flowers provides indirect evidence that these traits were specifically selected for to curb ant nectar-theft. This compliments the findings of Willmer *et al* (2009) that volatile ant-repellent traits are negatively traded-off against physical defences. Both studies point to predictable selection on floral traits that will prevent access to flowers by ants. The differences in findings between the studies also indicate that mechanisms are likely to vary with the likelihood of ant-visitation to flowers, which will differ greatly between species and habitat. Some evidence, however, does suggest that floral ant-repellence may indeed have been an exaptation in some groups and that these species are preadapted to repel ants. The prevalence of repellence within the acacias and related species suggests that the genus *Acacia* and perhaps even the Mimosidae as a whole are preadapted for ant-repellent traits.

The findings from Chapter 3 are consistent with the idea that pre-existing ant-repellent traits may be robust against changes in the ant community. However, the results would have been greatly enhanced with the addition of a common native nectar-thieving ant species to compliment the invasive nectar-thief and the native species that only rarely visited flowers. Despite this, the similarity of the response of both species is still informative and indicates that generalised ant-repellent traits may provide defences against the expanding dominance of invasive ant species.

My results also illustrate the need for experimental designs to take into account interactions at flowers at a very close range, as in my work tactile sensory trials were the most useful tools in the identification of floral ant-repellence. Physical defences, although primarily useful in defence against herbivory, can clearly have very efficient additional roles as floral ant-repellents and have, with the exception of Willmer *et al* (2009), been overlooked in recent studies. The role of physical defences, such as the trichomes of *Arbutus unedo*, in the potential disruption of ant-homopteran interactions is also one that deserves further attention.

Chapter 4 addressed the issue of ant-attendance at flowers from a different perspective, that of the pollinator. A number of recent studies have started to examine the influence invasive ants have on pollinator visitation at flowers due to their enormous ecological and economic importance (Blancafort & Gómez, 2005; Lach, 2008; Lach & Thomas, 2008; Hansen & Müller, 2009). However, we still know relatively little about how native ant species influence pollinator visitation. While confirming that ant presence at flowers can reduce visitation times of potential pollinators (as pointed out by others, including Junker *et al*, 2007) my results also indicate that ants are unlikely to indirectly influence visitation through ant-deposited scent-marks on many species of flowers, or at the very least on those three species tested here. Bees do not appear to associate ant-deposited scents with any significant costs. What we know about the flexibility of bee sensory capabilities and learning behaviour, however, implies that on flowers where such costs are imposed bees might avoid ant-marked flowers. How might selection for ant-repellent floral traits act differently on such species? Populations where nectar volumes are significantly reduced by ant nectar-thieves are expected to be avoided by bees, not just because their visits are regularly disrupted by ants but also because they may learn to associate ant-scent-marks with low nectar volumes. Precisely such populations will

benefit from floral ant-repellent traits. So the costs imposed by ants may be shared between plants and pollinators and where this occurs avoidance of ant-deposited scent-marks could be linked to the evolution of floral ant-repellent traits.

5.2 Unknowns in the Role of Ants in Pollination Biology

5.2a Floral ant-repellence

We are starting to understand how a reduction in nectar volumes due to theft can influence pollinator behaviour by promoting movement between plants (e.g. Biernaskie *et al*, 2002). The subsequent influence on plant fitness will vary greatly depending on plant sexual system and population densities, with self-incompatible pollen-limited species being most likely to suffer a loss of fitness (Gonçalves-Souza *et al*, 2008). As floral ant-repellence is found in such a wide range of plant species, with varying sexual systems, the advantages of reduced geitonogamy are perhaps realised only rarely.

What is not yet clear is the extent to which ants, acting as nectar-thieves, significantly alter nectar volumes in different habitats. Their impact is likely to be more severe in the tropics where ants are ubiquitous on all plants, while more likely to be patchy and focused around individual ant colonies in temperate areas. This will also depend on the cost of nectar due to water or energy constraints. Where ant-attendance does reduce fitness in plants without floral ant-repellence it would be interesting to see if any factors limit the evolution of repellence. Would chemical ant-repellence impose costs in the form of reduced pollinator attraction as seen in *Polemonium viscosum* (Galen *et al*, 2011)? Where no such limitations exist, the fitness costs may only be imposed sporadically or perhaps have little impact in terms of a plant's lifetime fitness.

While pleiotropy undoubtedly plays a role in shaping the form and intensity of floral ant-repellence, chemical forms of repellence have not been reported in species for which there are no potential benefits (physical barriers are still likely to restrict the movement of ant-sized herbivores). There is no evidence that chemical ant-repellence is found in nectarless or wind-pollinated species. Additionally, recent work by Junker *et al* (2010b) has found that native Hawaiian plant species, with no exposure to native ants, are less likely to repel ants from flowers, indicating that coevolution is needed

to promote selection for repellent traits. So coevolution between plants and ants in general is likely to be essential for some form of floral ant-repellence to arise and be maintained. But clearly not all chemical ant-repellence is effective across a wide range of ant species. If repellence also imposes costs on plant fitness it is likely to be lost when such plants grow in areas of lower ant density. The factors that structure the efficiency at the species-specific level are not yet clear. How closely do the players need to interact to promote repellence, and which forms of ant-repellence are most likely to also be effective against novel ant species?

Although plants are remarkably flexible in the emission of floral volatiles, changes selected for in the leaf or floral chemistry may have knock-on effects on other species and so the benefits produced by manipulation of ant behaviour may also be accompanied by costs. Junker and Blüthgen (2008) found terpenoids to be produced in greater quantities in those species with ant-repellent floral volatiles and suggested that they played a role in repellence. These compounds, normally “floral scented” to our senses, are commonly used as pollinator attractors and may function in a dual role if they do help to repel ant-nectar thieves. This contrasts with the two-tier trade-off discovered in *Polemonium viscosum*, where skunky flowers repel both ants and bumblebee pollinators when compared with sweet-smelling flowers (Galen, 1983); and within sweet-smelling flowers those that produce high levels of 2-phenylethanol are also less attractive to both ants and bumblebees (Galen *et al*, 2011). One way to avoid such trade-offs may be the use of analogs that mimic ant alarm pheromones, such as that produced by *Acacia seyal fistula* flowers (Willmer *et al*, 2009). However, it is not clear if E,E- α -farnesene alone is responsible for the repellence or what its effects are on potential pollinators.

To understand the specific chemical cues that mediate these interactions a more comprehensive integration of biochemistry and animal behaviour will be needed in the future (Bruna *et al*, 2008). This will involve experiments that analyse the floral scents of a wide range of ant-repellent and non-repellent species or morphs, isolating the fractions that influence both ant and pollinator behaviour. These experiments should be designed to detect volatiles that may only act at sufficient concentrations at very close range. As demonstrated in Chapter 2 of this thesis, repellence commonly acts only at close range, and this form of ant-repellence may be especially common in the tropics. If

the ant-repellent chemicals are not volatile in nature, as is probably the case in *Stachytarpheta jamaicensis*, additional simpler techniques will be needed to wash pollen or petals and collect surface chemicals.

Despite one of the goals of this thesis at the outset being to examine floral ant-repellence in a range of ant-plants I ultimately only surveyed one additional ant-plant, *Cordia alliodora*. A more comprehensive survey focusing on ant-plants would have involved numerous field trips to sites up and down Central and South America, catching flowering periods of different species. However, I was financially ill-equipped to carry out such an undertaking at the start of this project.

At this point we can predict which species are likely to prevent their ant-guards from accessing flowers. We have two clear examples of the floral ant-repellent traits in ant-plants that prevent different kinds of pollination disruption: as observed in other species of myrmecophytic *Acacia*, the ant-repellent pollen of *A. collinsii* prevents ant-guards from physically threatening pollinators, while in *Cordia alliodora* ant-repellent pollen prevents nectar theft by ant-residents. Some major groups of myrmecophytes are unlikely to need to repel ants for these reasons. *Cecropia* ant-plants are inhabited by highly aggressive ant-guards but these dioecious plants produce no nectar and are predominantly wind-pollinated (Bawa & Opler, 1975). While *Piper* ant-plants are pollinated by a range of visitors, most species produce spikes of minute apetalous flowers that do not produce nectar (Jaramillo & Manos, 2001). Additionally the *Pheidole* ants that patrol *Piper* ant-plants are passive defenders, mainly clearing herbivore eggs from leaves and perhaps reducing mould growth (Letourneau, 1983) so would pose no threat to potential pollinators. Where *Cecropia* and *Piper* ant-plants may benefit from ant-repellence is if ant contact with pollen significantly reduces pollen viability, in which case ant-repellence could be essential. This is perhaps unlikely as antibiotic secretions are less important to ant species that nest above ground in structures in or on plants, as their colonies and larvae are kept drier and so are less prone to infection (Blüthgen & Feldhaar, 2010). Where floral ant-repellence is more likely is in the genera that are insect-pollinated, produce nectar and potentially support aggressive ant-guards, such as *Hirtella*, *Tococa* and *Leonardoxa*. Other species of *Cordia*, especially *C. nodosa*, would also be good candidates for ant-repellence. The small flowers of *Macaranga* do produce nectar but probably only in quantities large enough to feed thrip pollinators (Moog *et al*, 2002).

5.2b Ant scent-marks on flowers

Any study analysing the responses of bees to ant scent-marks is unlikely to detect innate responses to specific components of the marks deposited. Instead such studies will be investigations into the mechanics of bee learning and decision-making, which previous studies suggest to be extensive flexibility (e.g. Saleh & Chittka, 2006; Raine & Chittka, 2008). This flexibility may lead to variation in bees' decision-making in response to ant-deposited scent-marks on the same species in different locations depending on both ant- and flower-density. So changes in ant communities may quickly change bee behaviour and it should not take long for them to learn when and where novel chemicals signal low reward levels when, for example, invasive ants are introduced to a new area. Even where no direct link between ant scent-marks and bee behaviour is found there is also the possibility that the ant-deposited chemicals may disguise or alter scent-marks deposited by bees on the same flower.

Additionally, it should also be remembered that ants may be capable pollinators in their own right. New examples of successful ant-pollinators are regularly discovered and researchers should keep an open mind when observing ant-activity at flowers. Although rarely involving specialised interactions, the role of ant-pollination in facilitating self-pollination and promoting seed-set may be underestimated in habitats or under conditions where flying visitors are rare (Gómez *et al*, 1996). Often during pollination studies pollinators are identified on the basis of morphological matches and frequency of visitation. Visitation networks can correspond well with pollen transport networks (Forup *et al*, 2008), but pollinator efficiency is still rarely proved experimentally (Vega *et al*, 2009).

5.3 Future Directions

5.3a Identifying the chemical basis of ant-repellence

Determining the precise chemical basis of floral ant-repellence is essential in determining which traits have been selected for and which compounds influence ant behaviour. It is also useful if we hope to understand how ant-repellent traits influence the behaviour of potential pollinators.

Olfactory and tactile signals from pollen will usually originate in the oily pollenkitt layer (Pacini

& Hesse, 2005). Floral volatiles can be efficiently collected using dynamic headspace collection of floral volatiles or, alternatively, solid phase microextraction. As floral ant-repellence is often only effective at very close range, all collection of volatiles should be done with this in mind. Methodology should be specialised to capture highly volatile compounds that may only be present in sufficient concentrations very close to the anthers. This will involve selecting appropriate adsorbents, storing and processing samples with care, and the use of suitable GC-MS settings with experienced analysis of the output against an established library. Where possible this should be combined with sampling from the surface of the repellent area. Pollenkitt composition can be determined using GC-MS by first washing pollen very rapidly with small volumes of a solvent such as hexane so that pollenkitt components are removed and internal lipids are excluded (Dobson & Bergström, 2000). It is also possible to analyse the compounds in situ using portable gas chromatography machines like the zNose (e.g. Tholl *et al*, 2006). If the ant species are available for laboratory work, individual components of the floral scent bouquet can be tested to determine which are eliciting repellent behaviour.

The most useful species to examine are clearly those that have been shown to have a dramatic effect on ant behaviour; for example, the floral scent of *Randia monantha*, the upper petal surface of *Stachytarpheta jamaicensis* and the pollen of *Malvaviscus arboreus*. A good candidate from a temperate habitat would be *Viburnum tinus*, known to repel a variety of native and non-native ant species. Comparisons between a larger number of *Acacia* species would build on what is already known about E,E- α -farnesene and test predictions concerning the homologous nature of floral ant-repellence within the group.

5.3b Identifying the role of coevolution in selection for ant-repellence

Testing interactions between flowers and novel plant species, with which there has been little or no coevolutionary history, will help us to understand how essential coevolution is in forming ant-repellent traits. Invasive ant activity at flowers provides natural experimental conditions for the study of novel interactions between potentially harmful ants and flowers.

The inclusion of native nectar-thieves in comparisons between invasive species and native

ants will be essential. Native species closest in habit to invasive species like *L. humile*, however, are unfortunately usually the first to be out-competed in invaded habitats, making comparisons in any particular area problematic. Only a small number of native ant species have been shown to be able to defend against *L. humile*, such as *Prenolepis imparis* (Formicinae) in North America that can kill *L. humile* with chemicals produced by the Dufour's gland (Sorrells *et al*, 2011). Alternatively, such practical difficulties can be overcome by the identification of populations of targeted plant species both inside and outside the invasive's range. Combined with chemical analysis of the compounds responsible, such trials will teach us a great deal about both selective pressures on floral ant-repellent traits and the specificity of ant sensory responses to particular compounds. They could also be used to investigate the responses of workers, or possibly different castes, to chemical stimuli at different times of day.

Additionally, it is also essential to find more examples of non-pollinating ants significantly effecting nectar volumes and therefore plant fitness. While it may be easier to identify cases involving invasive ant species, similar situations may also be found where native ants inflict costs, but do so only rarely, or selection for repellent traits is limited by other factors.

5.3c Identifying when and where ant scent-marks are informative in bee foraging decisions

While selection for ant-repellent floral traits may occur in species where ant-visitation could impose costs on bee visitors, bees are unlikely to avoid ant scent-marks experimentally deposited on the ant-repellent flowers, as bees will not associate the novel scent-marks with any costs. Much of the time costs will not be imposed on both partners as some plant species suffer no fitness costs from lower quality of visitation. Similarly when a plant species does suffer a fitness cost its pollinators may not suffer significant costs, especially if floral handling time is very low.

So to determine if, when and where ant scent-marks do influence bee behaviour the search should be focused on plant species where the costs ants could impose in terms of visits to individual flowers are maximised. Ideally, this will involve the study of flowers with low nectar volumes, long handling times and high local densities. Such flowers will be effectively drained by ants, involve a large

time-investment per flower on the part of a pollinator but not impose a great cost in terms of inter-flower movement. However, flowers with longer handling times typically have larger nectar volumes so compromises may have to be made when selecting study species. Avoidance of ant scent-marks may also be more common in habitats rich in ant numbers, like the tropics. Where ant visitation to flowers is more common, bees will be more likely to learn that ant scent-marks are an indicator of poor reward levels.

Such studies should be supported by work with captive bee colonies. Simple choice tests, where bees can choose between rewarding sugar sources and ant scent-marked unrewarding sources would easily demonstrate whether bees are capable of utilising the scent-marks in foraging decisions. Artificial flowers with low nectar volumes, long handling times and at high densities may then be used to test for the inclusion of ant-scent in bee foraging decisions under more realistic conditions.

5.4 Summary

As we expand our understanding of interactions between plants and animals it is becoming increasingly clear that it is essential to take into account all potential players. In pollination ecology this involves accounting for all pollinators, thieves, herbivores, predators and microbes that commonly interact with the flowers, as well as other flowering plants in the area. Many of these interactions have been overlooked in the past due to the understandable logistical constraints involved. Even basic tasks, like discerning a species' most efficient pollinator, involve time-consuming experiments. However, only by determining the nature of such interactions can we hope to understand how the different species have shaped floral evolution and how important the individual species are under a range of conditions. The fascinating role of ants in flower interactions should be considered an essential component of pollination ecology.

Bibliography

- Abramson CI (1986) Averse conditioning in honeybees (*Apis mellifera*). *Journal of Comparative Psychology* 100: 108-116.
- Adler LS (2000) The ecological significance of toxic nectar. *Oikos* 91: 409-420.
- Adler LS, Irwin RE (2005) Ecological costs and benefits of defences in nectar. *Ecology* 86: 2968-2978.
- Agarwal VM, Rastogi N (2008) Role of floral repellents in the regulation of flower visits of extrafloral nectary-visiting ants in an Indian crop plant. *Ecological Entomology* 33: 59-65.
- Agrawal AA (1998) Leaf damage and associated cues induce aggressive ant recruitment in a Neotropical ant-plant. *Ecology* 79: 2100-2112.
- Agrawal AA (2006) Macroevolution of plant defence strategies. *Trends in Ecology and Evolution* 22: 103-109.
- Agrawal AA, Fordyce JA (2000) Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. *Proceedings of the Royal Society of London B* 267: 1857-1861.
- Akino T, Morimoto M, Yamaoka R (2005) The chemical basis for trail recognition in *Lasius nipponensis* (Hymenoptera: Formicidae). *Chemoecology* 15: 13-20.
- Akino T, Yamaoka R (2005) Trail discrimination signal of *Lasius japonicus* (Hymenoptera: Formicidae). *Chemoecology* 15: 21-30.
- Alfaro-Tapia A, Verdugo JA, Astudillo LA, Ramírez CC (2007) Effect of epicuticular waxes of poplar hybrids on the aphid *Chaitophorus leucomelas* (Hemiptera: Aphididae). *Journal of Applied Entomology* 131: 486-492.
- Altshuler DL (1999) Novel interactions of non-pollinating ants with pollinators and fruit consumers in a tropical forest. *Oecologia* 119: 600-606.
- Amador-Vargas S (2008) Spartan defence in the Thermopylae Pass: Strategic defence by aggregations of *Pseudomyrmex spinicola* (Hymenoptera, Formicidae) on the trunks of *Acacia collinsii* (Mimosaceae). *Insectes Sociaux* 55: 241-245.
- Anikwe JC, Omoloye AA, Okelana FA (2007) Studies of the ant-plant mutualism in the Nigerian cocoa agroecology. *Middle-East Journal of Scientific Research* 2: 69-72.
- Apple JL, Feener DH (2001) Ant visitation of extrafloral nectaries of *Passiflora*: the effects of nectary attributes and ant behaviour on patterns in facultative ant-plant mutualisms. *Oecologia* 127: 409-416.
- Armbruster WS (1997) Exaptations link evolution of plant-herbivore and plant-pollinator interactions: a phylogenetic inquiry. *Ecology* 78: 1661-1672.
- Ashman T, Bradburn M, Cole DH, Blaney BH, Raguso RA (2005) The scent of a male: the role of floral volatiles in pollination of a gender dimorphic plant. *Ecology* 86: 2099-2105.
- Ashman T, King EA (2005) Are flower-visiting ants mutualists or antagonists? A study in a gynodioecious wild strawberry. *American Journal of Botany* 92: 891-895.
- Ashman T, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* 85: 2409-2421.
- Austerlitz F, Dick CW, Dutech C, Klein EK, Oddou-Muratorio, Smouse PE, Sork VL (2004) Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology* 13: 937-954.

- Axén AH, Leimar O, Hoffman V (1996) Signalling in a mutualistic interaction. *Animal Behaviour* 52: 321-333.
- Axén AH, Pierce NE (1998) Aggregation as a cost-reducing strategy for lycaenid larvae. *Behavioural Ecology* 9: 109-115.
- Baker HG, Baker I (1978) Ants and flowers. *Biotropica* 10: 80-80.
- Baldwin IT, Callahan P (1993) Autotoxicity and chemical defence: nicotine accumulation and carbon gain in Solanaceous plants. *Oecologia* 94: 534-541.
- Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA (2006) Volatile signalling in plant-plant interactions: "Talking Trees" in the Genomics Era. *Science* 311: 812-815.
- Banks CJ, Nixon HL (1958) Effects of the ant, *Lasius niger* L., on the feeding and excretion of the bean aphid, *Aphis fabae* Scop. *Journal of Experimental Biology* 35: 703-711.
- Barrows EM (1980) Robbing of exotic plants by introduced carpenter and honey bees in Hawaii, with comparative notes. *Biotropica* 12: 23-29.
- Bawa KS, Opler PA (1975) Dioecism in tropical trees. *Evolution* 29: 167-179.
- Beattie AJ, Turnbull C, Hough T, Jobson S, Knox RB (1985) The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *American Journal of Botany* 72: 606-614.
- Beattie AJ, Turnbull CL, Hough T, Knox RB (1986) Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). *Annals of the Entomological Society of America* 79: 448-450.
- Becerra JXI, Venable DL (1989) Extrafloral nectaries: a defence against ant-homoptera mutualisms? *Oikos* 55: 276-280.
- Bentley BL (1977) The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *The Journal of Ecology* 65: 27-38.
- Biesmeijer JC, Roberts SPM, Reemer M, Ohlemuller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351-354.
- Biernaskie JM, Cartar RV (2004) Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Functional Ecology* 18: 125-129.
- Biernaskie JM, Cartar RV, Hurly TA (2002) Risk-averse inflorescence departure in hummingbirds and bumble bees: could plants benefit from variable nectar volumes? *Oikos* 98: 98-104.
- Blancafort X, Gómez C (2005) Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of *Euphorbia characias* (L.) (Euphorbiaceae). *Acta Oecologica* 28: 49-55.
- Blüthgen N, Feldhaar H (2010) Food and shelter: how resources influence ant ecology. In *Ant Ecology* edited by Lach L, Parr CL, Abbott KL. Oxford University Press.
- Blüthgen N, Fiedler K (2004a) Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *Journal of Animal Ecology* 73: 155-166.
- Blüthgen N, Fiedler K (2004b) Competition for composition: lessons from nectar-feeding ant communities. *Ecology* 85: 1479-1485.
- Blüthgen N, Gebauer G, Fiedler K (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia* 137: 426-435.
- Blüthgen N, Gottsberger G, Fielder K (2004a) Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology* 29: 418-429.
- Blüthgen N, Mezger D, Linsenmair KE (2006) Ant-hemipteran trophobioses in a Bornean rainforest –

diversity, specificity and monopolisation. *Insectes Sociaux* 53: 194-203.

- Blüthgen N, Stork NE, Fiedler K (2004b) Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. 106: 344-358.
- Blüthgen N, Verhaagh M, Goitia W, Jaffé K, Morawetz W, Barthlott W (2000) How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. *Oecologia* 125: 229-240.
- Blüthgen N, Wesenberg J (2001) Ants induce domatia in a rain forest tree (*Vochysia vismiaeifolia*). *Biotropica* 33: 637-642.
- Bond W, Slingsby P (1984) Collapse of an ant-plant mutualism: the Argentine ant (*Iridomyrmex humilis*) and myrmecochorous Proteaceae. *Ecology* 65: 1031-1037.
- Bosch J, Retana J, Cerdá X (1997) Flowering phenology, floral traits and pollinator composition in a herbaceous Mediterranean plant community. *Oecologia* 109: 583-591.
- Bronstein JL (1986) The origin of bract liquid in a Neotropical *Heliconia* species. *Biotropica* 18: 111-114.
- Bronstein JL (1994) Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution* 9: 214-217.
- Bronstein JL (2001) The exploitation of mutualisms. *Ecology Letters* 4: 277-287.
- Brouat C, McKey D, Bessière J, Pascal L, Hossaert-McKey (2000) Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: preliminary results on *Leonardoxa* (Fabaceae: Caesalpinioideae) and *Petalomyrmex* (Formicidae: Formicinae). *Acta Oecologia* 21: 349-357.
- Brouat C, Garcia N, Andery C, McKey D (2001) Plant lock and ant key: pairwise coevolution of an exclusion filter in an ant-plant mutualism. *Proceedings of the Royal Society of London Series B* 268: 2131-2141.
- Bruna EM, Darrigo MR, Pacheco AMF, Vasconcelos HL (2008) Interspecific variation in the defensive responses of ant mutualists to plant volatiles. *Biological Journal of the Linnean Society* 94: 241-249.
- Bruna EM, Lapola DM, Vasconcelos HL (2004) Interspecific variation in the defensive responses of obligate plant-ants: experimental tests and consequences for herbivory. *Oecologia* 138: 558-565.
- Bryant JP, Provenza FD, Pastor J, Reichardt PB, Clausen TP, du Toit JT (1991) Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecological Systematics* 22: 431-446.
- Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review* 60: 83-139.
- Burkle LA, Irwin RE, Newman DA (2007) Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. *American Journal of Botany* 94: 1935-1943.
- Burns JG (2005) Impulsive bees forage better: the advantage of quick, sometimes inaccurate foraging decisions. *Animal Behaviour* 70: e1-e5.
- Búrquez A, Corbet SA (1991) Do flowers reabsorb nectar? *Functional Ecology* 5:369-379.
- Byk J, Del-Claro K (2010) Nectar- and pollen-gathering *Cephalotes* ants provide no protection against herbivory: a new manipulative experiment to test ant protective capabilities. *Acta Ethologica* 13: 33-38.
- Carlson JE, Harms KE (2007) The benefits of bathing buds: water calyces protect flowers from a microlepidopteran herbivore. *Biology Letters* 3: 405-407.
- Carroll CR, Janzen DH (1973) Ecology of foraging by ants. *Annual Review of Ecology and Systematics* 4: 231-257.

- Carter C, Thornburg RW (2004) Is the nectar redox cycle a floral defense against microbial attack? *Trends in Plant Science* 9: 320-324.
- Carvalheiro LG, Barbosa ERM, Memmott J (2008) Pollinator networks, alien species and the conservation of rare plants: *Trinia glauca* as a case study. *Journal of Applied Ecology* 45: 1419-1427.
- Castro S, Loureiro J, Santos C, Ater M, Ayensa G, Navarro L (2007) Distribution of flower morphs, ploidy level and sexual reproduction of the invasive weed *Oxalis pes-caprae* in the Western area of the Mediterranean region. *Annals of Botany* 99: 507-517.
- Castro S, Silveira P, Navarro L (2008) Consequences of nectar robbing for the fitness of a threatened plant species. *Plant Ecology* 199: 201-208.
- Castro S, Silveira P, Navarro L (2009) Floral traits variation, legitimate pollination, and nectar robbing in *Polygala vayredae* (Polygalaceae). *Ecological Research* 24: 47-55.
- Chamberlain SA, Holland (2008) Density-mediated, context-dependent consumer-resource interactions between ants and extrafloral nectar plants. *Ecology* 89: 1364-1374.
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237-268.
- Chittka L, Dyer AG, Bock F, Dornhaus A (2003) Bees trade off foraging speed for accuracy. *Nature* 424: 388.
- Chittka L, Skorupski P, Raine N (2009) Speed-accuracy tradeoffs in animal decision making. *Trends in Ecology and Evolution* 24: 400-407.
- Choh Y, Kugimiya S, Takabayashi J (2006) Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defence against spider mites. *Oecologia* 147: 455-460.
- Christian CE (2009) Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature* 413: 635-639.
- Cipollini D, Heil M (2010) Costs and benefits of induced resistance to herbivores and pathogens in plants. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 5: No. 005.
- Clement LW, Köppen SCW, Brand WA, Heil M (2008) Strategies of a parasite of the ant-*Acacia* mutualism. *Behavioural Ecology and Sociobiology* 62: 953-962.
- Cole FR, Medeiros AC, Loope LL, Zuehlke WW (1992) Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. *Ecology* 73: 1313-1322.
- Collins CM, Leather SR (2002) Ant-mediated dispersal of the black willow aphid *Pterocomma salicis* L.: does the ant *Lasius niger* L. judge aphid-host quality? *Ecological Entomology* 27: 238-241.
- Colwell RK (1995) Effects of nectar consumption by the hummingbird flower mite *Proctolaelaps kirmsei* on nectar availability in *Hamelia patens*. *Biotropica* 27: 206-217.
- Cook SC, Davidson DW (2006) Nutritional and functional biology of exudate-feeding ants. *Entomologia Experimentalis et Applicata* 118: 1-10.
- Cronin G (1998) Between-species and temporal variation in acacia-ant-herbivore interactions. *Biotropica* 30: 135-139.
- Cuautle M, Rico-Gray V (2003) The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Functional Ecology* 17: 417-423.
- Davidson DW (1997) The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biological Journal of the Linnean Society* 61: 153-181.

- Davidson DW, Cook SC, Snelling RR (2004) Liquid-feeding performances of ants (Formicidae): ecological and evolutionary implications. *Oecologia* 139: 255-266.
- Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationships. *Journal of Hymenopteran Research* 2: 13-83.
- Davidson DW, Snelling, Longino JT (1989) Competition among ants for myrmecophytes and the significance of plant trichomes. *Biotropica* 21: 64-73.
- de la Fuente MAS, Marquis RJ (1999) The role of ant-tended extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. *Oecologia* 118: 192-202.
- Defosse E, Selosse M, Mondolot L, Faccio A, Djieto-Lordon, McKey D, Blatrix R (2009) Ant-plants and fungi: a new threeway symbiosis. *New Phytologist* 182: 942-949.
- Dejean A, Grangier J, Leroy C, Orivel J (2009) Predation and aggressiveness in host plant protection: a generalization using ants from the genus *Azteca*. *Naturwissenschaften* 96: 57-63.
- Devigne C, Detrain C (2002) Collective exploration and area marking in the ant *Lasius niger*. *Insectes Sociaux* 49: 357-362.
- DeVries PJ, Baker I (1989) Butterfly exploitation of an ant-plant mutualism: adding insult to herbivory. *Journal of the New Entomological Society* 97: 332-340.
- Díaz-Castelazo C, Rico-Gray V, Ortega F, Ángeles (2004) Morphological and secretory characterization of extrafloral nectaries in plants of coastal Veracruz, Mexico. *Annals of Botany* 96: 1175-1189.
- Djieto-Lordon C, Dejean A (1999) Tropical arboreal ant mosaics: innate attraction and imprinting determine nest site selection in dominant ants. *Behavioural Ecology and Sociobiology* 45: 219-225.
- Djieto-Lordon C, Dejean A, Gibernau M, Hossaert-McKey M, McKey D (2004) Symbiotic mutualism with a community of opportunistic ants: protection, competition, and ant occupancy of the myrmecophyte *Barteria nigritana* (Passifloraceae). *Acta Oecologica* 26: 109-116.
- Djieto-Lordon C, Dejean A, Ring RA, Nkongmeneck BA, Lauga J, McKey D (2005). Ecology of an impossible association: the Pseudomyrmecine plant-ant *Tetraponera tessmanni* and the myrmecophytic lianar *Vitex thyrsoflora* (Lamiaceae) in Cameroon. *Biotropica* 37: 421-430.
- Dobson HEM, Bergström G (2000) The ecology and evolution of pollen odors. *Plant Systematics and Evolution* 222: 63-87.
- Domínguez CA, Dirzo R, Bullock SH (1989) On the function of floral nectar in *Croton suberosus* (Euphorbiaceae). *Oikos* 56: 109-114.
- Dreisig H (2000) Defence by exploitation in the Florida carpenter ant, *Camponotus floridanus*, at an extrafloral nectar resource. *Behavioural Ecology and Sociobiology* 47: 274-279.
- Duffield RM, Blum MS (1975) Methyl 6-methyl salicylate: identification and function in a ponerine ant (*Gnamptogenys pleurodon*). *Cellular and Molecular Life Sciences* 31: 466.
- Dukas R (2001) Effects of perceived danger on flower choice by bees. *Ecology Letters* 4: 327-333.
- Dukas R (2005) Bumble bee predators reduce pollinator density and plant fitness. *Ecology* 86: 1401-1406.
- Dukas R, Morse DH (2003) Crab spiders affect flower visitation by bees. *Oikos* 101: 157-163.
- Dworschak K, Blüthgen N (2010) Networks and dominance hierarchies: does interspecific aggression explain flower partitioning among stingless bees? *Ecological Entomology* 35: 216-225.
- Dyer LA, Dodson CD, Beihoffer J, Letourneau DK (2001) Trade-offs in antiherbivore defences in *Piper cenocladum*: Ant mutualists versus plant secondary metabolites. *Journal of Chemical Ecology* 27: 581-592.

- Eck G, Fiala B, Linsenmair KE, Bin Hashim, R, Proksch P (2001) Trade-off between chemical and biotic antiherbivore defence in the South East Asian plant genus *Macaranga*. *Journal of Chemical Ecology* 27: 1979-1996.
- Edwards DP (2009) The roles of tolerance in the evolution, maintenance and breakdown of mutualism. *Naturwissenschaften* 96: 1137-1145.
- Edwards DP, Andell FA, Woodcock P, Fayle TM, Chey VK, Hamer KC (2010) Can the failure to punish promote cheating in mutualism? *Oikos* 119: 45-52.
- Edwards DP, Frederickson ME, Shepard GH, Yu DW (2009) A plant needs ants like a dog needs fleas: *Myrmelachista schumanni* ants gall many tree species to create housing. *The American Naturalist* 174: 734-739.
- Edwards DP, Hassall M, Sutherland WJ, Yu DW (2006) Assembling a mutualism: ant symbionts locate their host plants by detecting volatile chemicals. *Insectes Sociaux* 53: 172-176.
- Eilms S, Heil M (2009) Bacterial associates of arboreal ants and their putative functions in an obligate ant-plant mutualism. *Applied and Environmental Microbiology* 75: 4324-4332.
- Eltz T (2006) Tracing pollinator footprints on natural flowers. *Journal of Chemical Ecology* 32: 907-915.
- Engel V, Fischer MK, Wäckers FL, Völkl W (2001) Interactions between extrafloral nectaries, aphids and ants: are there competitive effects between plant and Homopteran sugar sources? *Oecologia* 129: 577-584.
- Evison SEF, Petchey OL, Beckerman AP, Ratnieks FLW (2008) Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. *Behavioural Ecology and Sociobiology* 63: 261-267.
- Faegri & van der Pijl (1979) *The Principles of Pollination Biology*. Pergamon Press, Oxford.
- Federle W, Maschwitz, Fiala B, Riederer M, Hölldobler B (1997) Slippery ant-plants and skillful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). *Oecologia* 122: 217-224.
- Federle W, Riehle M, Curtis ASG, Full RJ (2002) An integrative study of insect adhesion: mechanics and wet adhesion of pretarsal pads in ants. *Integrative and Comparative Biology* 42: 1100-1106.
- Federle W, Rheindt FE (2005) *Macaranga* ant-plants hide food from intruders: correlation of food presentation and presence of wax barriers analysed using phylogenetically independent contrasts. *Biological Journal of the Linnean Society* 84: 177-193.
- Federle W, Rohrseitz K, Hölldobler B (2000) Attachment forces of ants measured with a centrifuge: better 'wax runners' have poorer attachment to a smooth surface. *The Journal of Experimental Biology* 203: 505-512.
- Feinsinger P, Swann LA (1978) How common are ant-repellent nectars? *Biotropica* 10: 238-239.
- Fernández-Marin H, Zimmerman JK, Rehner SA, Wcislo WT (2006) Active use of the metapleural glands by ants in controlling fungal infection. *Proceedings of the Royal Society London B* 273: 1689-1695.
- Ferrière R, Gauduchon M, Bronstein JL (2007) Evolution and persistence of obligate mutualists and exploiters: competition for partners and evolutionary immunization. *Ecology Letters* 10: 115-126.
- Fiala B, Jakob A, Maschwitz U, Linsenmair KE (1999) Diversity, evolutionary specialization and geographic distribution of a mutualistic ant-plant complex: *Macaranga* and *Crematogaster* in South East Asia. *Biological Journal of the Linnean Society* 66: 305-331.
- Fiala B, Maschwitz U (1992) Domatia as most important adaptations in the evolution of myrmecophytes in the paleotropical tree genus *Macaranga* (Euphorbiaceae). *Plant Systematics and Evolution* 180: 53-

- Fiedler K, Maschwitz U (1989) The symbiosis between the weaver ant, *Oecophylla smaragdina*, and *Anthe emolus*, an obligate myrmecophilous butterfly. *Journal of Natural History* 23: 833-846.
- Fincher RM, Dyer LA, Dodson CD, Richards JL, Tobler MA, Mather JE, Reid AJ, Rolig JS, Pidcock W (2008) Inter- and intraspecific comparisons of antiherbivore defences in three species of rainforest understory shrubs. *Journal of Chemical Ecology* 34: 1573-1561.
- Floate KD, Whitham TG (1994) Aphid-ant interaction reduces Chrysomelid herbivory in a cottonwood hybrid zone. *Oecologia* 97: 215-221.
- Folgarait PJ, Johnson HL, Davidson DW (1994) Responses of *Cecropia* to experimental removal of Müllerian bodies. *Functional Ecology* 8: 22-28.
- Fonseca CR, Ganade G (1996) Asymmetries, compartments and null interactions in an Amazonian ant-plant community. *Journal of Animal Ecology* 65: 339-347.
- Forister ML, Gompert Z, Nice CC, Forister GW, Fordyce JA (2010) Ant association facilitates the evolution of diet breadth in a lycaenid butterfly. *Proceedings of the Royal Society London B*: online.
- Forup ML, Henson KSE, Craze PG, Memmott J (2008) The restoration of ecological interactions: plant-pollinator networks on ancient and restored heathlands. *Journal of Applied Ecology* 45: 742-752.
- Fowler SV, MacGarvin M (1985) The impact of hairy wood ants, *Formica lugubris*, on the guild structure of herbivorous insects on birch, *Betula pubescens*. *Journal of Animal Ecology* 54: 847-855.
- Frederickson ME (2005) Ant species confer different partner benefits on two Neotropical myrmecophytes. *Oecologia* 143 387-395.
- Frederickson ME (2009) Conflict over reproduction in an ant-plant symbiosis: why *Allomerus octoarticulatus* ants sterilize *Cordia nodosa* trees. *The American Naturalist* 173: 675-681.
- Frederickson ME, Greene MJ, Gordon DM (2005) 'Devil's gardens' bedevilled by ants. *Nature* 437: 495-496.
- Freitas AVL, Oliveira PS (1996) Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. *Journal of Animal Ecology* 65: 205-210.
- Frey M, Stettner C, Paré W, Schmelz EA, Tumlinson JH, Gierl A (2000) An herbivore elicitor activates the gene for indole emission in maize. *Proceedings of the National Academy of Sciences* 97: 14801-14806.
- Fritz RS, Morse DH (1981) Nectar parasitism of *Asclepias syriaca* by ants: effect on nectar levels, pollinia insertion, pollinaria removal and pod production. *Oecologia* 50: 316-319.
- Galen C (1983) The effects of nectar thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. *Oikos* 41: 245-249.
- Galen C (1985) Regulation of seed-set in *Polemonium viscosum*: floral scents, pollination, and resources. *Ecology* 66: 792-797.
- Galen C (1999) Flowers and enemies: predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. *Oikos* 85: 426-434.
- Galen C, Butchart B (2003) Ants in your plants: effects of nectar-thieves on pollen fertility and seed-siring capacity in the alpine wildflower, *Polemonium viscosum*. *Oikos* 101: 521-528.
- Galen C, Cuba J (2001) Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. *Evolution* 55: 1963-1971.
- Galen C, Kaczorowski R, Todd SL, Geib J, Raguso RA (2011) Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists, and the potential for floral evolution in the alpine skypilot *Polemonium viscosum*. *The American Naturalist* 177: online.

- Galen C, Zimmer KA, Newport ME (1987) Pollination in floral scent morphs of *Polemonium viscosum*: A mechanism for disruptive selection on flower size. *Evolution* 41: 599-606.
- Gawleta N, Zimmermann Y, Eltz T (2005) Repellent foraging scent recognition across bee families. *Apidologie* 36: 325-330.
- Gegear RJ, Manson JS, Thomson JD (2007) Ecological context influences pollinator deterrence by alkaloids in floral nectar. *Ecology Letters* 10: 375-382.
- George N, Byrne M, Yan G (2008) Mixed mating with preferential outcrossing in *Acacia saligna* (Labill.) H. Wendl. (Leguminosae: Mimosoideae). *Silvae Genetica* 57: 139-145.
- Gentry G (2003) Multiple parasitoid visitors to the extrafloral nectaries of *Solanum adherens*. Is *S. adherens* an insectary plant? *Basic Applied Ecology* 4: 405-411.
- Ghazoul J (2001) Can floral repellents pre-empt potential ant-plant conflicts? *Ecology Letters* 4: 295-299.
- Gilbert F, Azmeh S, Barnard C, Behnke J, Collins SA, Hurst J, Shuker & The Behavioural Ecology Field Course. (2001) Individually recognizable scent marks on flowers made by a solitary bee. *Animal Behaviour* 61: 217-229.
- Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: The Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences* 99: 6075-6079.
- Giurfa M, Núñez J (1992) Honeybees mark with scent and reject recently visited flowers. *Oecologia* 89: 113-117.
- Giurfa M, Núñez J, Backhaus (1994) Odour and colour information in the foraging choice behaviour of the honeybee. *Journal of Comparative Physiology A* 175: 773-779.
- Golubov J, Mandujano MC, Montaña C, López-Portillo J, Eguiarte LE (2004) The demographic costs of nectar production in the desert perennial *Prosopis glandulosa* (Mimosoideae): a modular approach. *Plant Ecology* 170: 267-275.
- Gómez JM (2000) Effectiveness of ants as pollinators of *Lobularia maritima*: effects on main sequential fitness components of the host plant. *Oecologia* 122: 90-97.
- Gómez JM, Zamora R (1992) Pollination by ants: consequences of the quantitative effects on a mutualistic system. *Oecologia* 91: 410-418.
- Gómez JM, Zamora R, Hódar JA, García D (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia* 105: 236-242.
- Gonçalves-Souza T, Omena PM, Souza JC, Romero GQ (2008) Trait-mediated effects on flowers: Artificial spiders deceive pollinators and decrease plant fitness. *Ecology* 89: 2407-2413.
- González-Teuber M, Heil M (2009) The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *Journal of Chemical Ecology* 35: 459-468.
- González-Teuber M, Pozo MJ, Muck A, Svatos, Adame-Alvarez RM, Heil M (2010) Glucanases and chitinases as causal agents in the protection of *Acacia* extrafloral nectar from infestation by phytopathogens. *Plant Physiology* 152: 1705-1715.
- Goulson D, Hawson SA, Stout JC (1998) Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. *Animal Behaviour* 55:199–206.
- Goulson D, Stout JC, Langley J, Hughes WHO (2000) The identity and function of scent marks deposited by foraging bumblebees. *Journal of Chemical Ecology* 26: 2897-2911.
- Grangier J, Dejean A, Malé PG, Orivel J (2008) Indirect defence in a highly specific ant-plant mutualism. *Naturwissenschaften* 95: 1432-1904.
- Grangier J, Dejean A, Malé PG, Solano P, Orivel J (2009) Mechanisms driving the specificity of a

myrmecophyte-ant association. *Biological Journal of the Linnean Society* 97: 90-97.

- Grover CD, Dayton KC, Menke SB, Holway DA (2008) Effects of aphids on foliar foraging by Argentine ants and the resulting effects on other arthropods. *Ecological Entomology* 33: 101-106.
- Guerra TJ, Romero GQ, Benson WW (2010) Flower mites decrease nectar availability in the rain-forest bromeliad *Neoregelia johannis*. *Journal of Tropical Ecology* 26: 373-379.
- Guerrant EO, Fiedler PL (1981) Flower defences against nectar-pilferage by ants. *Biotropica* 13: 25-33.
- Guimarães PR, Rico-Gray V, Furtado dos Reis S, Thompson JN (2006) Asymmetries in specialization in ant-plant mutualistic networks. *Proceedings of the Royal Society of London B* 273: 2041-2047.
- Guimarães PR, Rico-Gray V, Furtado dos Reis S, Thompson JN (2007) Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. *Current Biology* 17: 1797-1803.
- Haber WA, Frankie GW, Baker HG, Baker I, Koptur (1981) Ants like floral nectar. *Biotropica* 13: 211-214.
- Hansen DM, Müller CB (2009) Invasive ants disrupt gecko pollination and seed dispersal of the endangered plant *Roussea simplex* in Mauritius. *Biotropica* 41: 202-208.
- Hargreaves AL, Harder LD, Johnson SD (2009) Consumptive emasculation: the ecology and evolutionary consequences of pollen theft. *Biological Review* 84: 259-276.
- Harriss FCL, Beattie AJ (1991) Viability of pollen carried by *Apis mellifera* L., *Trigona carbonaria* Smith and *Vespula germanica* (F.) (Hymenoptera: Apidae, Vespidae). *Journal of Australian Entomological Society* 30: 45-47.
- Heil M (2002) Ecological costs of induced resistance. *Current Opinion in Plant Biology* 5: 345-350.
- Heil M, Baldwin IT (2002) Fitness costs of induced resistance: Emerging experimental support for a slippery concept. *Trends in Plant Science* 7: 61-67.
- Heil M, Delsinne T, Hilpert A, Schürkens S, Andary C, Linsenmair KE, Sousa M, McKey D (2002) Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos* 99: 457-468.
- Heil M, Fiala B, Linsenmair KE, Boller T (1999) Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften* 86: 146-149.
- Heil M, Fiala B, Maschwitz U, Linsenmair KE (2001a) On benefits of indirect defence: Short- and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia* 126: 395-403.
- Heil M, McKey D (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution and Systematics* 34: 425-453.
- Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M (2009) Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Science* 106: 18091-18096.
- Heil M, Hilpert A, Fiala B, Linsenmair KE (2001b) Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia* 126: 404-408.
- Heil M, Rattke J, Boland W (2005) Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308: 560-563.
- Heil M, Staehelin C, McKey D (2000) Low chitinase activity in *Acacia* myrmecophytes: a potential trade-off between biotic and chemical defences? *Naturwissenschaften* 87: 555-558.
- Helms KR, Vinson SB (2002) Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. *Ecology* 83: 2425-2438.
- Henson KSE (2003) An investigation into the role of floral volatiles on the behaviour of Formicinae ants. Undergraduate dissertation, University of St Andrews.

- Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* 67: 283-335.
- Herrera CM, García IM, Pérez R (2008) Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology* 89: 2369-2376.
- Herrera CM, Herrera J, Espadaler X (1984) Nectar-thievery by ants from southern Spanish insect-pollinated flowers. *Insectes Sociaux* 31: 142-154.
- Herrera CM, Pérez R, Alonso C (2006) Extreme intraplant variation in nectar sugar composition in an insect-pollinated perennial herb. *American Journal of Botany* 93: 575-581.
- Hespenheide HA (1985) Insect visitors to extrafloral nectaries of *Byttneria aculeata* (Sterculiaceae): relative importance and roles. *Ecological Entomology* 10: 191-204.
- Hickman JC (1974) Pollination by ants: a low-energy system. *Science* 184: 1290-1292.
- Hodson AK, Gastreich KR (2006) Evidence for a novel mutualism in the tropical understory shrub *Piper urostachyum*. *Biotropica* 38: 127-131.
- Hojo MK, Wada-Katsumata A, Ozaki M, Yamaguchi S, Yamaoka R (2008) Gustatory synergism in ants mediates a species-specific symbiosis with lycaenid butterflies. *Journal of Comparative Physiology A* 194: 1043-1052.
- Holland JN, Chamberlain SA, Horn KC (2009) Optimal defence theory predicts investment in extrafloral nectar resources in an ant-plant mutualism. *Journal of Ecology* 97: 89-96.
- Holway DAH (1998) Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. *Oecologia* 116: 252-258.
- Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33: 181-233.
- Horvitz CC, Schemske DW (1990) Spatiotemporal variation in insect mutualists of a Neotropical herb. *Ecology* 71: 1085-1097.
- Howell AD, Alarcón R (2007) *Osmia* bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Animal Behaviour* 74: 199-205.
- Hrncir M, Jarau S, Zucchi R, Barth FG (2004) On the origin and properties of scent marks deposited at the food source by a stingless bee, *Melipona seminigra*. *Apidologie* 35: 3-13.
- Huang HT, Yang P (1987) The ancient cultured citrus ant. *BioScience* 37: 665-671.
- Hubbell SP, Howard JT, Wiemer DF (1984) Chemical leaf repellency to an Attine ant: Seasonal distribution among potential host plant species. *Ecology* 65: 1067-1076.
- Huhta A, Hellström K, Rautio P, Tuomi (2003) Grazing tolerance of *Gentianella amarella* and other monocarpic herbs: Why is tolerance highest at low damage levels? *Plant Ecology* 166: 49-61.
- Hull DA, Beattie AJ (1988) Adverse effects on pollen exposed to *Atta texana* and other North American ants: Implications for ant pollination. 75: 153-155.
- Human KG, Gordon DM (1997) Effects of Argentine ants on invertebrate biodiversity in Northern California. *Conservation Biology* 11: 1242-1248.
- Huntzinger M, Karban R, Young TP, Palmer TM (2004) Relaxation of induced indirect defences of acacias following exclusion of mammalian herbivores. *Ecology* 85: 609-614.
- Ings TC, Chittka L (2008) Speed-accuracy tradeoffs and false alarms in bee responses to cryptic predators. *Current Biology* 18: 1520-1524.
- Inouye DW (1980) The terminology of floral larceny. *Ecology* 61: 1251-1253.
- Inui Y, Itioka T (2007) Species-specific leaf volatile compounds of obligate *Macaranga* myrmecophytes

and host-specific aggressiveness of symbiotic *Crematogaster* ants. *Journal of Chemical Ecology* 33: 2054-2063.

- Inui Y, Itioka T, Murase K, Yamaoka R, Itino T (2001) Chemical recognition of partner plant species by foundress ant queens in *Macaranga-Crematogaster* myrmecophytism. *Journal of Chemical Ecology* 27: 2029-2040.
- Irwin RE (2003) Impact of nectar robbing on estimates of pollen flow: Conceptual predictions and empirical outcomes. *Ecology* 83: 485-495.
- Irwin RE, Adler LS, Brody AK (2004) The dual role of floral traits: pollinator attraction and plant defense. *Ecology* 85: 1503-1511.
- Irwin RE, Brody AK (1999) Nectar-robbing bumblebees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* 80: 1703-1712.
- Irwin RE, Brody AK (2000) Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* 81: 2637-2643.
- Irwin RE, Brody AK, Waser NM (2001) The impact of floral larceny on individuals, populations and communities. *Oecologia* 129: 161-168.
- Ishii HS, Hirabayashi Y, Kudo G (2008) Combined effects of inflorescence architecture, display size, plant density and empty flowers on bumble bee behaviour: experimental study with artificial flowers. *Oecologia* 156: 341-350.
- Itino T, Itioka T (2001) Interspecific variation and ontogenetic change in antiherbivore defense in myrmecophytic *Macaranga* species. *Ecological Research* 16: 765-774.
- Izzo TJ, Vasconcelos HL (2002) Cheating the cheater: domatia loss minimizes the effects of ant castration in an Amazonian ant-plant. *Oecologia* 133: 200-205.
- Jackson DE, Martin SJ, Ratnieks FLW, Holcombe M (2007) Spatial and temporal variation in pheromone composition of ant foraging trails. *Behavioural Ecology* 18: 444-450.
- Jaffé K, Hernandez JV, Goitia W, Osio A, Osborn F, Cerda H, Arab A, Rincones J, Gajardo R, Caraballo L, Andara C, Lopez H (2003) Flower ecology in the neotropics: A flower-ant love-hate relationship. *Arthropods of Tropical Forests*. Cambridge University Press. 231-219.
- James DG, Stevens MM, O'Malley KJ, Faulder RJ (1999) Ant foraging reduces the abundance of beneficial and incidental arthropods in citrus canopies. *Biological Control* 14: 121-126.
- Janzen DH (1973) Dissolution of mutualism between *Cecropia* and its *Azteca* ants. *Biotropica* 5: 15-28.
- Janzen DH (1977) Why don't ants visit flowers? *Biotropica* 9: 252.
- Janzen DH (1985) On ecological fitting. *Oikos* 45: 308-310.
- Jaramillo MA, Manos PS (2001) Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *American Journal of Botany* 88: 706-716.
- Jarau S, Hrncir M, Zucchi R, Barth FG (2005) Morphology and structure of the tarsal glands of the stingless bee *Melipona seminigra*. *Naturwissenschaften* 92: 147-150.
- Jeanson R, Ratnieks FW, Deneubourg J (2003) Pheromone trail decay rates on different substrates in the Pharaoh's ant, *Monomorium pharaonis*. *Physiological Entomology* 28: 192-198.
- Johnson SD, Hargreaves AL, Brown M (2006) Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology* 87: 2709-2716.
- Jones CE, Atallah YC, Shropshire FM, Luttrell J, Walker SE, Sandquist DR, Allen RL, Burk JH, Song Jr, LC (2010) Do native ants play a significant role in the reproductive success of the rare San Fernando Valley Spineflower, *Chorizanthe parryi* var. *fernandina* (Polygonaceae)? *Madroño* 57: 161-169.

- Josens RB, Farina WM, Roces F (1998) Nectar feeding by the ant *Camponotus mus*: Intake rate and crop filling as a function of sucrose concentration. *Journal of Insect Physiology* 44: 579-585.
- Junker RR, Bleil R, Daehler C, Blüthgen N (2010a) Responses to olfactory signals reflect network structure of flower-visitor interactions. *Journal of Animal Ecology* 79: 818-823.
- Junker RR, Blüthgen N (2008) Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research* 10: 295-308.
- Junker RR, Blüthgen N (2010) Floral scents repel facultative flower visitors, but attract obligate ones. *Annals of Botany* 105: 777-782.
- Junker RR, Chung AYC, Blüthgen N (2007) Interaction between flowers, ants and pollinators: additional evidence for floral repellence against ants. *Ecological Research* 22: 665-670.
- Junker RR, Daehler C, Bleil R, Blüthgen N, (2010b) COS 122-2: Defensive floral traits shape ant-flower networks in Hawai'i: Patterns and consequences for endemic species. 95th ESA Annual Meeting.
- Jürgens A, Feldhaar H, Feldmeyer B, Fiala B (2006) Chemical composition of leaf volatiles in *Macaranga* species (Euphorbiaceae) and their potential role as olfactory cues in host-localization of foundress queens of specific ant partners. *Biochemical Systematics and Ecology* 34: 97-113.
- Kaplan I, Eubanks MD (2005) Aphids alter the community-wide impact of fire ants. *Ecology* 86: 1640-1649.
- Kato M (1988) Bumblebee visits to *Impatiens* spp.: pattern and efficiency. *Oecologia* 76: 364-370.
- Kautz S, Lumbsch T, Ward PS, Heil M (2009) How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners. *Evolution* 63: 839-853.
- Keeler KH (1989) Ant-plant interactions. In *Plant-animal interactions*, ed. Abrahamson WG: 207-242. New York: McGraw-Hill.
- Kelly CA (1986) Extrafloral nectaries: Ants, herbivores and fecundity in *Cassia fasciculata*. *Oecologia* 69: 600-605.
- Kenrick J, Knox RB (1982) Function of the polyad in reproduction of *Acacia*. *Annals of Botany* 50: 721-727.
- Kerner AJ (1878, reprinted 2008) *Flowers and their Unbidden Guests*. Biblio Bazaar.
- Kessler A, Halitschke R (2009) Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. *Functional Ecology* 23: 901-912.
- Kessler D, Baldwin IT (2006) Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. *The Plant Journal* 49: 840-854.
- Kessler D, Diezel C, Baldwin IT (2010) Changing pollinators as a means of escaping herbivores. *Current Biology* 20: 237-242.
- Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudas MR, Johnston MO, Mitchell RJ, Ashman (2005) Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology and Evolutionary Systematics* 36: 467-497.
- Knudsen JT, Eriksson R, Gershenzon J, Ståhl (2006) Diversity and distribution of floral scent. *The Botanical Review* 72: 1-120.
- Koopowitz H, Marchant TA (1998) Postpollination nectar reabsorption in the African epiphyte *Aerangis verdickii* (Orchidaceae). *American Journal of Botany* 85: 508-512.
- Koptur S (1985) Alternative defences against herbivores in *Inga* (Fabaceae: Mimosideae) over an elevational gradient. *Ecology* 66: 1639-1650.
- Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. in E. Bernays,

editor. Insect–Plant Interactions. Volume IV: 81–129. CRC Press, Boca Raton, Florida, USA.

- Koptur S, Truong N (1998) Facultative ant-plant interactions: nectar sugar preferences of introduced pest ant species in South Florida. *Biotropica* 30: 179-189.
- Kost C, Heil M (2005) Increased availability of extrafloral nectar reduces herbivory in lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology* 6: 237-248.
- Kost C, Heil M (2006) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology* 94: 619-628.
- Lach L (2008a) Argentine ants displace floral arthropods in a biodiversity hotspot. *Diversity and Distributions* 14: 281-290.
- Lach L (2008b) Floral visitation patterns of two invasive ant species and their effects on other hymenopteran visitors. *Ecological Entomology* 33: 155-160.
- Lach L, Hobbs RJ, Majer JD (2009) Herbivory-induced extrafloral nectar increases native and invasive ant worker survival. *Population Ecology* 51: 237-243.
- Lach L, Hoffman BD (2011) Are invasive ants better plant-defense mutualists? A comparison of foliage patrolling and herbivory in sites with invasive yellow crazy ants and native weaver ants. *Oikos* 120: 9-16.
- Lach L, Thomas ML (2008) Invasive ants in Australia: documented and potential ecological consequences. *Australian Journal of Entomology* 47: 275-288.
- Lanza J (1988) Ant preferences for *Passiflora* nectar mimics that containing amino acids. *Biotropica* 20: 341-344.
- LaPierre L, Hespenheide H, Dejean A (2007) Wasps robbing food from ants: a frequent behaviour? *Naturwissenschaften* 94: 997-1001.
- Lapola DM, Bruna EM, Vasconcelos HL (2003) Contrasting responses to induction cues by ants inhabiting *Maieta guianensis* (Malastomataceae). *Biotropica* 35: 295-300.
- Lara C, Ornelas JF (2001a) Preferential nectar robbing of flowers with long corollas: Experimental studies of two hummingbird species visiting three plant species. *Oecologia* 128: 263-273.
- Lara C, Ornelas JF (2001b) Nectar “theft” by hummingbird flower mites and its consequences for seed set in *Moussonia deppena*. *Functional Ecology* 15: 78-84.
- Lara C, Ornelas JF (2002) Effects of nectar theft by flower mites on hummingbird behaviour and the reproductive success of their host plant, *Moussonia deppena* (Gesneriaceae). *Oikos* 96: 470-480.
- Leal IR, Fischer E, Kost C, Tabarelli M, Wirth R (2006) Ant protection against herbivores and nectar thieves in *Passiflora coccinea* flowers. *Ecoscience* 13: 431-438.
- Lehmberg L, Dworschak K, Blüthgen N (2008) Defensive behaviour and chemical deterrence against ants in the stingless bee genus *Trigona* (Apidae, Meliponini). *Journal of Apicultural Research and Bee World* 47: 17-21.
- Leiss KA, Vrieling K, Klinkhamer PGL (2004) Heritability of nectar production in *Echium vulgare*. *Heredity* 92: 446-451.
- Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR (2009) Ants sow the seeds of global diversification in flowering plants. *PLoS ONE* 4: e5480.
- Lennartsson T (2002) Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. *Ecology* 83:3060-3072.
- Léotard G, Saltmarsh A, Kjellberg F, McKey D (2008) Mutualism, hybrid inviability and speciation in a tropical ant-plant. *Journal of Evolutionary Biology* 21: 1133-1143.
- Letourneau DK (1983) Passive aggression: an alternative hypothesis for the *Piper-Pheidole* association.

Oecologia 60: 122-126.

- Letourneau DK (1990) Code of ant-plant mutualism broken by parasite. *Science* 248: 215-217.
- Limburg DD, Rosenheim JA (2001) Extrafloral nectar consumption and its influence on survival and development of an omnivorous predator, larval *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). *Environmental Entomology* 30: 595-604.
- Liu F, Zhang X, Chai J, Yang D (2006) Pollen phenolics and regulation of pollen foraging in honeybee colony. *Behavioural Ecology and Sociobiology* 59: 582-588.
- Llandres AL, Rodríguez-Gironés, Dirzo R (2010) Plant stages with biotic, indirect defences are more palatable and suffer less herbivory than their undefended counterparts. *Biological Journal of the Linnean Society* 101: 536-543.
- Luyt R, Johnson SD (2002) Postpollination nectar reabsorption and its implications for fruit quality in an epiphytic orchid. *Biotropica* 34: 442-446.
- Machado CA, Jouselin E, Kjellberg F, Compton SG, Herre EA (2001) Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proceedings of the Royal Society London B* 268: 685-694.
- Machado IC, Lopes AV, Sazima M (2006) Plant sexual systems and a review of the breeding system studies in the Caatinga, a Brazilian tropical dry forest. *Annals of Botany* 97: 277-287.
- Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists? *Ecology* 81: 2651-2661.
- Maramorosch K (1963) Arthropod transmission of plant viruses. *Annual Review of Entomology* 8: 369-414.
- Mauck WM, Burns KJ (2009) Phylogeny, biogeography, and recurrent evolution of divergent bill types in the nectar-stealing flowerpiercers (Thraupini: *Diglossa* and *Diglossopsis*). *Biological Journal of the Linnean Society* 98: 14-28.
- May PG (1985) Nectar uptake rates and optimal nectar concentrations of two butterfly species. *Oecologia* 66: 381-386.
- McDade LA, Kinsman S (1980) The impact of floral parasitism in two Neotropical hummingbird-pollinated plant species. *Evolution* 34: 944-958.
- McKey D (1974) Adaptive patterns in alkaloid physiology. *The American Naturalist* 108: 305-320.
- Meehan CJ, Olson EJ, Reudink MW, Kyser TK, Curry RL (2009) Herbivory in a spider through exploitation of an ant-plant mutualism. *Current Biology* 19: R892-R893.
- Memmott J, Waser NM, Price MV (2004) Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society London B* 271: 2605-2611.
- Menzel F, Pokorny T, Blüthgen N, Schmitt T (2010) Trail-sharing among tropical ants: interspecific use of trail pheromones? *Ecological Entomology* 35: 495-503.
- Mody K, Linsenmair KE (2004) Plant-attracted ants affect arthropod community structure but not necessarily herbivory. *Ecological Entomology* 29: 217-225.
- Mondor EB, Addicott JF (2007) Do exaptations facilitate mutualistic associations between invasive and native species? *Biological Invasions* 9: 623-628.
- Moog J, Saw LG, Hashim R, Maschwitz U (2005) The triple alliance: how a plant-ant, living in an ant-plant, acquires the third partner, a scale insect. *Insectes Sociaux* 52: 169-176.
- Moog U, Fiala B, Federle W, Maschwitz (2002) Thrips pollination of the dioecious ant plant *Macaranga hullettii* (Euphorbiaceae) in Southeast Asia. *American Journal of Botany* 89: 50-59.
- Mooney KA, Agrawal AA (2008) Plant genotype shapes ant-aphid interactions: implications for

community structure and indirect plant defence. *The American Naturalist* 171: 195-205.

- Moraes SC, Vasconcelos HL (2009) Long-term persistence of a Neotropical ant-plant population in the absence of obligate palnt-ants. *Ecology* 90: 2375-2383.
- Morales MA, Barone JL, Henry CS (2008) Acoustic alarm signalling facilitates predator protection of treehoppers by mutualist ant bodyguards. *Proceedings of the Royal Society London B* 275: 1935-1941.
- Morgan DE (2009) Trail pheromones of ants. *Physiological Entomology* 34: 1-17.
- Morris WF (1996) Mutualism denied? Nectar-robbing bumble bees do not reduce female or male success of bluebells. *Ecology* 77: 1451-1462.
- Morse DH (1986) Inflorescence choice and time allocation by insects foraging on milkweed. *Oikos* 46: 229-236.
- Moya-Raygoza G, Larsen KJ (2000) Temporal resource switching by ants between honeydew produced by the fivespotted gama grass leafhopper (*Dalbulus quinquenotatus*) and nectar produced by plants with extrafloral nectaries. *American Midland Naturalist* 146: 311-320.
- Muñoz AA, Arroyo MTK (2004) Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub. *Oecologia* 138: 66-73.
- Nagamitsu T, Inoue T (1997) Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia* 110: 432-439.
- Navarro L (2001) Reproductive biology and effect of nectar robbing on fruit production in *Macleania bullata* (Ericaceae). *Plant Ecology* 152: 59-65.
- Ne'eman G, Jürgens A, Newstrom-Lloyd, Potts SG, Dafni A (2010) A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Review* 85: 435-451.
- Nepi M, Guarnieri M, Pacini E (2001) Nectar secretion, reabsorption, and sugar composition in male and female flowers of *Cucurbita pepo*. *International Journal of Plant Science* 162: 353-358.
- Ness JH (2006) A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. *Oikos* 113: 506-514.
- Ness JH, Bronstein JL (2004) The effects of invasive ants on prospective ant mutualists. *Biological Invasions* 6: 445-461.
- Ness JH, Morin DF, Giladi I (2009) Uncommon specialization in a mutualism between a temperate herbaceous plant guild and an ant: are *Aphaenogaster* ants keystone mutualists? *Oikos* 118: 1793-1804.
- Newman DA, Thomson JD (2005) Interactions among nectar robbing, floral herbivory, and ant protection in *Linaria vulgaris*. *Oikos* 110: 497-506.
- Nicklen EF, Wagner D (2006) Conflict resolution in an ant-plant interaction: *Acacia constricta* traits reduce ant costs to reproduction. *Oecologia* 148: 81-87.
- Nicolson SW (1995) Direct demonstration of nectar reabsorption in the flowers of *Grevillea robusta* (Proteaceae). *Functional Ecology* 9: 584-588.
- Nieh JC (2010) A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Current Biology* 20: 310-315.
- Nieh JC, Barreto LS, Contrera FAL, Imperatriz-Fonseca (2004) Olfactory eavesdropping by a competitively foraging stingless bee, *Trigona spinipes*. *Proceedings of the Royal Society London B* 271: 1633-1640.
- Nielsen C, Agrawal AA, Hajek AE (2010) Ants defend aphids against lethal disease. *Biology Letters* 6: 205-208.
- Nomura M, Hatada A, Itioka T (2011) Correlation between the leaf turnover rate and anti-herbivore

- defence strategy (balance between ant and non-ant defences) amongst ten species of *Macaranga* (Euphorbiaceae). *Plant Ecology* 212: 143-155.
- Norment CJ (1988) The effect of nectar-thieving ants on the reproductive success of *Frasera speciosa* (Gentianaceae). *American Midland Naturalist* 120: 331-336.
 - O'Dowd DJ, Greent PT, Lake PS (2003) Invasional "meltdown" on an oceanic island. *Ecology Letters* 6: 812-817.
 - Offenberg J (2000) Correlated evolution of the association between aphids and ants and the association between aphids and plants with extrafloral nectaries. *Oikos* 91: 146-152.
 - Offenberg J (2001) Balancing between mutualism and exploitation: the symbiotic interaction between *Lasius* ants and aphids. *Behavioural Ecology and Sociobiology* 49: 304-310.
 - Offenberg J, Nielsen MG, Macintosh DJ, Havanon S, Aksornkoae S (2004) Evidence that insect herbivores are deterred by ant pheromones. *Proceedings of the Royal Society of London B* 271: S433-S435.
 - Offenberg J, Nielsen MG, Macintosh DJ, Havanon S, Aksornkoae (2005) Lack of ant attendance may induce compensatory plant growth. *Oikos* 111: 170-178.
 - Oliveira PS, Freitas AVL (2004) Ant-plant-herbivore interactions in the Neotropical cerrado savanna. *Naturwissenschaften* 91: 557-570.
 - Oliver TH, Cook JM, Leather SR (2007) When are ant-attractant devices a worthwhile investment? *Vicia faba* extrafloral nectaries and *Lasius niger* ants. *Population Ecology* 49: 265-273.
 - Ōmura H, Honda K, Hayashi N (2000) Floral scent of *Osmanthus fragrans* discourages foraging behaviour of cabbage butterfly, *Pieris rapae*. *Journal of Chemical Ecology* 26: 655-666.
 - Opler PA, Baker HG, Frankie GW (1975) Reproductive biology of some Costa Rican *Cordia* species (Boraginaceae). *Biotropica* 7: 234-247.
 - Ordano M, Ornelas JF (2004) Generous-like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. *Oecologia* 140: 495-505.
 - Ordano M, Ornelas JF (2005) The cost of nectar replenishment in two epiphytic bromeliads. *Journal of Tropical Ecology* 21: 541-547.
 - Ornelas JF (1994) Serrate tomia: an adaptation for nectar robbing in hummingbirds? *The Auk* 111: 703-710.
 - Pacini E, Hesse M (2005) Pollenkitt: its composition, forms and functions. *Flora* 2000: 399-415.
 - Paciorek CJ, Moyer BR, Levin RA, Halpern SL (1995) Pollen consumption by the hummingbird flower mite *Proctolaelaps kirmsei* and possible fitness effects on *Hamelia patens*. *Biotropica* 27: 258-262.
 - Palmer TM, Stanton ML, Young TP, Goheen JR, Pringle RM, Karban R (2008) Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. *Science* 319: 192-195.
 - Palmer TM, Young TP, Stanton ML (2002) Burning bridges: priority effects and the persistence of a competitively subordinate acacia-ant in Laikipia, Kenya. *Oecologia* 133: 372-379.
 - Palmer TM, Young TP, Stanton ML, Wenk E (2000) Short-term dynamics of an acacia ant community in Laikipia, Kenya. *Oecologia* 123: 425-435.
 - Peakall R, Angus CJ, Beattie AJ (1990) The significance of ant and plant traits for ant pollination in *Leporella fimbriata*. *Oecologia* 84: 457-460.
 - Peakall R, Beattie AJ (1989) Pollination of the orchid *Microtis parviflora* R. Br. by flightless worker ants. *Functional Ecology* 3: 515-522.
 - Pellmyr O, Leebens-Mack J, Huth CJ (1996) Non-mutualistic yucca moths and their evolutionary

consequences. *Nature* 380: 155-156.

- Pellmyr O, Thien LB (1986) Insect reproduction and floral fragrances: keys to the evolution of the angiosperms? *Taxon* 35: 76-85.
- Pemberton RW (1992) Fossil extrafloral nectaries, evidence for the ant-guard antiherbivore defence in an Oligocene *Populus*. *American Journal of Botany* 79: 1242-1246.
- Perry JC, Mondor EB, Addicott JF (2004) An indirect mutualism: ants deter seed predators from ovipositing in yucca fruit. *Canadian Journal of Zoology* 82: 823-827.
- Peterson A, Bartish IV, Peterson J (2002) Genetic structure detected in a small population of the endangered plant *Anthericum liliago* (Anthericaceae) by RAPD analysis. *Ecography* 25: 677-684.
- Pierce NE (1995) Predatory and parasitic Lepidoptera: carnivores living on plants. *Journal of the Lepidopterists' Society* 49: 412-453.
- Pierce NE, Braby MF, Heath A, Lohman DJ, Mathew J, Rand DB, Travassos MA (2002) The ecology and evolution of ant associations in the Lycaenidae (Lepidoptera). *Annual Review of Entomology* 47: 733-771.
- Pierce NE, Elgar MA (1985) The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. *Behavioural Ecology and Sociobiology* 16: 209-222.
- Pimentel de Carvalho AC, Message D (2004) A scientific note on the toxic pollen of *Stryphnodendron polyphyllum* (Fabaceae, Mimosoideae) which causes sacbrood-like symptoms. *Apidologie* 35: 89-90.
- Pleasants JM, Chaplin SJ (1983) Nectar production rates of *Asclepias quadrifolia*: causes and consequences of individual variation. *Oecologia* 59: 232-238.
- Pontin AJ (1978) The numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.) *Ecological Entomology* 3: 203-207.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution* 25: 345-353.
- Poulsen M, Bot ANM, Nielsen MG, Boomsma JJ (2002) Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. *Behavioural and Ecological Sociobiology* 52: 151-157.
- Pozo MI, Herrera CM, Bazaga P (2011) Species richness of yeast communities in floral nectar of Southern Spanish plants. *Microbial Ecology* 61: 82-91.
- Praz CJ, Müller A, Dorn S (2008) Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen? *Ecology* 89: 795-804.
- Prýs-Jones OE, Willmer PG (1992) The biology of alkaline nectar in the purple toothwort (*Lathraea clandestina*): ground level defences. *Biological Journal of the Linnean Society* 45: 373-388.
- Pudlo RJ, Beattie AJ, Culver DC (1980) Population consequences in an ant-seed mutualism in *Sanguinaria canadensis*. *Oecologia* 146: 32-37.
- Puterbaugh MN (1998) The roles of ants as flower visitors: experimental analysis in three alpine species. *Oikos* 83: 36-46.
- Pyke GH (1979) Optimal foraging in bumblebees: Rule of movement between flowers within inflorescences. *Animal Behaviour* 27: 1167-1181.
- Pyke GH (1991) What does it cost a plant to produce floral nectar? *Nature* 350: 58-59.
- Raguso RA (2004) Why are some floral nectars scented? *Ecology* 85: 1486-1494.
- Raine NE, Chittka L (2008) The correlation of learning speed and natural foraging success in bumblebees. *Proceedings of the Royal Society London B* 275: 803-808.

- Raine NE, Gammans N, Macfadyen IJ, Scrivner GK, Stone GN (2004) Guards and thieves: antagonistic interactions between two ant species coexisting on the same ant-plant. *Ecological Entomology* 29: 345-352.
- Raine NE, Pierson AS, Stone GN (2007) Plant-pollinator interactions in a Mexican *Acacia* community. *Arthropod-Plant Interactions* 1: 101-117.
- Raine NE, Willmer PG, Stone GN (2002) Spatial structuring and floral avoidance behaviour prevent ant-pollinator conflict in a Mexican ant-acacia. *Ecology* 83: 3086-3096.
- Ramsey M (1995) Ant pollination of the perennial herb *Blandfordia grandiflora* (Liliaceae). *Oikos* 74: 265-272.
- Rashbrook VK, Compton SG, Lawton JH (1992) Ant-herbivore interactions: reasons for the absence of benefits to a fern with foliar nectaries. *Ecology* 73: 2167-2174.
- Reader T, Higginson AD, Barnard CJ, Gilbert FS (2006) The effects of predation risk from crab spiders on bee foraging behaviour. *Behavioural Ecology* 17: 933-939.
- Reader T, MacLeod I, Elliott PT, Robinson OJ, Manica A (2005) Inter-order interactions between flower-visiting insects: foraging bees avoid flowers previously visited by hoverflies. *Journal of Insect Behaviour* 18: 51-57.
- Rickson FR (1971) Glycogen plastids in mullerian body cells of *Cecropia peltata* – a higher green plant. *Science* 173: 344-347.
- Rico-Gray V (1993) Use of plant-derived food resources by ants in the dry tropical lowlands of coastal Veracruz, Mexico. *Biotropica* 25: 301-315.
- Rico-Gray V, Oliveira PS (2007) *The Ecology and Evolution of Ant-Plant Interactions*. The University of Chicago Press.
- Rico-Gray V, Sternberg LSL (1991) Carbon isotopic evidence for seasonal change in feeding habits of *Camponotus planatus* Roger (Formicidae) in Yucatan, Mexico. *Biotropica* 23: 93-95.
- Rios RS, Marquis RJ, Flunker JC (2008) Population variation in plant traits associated with ant attraction and herbivory in *Chamaecrista fasciculata* (Fabaceae). *Oecologia* 156: 577-588.
- Rodriguez-Cabal MA, Stuble KL, Nuñez MA, Sander NJ (2009) Quantitative analysis of the effects of the exotic Argentine ant on seed-dispersal mutualisms. *Biology Letters* 5: 499-502.
- Romero GQ, Benson WW (2004) Leaf domatia mediate mutualism between mites and a tropical tree. *Oecologia* 140: 609-616.
- Romero GQ, Izzo TJ (2004) Leaf damage induces ant recruitment in the Amazonian ant-plant *Hirtella myrmecophila*. *Journal of Tropical Ecology* 20: 675-682.
- Romero GQ, Vasconcellos-Neto J (2004) Beneficial effects of flower-dwelling predators on their host plant. *Ecology* 85: 446-457.
- Rosumek FB, Silveira FAO, Neves FS, Barbosa NPU, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160: 537-549.
- Roubik DW (1982) The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. *Ecology* 63: 354-360.
- Rowles AD, O'Dowd DJ (2009) New mutualism for old: indirect disruption and direct facilitation of seed dispersal following Argentine ant invasion. *Oecologia* 158: 709-716.
- Rudgers JA (2004) Enemies of herbivores can shape plant traits: selection in a facultative ant-plant mutualism. *Ecology* 85: 192-205.

- Rudgers JA, Gardener MC (2004) Extrafloral nectar as a resource mediating multispecies interactions. *Ecology* 85: 1495-1502.
- Ruhren S (2003) Seed predators are undeterred by nectar-feeding ants on *Chamaecrista nictitans* (Caesalpineaceae). *Plant Ecology* 166: 189-198.
- Ruhren S, Handel SN (1999) Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia* 119: 227-230.
- Rutter MT, Rausher MD (2004) Natural selection on extrafloral nectar production in *Chamaecrista fasciculata*: the costs and benefits of a mutualism trait. *Evolution* 58: 2657-2668.
- Saarinen EV (2006) Differences in worker caste behaviour of *Oecophylla smaragdina* (Hymenoptera: Formicidae) in response to larvae of *Anthene emolus* (Lepidoptera: Lycaenidae). *Biological Journal of the Linnean Society* 88: 391-395.
- Sachs JL, Simms EL (2006) Pathways to mutualism breakdown. *Trends in Ecology and Evolution* 21: 585-592.
- Sagers CL, Ginger SM, Evans RD (2000) Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia* 123: 582-586.
- Sakata H, Hashimoto Y (2000) Should aphids attract or repel ants? Effect of rival aphids and extrafloral nectaries on ant-aphid interactions. *Population Ecology* 42: 171-178.
- Saleh N, Chittka L (2006) The importance of experience in the interpretation of conspecific chemical signals. *Behavioral and Ecological Sociobiology* 61: 215-220.
- Saleh N, Ohashi K, Thomson JD, Chittka L (2006) Facultative use of the repellent scent mark in foraging bumblebees: complex versus simple flowers. *Animal Behaviour* 71: 847-854.
- Schatz B, Djieto-Lordon C, Dormont L, Bessière J, McKey D, Blatrix R (2009) A simple non-specific chemical signal mediates defence behaviour in a specialised ant-plant mutualism. *Current Biology* 19: R361-R362
- Schatz B, Kjellberg F, Nyawa S, Hossaert-McKey (2008) Fig wasps: a staple food for ants on *Ficus*. *Biotropica* 40: 190-195.
- Schatz B, Wcislo WT (1999) Ambush predation by the ponerine ant *Ectatomma ruidum* Roger (Formicidae) on a sweat bee *Lasioglossum umbripenne* (Halictidae), in Panama. *Journal of Insect Behaviour* 12: 641-663.
- Schemske DW (1980) The evolutionary significance of extrafloral nectar production by *Costus woodsonii* (Zingiberaceae): an experimental analysis of ant protection. *Journal of Ecology* 68: 959-967.
- Schiestl FP, Ayasse M (2001) Post-pollination emission of a repellent compound in a sexually deceptive orchid: a new mechanism for maximising reproductive success? *Oecologia* 126: 531-534.
- Schmidt VM, Zucchi R, Barth FG (2005) Scent marks left by *Nannotrigona testaceicornis* at the feeding site: cues rather than signals. *Apidologie* 36: 285-291.
- Schmitt U, Bertsch A (1990) Do foraging bumblebees scent-mark food sources and does it matter? *Oecologia* 82: 137-144.
- Schorkopf DLP, Jarau S, Francke W, Twele R, Zucchi R, Hrncir M, Schmidt VM, Ayasse M, Barth FG (2007) Spitting out information: *Trigona* bees deposit saliva to signal resource locations. *Proceedings of the Royal Society London B* 274: 895-898.
- Schubart HOR, Anderson AB (1978) Why don't ants visit flowers? A reply to D. H. Janzen. *Biotropica* 10: 310-311.
- Schürch S, Pfunder M, Roy BA (2000) Effects of ants on the reproductive success of *Euphorbia*

cyparissias and associated pathogenic rust fungi. *Oikos* 88: 6-12.

- Segraves KA, Althoff DM, Pellmyr O (2005) Limiting cheaters in mutualism: evidence from hybridisation between mutualist and cheater yucca moths. *Proceedings of the Royal Society London B* 272: 2195-2201.
- Seidel JL, Epstein WW, Davidson DW (1990) Neotropical ant gardens I. Chemical constituents. *Journal of Chemical Ecology* 16: 1791-1816.
- Sendoya SF, Freitas AVL, Oliveira PS (2009) Egg-laying butterflies distinguish predaceous ants by sight. *The American Naturalist* 174: 134-140.
- Shenoy M., Borges RM (2008) A novel mutualism between an ant-plant and its resident pollinator. *Naturwissenschaften* 95: 61-65.
- Shorey HH, Gaston LK, Gerber RG, Sisk CB, Phillips PA (1996) Formulating farnesol and other ant-repellent semiochemicals for exclusion of Argentine ants (Hymenoptera: Formicidae) from citrus trees. *Environmental Entomology* 25: 114-119.
- Shuttlesworth A, Johnson SD (2009) Specialized pollination in the African milkweed *Xysmalobium orbiculare*: a key role for floral scent in the attraction of spider-hunting wasps. *Plant Systematics and Evolution* 280: 37-44.
- Smith BH, Abramson CI, Tobin TR (1991) Conditional withholding of proboscis in honeybees (*Apis mellifera*) during discriminative punishment. *Journal of Comparative Psychology* 4: 345-356.
- Smith LL, Lanza J, Smith GC (1990) Amino acid concentrations in extrafloral nectar of *Impatiens sultani* increase after simulated herbivory. *Ecology* 71: 107-115.
- Sorrells TR, Kuritzky LY, Kauhanen PG, Fitzgerald K, Sturgis SJ, Chen J, Dijamco CA, Basurto KN, Gordon DM (2011) Chemical defense by the native Winter ant (*Prenolepis imparis*) against the invasive Argentine ant (*Linepithema humile*). *Public Library of Science ONE* 6: e18717.
- Southwick EE (1984) Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology* 65: 1775-1779.
- Spangler HG, Taber S (1970) Defensive behaviour of honey bees toward ants. *Psyche* 77: 184-188.
- Stadler B, Fiedler K, Kawecki TJ, Weisser WW (2001) Costs and benefits for phytophagous myrmecophiles: when ants are not always available. *Oikos* 92: 467-478.
- Stanton ML (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. *The American Naturalist* 162: S10-S23.
- Stanton ML, Palmer TM, Young TP (2002) Competition-colonization trade-offs in a guild of African acacia-ants. *Ecological Monographs* 72: 347-363.
- Stanton ML, Palmer TM, Young TP, Evans A, Turner ML (1999) Sterilization and canopy modification of a swollen thorn acacia tree by a plant-ant. *Nature* 401: 578-581.
- Stephenson AG (1981) Toxic nectar thieves of *Catalpa speciosa*. *American Midland Naturalist* 105: 381-383.
- Stephenson AG (1982) Iridoid glycosides in the nectar of *Catalpa speciosa* are unpalatable to nectar thieves. *Journal of Chemical Ecology* 8: 1025-1034.
- Stone G, Willmer P, Nee S (1996) Daily partitioning of pollinators in an African acacia community. *Proceedings of the Royal Society of London B* 263: 1389-1393.
- Stout JC, Goulson D (2001) The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Animal Behaviour* 62: 183-189.
- Stout JC, Goulson D (2002) The influence of nectar secretion rates on the responses of bumblebees

- (*Bombus* spp.) to previously visited flowers. Behavioural and Ecological Sociobiology 52: 239-246.
- Stout JC, Goulson D, Allen JA (1998) Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.). Behavioural Ecology and Sociobiology 43: 317-326.
 - Stpicyńska M (2003) Nectar resorption in the spur of *Platanthera chlorantha* Custer (Rchb.) Orchidaceae – structural and microautoradiographic study. Plant Systematics and Evolution 238: 119-126.
 - Strauss SY, Rudgers JA, Lau JA, Irwin RE (2002) Direct and ecological costs of resistance to herbivory. Trends in Ecology & Evolution 17: 278-285.
 - Styrsky JD, Eubanks MD (2007) Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings of the Royal Society London B 274: 151-164.
 - Styrsky JD, Eubanks MD (2010) A facultative mutualism between aphids and an invasive ant increases plant reproduction. Ecological Entomology 35: 190-199.
 - Suarez AV, De Moraes C, Ippolito A. (1997) Defense of *Acacia collinsii* by an obligate and nonobligate ant species: the significance of encroaching vegetation. Biotropica 30:480–482.
 - Sugiura N, Miyazaki S, Nagaishi S (2006) A supplementary contribution of ants in the pollination of an orchid, *Epipactis thunbergii*, usually pollinated by hoverflies. Plant Systematics and Evolution 258: 17-26.
 - Suttle KB (2003) Pollinators as mediators of top-down effects on plants. Ecology Letters 6: 688-694.
 - Szilágyi A, Scheuring I, Edwards DP, Orivel J, Yu DW (2009) The evolution of intermediate castration virulence and ant coexistence in a spatially structured environment. Ecology Letters 12: 1306-1316.
 - Takeda S, Kinomura K, Sakurai H (1982) Effects of ant-attendance on the honeydew excretion and larviposition of the cowpea aphid *Aphis craccivora* Koch. Japanese Society of Applied Entomology and Zoology 17: 133-135.
 - Taki H, Okabe K, Makino S, Yamaura Y, Sueyoshi M (2009) Contribution of small insects to pollination of common buckwheat, a distylous crop. Annals of Applied Biology 155: 121-129.
 - Tepe EJ, Kelley WA, Rodriguez-Castañeda, Dyer LA (2009) Characterizing the cauline domatia of two newly discovered Ecuadorian ant plants in *Piper*: an example of convergent evolution. Journal of Insect Science 9: 27.
 - Tepe EJ, Vincent MA, Watson LE (2007) Stem diversity, cauline domatia, and the evolution of ant-plant associations in *Piper* sect. *Macrostachys* (Piperaceae). American Journal of Botany 94: 1-11.
 - Thaler JS (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399: 686-688.
 - Thiele KR, Funk VA, Iwatsuki K, Morat P, Peng C, Raven PH (2011) The controversy over the retypification of *Acacia* Mill. with an Australian type: a pragmatic view. Taxon 60: 194-198.
 - Thien LB, Azuma H, Kawano S (2000) New perspectives on the pollination biology of basal angiosperms. International Journal of Plant Science 161: S225-S235.
 - Tholl D, Boland W, Hansel A, Loreto F, Röse USR, Schnitzler J (2006) Practical approaches to plant volatile analysis. The Plant Journal 45: 540-560.
 - Thomas JA, Elmes GW (1993) Specialized searching and the hostile use of allomones by a parasitoid whose host, the butterfly *Maculinea rebeli*, inhabits ant nests. Animal Behaviour 45: 593-602.
 - Thompson JD, Plowright RC (1980) Pollen carryover, nectar rewards, and pollinator behaviour with special reference to *Diervilla lonicera*. Oecologia 46: 68-74.
 - Thompson JN (1982) Interaction and Coevolution. Wiley New York (referenced in Becerra & Venable, 1989).
 - Thompson JN (1988) Variation in interspecific interactions. Annual Review of Ecology and Systematics

19: 65-87.

- Tillberg CV (2004) Friend or foe? A behavioural and stable isotopic investigation of an ant-plant symbiosis. *Oecologia* 140: 506-515.
- Tobin JE (1994) Ants as primary consumers: Diet and abundance in the Formicidae. In *Nourishment and evolution in insect societies*. Ed. Hunt JH and Napela CA, 279-307. Boulder, CO: Westview Press
- Torres-Hernandez L, Rico-Gray V, Castillo-Guevara C, Vergara JA (2000) Effect of nectar-foraging ants and wasps on the reproductive fitness of *Turnera ulmifolia* (Turneraceae) in a coastal sand dune in Mexico. *Acta Zoologica Mexicana* 81: 13-21.
- Trager MD, Bruna EM (2006) Effects of plant age, experimental nutrient addition and ant occupancy on herbivory in a neotropical myrmecophyte. *Journal of Ecology* 94: 1156-1163.
- Travassos MA, Pierce NE (2000) Acoustics, context and function of vibrational signalling in a lycaenid butterfly-ant mutualism. *Animal Behaviour* 60: 13-26.
- Traveset A, Willson MF, Sabag (1998) Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. *Functional Ecology* 12: 459-464.
- Troncoso AJ, Cabezas NJ, Faúndez EH, Urzúna A, Niemeyer HM (2010) Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. *Oecologia* 162: 413-425.
- Tsuji K, Hasyim A, Nakamura H, Nakamura K (2004) Asian weaver ants, *Oecophylla smaragdina*, and their repelling of pollinators. *Ecological Research* 19: 669-673.
- Tutin CEG, Telfer P, Lahm SA (2004) The *Azteca-Cecropia* association: are ants always necessary for their host plants? *Biotropica* 36:641-646.
- Urbani CB, de Andrade ML (1997) Pollen eating, storing, and spitting by ants. *Naturwissenschaften* 84: 256-258.
- Valdivia CE, González-Gómez PL (2006) A trade-off between the amount and distance of pollen dispersal triggered by the mixed foraging behaviour of *Sephanoides sephaniodes* (Trochilidae) on *Lapageria rosea* (Philesiaceae). *Acta Oecologia* 29: 324-327.
- Vallet A, Cassier P, Lensky Y (1991) Ontogeny of the fine structure of the mandibular glands of the honeybee (*Apis mellifera* L.) workers and the pheromonal activity of 2-heptanone. *Journal of Insect Physiology* 37: 789-804.
- Van der Pijl L (1955) Some remarks on myrmecophytes. *Phytomorphology* 5: 190-200.
- Van der Pijl L (1960) Ecological aspects of flower evolution. I. Phyletic evolution. *Evolution* 14: 403-416.
- Van Rijn PC, Tanigoshi LK (1999) The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius deganerans* on *Ricinus communis*. *Experimental and Applied Acarology* 23: 281-296.
- Vázquez DP, Blüthgen N, Canolo L, Chacoff NP (2009) Uniting pattern and process in plant-animal mutualistic networks: a review. *Annals of Botany* 103: 1445-1457.
- Vega C, Arista M, Ortiz PL, Herrera CM, Talavera S (2009) The ant-pollination system of *Cytinus hypcistis* (Cytinaceae), a Mediterranean root holoparasite. *Annals of Botany* 103: 1065-1075.
- Völkl W (1992) Aphids of their parasitoids: Who actually benefits from ant-attendance? *Journal of Animal Ecology* 61: 273-281.
- Wäckers FL, Zuber D, Wunderlin R, Keller F (2001) The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany* 87: 365-370.
- Wagner D (2000) Pollen viability reduction as a potential cost of ant association for *Acacia constricta* (Fabaceae). *American Journal of Botany* 87: 711-715.

- Wagner D, Kay A (2002) Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evolutionary Ecology Research* 4: 293-305.
- Walker TG (1986) The ant-fern, *Lecanopteris mirabilis*. *Kew Bulletin* 41: 533-545.
- Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology* 8: 307-344.
- Way MJ, Cammell ME, Paiva MR, Collingwood CA (1997) Distribution and dynamics of the Argentine ant *Linepithema (Iridomyrmex) humile* (Mayr) in relation to vegetation, soil conditions, topography and native competitor ants in Portugal. *Insectes Sociaux* 44: 415-433.
- Way MJ, Paiva MR, Cammell ME (1999) Natural biological control of the pine processionary moth *Thaumetopoea pityocampa* (Den. & Schiff.) by the Argentine ant *Linepithema humile* (Mayr) in Portugal. *Agricultural and Forest Entomology* 1: 27-31.
- Weatherwax PB (1986) Why do honeybees reject certain flowers? *Oecologia* 69: 567-570.
- Wheeler WM (1914) The ants of the Baltic amber. *Schriften Physikalisch-Okonomischen Gesellschaft Königsberg* 55: 1-42. (Referenced in Pemberton, 1988)
- Whitney HM, Chittka L, Bruce TJA (2009) Conical epidermal cells allow bees to grip flowers and increase foraging efficiency. *Current Biology* 19: 948-953.
- Williams CS (1998) The identity of the previous visitor influences flower rejection by nectar-collecting bees. *Animal Behaviour* 56: 673-681.
- Williams K, Koch GW, Mooney HA (1985) The carbon balance of flowers of *Diplacus aurantiacus* (Scrophulariaceae). *Oecologia* 66: 530-535.
- Willmer PG (1983) Thermal constraints on activity patterns in nectar-feeding insects. *Ecological Entomology* 8: 455-469.
- Willmer PG (1986) Foraging patterns and water balance: problems of optimization for a xerophilic bee, *Chalicodoma sicula*. *Journal of Animal Ecology* 55: 941-962.
- Willmer PG (2011) *Pollination and Floral Ecology*. Princeton University Press.
- Willmer PG, Nuttman CV, Raine NE, Stone GN, Patrick JG, Henson K, Stillman P, McIlroy L, Potts S, Knudsen JT (2009) Floral volatiles controlling ant behaviour. *Functional Ecology* 23: 888-900.
- Willmer PG, Stone GN (1997) How aggressive ant-guards assist seed-set in *Acacia* flowers. *Nature* 388: 165-167.
- Willott SJ, Compton SG, Incoll LD (2000) Foraging, food selection and worker size in the seed harvesting ant *Messor bouvieri*. *Oecologia* 125L 35-44.
- Wimp GM, Whitham TG (2001) Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism 82: 440-452.
- Witjes S, Eltz T (2007) Influence of scent deposits on flower choice: experiments in an artificial flower array with bumblebees. *Apidologie* 38: 12-18.
- Witjes S, Eltz T (2009) Hydrocarbon footprints as a record of bumblebee flower visitation. *Journal of Chemical Ecology* 35: 1320-1325.
- Witjes S, Witsch K, Eltz T (2010) Reconstructing the pollinator community and predicting seed set from hydrocarbon footprints on flowers. *Oecologia* 165: 1017-1029.
- Wootton JT, Sun I (1990) Bract liquid as a herbivore defence mechanism for *Heliconia wagneriana* inflorescences. *Biotropica* 22: 155-159.
- Writh R, Leal IR (2001) Does rainfall affect temporal variability of ant protection in *Passiflora coccinea*? *Ecoscience* 8: 450-453.

- Wyatt R (1980) The impact of nectar-robbing ants on the pollination system of *Asclepias curassavica*. Bulletin of the Torrey Botanical Club 107: 24-28.
- Wyatt R, Stoneburner A (1981) Patterns of ant-mediated pollen dispersal in *Diamorpha smallii* (Crassulaceae). Systematic Botany 6: 1-7.
- Yamashiro A, Yamashiro T (2008) Utilization on extrafloral nectaries and fruit domatia of *Canavalia lineata* and *C. cathartica* (Leguminosae) by ants. Arthropod-Plant Interactions 2: 1-8.
- Yang M, Radloff, Tan K, Hepburn R (2009) Anti-predator fan-blowing in guard bees, *Apis mellifera capensis* Esch. Journal of Insect Behaviour 23:12-18.
- Yano S (1994) Flower nectar of an autogamous perennial *Rorippa indica* as an indirect defence mechanism against herbivorous insects. Researches on Population Ecology 36: 63-71.
- Yao I, Shibao H, Akimoto S (2000) Costs and benefits of ant attendance to the Drepanosiphid aphid *Tuberculatus quercicola* Oikos 89: 3-10.
- Yao I, Akimoto S (2002) Flexibility in the composition and concentration of amino acids in honeydew of the Drepanosiphid aphid *Tuberculatus quercicola*. Ecological Entomology 27: 745-752.
- Yokoi T, Fujisaki (2007) Repellent scent-marking behaviour of the sweat bee *Halictus (Seladonia) aerarius* during flower foraging. Apidologie 38: 474-481.
- Yokoi T, Fujisaki K (2009a) Recognition of scent marks in solitary bees to avoid previously visited flowers. Ecological Research 24: 803-809.
- Yokoi T, Fujisaki K (2009b) Hesitation behaviour of hoverflies *Sphaerophoris* spp. to avoid ambush by crab spiders. Naturwissenschaften 96: 195-200.
- Yokoi T, Goulson D, Fujisaki K (2007) The use of heterospecific scent marks by the sweat bee *Halictus aerarius*. Naturwissenschaften 94: 1021-1024.
- Young TP, Stubblefield CH, Isbell LA (1997) Ants on swollen-thorn acacias: species coexistence in a simple system. Oecologia 109: 98-107.
- Youngsteadt E, Nojima S, Häberlein C, Schulz S, Schal C (2008) Seed odor mediates an obligate ant-plant mutualism in Amazonian rainforests. Proceedings of the National Academy of Sciences 105: 4571-4575.
- Yu DW, Pierce NE (1998) A castration parasite of an ant-plant mutualism. Proceedings of the Royal Society of London B 265: 375-382.
- Yu DW, Wilson HB, Pierce NE (2001) An empirical model of species coexistence in a spatially structured environment. Ecology 82: 1761-1771.
- Zangerl AR, Rutledge CE (1996) The probability of attack and patterns of constitutive and induced defence: a test of optimal defence theory. The American Naturalist 147: 599-608.