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THEMA:

Smell and Repel:
**Resin based defense mechanisms and interactions
between Australian ants and stingless bees**

Wie stachellose Bienen in Australien mit Ameisen interagieren und sich mit Hilfe von Harzen verteidigen

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“Nature will bear the closest inspection. She invites us to lay our eye level with her smallest leaf, and take an insect view of its plain.”

Henry David Thoreau

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Summary

Bees are subject to permanent threat from predators such as ants. Their nests with large quantities of brood, pollen and honey represent lucrative targets for attacks whereas foragers have to face rivalry at food sources. This thesis focused on the role of stingless bees as third party interactor on ant-aphid-associations as well as on the predatory potential represented by ants and defense mechanisms against this threat. Regular observations of an aphid infested *Podocarpus* for approaching stingless bees yielded no results. Another aim of this thesis was the observation of foraging habits of four native and one introduced ant species for assessment of their predatory potential to stingless bees. All species turned out to be dietary balanced generalists with one mostly carnivorous species and four species predominantly collecting nectar roughly according to optimal foraging theory. Two of the species monitored, *Rhytidoponera metallica* and *Iridomyrmex rufoniger* were considered potential nest robbers.

As the name implies, stingless bees lack the powerful weapon of their distant relatives; hence they specialized on other defense strategies. Resin is an important, multipurpose resource for stingless bees that is used as material for nest construction, antibiotic and for defensive means. For the latter purpose highly viscous resin is either directly used to stick down aggressors or its terpenic compounds are included in the bees cuticular surface. In a feeding choice experiment, three ant species were confronted with the choice between two native bee species - *Tetragonula carbonaria* and *Austroplebeia australis* - with different cuticular profiles and resin collection habits. Two of the ant species, especially the introduced *Tetramorium bicarinatum* did not show any preferences. The carnivorous *R. metallica* predominantly took the less resinous *A. australis* as prey. The reluctance towards *T. carbonaria* disappeared when the resinous compounds on its cuticle had been washed off with hexane. To test whether the repulsive reactions were related to the stickiness of the resinous surface or to chemical substances, hexane extracts of bees' cuticles, propolis and three natural tree resins were prepared. In the following assay responses of ants towards extract treated surfaces were observed. Except for one of the resin extracts, all tested substances had repellent effects to the ants. Efficacy varied with the type of extract and species. Especially to the introduced *T. bicarinatum* the cuticular extract had no effect. GCMS-analyses showed that some of the resinous compounds were also found in the cuticular profile of *T. carbonaria* which featured reasonable analogies to the resin of *Corymbia torelliana* that is highly attractive for stingless bees. The results showed that repellent effects were only partially related to the sticky quality of resin but were rather caused by chemical substances, presumably sesqui- and diterpenes. Despite its efficacy this defense strategy only provides short time repellent effects sufficient for escape and warning of nest mates to initiate further preventive measures.

Zusammenfassung

Bienen sind permanent Gefahren ausgesetzt, ihre Nester voll Brut, Pollen und Honig bieten ein ertragreiches Ziel für Räuber und auch bei der Nahrungssuche droht Konkurrenz an den Futterquellen, beispielsweise durch Ameisen. Ziel dieser Arbeit war es zu untersuchen, welche Rolle stachellose Bienen in Australien als dritter Interaktionspartner an Ameisen-Blattlaus-Assoziationen einnehmen, welcher Bedrohung sie durch räuberische Ameisen ausgesetzt sind und wie sie sich gegen diese verteidigen. Regelmäßige Beobachtungen einer von Blattläusen befallenen Steineibe auf Besuche von stachellosen Bienen blieben erfolglos, es wurden keine Anflüge erfasst. Ein weiterer Fokus dieser Arbeit lag auf der Untersuchung des Nahrungseintrags von vier heimischen, sowie einer eingeschleppten Ameisenart zur Erfassung des räuberischen Potenzials gegenüber stachellosen Bienen. Alle Ameisenarten stellten sich als Generalisten mit ausgewogenem Nahrungseintrag heraus. Eine der Arten ernährte sich hauptsächlich räuberisch, während der Eintrag von Nektar für vier Arten die Hauptressource darstellte und annäherungsweise gemäß der „optimal foraging theory“ erfolgte. Zwei der untersuchten Arten, *Rhytidoponera metallica* und *Iridomyrmex rufoniger*, wurden als potenzielle Nesträuber eingestuft.

Stachellose Bienen können sich nicht durch Stiche verteidigen, sie nutzen daher andere Strategien. Pflanzenharz stellt für Bienen eine vielseitige Ressource dar, welche als Baumaterial, Desinfiziens und auch zur Verteidigung eingesetzt wird. Das Harz wird entweder in zähflüssiger Form dazu verwendet, um Angreifer zu verkleben oder die darin enthaltenen Terpene gelangen in Bestandteilen auf die Oberfläche der Bienen. In einem Futterwahl-Experiment wurden *Tetragonula carbonaria* und *Austroplebeia australis*, zwei heimische Bienenarten mit unterschiedlichen Harzsammel-Gewohnheiten und Oberflächenprofilen, drei Ameisenarten als Beute vorgelegt. Während zwei der Ameisenarten, insbesondere die eingeführte *Tetramorium bicarinatum*, keinerlei Präferenzen zeigte, entschieden sich die karnivoren *R. metallica* vorrangig für *A. australis*, deren Oberflächenprofil weniger Harzkomponenten aufwies. Wurden die Oberflächenbestandteile von *T. carbonaria* durch Waschen mit Hexan entfernt, verschwand auch die Zurückhaltung der Räuber. Um zu untersuchen ob diese Abwehrreaktion durch die Klebrigkeit der Oberfläche oder durch chemische Substanzen verursacht wurde, wurden Hexan-Extrakte der Bienenoberflächen sowie von drei Baumharzen und Nestmaterial angefertigt. Die nachfolgenden Untersuchungen richteten sich daraufhin auf die Beobachtung der Reaktion von Ameisen bei Kontakt mit Extrakt-behandelten Oberflächen. Bis auf einen der Harzextrakte zeigten alle untersuchten Substanzen unterschiedlich stark abstoßende Effekte auf Ameisen. Die eingeführte *T. bicarinatum* wurde jedoch nicht durch Bienenextrakt in ihrem Verhalten beeinflusst. Eine GCMS-Analyse ergab, dass einige der Harzsubstanzen auch im Oberflächenprofil von *T. carbonaria* zu finden waren, welches vor allem Übereinstimmungen mit dem Harz von *Corymbia torelliana*

aufwies, einer Pflanze deren Harz für Bienen besonders attraktiv ist. Es zeigte sich, dass nicht nur die Klebrigkeit, sondern auch chemische Substanzen, vermutlich Sesqui- und Diterpene, für abstoßende Effekte verantwortlich sind. Trotz der Effektivität dieses Mechanismus sorgt er nur für eine kurzzeitige Abwehrreaktion, ermöglicht jedoch die Gelegenheit zur Flucht und Warnung von Nestgenossen, sowie zur Einleitung weiterer Gegenwehr.

Table of contents

| | |
|---|------|
| Acknowledgments / Danksagungen | III |
| Summary | V |
| Zusammenfassung | VI |
| Table of contents | VIII |
| 1 Introduction | 1 |
| 1.1 Social life – a successful strategy relying on cooperative interaction..... | 1 |
| 1.2 Hustle and bustle - ecological aspects of ants | 2 |
| 1.3 Some fuss about the buzz - ecological aspects of stingless bees..... | 5 |
| 1.4 Interactions and olfactory traits - the role of resin | 10 |
| 1.5 Introducing the protagonists..... | 14 |
| 1.6 Thesis outline | 17 |
| 2 Methods..... | 18 |
| 2.1 Study site..... | 18 |
| 2.2 Experimental animals | 19 |
| 2.3 Food source monitoring | 20 |
| 2.4 Feeding choice assay | 22 |
| 2.5 Extract assay | 24 |
| 3 Results..... | 29 |
| 3.1 Food monitoring | 29 |
| 3.2 Feeding choice assay | 33 |
| 3.3 Extract assay | 34 |
| 4 Discussion..... | 44 |
| 4.1 Foraging habits of different ant species | 44 |
| 4.2 Trophobiotic interactions..... | 46 |
| 4.3 Ant repellent effects of stingless bees..... | 46 |
| 4.4 Efficacy of resinous compounds as ant repellents..... | 48 |
| 4.5 Prospect..... | 53 |
| 5 Bibliography..... | 56 |
| Appendix | 62 |
| Declaration / Erklärung..... | 69 |

1 Introduction

1.1 Social life – a successful strategy relying on cooperative interaction

Social insects such as bees and ants are among the most successful creatures to have evolved on earth. Even though they only account for about 2% of all known insect species they contribute to roughly half of the insect biomass worldwide. Their effectiveness is based on several evolutionary adaptations related to cooperative living. Whereas (apart from a few exceptions) all ants are eusocial (Hölldobler *et al.* 1990, 2009), a broad variety of life cycles from solitary to facultative or obligate social can be found in bees (Michener 1974; Roubik 1989). Eusocial associations in hymenopterans, often referred to as superorganisms, show a broad spectrum of complexity and are characterized by several traits which contribute to their success: The first point is a caste system based on the division of labor amongst adults such as reproductive (e.g. queens or drones) and worker castes with reduced or lacking fertility. Secondly, two or more generations share a nest and workers cooperate in nest building as well as in brood care and feeding (Hölldobler *et al.* 2009). These traits result in a high efficiency of social life due to a large number of workers that subordinate their own life to the well-being of the colony and the reproductive caste. Social life also allows more effective foraging and defense as well as the building of complex nest structures which provide food and shelter (Blüthgen *et al.* 2010). Overall these features contribute to the fact that social insects dominate almost all habitats on land. The variability and complexity of interactions of social insects with nest mates but also with other species has drawn the attention and fascination of scientists around the globe to these little but mighty creatures (Roubik 1989; Hölldobler *et al.* 2009).

The cooperative way of life in a social colony puts high demands on communicational skills in order to coordinate adequate responses to environmental stimuli. Besides visual, tactile and auditory signals, olfactory or chemical cues form a crucial foundation for interactions in a social insect community. They play an important role in communication, division of labor, nest mate recognition, resource detection and defense (Roubik 1989; Hölldobler *et al.* 1990; Hölldobler 1995; Hölldobler *et al.* 2009). Studying the interface of two eusocial insect species with different foraging ecologies is subject of this thesis. It focuses on the interaction of Australian stingless bees and ants, with special attention to food related contacts and the use of resin as an olfactory defense mechanism in stingless bees.

1.2 Hustle and bustle - ecological aspects of ants

GENERAL ECOLOGY

Ants (Formicidae) represent one of the most prosperous groups of organisms that have colonized almost all terrestrial habitats on Earth. Worldwide 16 subfamilies, about 300 genera and about 15,000 described species and subspecies of ants are known to exist with numbers of new species discovered constantly rising (Bolton 1995). Australia offers a quite diverse ant fauna with currently 10 subfamilies, 101 genera and 1275 described species (Bolton 1995).

Due to its size Australia provides a wide range of habitats which strongly influence the distribution patterns of ants (Figure 1.). The highest variety in genera can be found along the northern east coast with its warm, humid forest areas, whereas the dry central regions comprise comparatively fewer ant genera (Shattuck 1999). However, species numbers are distributed quite evenly and vary between 80 and 100 due to the fact that rainforest genera only contain a few species whereas the number of species rises within the genera of more arid regions. The highest diversity is presumably found in the eastern semi-arid transition areas with several hundreds of species assumed (Shattuck 1999).

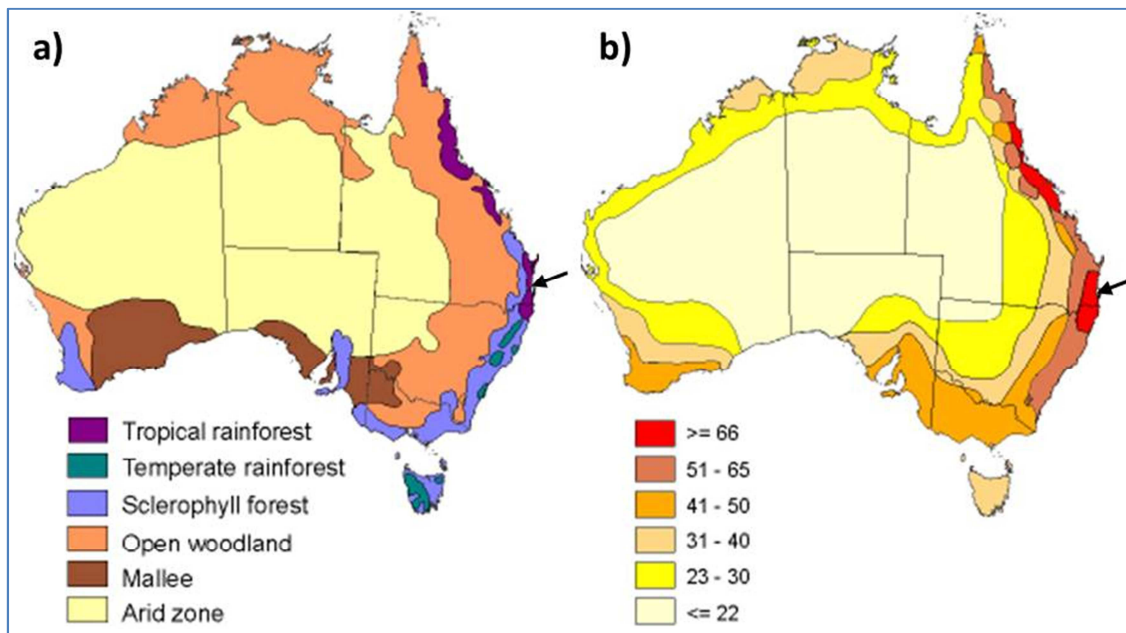


Figure 1. Habitat and ant genera distribution in Australia, arrow marks the study site: a) Major habitat types in Australia; b) Generic distribution of ant diversity¹

Colonies can exist for several years and usually consist of at least three different castes: Diploid, female queens (fertile), workers (sterile, wingless) and haploid males. Colony sizes vary from less than 50 up to several hundreds or thousands of workers

¹ CSIRO, 2011. Ants Down Under, viewed 24 August 2011, <http://anic.ento.csiro.au/ants>

(Hölldobler *et al.* 1990). A typical life cycle of a colony can be divided into three phases. In the founding stage a new queen mates with one or several males and starts constructing a first nest cell at a suitable nest site where she rears the first worker generation from her own body tissue. The workers then start foraging, enlarging the nest and caring for the brood. The queen confines herself to egg laying, and the population grows continually, sometimes with further caste differentiation. From this ergonomic stage, the colony passes into the reproductive stage where new queens and males are produced (Hölldobler *et al.* 1990).

Task duties often depend on the age of workers (Hölldobler *et al.* 1990; Shattuck 1999): Whereas younger workers tend to care for the brood, older ones engage in nest construction and excavation before finally becoming foragers that leave the nest in search for food. However, colonies where workers equally perform all tasks depending on the necessity of individual duties can also be found. A more distinct division of labor occurs in colonies with physical divergent sub-castes such as majors and minors where the size of the worker determines its activities (Hölldobler *et al.* 1990; Shattuck 1999; Hölldobler *et al.* 2009). In contrast to monomorphic colonies where all workers show the same size, these sub-castes can reach from dimorphism (e.g. minors and majors) to a high variability of worker sizes (polymorphism) as can be observed in leaf cutting ants of the genus *Atta*, honeypot ants like *Myrmecocystus*, or the hyperdiverse *Pheidole* and several other genera (Hölldobler *et al.* 1990). This diversification is highly linked to allometry, the nonisometric, disproportional growth of body parts (Hölldobler *et al.* 1990, 2009).

FOOD SOURCES, FORAGING AND NESTING

Most ants are omnivores feeding on a broad spectrum of plant based resources, as well as predating and scavenging on dead animals and feces or sometimes also fungi (Shattuck 1999; Blüthgen *et al.* 2010). Whereas adult ants only feed on liquefied food that is rich in carbohydrates (especially sucrose), their larvae often need more protein and lipid rich food sources such as solid, dead animals or seeds (Shattuck 1999). Queens and workers that stay in the nest mostly receive their food from foragers by trophallaxis (Shattuck 1999). In many cases the larval secretions play an important role in predigesting and fragmenting food which is then redistributed among the workers (Hölldobler *et al.* 1990). Food can be stored internally, for example in the fat bodies and the crop, or in the nest as dried insects or seeds (reviewed by Blüthgen *et al.* 2010). Nutrient intake, especially the carbohydrate to protein ratio is generally well balanced. A lack of one component may affect mortality, worker size, colony growth, aggressiveness and the body composition of larvae; the latter will be retained to the adult stage (Kay *et al.* 2006; Blüthgen *et al.* 2010). Therefore omnivorous ants

actively control their food intake by altering their preferences for certain food sources, a process called compensatory foraging (Kay 2002; Christensen *et al.* 2010).

The main carbohydrate source for many ants is nectar obtained from extrafloral nectaries or honeydew obtained from plant sucking hemipterans which usually contains a higher spectrum of nutrients such as valuable nitrogen than plain nectar (Davidson *et al.* 2003; Blüthgen *et al.* 2004a, b). This has led to the development of manifold, mostly mutualistic interactions where ants receive food from animals (trophobiosis) or plants in exchange for defense from predators or herbivores (Way 1963; Hölldobler *et al.* 1990; Ness *et al.* 2010). However nectar bearing flowers are less frequently visited by ants due to the emission of repellent, terpenoid containing odors (Junker *et al.* 2008). Whereas the use of liquid food sources is mainly found in the subfamilies Dolichoderinae, Formicinae and Myrmicinae a primarily predatory lifestyle is found in the subfamilies Ponerinae, Leptanillinae and various Myrmicinae (Blüthgen *et al.* 2010). Typical food sources comprise invertebrates such as Collembolans, other ants and nitrogen rich feces. Food is either captured by single workers or by collaborative associations to the size of swarms in order to overwhelm and transport larger prey (Blüthgen *et al.* 2010). The sting (if existent) is often used to paralyze the prey (Hölldobler *et al.* 1990). Seeds often bear protein and fat rich elaiosomes that make them an attractive food source for many ant genera and contribute to the distribution of plants via myrmecochory (Ness *et al.* 2010). The seeds themselves are rarely consumed by only a few species in more arid regions. Fungi are either gardened or harvested from natural habitats (Blüthgen *et al.* 2010).

Foraging strategies include solitary foraging, recruitment via (simulated) trophallaxis with subsequent tandem runs or carrying of workers, group recruitment by pheromones (e.g. trunk trails – a single path that splits into smaller ones like a tree trunk and covers a wide area) as well as swarm raids of army ants (Reviewed by Hölldobler *et al.* 1990; Dornhaus *et al.* 2010). Retrieval of prey is either accomplished by a single worker, team transport for larger items or ‘bucket brigades’ where items are only carried for a short distance before being turned over to the next ant (Dornhaus *et al.* 2010). A more detailed list can be found in Hölldobler *et al.* (1990, p. 280 f.) and Dornhaus *et al.* (2010, p.212 f.). Bigger ant colonies exploiting profitable and large resources like other nests benefit from the mass recruitment of workers (e.g. with pheromone trails) whereas smaller colonies hunting for single prey items are less likely to make use of a mass recruitment system (Hölldobler *et al.* 1990; Edelsteinkeshet *et al.* 1995; Beekman *et al.* 2001).

Ants mostly are central place foragers with a nest that limits foraging range but also offers lots of advantages like shelter for the queen and brood as well as room for the storage, cultivation and exchange of food (Blüthgen *et al.* 2010). Nest structures and sites can be found in a considerable variety of places: most frequently found are ground nests, nests in wooden cavities and carton or silk nests (Blüthgen *et al.* 2010).

DEFENSE STRATEGIES

Defense mechanisms in ants include a high variety of individual and group strategies (Reviewed by Hölldobler *et al.* 1990; Dornhaus *et al.* 2010). The most common individual defense mechanisms include morphological features such as mandibles, armor, spines, hair, warning or camouflaging coloration and chemicals. The latter comprise powerful venomous stings or if the sting is reduced, other poisonous, repellent, pungent or caustic secretions such as formic acid from various glands (Buschinger 1984; Dornhaus *et al.* 2010). Further behavioral methods like speed bursts, freezing in combination with camouflage or jumping and dropping from branches add to the repertory of defense mechanisms. Group defense strategies including more than one individual, are often used for the defense of the whole colony and attain higher effectiveness than the summed up individual performances. They comprise the use of specialized soldiers, blocking of the nest entrance, (temporary) nest evacuation, ritualized fights, cooperative use of chemicals such as alarm pheromones or chemical weapons and the establishment of guarded territorial borders. Structural and architectural means like redundant dummy chambers and barriers additionally help to block nest entrances, hide trails and generally enhance the defensibility of the nest (Reviewed by Dornhaus *et al.* 2010).

1.3 Some fuss about the buzz - ecological aspects of stingless bees

GENERAL ECOLOGY

Stingless Bees (Meliponini: Apidae) are a widespread highly social group of insects found in all tropical and subtropical regions of the world being most abundant in South and Central America (Roubik 1989). Of the more than 600 described species in about 60 genera only two genera, *Tetragonula* and *Austroplebeia*, comprising about 14 species (5 *Tetragonula*, 9 *Austroplebeia*) can be found mostly in the northern and eastern areas of Australia (Michener *et al.* 1990; Heard 1996; Rasmussen *et al.* 2010). All species are highly social and live in large, perennial colonies with usually hundreds or thousands of workers under a physogastric queen that is unable to fly when gravid (Roubik 1989, 2006). Another common trait of stingless bees is the reduction of the sting – hence the name (Wille 1983).

The Australian species are of a dark color, less than 4mm long and naturally live in crevices or cavities as offered by hollow trees, logs and rocks as well as human built structures (Heard 1996; Klumpp 2007). Yet, within the past 25 years keeping of native stingless bees by humans has become common in Australia and is evolving into a more and more important industry especially with regard to crop pollination, with macadamia and mango being the most common crops to profit from pollination by stingless bees in Australia (Heard 1999). The bees also play an important role as pollinators for at least 7 other agricultural plants and partially contribute to the pollination of about 60-90 more crop species around the globe (Heard 1999). *Tetragonula carbonaria* and *Austroplebeia australis* are the most common species held by beekeepers in Australia (Heard *et al.* 2000).

NESTING ECOLOGY

Nest structures vary considerably between species but are usually constructed with cerumen, a propolis mixture of glandular wax components, resin and various other compounds like mud or plant fibers, as main building material (Wille 1983; Roubik 2006). The brood cells are separated from the rest of the nest by an involucre (one or several layers of cerumen) that forms the brood chamber (Wille 1983). Storage pots for pollen, nectar and honey are located around the brood chamber and surrounded by a protective layer of batumen (hard cerumen) that forms the outline of the nest and also serves to seal hollows (Wille 1983). Nests of *A. australis* and *T. carbonaria* can be distinguished by the arrangement of their brood cells (Figure 1.): Whereas larval cells of *T. carbonaria* are constructed in a horizontal spiral comb, they accumulate in an irregular, loose cluster in *A. australis* (Franck *et al.* 2004; Roubik 2006).

Nest entrances are often extended by a characteristic entrance tube that can reach considerable sizes of 50 cm to one meter (Wille 1983; Roubik 2006). It channels entering and departing bees and reduces the entrance size making it easier to defend (Roubik 2006). Guard bees can often be seen keeping watch for potential predators (Figure 1.d, e) (Wille 1983; Roubik 2006). However in *A. australis* and *T. carbonaria* entrance tubes are quite small or completely absent (Franck *et al.* 2004). Typical for *T. carbonaria* is a dark, spread out mixture of resin surrounding the entrance hole which can pile up to a respectable size due to the collection of *Corymbia torelliana* resin and associated seed dispersal (Figure 1.d, f) (Wallace *et al.* 1995; Franck *et al.* 2004; Leonhardt *et al.* 2011b). A small, but often also missing or cryptic entrance tube can be found in *A. australis* and is usually sealed at night with a loose netlike curtain of resin or propolis to provide an effective barrier against parasites and ants (own observation; Roubik 2006; Klumpp 2007).

Unlike several *Apis* species stingless bees do not migrate due to their queens' disability to fly (Heard 1996; Roubik 2006). A new colony is founded by a virgin queen raised in special queen cells (Figure 1.c) that leaves the nest together with a swarm of workers who transport building material and food provisions for the new nesting site which is usually established within a few hundred meters from the mother's nest (Wille 1983). After having built the new nest, the virgin queen leaves for a mating flight, followed by often hundreds of males (Reviewed by Roubik 2006). The dependence on material exchange between mother and daughter colony has been documented to last from several weeks up to six months (Wille 1983; Roubik 2006).

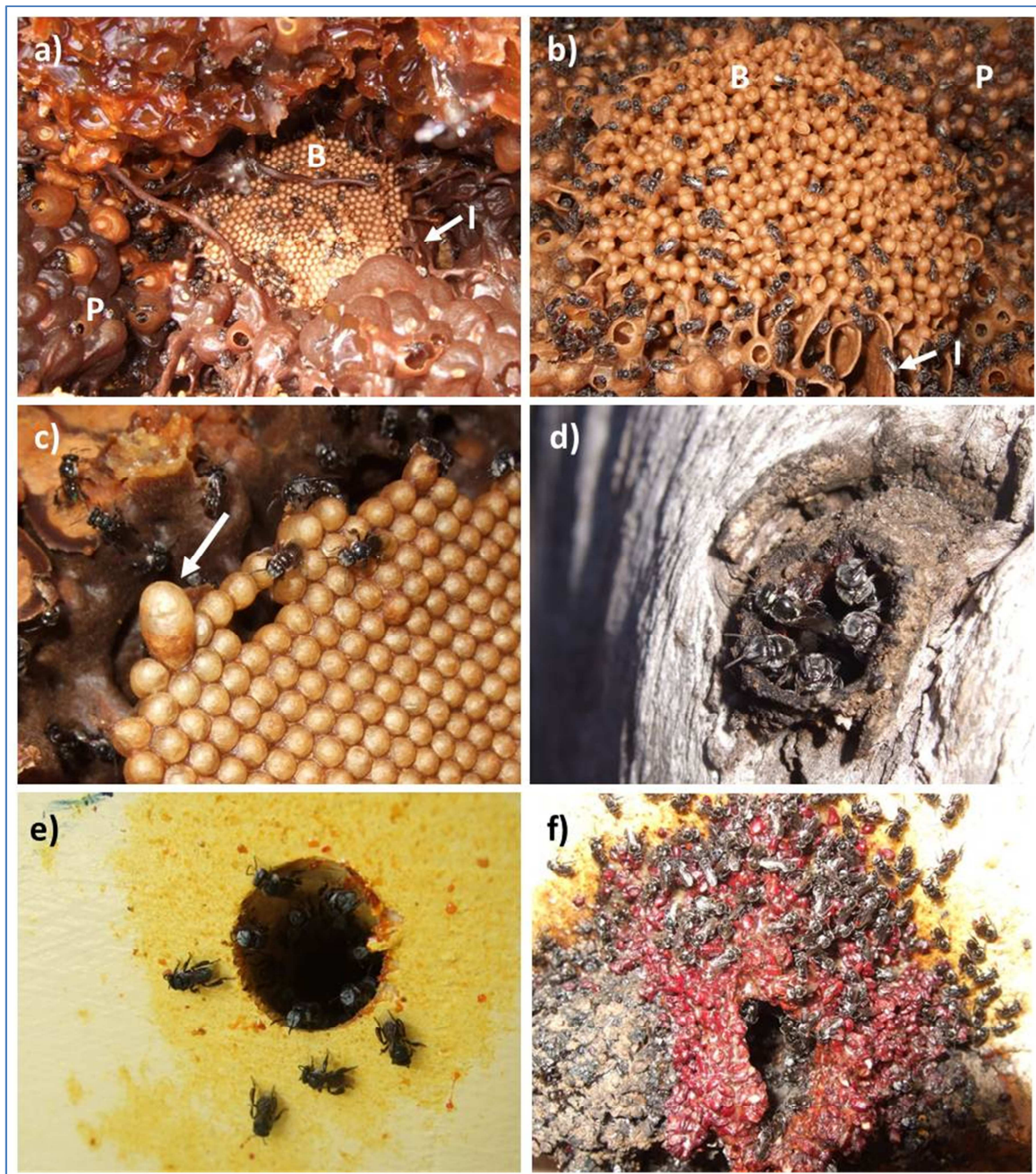


Figure 1. Nesting characteristics of stingless bees: a) hive of *T. carbonaria*: B = brood cells, I = involucrum, P = pots for honey and pollen; b) hive of *A. australis*; c) queen cell in a *T. carbonaria* hive; d) guard bees protecting the entrance tube of an *A. australis* nest; e) entrance tube of a recently split *T. carbonaria* hive; f) entrance tube of an older *T. carbonaria* colony covered with resin and seeds (red)

FORAGING AND USE OF RESOURCES

Stingless bees make use of a broad variety of materials. Among the most important resources collected are pollen, nectar and resin (Roubik 1989). Pollen is an important protein source and therefore also used for larval provisions in brood cells. The most important source of carbohydrates is nectar with sugar concentrations ranging from about 10% up to 72% (Roubik 1989). Both, nectar and pollen are stored in separate pots inside the nest (Figure 1.a, b) and therefore represent a potential target for predators, parasites and robbers (Wilson 1971; Roubik 1989). Another important resource used by stingless bees is resin (Roubik 1989). Resin plays a major role in nest construction, but also in defense (Roubik 2006). Further resources gathered by bees comprise oils, honeydew, sap, gums, wax, plant parts, mud, feces, fungi, spores, hairs, carrion, urine and water (Roubik 1989).

Materials are transported using mandibles, legs, tarsi, other more or less hairy body parts, and by internal means – for example as a mix of pollen and nectar in the crop (Roubik 1989). The most important way of transportation for pollen and resin are the corbiculae, concave, mostly hairy areas on the hind tibiae (Roubik 1989). Depending on the stickiness of the collected material, bees can also add nectar to increase adhesive qualities (Leonhardt *et al.* 2007).

To navigate stingless bees rely on various senses and use information like position of the sun, polarized light, landmarks and geomagnetic cues (Reviewed by Roubik 1989). Chemical guidelines or trails from the nest to a resource have also been reported for some stingless bee species (Roubik 1989). Foraging radii range from about 100 m in small species up to about 2.5 km, depending on the size of the bee (Roubik 1989). Nectar in the crop of departing foragers were suggested to serve as fuel for the flights (Leonhardt *et al.* 2007). Recruitment of nest mates occurs via jostling, vibrations and auditory cues; outside the nest new recruits are guided by scent trails or guiding flights where they directly follow a recruiting forager to the resource (Lindauer *et al.* 1960; reviewed by Barth *et al.* 2008).

Effectiveness in finding new supplies varies among different species (Hubbell *et al.* 1978). The same holds true for the level of aggression which also depends on the attractiveness of the resource (Hubbell *et al.* 1978; Biesmeijer *et al.* 2004). Successful monopolization of attractive resources can be accomplished by high recruitment rates and aggressive behavior (Hubbell *et al.* 1978; Biesmeijer *et al.* 2004). Simultaneous exploitation of resources by various species using different strategies, such as scrambling, bustling, extirpation and opportunism can be observed in many cases (Biesmeijer *et al.* 2004). Other less aggressive species show a higher efficiency in finding new, widespread or isolated resources (Hubbell *et al.* 1978; Biesmeijer *et al.* 2004; Leonhardt *et al.* 2009a).

DEFENSE MECHANISMS

Given that bee nests host large quantities of food (brood, pollen, nectar and adult bees) compared to most natural resources, it is not surprising that a broad range of predators is attracted and that bees possess an elaborate repertory of defense strategies to protect their own lives and the well-being of their colonies (Roubik 1989; Breed *et al.* 2004). Australia lacks serious large mammal predators such as badgers, bears or apes (Roubik 2006). Natural enemies of individual stingless bees foraging on flowers comprise birds, lizards, geckoes, spiders, mantids, wasps, ants, assassin bugs and flies (Roubik 1989). Nests often fall prey to parasites such as wasps, flies, beetles, mites and ants that consume honey, bee larvae, eggs, pupae or living bees and – once they have entered the nest – often destroy its whole internal structure (Roubik 1989; Klumpp 2007). Sometimes other (even conspecific) bee colonies try to usurp or rob nests (Gloag *et al.* 2008). However, a well-established colony is generally able to defend itself against most predators and ant raids, but any disturbances can facilitate access to the colony (Roubik 1989; Klumpp 2007).

Since the sting is depauperate, other strategies have evolved in stingless bees. Aggressive worker defense and biting with mandibles is the most common tactic (Roubik 2006). Retreat into the nest is also observed as a first response to hide in the camouflaged nest entrance, especially from larger predators that tend to look out for hovering bees in order to locate nests (Roubik 2006). Sometimes, defending or fighting swarms form when a conspecific threat has been detected by guard bees who assess the smell of entering individuals and either block the entrance, show aggressive behavior or recruit further defenders via alarm pheromones if chemical profiles do not match (Roubik 1989, 2006; Gloag *et al.* 2008; Lehmberg *et al.* 2008). Few species, for instance *Teragonisca angustula*, even have permanently hovering guards in front of their nests (Wittmann 1985).

Chemical defenses include alarm pheromones (Johnson *et al.* 1985), repellent or pungent glandular secretions like citral or formic acid (Roubik 1989) and cuticular compounds occasionally comprising terpenoids of resinous origin with repellent effects (Debboun *et al.* 2007; Lehmberg *et al.* 2008; Leonhardt *et al.* 2009b). The most mentionable strategy is the use of external materials for nest defense with resin playing a key role: Sticky resin droplets are either sprinkled or evenly distributed around the nest entrance tube and surrounding branches to prevent access or are actively applied to intruders to immobilize them as has been reported for intruding parasites such as the small hive beetle (Roubik 2006; Greco *et al.* 2010b; Halcroft *et al.* 2011).

Fresh resin is reapplied regularly to keep the viscous barrier upright and resin foraging increases after ant attacks (Howard 1985; Khoo *et al.* 1987; Duangphakdee *et al.* 2009; Leonhardt *et al.* 2009a). Blocking of the nest entrance with spheres of hardened resin has also been reported (Roubik 2006)

The nest structure itself provides further tactics. Hidden or small entrances prevent nests from being detected while long entrance tubes provide a good basis for stationing of many defenders and ample application of resin. Narrow tubes serve as bottleneck and can easily be blocked or closed off with resin. Most bees build resin deposits inside the nest near the entrance to allow easy access to resin stores in case of danger (Roubik 1989, 2006).

1.4 Interactions and olfactory traits - the role of resin

INTERACTIVE RELATIONSHIPS IN ANTS

Interactions between social insects and other organisms are just as manifold as their individual lifestyles and offer a broad field for studies. The scale reaches from simple predator-prey relationships to nest associations with varying degrees of interconnection (Hölldobler *et al.* 1990, p.445-6) and other forms of temporary social parasitism. More complex forms such as slavery (dulosis) and permanent parasitism (inquilinism) are found in mixed colonies among different ant species (Hölldobler *et al.* 1990). Mutualisms exist between ants and plants in multifarious relationships, such as offering food or protection for either partner, seed dispersal via myrmecochory and pollination. Very complex interactions between plants, ants, microorganisms and fungi are found in the leaf cutting Attini (Hölldobler *et al.* 1990, 2009). Interactions with other arthropods comprise more or less parasitic myrmecophiles (ant guests that depend on ant societies), as well as mutualistic trophobionts (Hölldobler *et al.* 1990, p.471). The latter comprise mainly hemipterans, but also a few lycaenid larvae, whose honeydew or other nutritious secretions are used by ants. In return the ants offer protection and in some extreme forms even breed and herd aphids (Hölldobler *et al.* 1990). The high relevance of honeydew as food supply is demonstrated by the ants' successful monopolization of most honeydew resources (Way 1963; Blüthgen *et al.* 2006).

INTERACTIVE RELATIONSHIPS IN BEES

Bees also interact with a variety of organisms in parasitic, commensal or even mutualistic relationships. Mites and microorganisms are among the most common parasites with the latter also being mutualists in many cases (Roubik 1989). Interactions with other insects besides some commensals mostly comprise parasitism, predominately by beetles, wasps and flies (e.g. *Phoridae*), but also social parasitism

by other bees (Roubik 1989, 2006; Klumpp 2007). Furthermore, scarcely obligate associations with termites and ants or even birds are common, mainly for protective means on either side, or for the use of existing cavities as nest locations (Wille 1983; Roubik 2006).

Unlike ants, stingless bees are rarely found engaged in trophobioses with hemipterans, but few records found interactions between Coccidae or membracid treehoppers and *Oxytrigona* or *Trigona* species in South- and Central America (Salt 1929; Castro 1975; Laroca *et al.* 1976; Schuster 1981; Figueiredo 1996) as well as between mealybugs (Pseudococcidae) and *Liotrigona* in Africa (Koch *et al.* 2011). One species of *Schwarzula* even hosts Coccidae as wax and honeydew producers inside their nests (Camargo *et al.* 2002). Interactions between honeydew collecting stingless bees and ants have been observed by Almeida-Neto (2003). Other bee species, predominantly apid bees are also known to collect honeydew, albeit most interactions (also for stingless bees) are of opportunistic character collecting excess droplets in the vicinity (Roubik 1989). However, these resources are limited due to competition with wasps and ants protecting hemipterans (Wagner *et al.* 1985; Roubik 1989). Although ants are generally able to outcompete bee rivals (Koch *et al.* 2011) and tend to monopolize their honeydew supply (Blüthgen *et al.* 2006), stingless bees occasionally seem to be capable of holding their ground or, at least, protecting or even defending hemipteran resources against ants, termites or other flying hymenopterans (Figueiredo 1996; Almeida-Neto *et al.* 2003). Presumably chemical means are used for this purpose (Wilson 1971; Castro 1975; Roubik 1989). Protection of nests against ants by chemical deterrents has been recorded by Lehmborg *et al.* (2008) and Duangphakdee *et al.* (2009). However, it is not clear whether these mechanisms also apply for external resource protection.

CHEMICAL COMMUNICATION - A BASIC PRINCIPLE FOR SOCIAL INTERACTION

Social life requires a high efficacy of information processing and transmission among individuals. Reliable communication systems therefore play a crucial role in social insects. Typical signal channels comprise auditory, visual, vibrational, tactile and most important chemical cues (Lindauer *et al.* 1960; Roubik 1989; Hölldobler *et al.* 1990). Due to their terrestrial habitat ants predominantly rely on a highly complex chemical communication system (Hölldobler *et al.* 1990, 2009). As “walking batteries of exocrine glands” (Hölldobler *et al.* 1990; p. 229) they produce pheromones that serve for attraction, alarm, aggressive behavior, recruitment, nest mate recognition, defense, sexual communication, caste determination, food exchange, territorial marking, grooming and further purposes (Hölldobler *et al.* 1990). The complexity of the communicational signal design is further modulated by different pheromones with

concentration depending differences in their meaning. The combination of pheromones with tactile and/or other signals generates a complex multicomponent message (Hölldobler *et al.* 1990; Hölldobler 1999).

Stingless bees also make use of a broad variety of chemical, glandular compounds that serve similar purposes, i.e. recruitment (Barth *et al.* 2008), trail marking (Hrncir *et al.* 2004; Jarau 2009), defense or alarm (Johnson *et al.* 1985), nest mate recognition and sexual communication (Ayasse *et al.* 2001) as well as for various further types of interactions, for instance for feeding larvae or even for nest construction (Blum *et al.* 1972; Smith *et al.* 1983; Roubik 1989; Da Cruz-Landim *et al.* 2005).

Olfactory cues particularly from cuticular profiles play a key role for kin, nest mate and species recognition in many insects, because they enable individuals to differentiate between friend and foe (Wilson 1971; Fletcher *et al.* 1987; Howard 1993). Cuticles mostly consist of waxy, long-chain hydrocarbon (20-35 C-atoms) compounds which are produced in epithelial gland cells and protect insects from physical damages, desiccation or infections (Lockey 1988; St. Leger 1995). The composition of cuticular compounds varies between species and colonies but is relatively uniform within one colony, which renders them ideal cues to be used for recognition (Blomquist *et al.* 1998; D'Ettoire *et al.* 2010). The major part of cuticular substance classes comprises non-polar long chain linear alkanes, alkenes, methyl-branched alkanes and compounds bearing further functional groups, such as carboxylic acids, esters, long-chain alcohols and aldehydes (Buckner 1993; Howard 1993). Whereas the cuticles of ants mainly consist of the aforementioned non-polar compounds (Martin *et al.* 2009), additional polar compounds with functional groups are common in cuticular profiles of bees (Ayasse *et al.* 1999; Abdalla *et al.* 2003; Jungnickel *et al.* 2004; Kerr *et al.* 2004; Nunes *et al.* 2008).

In stingless bees, however, compounds with functional groups are less frequently found, while non-polar aliphatic hydrocarbons dominate their profiles (Abdalla *et al.* 2003; Jungnickel *et al.* 2004; Kerr *et al.* 2004; Nunes *et al.* 2008). Leonhardt *et al.* (2009b; 2011a; 2011b) further showed that, in Southeast Asian stingless bees, resin derived compounds are actively filtered and included into cuticular profiles, with terpenoids (especially sesquiterpenes) being the most prominent components. Terpenoids are presumably responsible for interspecific recognition (Buchwald *et al.* 2005), tolerance or aggression (Leonhardt *et al.* 2010a) as well as for defensive applications against predators (Duangphakdee *et al.* 2009) or microbes (Messer 1985).

STICKY THUS TRICKY – RESIN HANDLING AND USE IN ANTS AND BEES

Resin is a viscous plant secretion that serves to seal wounds after injury as it polymerizes on exposure to oxygen (Langenheim 2003). Besides, it also protects plants from microbial infections and shows toxic or at least repellent effects to a broad spectrum of organisms such as bacteria, fungi (resin containing propolis: Ghisalberti 1979; Messer 1985; Lokvam *et al.* 1999), ants (Codella *et al.* 1995), termites (Bultman *et al.* 1998) or herbivorous insects (Langenheim *et al.* 1983; Langenheim 2003). Resin contains an extraordinary variety of chemical substances with its main constituents being isoprenoid polymers known as mono-, sesqui-, di- and triterpenes (Ghisalberti 1979; Langenheim 2003). Monoterpenes with ant repellent (Eisner *et al.* 1986) and antifungal properties (Langenheim 1994; Gershenzon *et al.* 2007) are believed to serve as solvents for transport of longer terpenoids that are responsible for polymerization and (just like some monoterpenes) for deterrence of herbivores (Langenheim 2003; Debboun *et al.* 2007; Gershenzon *et al.* 2007).

Despite and particularly due to its toxicity for many organisms resin is utilized and taken advantage of by multiple insects. Sawfly larvae deter predators using terpenes of its host plant (Eisner *et al.* 1974) whereas ants mostly use it for self-medication (Christe *et al.* 2003; Castella *et al.* 2008a). Wood ants (*Formica paralugubris*) are known to carry solidified conifer resin into their nests to inhibit the growth of bacteria and fungi with the side effect of reducing their immune activity (Chapuisat *et al.* 2007; Castella *et al.* 2008b). Resin is further used for nest construction in *Vollenhovia* ants (Brühl 2003) but also represents a common nest building material in stingless bees (Roubik 2006). Being a constituent of propolis (Ghisalberti 1979) resin serves as an important preservative for food stock and brood (Roubik 2006; Simone *et al.* 2009). Resin sources are located via terpenes emanating from resin wounds, acting as attractants (Leonhardt *et al.* 2010b). The importance of resin as a limiting resource for stingless bees is reflected in their high foraging density and aggressive behavior at resin wounds (Howard 1985).

As mentioned above, resin is frequently used for defense, partly because of its volatile irritants but also because of its sticky properties which impair movement of arthropod predators and glue their mouthparts or sense organs (Pasteels *et al.* 1983). So far little is known about how stingless bees are able to handle this adhesive material without having to suffer the fate of their offenders. Recent research by Gastauer *et al.* (2011) proposes temporary resin repellent areas on the bees' mandibles that are used for cleaning whereas removable cuticular microfilms (S.D. Leonhardt, personal communication) are also taken into consideration. Apart from the research of Duangphakdee *et al.* (2009), Lehmberg *et al.* (2008) it is not known to which extent resinous compounds on bee cuticles (see p.12) deter predators or whether deterrence is related to the stickiness of some bee species

1.5 Introducing the protagonists

BEEES

Increasing popularity of stingless beekeeping in Australia has increased the availability of stingless bees for research purposes. The two most common species *Tetragonula carbonaria* and *Austroplebeia australis* belong to the two genera found in Australia (Heard *et al.* 2000; Klumpp 2007).

Both species vary significantly in their cuticular profiles (Leonhardt *et al.* 2011b). Whereas *A. australis* bees only collect small amounts of resin and hardly show resin derived compounds in their cuticle, *T. carbonaria* acquires comparatively large amounts of resin in their cuticle, to the extent that the whole body is sticky (personal observation; Michener 2000; Klumpp 2007; Leonhardt *et al.* 2011b). Both species and particularly *T. carbonaria* are strongly attracted by resin from seed capsules of the eucalypt *Corymbia torelliana*. Resin from these capsules is completely soluble in hexane and contains a characteristic but hitherto unknown polar compound which can also be found in the cuticular profiles of *T. carbonaria* (Leonhardt *et al.* 2011b). Why stingless bees acquire *C. torelliana* resin or this particular compound is still unknown.

ANTS

Camponotus aeneopilosus, *Iridomyrmex rufoniger* *gp.*, *Polyrhachis australis*, *Rhytidoponera metallica*, and *Tetramorium bicarinatum* were studied in this project. All these ant species are abundant in Queensland and commonly occur in gardens of settlements. An overview on the ant species this work focuses on is given in Figure 1. and Table 1..

Polyrhachis australis is also known as “Rattle ant” because, if disturbed, it bangs its abdomen on the hard leaf surface, making a distinct rattling noise while swarming out. Its queens show a particular dimorphism with worker like microgyne queens in small nests and larger macrogyne queens being found in notably bigger nest aggregations (Heinze *et al.* 1993). Both types of nests have been found on the study site.

Rhytidoponera Metallica has puzzled the myrmecologists’ understanding of kin recognition due to its frequent absence of functional queens and the low genetic relatedness of nest mates due to ergatoid workers (Ward 1986; Chapuisat *et al.* 2001). In this species, colony size affects the division of labor as well as decisions on foraging range and time (Thomas *et al.* 2003; Thomas *et al.* 2005).



Figure 1. Ant species found at the study site: a) *Polyrhachis australis* on a leaf nest; b) *Camponotus aeneopilosus* at its nest entrance that is heavily guarded by fellow workers; c) *Iridomyrmex rufoniger* on an aphid covered bud of *Podocarpus elatus*; d) *I. rufoniger* tending a lepidopteran larvae ©Steve Shattuck¹; e) *Rhytidoponera metallica* carrying a small flower ©Steve Shattuck¹; f) *Tetramorium bicarinatum* ©Peter Chew²

¹ http://www.flickr.com/photos/steve_shattuck (retrieved on 01.09.2011)

² http://www.brisbaneinsects.com/brisbane_ants/BicolouredPennantAnt.htm (retrieved on 01.09.2011)

Table 1. Overview of the ant species addressed in this thesis and their characteristic traits (Burwell, personal communication; Burwell 2007 - unless stated otherwise)

| Species | Subfamily | Morphology | Habitat and Nest | Foraging Activity and Diet |
|--|----------------|---|--|--|
| <i>Rhytidoponera metallica</i> 'Green Head Ant' | Ponerinae | 4.5-6 mm – Ergatogyne Heavily armored - powerful sting Black with metallic green, blue and purple reflections | Australia wide - Open and disturbed habitats, forest, wood- and grassland Nest in soil, under rocks and logs or open ground | Mostly diurnal, foraging on ground or low vegetation Omnivorous, general scavengers and predators of invertebrates Also feed on honeydew and elaiosome bearing seeds |
| <i>Polyrhachis australis</i> 'Dome-backed Spiny Ant' | Formicinae | 4-6 mm – Monomorphic; Queen dimorphism Shiny, jet black color, two short spines on mesosoma Distinct smell and rattling noise while swarming out when nest is disturbed | Coastal Queensland - Rainforest edges, open forest, woodland and gardens Nest among vegetation of trees and shrubs or in pre-existing cavities Nest is woven together with silk produced by larvae and fragments of vegetation. Entrance sealed at night | Diurnal, foraging along tree branches Floral or extra floral nectaries, sugary saps of fruits and trees, food bodies, dead or living insect prey or electrolytes in form of vertebrate or bird excrement or urine (Liefke <i>et al.</i> 1998) |
| <i>Iridomyrmex rufoniger</i> <i>gp.</i> 'Tufted Tyrant Ant' | Dolichoderinae | 2-3.5 mm Dark, grey brown color Strong odor when crushed Very active | South-east Queensland – Open forest, woodland and gardens Nest in soil, open or beneath rocks and logs, between bricks/pavers or in posts Citrus pest in south-eastern Australia (Stevens <i>et al.</i> 2002) | Mostly diurnal, foraging in trails Generalists, scavengers, honeydew |
| <i>Tetramorium bicarinatum</i> 'Bicolored Pennant Ant' | Myrmicinae | 3.5-4.5 mm - Monomorphic Yellowish-brown head and thorax, dark brown to black abdomen, waist with two segments Introduced from South-east Asia | Invasive, urban areas and disturbed environments – parks and gardens Does not seem to invade natural bushland Nest in soil, frequently introduced via potted plants | Diurnal, usually in cooler morning or evening hours Generalists, scavengers |
| <i>Camponotus aeneopilosus</i> 'Golden Tailed Sugar Ant' | Formicinae | 5-9 mm - Polymorphic Black with a thick cover of golden hair on dorsal surface of the gaster | Eastern Australia, North Qld.to northern Victoria - Open forest and woodland Nest in soil, beneath rocks or logs or at the bases of tree roots | Diurnal, foraging on ground and vegetation Generalists, leaning towards nectar and honeydew |

1.6 Thesis outline

Central aim of this thesis was to assess the following aspects related to interaction and defense of Australian stingless bees with/ against ants:

1. *FORAGING HABITS OF DIFFERENT ANT SPECIES*

Five different ant species were monitored for intake of different food sources to evaluate their feeding preferences and hence potential as predators of stingless bees. Food items carried in by returning foragers were observed and categorized on different times of the day over several weeks. Nectar entries were also analyzed for their volume and sugar content.

2. *TROPHOBIOTIC INTERACTIONS*

An aphid infested tree, attended by honeydew harvesting ants was monitored for the occurrence of stingless bees as a third party in trophobiotic interactions.

3. *ANT REPELLENT EFFECTS OF STINGLESS BEES' CUTICULAR PROFILES*

To investigate preferences of predatory ants for stingless bees, two bee species from different genera with different degrees of resin collection activity, were offered in a choice experiment. To further clarify the influence of resin-derived compounds in the bees' cuticular profiles on the feeding choice by ants, the bee species with a resinous chemical profile was washed in hexane and offered to ants with unwashed individuals of the other species. Cuticular profiles of washed and untreated bees were analyzed and compared using GC-MS.

4. *EFFICACY OF RESINOUS COMPOUNDS AS ANT REPELLENTS*

To find out whether preferences in the feeding choice assay were related to the sticky appearance of *T. carbonaria* or to resin derived cuticular compounds, hexane extracts of the two bee species were prepared. Both extracts were then tested and compared for their effect on ant behavior. Further extracts of four different natural resin sources were additionally investigated for their repellent effects on ants and compared to the two extracts of bee cuticles. Chemical composition of all extracts was analyzed and compared via GCMS.

2 Methods

2.1 Study site

All studies were conducted from 16.02.2011-25.04.2011 at Forest Glen (26°41'S 153°00'E), Queensland, Australia. The area has a subtropical climate with a mean annual temperature of 18-21°C and yearly rainfall from 1200-1600mm (Australian Bureau of Meteorology 2011). The study site covers about 0.5 ha of disturbed garden area surrounded by eucalypt forest which is interspersed by sporadic conifers. Due to human activities such as gardening and landscaping, several non-native plants such as various palm species, bamboo, and *Pinus caribea* were found in the closer vicinity of the study site.

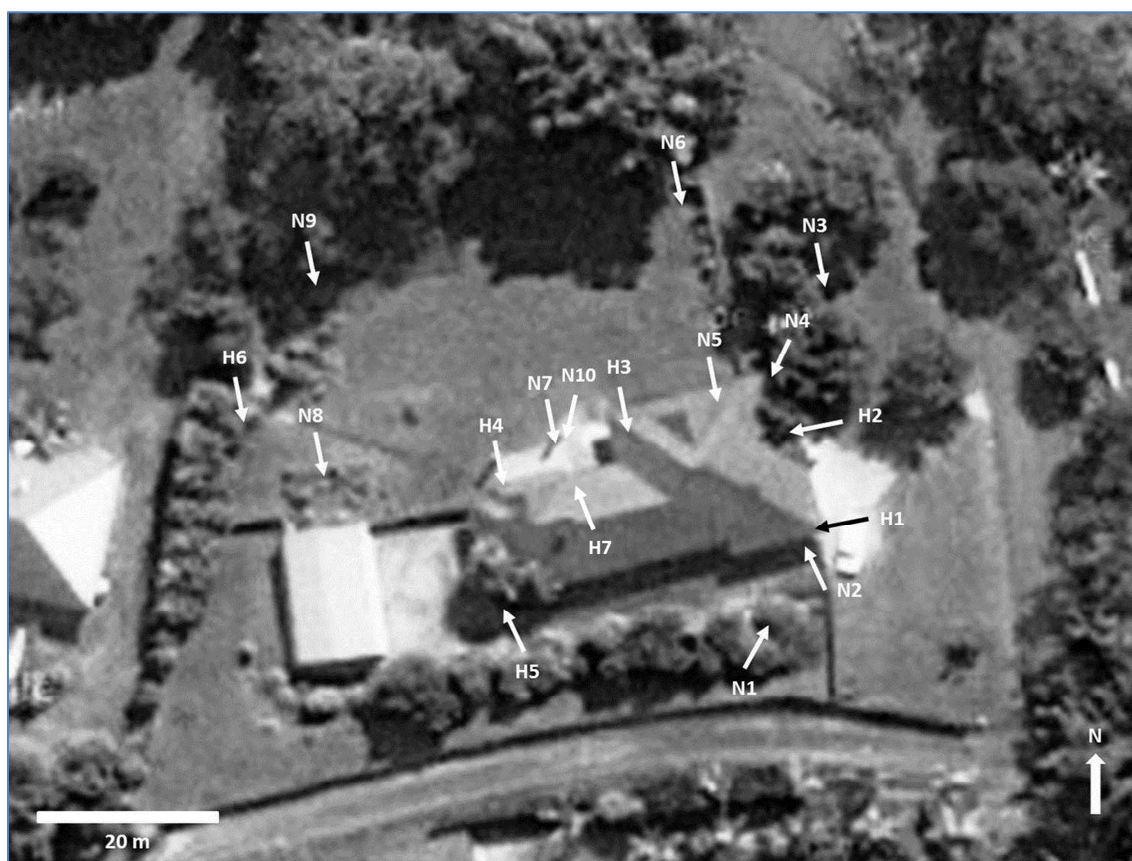


Figure 2. Aerial view of the study site. Ant nest (N1-10) and bee hive (H1-7) locations marked by arrows.
© Google Maps

2.2 Experimental animals

BEEES

For the experiments two species of eusocial native Australian bees, were used: *Austroplebeia australis* and *Tetragonula carbonaria*.

Both species were kept in standard wooden hive boxes (200mm wide x 280mm long), consisting of two halves with a height of about 95mm, made out of 45mm thick Cypress Pine wood. These boxes constitute the most common hive designs used for stingless beekeeping in Australia (Heard *et al.* 2000). Altogether six hives of *T. carbonaria* and one hive of *A. australis* (H4) were arranged around the house and garden area (Figure 2.). All hives had been established at least 4 weeks ago and were well accustomed to the area except for the *A. australis* colony which was put up at the study site in early March and given a week to adapt to the new environment.

Bees for behavioral experiments and extracts were obtained by fixing a resealable, clear plastic bag in front of the entrance hole to catch the required number of bees (Figure 2.). Bees were then put in the freezer for at least three hours before using them for experiments. Depending on the weather and flight conditions which limited the catch quota of new bees, the dead bees were used as soon as possible, not exceeding one week. In order to avoid variations in the chemical composition of hive fragrances, due to colony specific differences in resource allocation and genetically determined waxy compounds, bees were collected from hive H3 (*T. carbonaria*) and H4 (*A. australis*) solely. Contamination of bees with pollen or resin was ruled out by only catching departing foragers.



Figure 2. Collection of *A. australis* from a hive-box using a clear plastic bag

ANTS

All ants used for experiments or observations nested in different areas of the garden and house (Figure 2.). Altogether five different species were used in this study (number of monitored colonies / nests in brackets):

Polyrhachis australis (2), *Rhytidoponera metallica* (3), *Iridomyrmex rufoniger* gp. (3), *Camponotus aeneopilosus* (1) and *Tetramorium bicarinatum* (1) - also see Table 2..

All species were identified by Dr. Chris Burwell, Curator (Entomology) at the Queensland Museum, Brisbane.

Table 2. List of ant nests used in this study (see also Figure 2.). GPS data was recorded with a GARMIN-GPSmap 60Cx, accuracy $\pm 3\text{m}$. All ant species were native to Australia except for one introduced species (indicated by asterisk)

| Nest No. | Ant species | Subfamily | Location | Coordinates | |
|----------|-------------------------|----------------|--------------|-------------|-----------|
| | | | | S 26°41' | E 153°00' |
| N1 | <i>P. australis</i> | Formicinae | Tree | 02.8" | 52.6" |
| N2 | <i>I. rufoniger</i> gp. | Dolichoderinae | Wall | 02.3" | 51.0" |
| N3 | <i>C. aeneopilosus</i> | Formicinae | Soil | 01.9" | 51.0" |
| N4 | <i>P. australis</i> | Formicinae | Window frame | 02.8" | 52.6" |
| N5 | <i>I. rufoniger</i> gp. | Dolichoderinae | Wall | 01.9" | 50.8" |
| N6 | <i>I. rufoniger</i> gp. | Dolichoderinae | Soil | 01.9" | 52.5" |
| N7 | <i>R. metallica</i> | Ponerinae | Wall/pillar | 02.0" | 52.2" |
| N8 | <i>R. metallica</i> | Ponerinae | Soil | 01.5" | 52.3" |
| N9 | <i>R. metallica</i> | Ponerinae | Soil | 01.7" | 52.6" |
| N10 | <i>T. bicarinatum</i> * | Myrmicinae | Plant pot | 01.9" | 52.3" |

2.3 Food source monitoring

FOOD INTAKE

In order to get an overview of the resources collected by the different ant colonies, foragers returning to the nest were caught and the type and quality of food carried in their mandibles and crops analyzed. Overall about 90 returning foragers per colony were picked up with forceps, put into a drosophila-glass and kept in the freezer for 10 minutes. The so immobilized ants were then studied for food items held in their mandibles or nectar in their crops which was collected by gently applying pressure to the ants' gaster using forceps (Figure 2.). Ants were caught across several days and within different time blocks: morning 6:00-10:30 (30 ants), noon 10:30-14:30 (30 ants) and evening 14:30-18:00 (30 ants) to account for daytime related differences in resource intake. About ten ants were caught within each time period on a given day.

If less than 10 ants were collected within 40 minutes only the number of ants obtained by this time were studied.

Ants were returned to their nests after having been screened for resources and their nests were not sampled for at least one day.

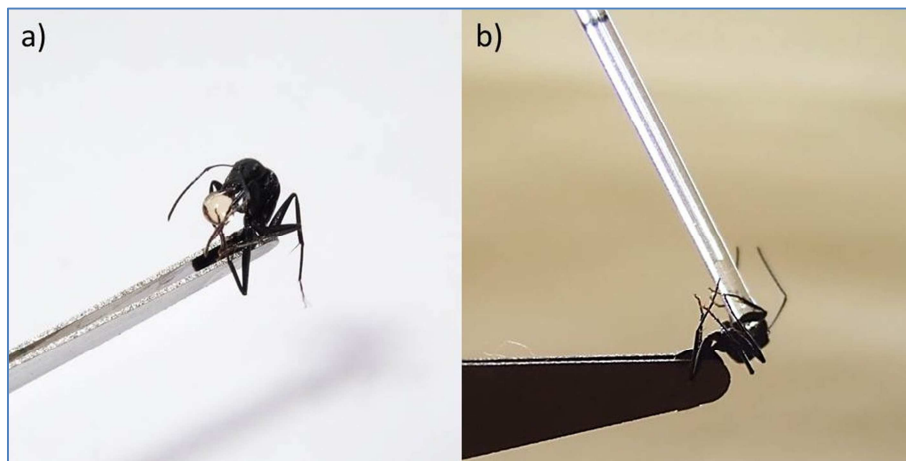


Figure 2. Sampling nectar from returning ant foragers: a) *P. australis* regurgitating nectar; b) collection of nectar with a micro-capillary

Resources carried by returning foragers were classified according to 5 categories: nothing, nectar (fluid substances like honeydew, nectar and sap), protein/animal-material (insects, insect parts, larvae, other animal meat), plant-parts (including seeds), and miscellaneous (comprising all non-identifiable objects as well as human food, like breadcrumbs etc.).

To test whether the different ant species studied generally foraged on resources rich in protein or carbohydrates and whether they showed preferences for one or the other, colonies were provided with a honey/sugar-water solution and tuna bits in Eppendorf tube lids placed near the nest. All species, even the more carnivorous *R. metallica* consumed the honey-water solution. Hence, honey-water was used as bait in all following assays. *R. metallica* and *I. rufoniger gp* were assessed to have the highest predatory potential for stingless bees (c.f. 4.1 p. 45). For this reason focus was predominantly put on these two species in the subsequent feeding choice and extract experiments.

NECTAR

The following species were found collecting nectar in their crops and therefore used for nectar quality assessment: *C. aeneopilosus*, *I. rufoniger gp.*, *P. australis* and *T. bicarinatum*. To analyze nectar quality and volume, randomly picked samples of 7-19 forager crop loads per colony were collected with a 5 μ L micro-capillary (Blaubrand IntraMark \pm 0.1 μ L) and their volumes measured (see Figure 2.). The sucrose content of crop loads was measured using a hand-held refractometer (Eclipse,

Bellingham & Stanley). The first samples were measured with a refractometer ranging only to a sucrose concentration of 50 % (°Brix), later higher concentrations were measured with a second refractometer (Eclipse, Bellingham & Stanley) featuring a wider ranged scale.

APHID INTERACTION

In order to check for potential trophobiotic aphid-bee interactions as well as the response of *I. rufoniger* ants tending these aphids one aphid affected tree of *Podocarpus elatus* (Podocarpaceae) was monitored for potential visits by stingless bees. Observations were carried out three times within the aforementioned time blocks for 10 minutes each. Additionally, the number of ants visiting the plant as well as the mean sucrose concentration of five randomly picked ants that had collected honeydew was determined.

STATISTICAL ANALYSIS

The collected data were tested for differences in nectar and protein entry between the five species as well as between colonies of the same species in different nest locations. Furthermore, daytime differences in resource collection were tested for each species separately. The data collected over time was therefore added up for each species and analyzed with Pearson's Chi-squared test in R using a post hoc Bonferroni correction factor of 5 for multiple testing.

Species depending preferences for a certain food source (nectar or protein) and probabilities for finding the respective item were tested with the exact binomial test in R.

In addition to that, general interspecific differences in nectar volume as well as in concentration were tested with a Kruskal-Wallis rank sum test. Further pairwise comparisons between species were done with the Wilcoxon rank sum test, including a post hoc significance correction.

2.4 Feeding choice assay

To test whether cuticular compounds on the bees' surface affected the behavior of ants approaching a bee as potential prey, *A. australis* and *T. carbonaria* were offered to *R. metallica*, *I. rufoniger* and *T. bicarinatum* in a feeding choice experiment. Whereas *T. carbonaria* has a sticky cuticle with a high density of resin-derived compounds in addition to the cuticular hydrocarbons produced by the bees themselves, *A. australis* has only few or none resin-derived compounds on its body surface (Leonhardt *et al.* 2011b). Bees of both species leaving the nest were caught and put in the freezer for at least three hours. For every trial five bees of each species

were arranged in a circle shaped arena (\varnothing 10cm) which could be entered by the ants through a hole in its center (Figure 2.; also see Lehmberg *et. al.* (2008) for a similar setup). The arena consisted of a circular Styrofoam plate, surrounded by a 5 cm high paper strip preventing the bees from being blown off the platform. This platform was put on a 15 cm long stick to lift the arena off the ground and force the ants to enter through the hole. To rule out that the ants' choices were influenced by chemical compounds from the bees' cuticle or ant pheromones sticking to the bottom of the arena the bees were put on a circular filter paper (divided into ten segments) in alternating order. The filter paper was then pinned onto a cardboard support which could easily be put on the arena. After each trial filter paper and bees were discarded and the cardboard was kept on open air for at least two hours to allow evaporation of potential odorants before being reused.

The arena was placed into or next to an established ant trail near the nest. Honey-water solution (about 50% sugar content) was used as bait to generally attract ants to the arena. When a sufficient number of ants had found the food source and established a trail with at least one ant per minute entering the arena, the plate with sugar was exchanged by the one with bees. The order in which ants chose bees was recorded. A successful choice required that a bee had been dragged out of its segment or carried off the plate by an ant. Each ant that had picked up a bee was subsequently removed and put in a drosophila-cup to rule out double hits. Trials were stopped after five bees had been taken or after a time period of 40 minutes had passed. Each trial was repeated seven times. Combinations of 5 *T. carbonaria* and 5 *A. australis* were fed to *I. rufoniger*, *R. metallica* and *T. bicarinatum*, the latter is an introduced species from Southeast Asia and used to test if foreign ant species are also affected by cuticular compounds of Australian bees. Finally, a fourth control combination was tested using *A. australis* and washed *T. carbonaria* with *R. metallica* to confirm that differences in choices were related to resinous compounds on the bees cuticle.

In order to remove these cuticular compounds of *T. carbonaria* the bees were put into 5 ml hexane, shaken and left standing for 2 minutes. Each bee was then rinsed off again in 5 ml hexane and finally left to dry on a filter paper for 10 minutes before being put on the feeding plate. The effectiveness of the washing procedure was confirmed by analyzing the cuticular compounds of washed and untreated bees with coupled gas chromatography and mass spectrometry (as described on page 27).

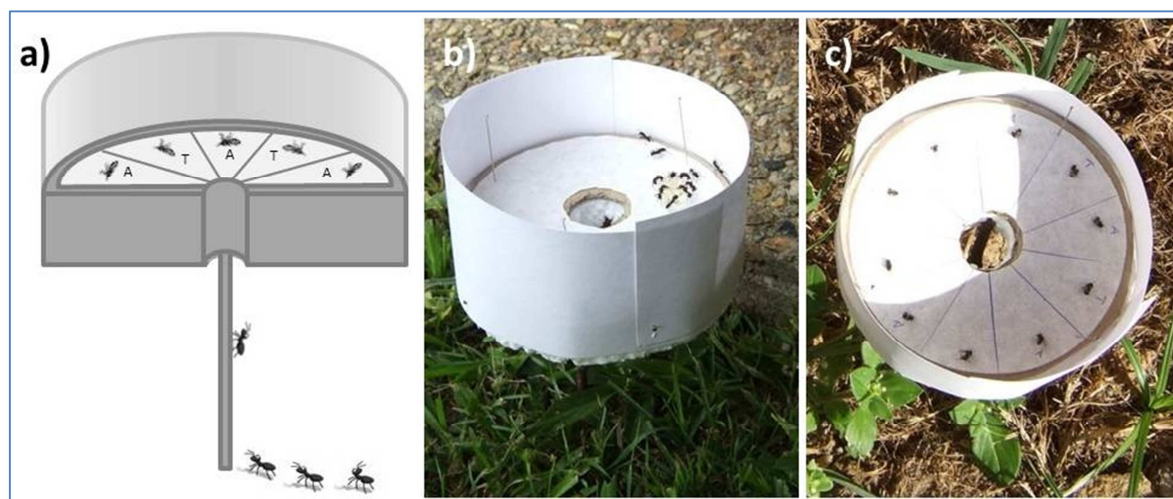


Figure 2. Feeding choice setup (A = *A. australis*; T = *T. carbonaria*): a) schematic of the arena; b) baiting the ants with a cup of honey-water solution; c) prepared feeding plate ready for the ants to attack

STATISTICAL ANALYSIS

To check whether the ants do prefer one of the bee species a Wilcoxon signed rank test with continuity correction was used. Therefore taken numbers of each bee species in a trial were pooled and tested against each other.

2.5 Extract assay

SETUP

The influence of resinous compounds on ant behavior was tested by observing responses of ants approaching a surface treated with different extracts. The aim of these tests was to examine whether preferences for any bee species in the feeding choice assay were related to the stickiness of the bees' body surface or to the smell of cuticular chemical substances. *T. carbonaria*, in contrast to *A. australis* has a high density of resin derived compounds on its cuticle and is therefore way stickier which might lead to the gumming up of predator's mandibles. Further, extracts of pure resin were also tested to assess and compare their effect on ant's behavior.

Hexane extracts were prepared from the following components:

- *Tetragonula carbonaria* cuticle
- *Austroplebeia australis* cuticle
- *Pinus caribea* resin from the trunk
- *Araucaria bidwillii* resin from the trunk
- *Corymbia torelliana* seed capsules
- Propolis of a *T. carbonaria* hive (comprising mainly *C. torelliana* resin)

Resin from the trees was obtained by inflicting artificial wounds upon the trunk using a knife. The resin was collected with a glass rod, transferred to a 6 ml glass vial until it was filled up to about 0.7 cm (which is equivalent to approx. 1 ml resin) and filled up with 5 ml hexane to solve the nonpolar compounds. For the *C. torelliana* extracts, the resin of 5 seed capsules (approx. 0.15 ml resin) and about 1.5 ml of propolis from a *T. carbonaria* nest were solved in 5 ml hexane. Bee cuticular compounds were extracted using 50-60 bees that had been caught in a plastic bag and killed in the freezer overnight, and 5 ml hexane. All extracts (Figure 2.) were kept in the freezer while not being used. The different extracts were subsequently analyzed by gas chromatography and mass spectrometry to characterize differences in their chemical composition (see next subsection on page 27).



Figure 2. Hexane extracts of (from left to right): *C. torelliana* (seed capsules), propolis from a *T. carbonaria* nest, *A. bidwillii*, *P. caribea*, *T. carbonaria*, *A. australis*.

The setup used here was inspired by the bioassay of Duangphakdee *et. al.* (2009) and modified to meet the requirements of this study. Behavioral responses of ants were tested on a Styrofoam feeding dish (\varnothing 10 cm) which was lifted about 10 cm off the ground by a stick anchored in another Styrofoam disk serving as base. A 5 mm wide trench was carved into the base disk and filled with water so that the ants could only enter the feeding dish via a stick used as bridge. Like in the feeding choice assay a cardboard circle with a filter paper pinned to it was put on the dish and used as platform to facilitate an exchange between the different trials as well as to remove pheromone trails and odorous resin leftovers after each trial. In addition, another, smaller filter paper (\varnothing 4.5 cm) was placed in the middle of the platform and equipped with an E-tube lid filled with honey-water solution used as bait to attract ants. Before putting it on the platform the smaller filter paper was soaked with either pure hexane as control or one of the hexane-extracts. Therefore about 10 drops of hexane or extract were applied to the filter until it was evenly soaked. It was left for 30 seconds to allow hexane to evaporate before being put on the dish and equipped with the honey pot.

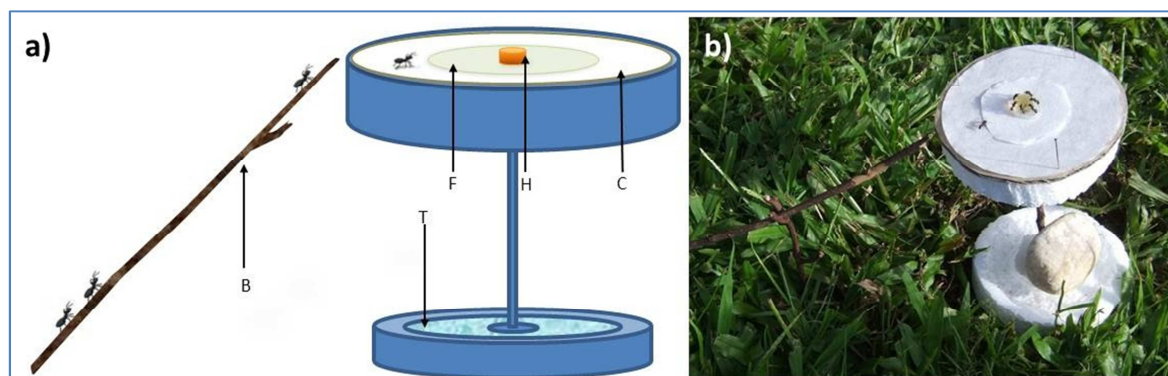


Figure 2. Experimental setup for extract assays: a) schematic of the feeding platform: B = bridge, T = trench with water, F = small filter soaked with chemicals, H = honey-pot, C = cardboard dish with large filter; b) ants feeding on the honey-pot in a control trial, with one ant approaching the smaller filter which had previously been soaked in hexane

To initiate a trial the arena was equipped with bait and put next to an ant trail until a sufficient number of ants had found the food source and established a new trail with at least two ants per minute crossing the bridge. After the trail had been established, the platform was exchanged for one equipped with a hexane or extract filter paper. Hexane and extract filter paper were provided in alternating order. Each platform was left for 5 minutes before being exchanged, to avoid that all highly volatile substances in extracts evaporated and that the ant pheromone trails (enhanced by each visiting ant) interfered with the extracts. Used filter papers were disposed. The cardboard dish was left lying on open air for at least two hours to allow evaporation of potential odorants before being reused. To further avoid chemical substances being carried over by the honey-pots that touched the filter paper, new pots were used for each extract.

Ants entering the platform were then observed for their behavioral response when making first contact with the smaller filter paper. Three main types of behavior could be distinguished:

- Touch and turn: The ant backed up fitfully or turned around immediately after touching the filter paper with the antennae.
- Run for food: The filter paper was accessed without any obvious response to the applied chemicals, the ant headed straight for the food source.
- Cross or explore: Indifferent behavior. The filter paper was accessed and crossed without approaching the food or responding to the chemicals, the ant continued to explore the platform. Further behavioral responses that could not exactly be classified were also sorted in this category.

Each trial was conducted in a paired approach by first monitoring reactions towards the hexane control filter paper, and then towards the extract filter paper for five minutes each.

Extract - species combinations tested are provided in Table 2.:

Table 2. Number of trials tested for each ant species – extract combination

| Extracts | Number of trials for each species | | | |
|-----------------------------|-----------------------------------|---------------------|---------------------|-----------------------|
| | <i>R. metallica</i> | <i>I. rufoniger</i> | <i>P. australis</i> | <i>T. bicarinatum</i> |
| <i>T. carbonaria</i> | 7 | 5 | 7 | 7 |
| <i>C. torelliana</i> (seed) | 7 | 5 | - | - |
| <i>A. bidwillii</i> | 7 | 5 | - | - |
| <i>P. caribea</i> | 7 | 5 | - | - |
| <i>Propolis</i> | 7 | - | - | - |
| <i>A. australis</i> | 7 | - | - | - |

CHEMICAL ANALYSIS

The extracts of bee surfaces, resin and nest samples were analyzed for their chemical compounds by their mass spectra and retention times as described in (Leonhardt 2010). For characterization of the different compounds a Hewlett Packard HP 6890 Series GC System coupled to a Hewlett Packard HP 5973 Mass Selective Detector (Agilent Technologies, Böblingen, Germany) was used. The GC was equipped with a J & W, DB-1 fused silica capillary column (30m x 0.25 mm ID; df = 0.25 μ m; J & W, Folsom, CA, USA). Temperature was programmed from 60°C to 300°C with a 5°C/min heating rate. It was held for 10 min at 300°C. Helium was used as carrier gas (constant flow of 1 ml/min). Injection was carried out at 250°C in the splitless mode for 1 min. Electron impact mass spectra (EI-MS) were recorded at an ionization voltage of 70 eV and a source temperature of 230°C. The Windows version of the ChemStation software package (Agilent Technologies, Böblingen, Germany) was used for data acquisition.

STATISTICAL ANALYSIS

Differences between behavioral responses of ants towards hexane extracts were analyzed in R, using Pearson's Chi-squared test and post hoc Bonferroni correction with factor 6. Expected results and residuals were also taken into consideration.

First, differences in behavioral responses between control and extract were tested for each species.

Second, the ants' responses towards different extracts tested were compared within *R. metallica* and *I. rufoniger*. Since only extracts of *T. carbonaria*, *A. australis* and *C. torelliana*-capsules had similar concentrations (c.f. 'Chemical analysis' p. 34), these were compared against each other as well. Further the hexane controls were also analyzed for behavioral variations within each species.

Finally, responses to *T. carbonaria* extract were compared between the four species. Extracts of *T. carbonaria*, *C. torelliana*, *P. caribea* and *A. bidwili* were further compared between *Rhytidoponera metallica* and *Iridomyrmex rufoniger* *gp.*

3 Results

3.1 Food monitoring

OVERVIEW

Altogether 952 ants were monitored for this assay and 474 collected food items were found. A summary of food allocation is given in Table 3. and Figure 3., a more detailed table can be found in the appendix (App Table) and original data are provided on the enclosed data CD (Ants-Monitoring.xlsx). Nectar was collected by at least 3 % of foragers in all species and so was protein (>6 %) with *C. aeneopilosus* collecting the most nectar (94 %) and least protein whereas *R. metallica* brought in comparatively little nectar and lots of protein (81 %; Table 3.). Unlike the other species, *R. metallica* workers did not carry nectar in their crop but between their mandibles. Intake of plant parts was observed in 3 out of 5 species whereas 2-15 % of miscellaneous items were gathered by all of them (Table 3.). All significance levels given in the following figures and tables already include a Bonferroni correction by factor 5, except for the nectar quality assessment where different data were used.

The most frequently found nectar portions (72 %) were clear in color but milky and brown or yellowish crop loads were also found (Table 3.). It could not be distinguished where the fluid came from (e.g. honeydew, sap or extrafloral nectaries). Plant parts mostly comprised seeds but also pollen and (dry) leaf/grass parts were found. The latter were probably used for nest construction since no leaf cutter ants are found in Australia (Shattuck 1999). Animal prey items comprised primarily insects or insect parts such as ants (Formicidae: Hymenoptera), various larvae and land hoppers (Talitridae: Amphipoda) but also caterpillars (Lepidoptera), crickets/grasshoppers (Orthoptera), woodlice (Isopoda: Oniscidea), lacewings (Neuroptera), beetles (Coleoptera), flies (Diptera), aphids (Aphidoidea: Hemiptera), springtails (Collembola) and earwigs (Dermaptera). Further non-insect prey such as small spiders (Arachnida), snails (Pulmonata), an earthworm (Oligochaeta) and a leech (Hirudinea) were found. Sizes of prey items varied between 0.25 mm to 15 mm, with the most frequent objects being found in the range of 2-5 mm.

Although no stingless bees were found as prey in the actual monitoring experiment, *Rhytidoponera metallica* ants were observed carrying *T. carbonaria* bees in two cases (independent from the feeding choice assay).

Table 3. Summarized overview of food items collected by the different species. Depicted are the numbers of nests observed (N Nests), total number of foragers monitored (N Ants) and the number of food items brought in per category (Misc = Miscellaneous)

| Species | N _{Nests} | N _{Ants} | Nothing | Nectar | Protein | Plants | Misc |
|----------------------------------|--------------------|-------------------|---------|--------|---------|--------|------|
| <i>Camponotus aeneopilosus</i> | 1 | 81 | 32 | 46 | 3 | 0 | 1 |
| <i>Polyrhachis australis</i> | 2 | 180 | 72 | 94 | 9 | 3 | 6 |
| <i>Tetramorium bicarinatum</i> | 1 | 90 | 49 | 35 | 7 | 0 | 3 |
| <i>Iridomyrmex rufoniger</i> gp. | 3 | 296 | 165 | 102 | 22 | 2 | 7 |
| <i>Rhytidoponera metallica</i> | 3 | 305 | 175 | 4 | 105 | 5 | 20 |
| Total for all species | 10 | 952 | 493 | 281 | 146 | 10 | 37 |

Table 3. Color variation in collected nectar and the number of workers found with the according item, pooled for all species. A species specific list is given in App Table .

| Total | Clear | Opaque | Milky | Brown clear | Brown milky | Yellow milky | Other |
|-------|-------|--------|-------|-------------|-------------|--------------|-------|
| 281 | 212 | 26 | 8 | 14 | 9 | 6 | 6 |

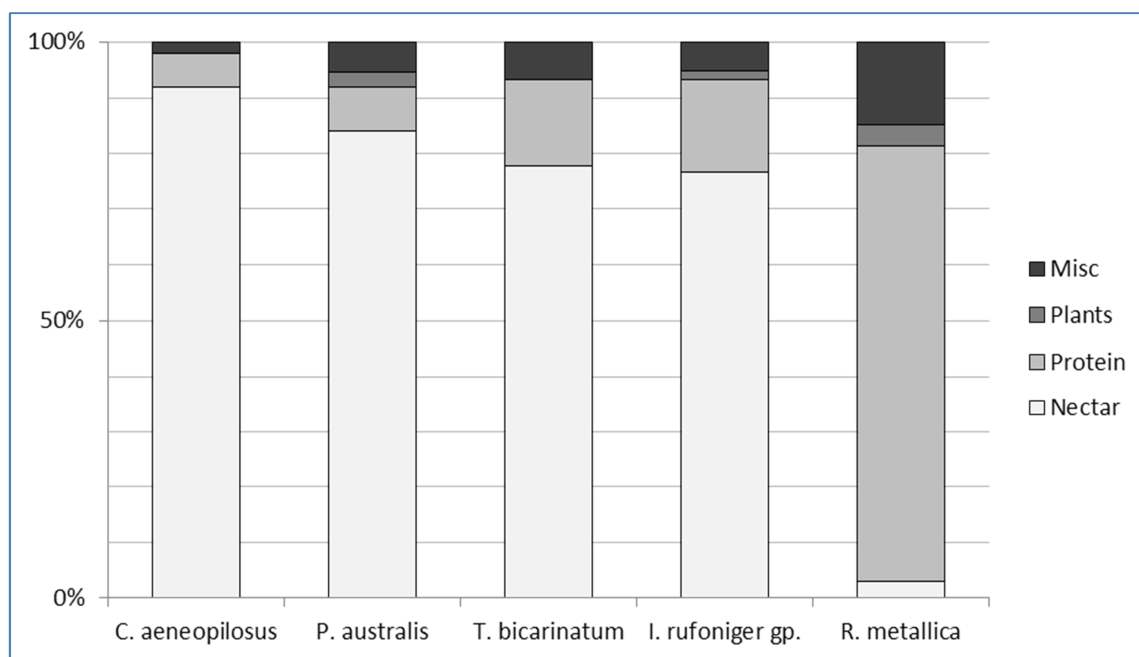


Figure 3. Overview of the percentage of different food items collected by each species (ants without food are not depicted)

The five ant species collected different types of food items (Pearson's chi-squared: $\chi^2 = 254.51$; $df = 4$; $p < 0.0001$, significant after correction for multiple testing). However, when *R. metallica* was excluded from the analysis, no significant differences in food intake could be found between the other four species (Pearson's chi-squared: $\chi^2 = 6.71$; $df = 3$; $p = 0.08$).

PREFERENCES FOR ONE FOOD SOURCE

An exact binomial test for the ants' preferences for certain food sources showed that all ant species highly preferred nectar, except for *R. metallica* that preferred protein (Table 3.).

Table 3. Overview over species specific preferences for a certain food source and probability for finding an ant carrying the preferred item (here and in the following tables, p-values significant after Bonferroni correction are indicated in bold)

| Species | Preferred food | <i>p</i> | Probability for taking nectar |
|-------------------------|----------------|-----------------|-------------------------------|
| <i>C. aeneopilosus</i> | Nectar | < 0.0001 | 93.9 % |
| <i>I. rufoniger gp.</i> | Nectar | < 0.0001 | 82.3 % |
| <i>P. australis</i> | Nectar | < 0.0001 | 91.3 % |
| <i>R. metallica</i> | Protein | < 0.0001 | 3.7 % |
| <i>T. bicarinatum</i> | Nectar | < 0.0001 | 83.3 % |

NEST AND DAYTIME RELATED DIFFERENCES:

Table 3. shows that no significant variations in resource allocation could be found among the different nests of a given species.

Table 3. Variation in food item collection among different colonies of the three species where between 2 and 3 nests had been found

| Species | χ^2 | df | <i>p</i> |
|-------------------------|----------|----|----------|
| <i>I. rufoniger gp.</i> | 6.71 | 2 | 0.01 |
| <i>P. australis</i> | 0.02 | 2 | 0.9 |
| <i>R. metallica</i> | 0.68 | 2 | 0.7 |

The time of the day did not affect variation in food item collection in any of the five species (Table 3.).

Table 3. Differences in food item allocation between different times of the day

| Species | χ^2 | df | <i>p</i> |
|-------------------------|----------|----|----------|
| <i>C. aeneopilosus</i> | 0.39 | 2 | 0.82 |
| <i>I. rufoniger gp.</i> | 1.66 | 2 | 0.44 |
| <i>P. australis</i> | 0.83 | 2 | 0.66 |
| <i>R. metallica</i> | 1.75 | 2 | 0.42 |
| <i>T. bicarinatum</i> | 7.14 | 2 | 0.03 |
| All species pooled | 0.11 | 2 | 0.94 |

NECTAR ASSESSMENT

The five species differed significantly in the volume of nectar carried by returning foragers (Kruskal-Wallis rank sum: $H = 25.31$; $df = 3$; $p < 0.0001$). *C. aeneopilosus* carried the highest volume in its crop (0.1-4.0 μl) whereas *T. bicarinatum* collected the smallest amounts (0.1-0.2 μl). Nectar crop loads comprised similar volumes in *C. aeneopilosus* and *I. rufoniger* gp. or *P. australis* respectively. All other combinations of species carried in significantly different amounts of nectar (Figure 3.a).

All five species also collected nectar with significantly varying sucrose concentrations (Kruskal-Wallis rank sum: $H = 14.53$; $df = 3$; $p = 0.002$). *C. aeneopilosus* carried in the highest ($45 \pm 20\%$) and *T. bicarinatum* the lowest ($17 \pm 3\%$) concentrations. These two species were the only ones whose sucrose concentrations differed significantly from each other (Figure 3.b). More detailed tables and results can be found in the appendix (App Table -6).

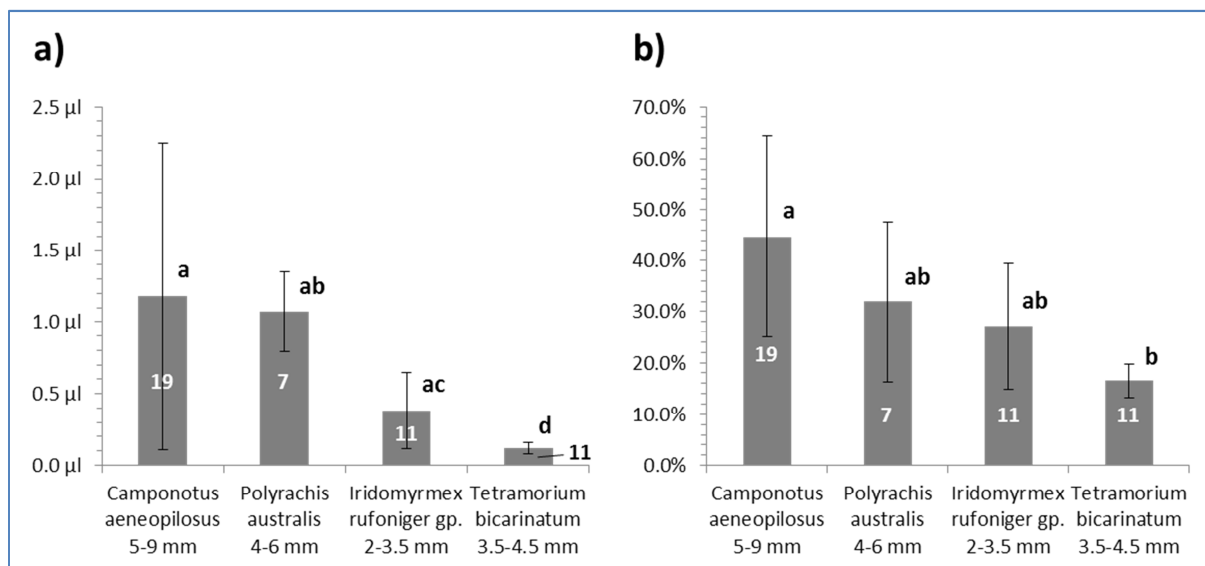


Figure 3. Overview over nectar quality and quantity found for the monitored species: a) Mean nectar volume; b) Mean sucrose concentration ($^{\circ}\text{Brix}$). Numbers given in the bars indicate the number of ants surveyed; different letters indicate significantly different results; size range of each ant species is given below its species name

Body sizes of the ants correlated positively with carried nectar volumes (Spearman's rank correlation: $S = 7174.53$, $p = 0.04$, $r = 0.33$; not significant after correction for multiple testing) but not with sucrose concentration ($S = 8895.76$, $p = 0.31$; $r = 0.17$). Nectar volume and sucrose concentration correlated moderately ($S = 4883.32$, $p = 0.0003$, $r = 0.54$). Hence bigger ants tended to carry more nectar in their crops and larger crop loads were prevalently higher concentrated in sucrose.

APHID INTERACTION

A branch of *Podocarpus elatus* (Podocarpaceae) with 70-80 aphids was attended by about 5 *I. rufoniger* *gp.* ants per minute. A random sample of five workers leaving the tree gave a mean sucrose content of 19.8 ± 4.4 % (ranging from 15-27 %).

No stingless bees were found to attend the aphid infested tree within the observation period. Further, 18 other flying insects and one other ant species were observed on the tree but not monitored for their interactions with aphids or ants. A table of observation times and insect-visitors can be found in the appendix (App Table).

3.2 Feeding choice assay

Altogether 35 bees were fed per test and ant species. Only *R. metallica* had a significant preference for *A. australis* over untreated *T. carbonaria* workers (Wilcoxon signed rank: $V = 28$; $p = 0.02$) whereas their preference for *T. carbonaria* workers disappeared when the bees had been washed prior to testing. ($V = 25$; $p = 0.06$). Neither *T. bicarinatum* ($V = 25$; $p = 0.06$) nor *I. rufoniger* *gp.* ($V = 21$; $p = 0.24$) favored any of the two bee species (Figure 3.). A detailed list of the choices made in each trial is provided on the enclosed data CD (Feeding-Choice.xlsx)

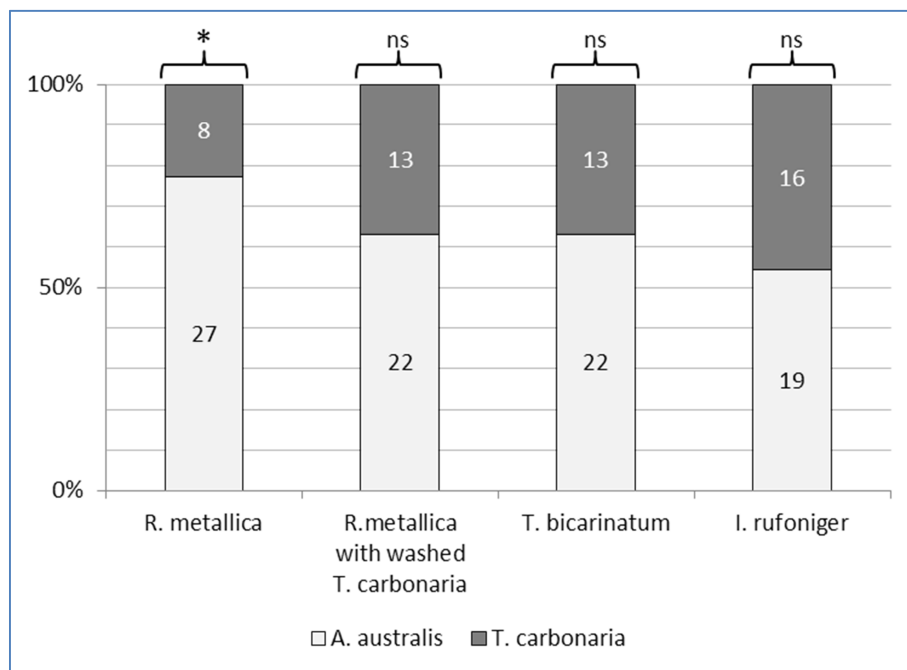


Figure 3. Overview of the choices made by different ant species; numbers in bars give the numbers of bee individuals taken. (significance levels as follows: * $p < 0.05$; ns = not significant)

GC-MS analyses of *A. australis* as well as unwashed and washed *T. carbonaria* workers showed that the washing procedure with hexane had successfully removed almost all cuticular compounds from *T. carbonaria* (Figure 3., p. 35 - as further indicated by the differing height relations of the standard peak). Figure 3. also shows that both bee species have quite different chemical profiles with *A. australis* possessing lower concentrations of chemicals. The most apparent differences are found in the missing peaks at 21.5 and 37 min in *A. australis* where large peaks are found at 33 and 36.5 min instead. The peak at 21.5 min in *T. carbonaria* marks a characteristic, but hitherto unknown compound that is derived from *Corymbia torelliana* resin which has so far not been found in cuticular profiles of *A. australis* (c.f. Section 1.5. and Leonhardt *et al.* 2011). Also terpenoids compounds, present in *T. carbonaria* were not found in *A. australis*. Further cuticular compounds comprise esters, alkanes and in the case of *A. australis* also alkenes.

3.3 Extract assay

CHEMICAL ANALYSIS

Chemical profiles of the different extracts are shown in Figure 3. (p. 36). A comparison of the C 18 standard peak heights showed that *P. caribea* and *A. bidwili* had roughly the same concentrations, whereas *C. torelliana* resin was less concentrated (also see description in Figure 3.). The standard for Propolis is not visible due to overlap, but this extract is most likely much more strongly concentrated than any of the other extracts. Further, extracts of *T. carbonaria*, *A. australis* bees (not shown, c.f. Figure 3.) and *C. torelliana* had similar concentrations.

All four extracts contained mono-, sesqui-, and diterpenes in varying amounts. Additional alkanes and triterpenes were found in propolis which as a mixture of wax and resin showed the highest number of different compound classes. *C. torelliana* resin and propolis are reasonably similar in mono-, sesqui- and diterpene composition. In both extracts two large peaks of monoterpenes were found, whereas *P. caribea* and *A. bidwili* comprised a third monoterpenic compound. Among sesquiterpenes the unknown *C. torelliana* compound was exclusively found in its seed capsules and in propolis of *T. carbonaria*. This particular compound was further found in *T. carbonaria* extract (not shown, c.f. Figure 3.). Generally, many compounds found in *C. torelliana* resin (except for monoterpenes) were also apparent on the cuticle of *T. carbonaria* (and in propolis) but not in *A. australis* extracts (Figure 3.). Whereas *C. torelliana* resin completely dissolved in hexane and propolis as well as *P. caribea* resin dissolved for the most part, resin of *A. bidwili* remained mostly undissolved.

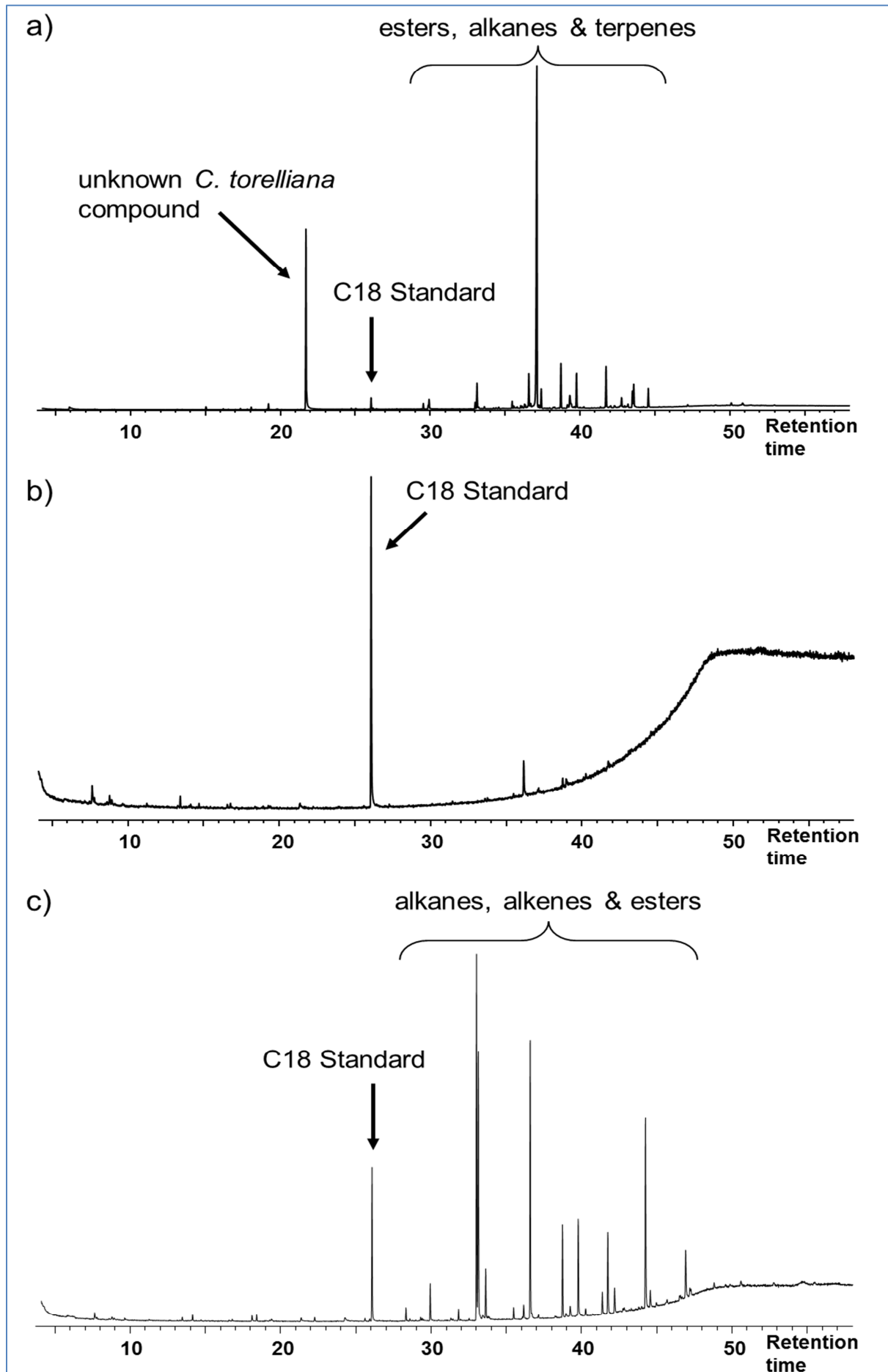


Figure 3. Chemical, cuticular profiles of three stingless bee workers: a) *T. carbonaria* worker – natural; b) *T. carbonaria* worker – washed in hexane; c) *A. australis* worker – natural; Retention time is given in minutes

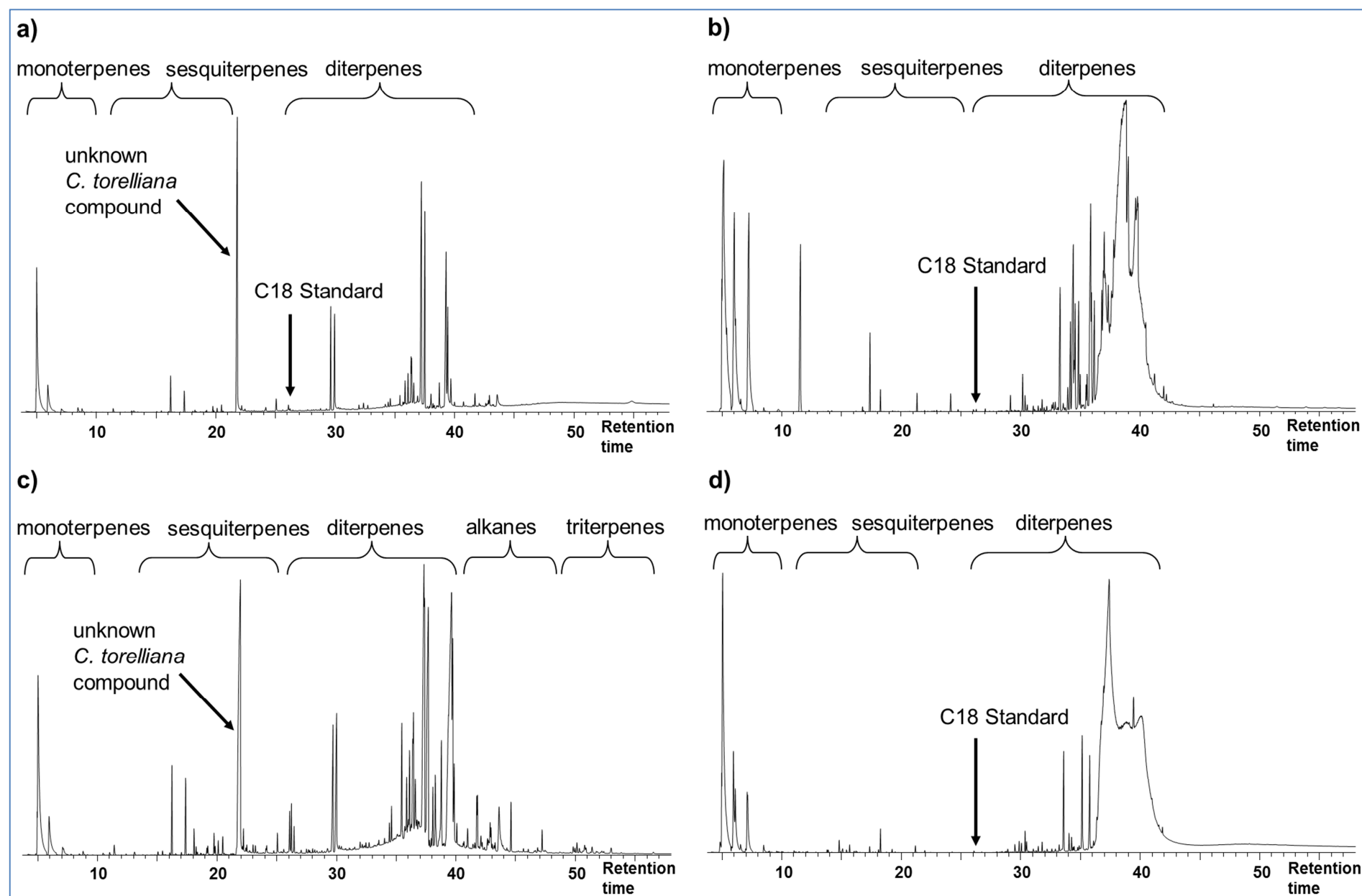


Figure 3. Overview and comparison of different resinous extracts and their compounds: a) *C. torelliana* seed capsules, 5 capsules (≈ 0.15 ml) in 5 ml hexane; b) *P. caribea* resin, ≈ 1 ml in 5 ml hexane; c) Propolis from *T. carbonaria* hive, ≈ 1.5 ml in 5 ml hexane (C 18 standard is not visible); d) *A. bidwillii* resin, ≈ 1 ml in 5 ml hexane; Retention time is given in minutes

COMPARISON OF BEHAVIORAL RESPONSES TOWARDS EXTRACTS AND HEXANE

The behavior of more than 4050 ants towards first contact with different extract treated surfaces was observed. In comparison with hexane, *Rhytidoponera metallica* ants showed highly significant differences in behavior when they made contact with extract treated surfaces except for the *A. australis* (less significant) and *Araucaria bidwillii* extract (not significant; Figure 3.; exact test results in appendix: App Table). Workers turned away more often from filter papers treated with extract and thus did not enter the filter paper to explore it or get food as often as on hexane treated surfaces (Figure 3.).

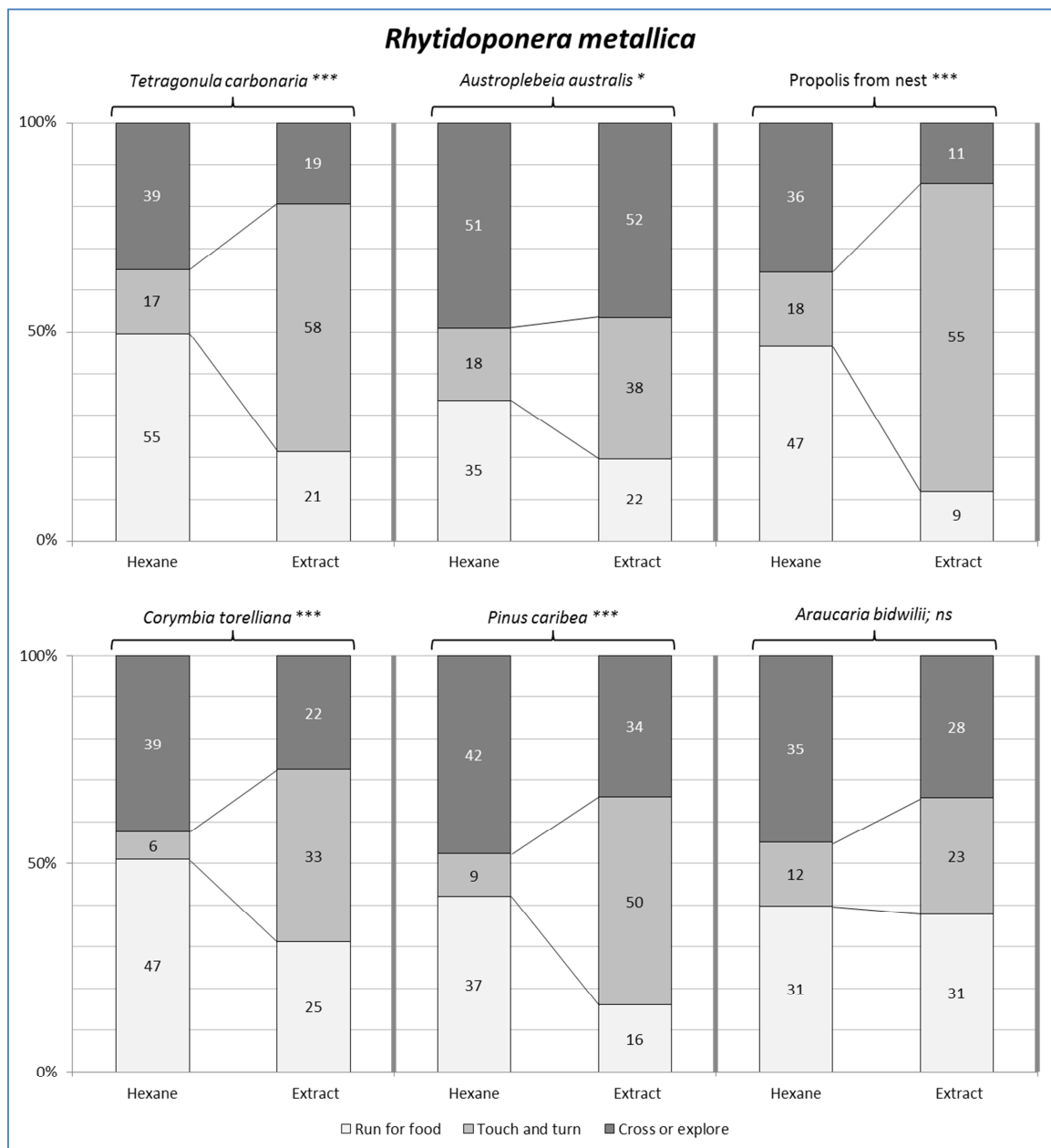


Figure 3. Comparison of *R. metallica* foragers' responses towards hexane and different extracts; numbers in bars give the numbers of ant individuals showing a particular behavioral response (significance levels as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)

Workers of *Iridomyrmex rufoniger* gp. turned significantly more often away from filter papers treated with extracts of *T. carbonaria* and *P. caribea*. Hence these ants did not enter the treated filter for exploration or feeding as frequently as the control filter paper. Extracts of *A. bidwillii* and *C. torelliana* did not affect these ants' behavior (Figure 3.; exact test results in appendix: App Table).

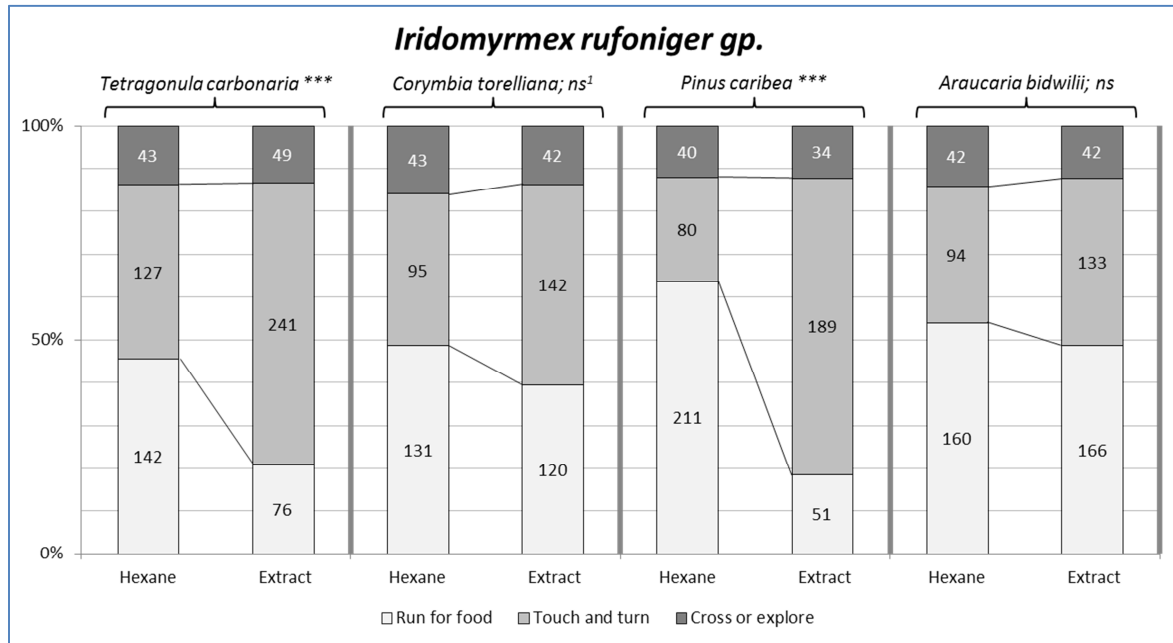


Figure 3. Comparison of *I. rufoniger* gp. foragers' responses towards hexane and different extracts; numbers in bars give the numbers of ant individuals showing a particular behavioral response (significance levels as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)
¹not significant after correction for multiple testing

Workers of *Polyrhachis australis* were highly significantly influenced in their behavior by extract of *T. carbonaria* bees. They prevalently backed off from bee extract but also explored the filter paper more often than the control whereas the food was less frequently touched. On workers of *Tetramorium bicarinatum* the bee extract had no significant behavioral impact in comparison with the hexane control (Figure 3.; exact test results in appendix: App Table).

Although some ants did turn away after first contact with the filter paper, most ants were found to enter it and go for the honey after a while or after several contacts. Detailed data for each trial are provided on the enclosed data CD (Extract-Assay.xlsx).

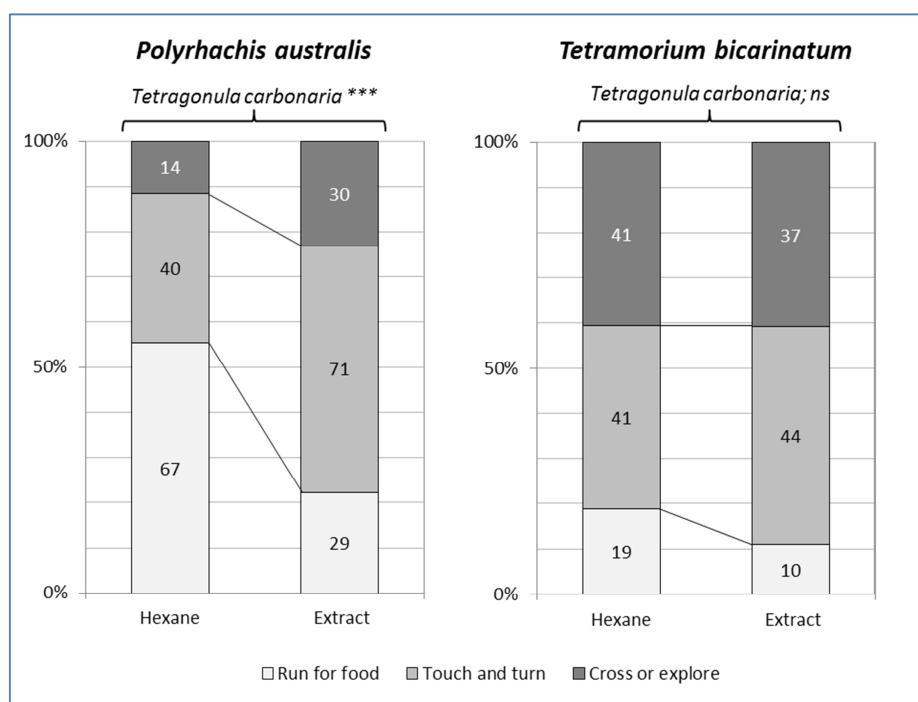


Figure 3. Comparison of responses of *P. australis* and *T. bicarinatum* towards hexane and *T. carbonaria* extract; numbers in bars give the numbers of ant individuals showing a particular behavioral response (significance levels as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)

COMPARISON OF DIFFERENT EXTRACTS WITHIN EACH SPECIES

For workers of *R. metallica* highly significant differences in behavior were found when all extracts were compared (Pearson's Chi-squared: $\chi^2 = 62.93$; $df = 10$; $p < 0.0001$). The residuals in Table 3. show that workers primarily turned away from filter papers treated with propolis extract, followed by *T. carbonaria* and *P. caribea* extract, hence the filter was not entered for feeding or exploration very often. Less ants than expected backed off from *Austroplebeia australis* extract but prevalently went to explore the treated filter instead. Filter papers treated with extracts of *C. torelliana* capsules and *A. bidwili* were entered for feeding more regularly than expected and workers drew back from the filter in fewer cases (also see Figure 3.).

Extracts of *T. carbonaria*, *A. australis* and *C. torelliana* nearly had the same concentrations of chemical compounds (c.f. 'Chemical analysis' p.34). A comparison of behavior between these extract showed that *R. metallica* workers turned away significantly more often from *T. carbonaria* extract than they did from filter papers with *A. australis* extract (Pearson's Chi-squared: $\chi^2 = 18.68$; $df = 2$; $p < 0.0001$). Behavioral differences were less pronounced between *C. torelliana* and *A. australis* ($\chi^2 = 7.58$; $p = 0.02$, not significant after correction for multiple testing) or *C. torelliana* and *T. carbonaria* ($\chi^2 = 5.67$; $p = 0.06$; Figure 3.).

Table 3. Overview of the number of *R. metallica* ants showing one of the three responses (R = Run for food; T = Touch and turn; C = Cross or Explore) towards different extracts; based on the total number of workers observed to perform a particular behavior residuals give the degree of deviation of the observed behavior from values expected for this behavior if extracts had no effect (calculated with Pearson's Chi-squared test). Considerable deviations (values $\geq \pm 2.0$ are thought to be extremely rare and contribute severely to the significance level) are marked in red (+) and blue (-)

| Extract | Counted number of ants | | | Expected number of ants | | | Residuals | | |
|-------------------------------|------------------------|----|----|-------------------------|----|----|-----------|-------|-------|
| | R | T | C | R | T | C | R | T | C |
| <i>T. carbonaria</i> | 21 | 58 | 19 | 22 | 46 | 30 | -0.26 | 1.76 | -1.97 |
| <i>A. australis</i> | 22 | 38 | 52 | 25 | 53 | 34 | -0.67 | -2.02 | 3.09 |
| Propolis from Nest | 9 | 55 | 11 | 17 | 35 | 23 | -1.94 | 3.33 | -2.47 |
| <i>C. torelliana</i> capsules | 25 | 33 | 22 | 18 | 38 | 24 | 1.61 | -0.75 | -0.46 |
| <i>P. caribea</i> | 16 | 50 | 34 | 23 | 47 | 30 | -1.40 | 0.44 | 0.66 |
| <i>A. bidwili</i> | 31 | 23 | 28 | 19 | 39 | 25 | 2.88 | -2.50 | 0.62 |

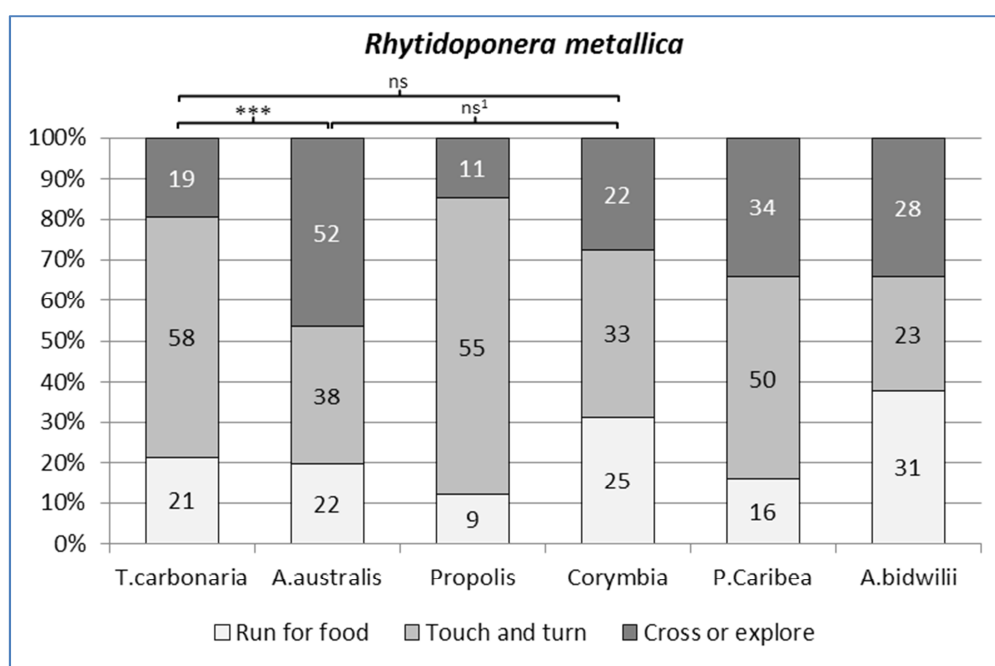


Figure 3. Behavioral responses of *R. metallica* workers towards different extracts; numbers in bars give the numbers of ant individuals showing a particular behavioral response; degree of behavioral differences between three selected extracts is indicated by brackets (significance levels as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)
¹ not significant after correction for multiple testing

Workers of *Iridomyrmex rufoniger* *gp.* also showed highly significant behavioral differences between all extracts (Pearson's Chi-squared: $\chi^2 = 102.16$; $df = 6$; $p < 0.0001$). The residuals (Table 3.) show that *P. caribea* extract caused the most ants to turn away from the filter paper, followed by *T. carbonaria* extract. In turn, more ants entered the filter papers for feeding when these were treated with *C. torelliana* and especially with *A. bidwili* extract.

The numbers of workers exploring the filter papers did not vary a lot but a high number of ants to run for food resulted in a low number to back away from the filter and vice versa (also see Figure 3.). A comparison between the similarly concentrated extracts of *C. torelliana* and *T. carbonaria* showed that ants backed off significantly more often from the bee extract whereas they were more ready to enter a filter paper treated with *C. torelliana* resin to feed (Pearson's Chi-squared: $\chi^2 = 30.53$; $df = 2$; $p < 0.0001$; Figure 3.).

Table 3. Overview of the number of *I. rufoniger* *gp.* ants showing one of the three responses (R = Run for food; T = Touch and turn; C = Cross or Explore) towards different extracts; based on the number of workers showing a particular behavior residuals give the degree of deviation of the observed behavior from values expected for this behavior if extracts had no effect (calculated with Pearson's Chi-squared test). Considerable deviations (values $> \pm 2.0$ are thought to be extremely rare and contribute severely to the significance level) are marked in red (+) and blue (-)

| Extract | Counted number of ants | | | Expected number of ants | | | Residuals | | |
|-------------------------------|------------------------|-----|----|-------------------------|-----|----|-----------|-------|-------|
| | R | T | C | R | T | C | R | T | C |
| <i>T. carbonaria</i> | 76 | 241 | 49 | 118 | 201 | 48 | -3.84 | 2.84 | 0.21 |
| <i>C. torelliana</i> capsules | 120 | 142 | 42 | 98 | 167 | 40 | 2.26 | -1.92 | 0.40 |
| <i>P. caribea</i> | 51 | 189 | 34 | 88 | 150 | 36 | -3.95 | 3.15 | -0.27 |
| <i>A. bidwillii</i> | 166 | 133 | 42 | 110 | 187 | 44 | 5.39 | -3.95 | -0.35 |

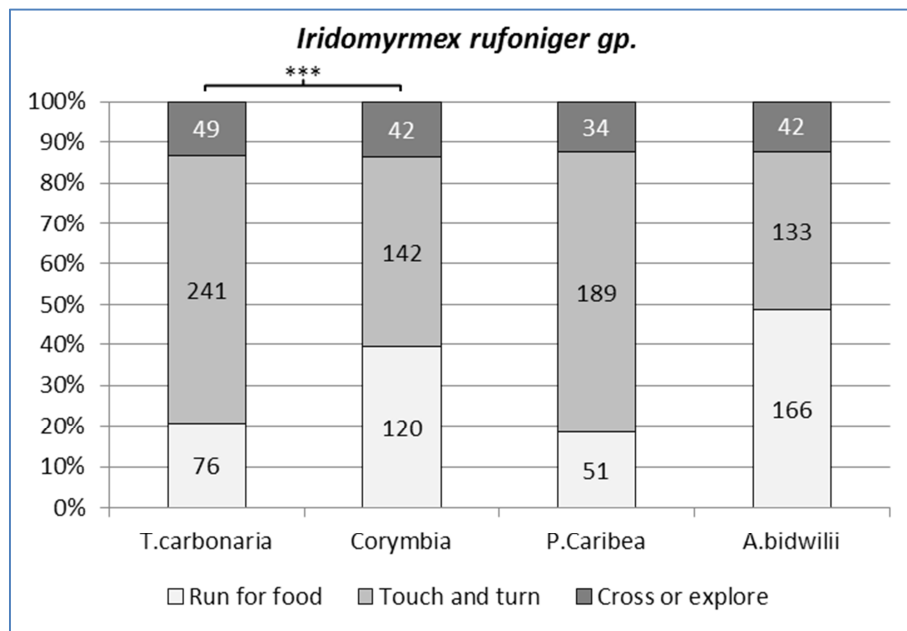


Figure 3. Behavioral responses of *I. rufoniger* *gp.* workers towards different extracts; numbers in bars give the numbers of ant individuals showing a particular behavioral response; degree of behavioral differences between two selected extracts is indicated by brackets (significance levels as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)

A comparison of the behavioral responses towards the hexane controls within each species showed that *R. metallica* workers did not differ in their behavior towards different hexane filters (Pearson's Chi-squared: $\chi^2 = 15.89$; df = 10; $p = 0.10$) whereas workers of *I. rufoniger* gp. showed a significant variability in their reactions to hexane treated filter papers (Pearson's Chi-squared: $\chi^2 = 27.16$; df = 6; $p = 0.0001$).

COMPARISON OF RESPONSES TOWARDS THE SAME EXTRACTS AMONG DIFFERENT SPECIES

Responses to the *T. carbonaria* bee extract were compared between all four ant species, as well as separately between *R. metallica* and *I. rufoniger* gp. whereas the other resinous extracts were compared between the latter two ant species only (detailed test results in App Table).

All four species differed highly significantly in their reactions towards *T. carbonaria* extract. *I. rufoniger* gp. workers backed off the filter most frequently, followed by *R. metallica* and *P. australis*. *T. bicarinatum* did not turn away from the filter as often as the other species but also went to feed from the honey the least. Instead *T. bicarinatum* workers tended to prevalently explore the filter (Figure 3.). A comparison of the residuals showed that this deviant behavior of *T. bicarinatum* mostly accounted for the high significance level, whereas the other three species showed only marginal deviations from responses expected if the extracts had no effect.

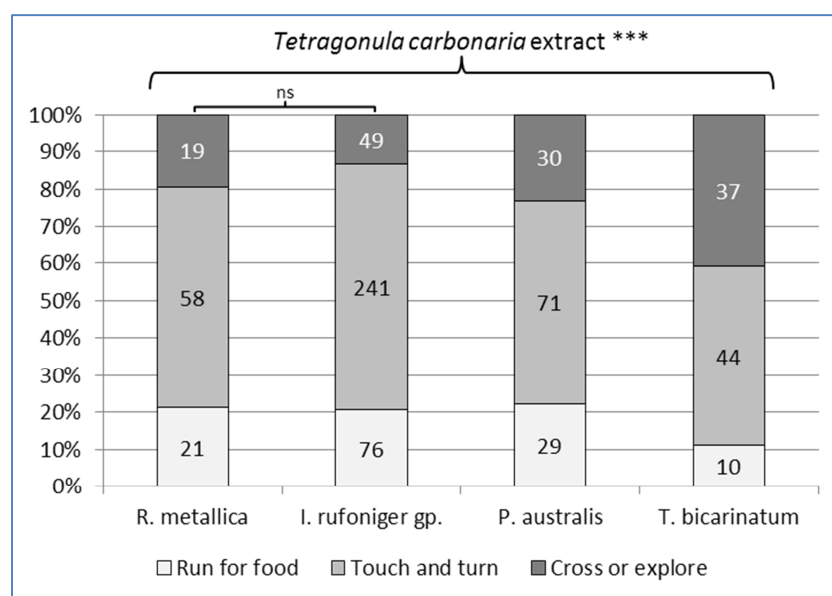


Figure 3. Behavioral responses of different ant species towards *T. carbonaria* extract; numbers in bars give the numbers of ant individuals showing a particular behavioral response; Significance level of behavioral differences between two selected species (small bracket) and over all species pooled (big brackets) as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)

R. metallica and *I. rufoniger gp.* differed highly significantly in their behavioral responses towards *P. caribea* (Pearson's Chi-squared: $\chi^2 = 23.19$; $df = 2$; $p < 0.0001$) and *A. bidwillii* ($\chi^2 = 22.87$; $p < 0.0001$). In both cases a greater number of *I. rufoniger gp.* workers turned away on first contact with the filter paper than did workers of *R. metallica*. Those ants of *I. rufoniger gp.* that entered the filter did not explore or cross it as often as *R. metallica* but went straight for the food more frequently (Figure 3.). No behavioral differences between both species were found for *T. carbonaria* ($\chi^2 = 2.45$; $p = 0.29$; Figure 3.) and *C. torelliana* extract ($\chi^2 = 8.66$; $p = 0.01$, not significant after correction for multiple testing; Figure 3.).

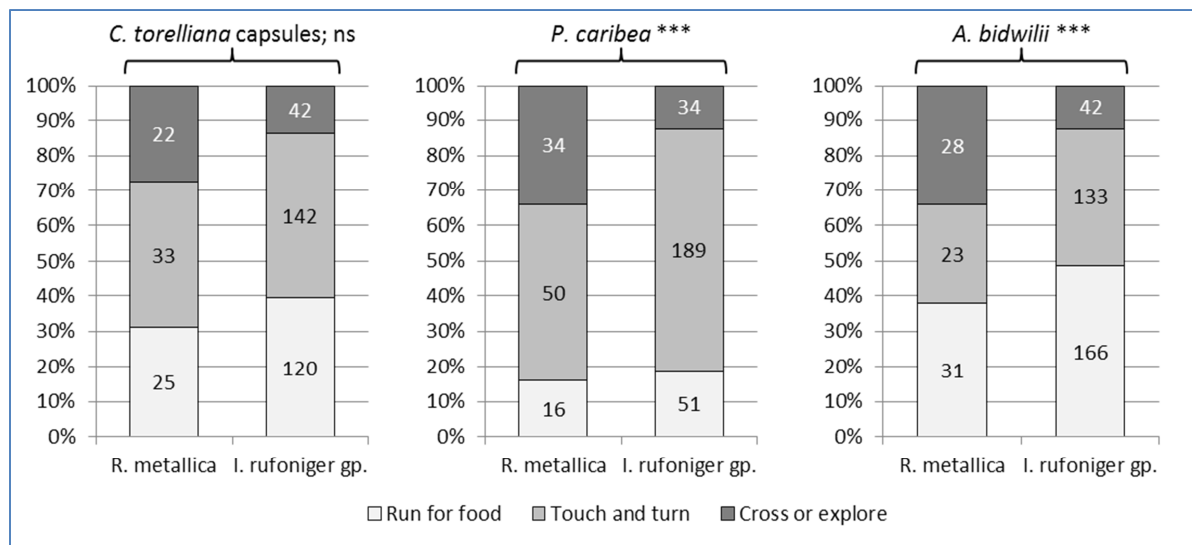


Figure 3. Behavioral responses of *R. metallica* and *I. rufoniger gp.* workers towards three different resin extracts; numbers in bars give the numbers of ant individuals showing a particular behavioral response; (significance levels as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant)

4 Discussion

Considering the fact, that stingless bees host substantial amounts of food in their nest, it is likely that those nests are object to attacks from predators and parasites. All ant species observed in this survey consumed variable amounts of nectar and insects. When being offered two different bee species, all of the tested ants accepted them as prey but preferred bees with less resinous compounds on their cuticle. Aversive responses of ants were found towards one cuticular and three resinous extracts, with different ant species varying in response intensity. Interactions of stingless bees and ants on aphid honeydew sources could not be found.

4.1 Foraging habits of different ant species

Four of the five observed ant species notably preferred nectar as a food source but also collected a variety of other items. Only *R. metallica* had a substantially higher preference for insects and other protein sources. This reflects and quantifies in more detail the dietary habits found by Shattuck (1999) and Burwell (2007) who described all monitored ant species as generalists with disposition to either nectar or protein (c.f. Table 1.). It further matches with the fact that most ants are omnivorous and do not often solely rely on nectar as food (Stradling 1978).

The proportions of food composition did not differ between species (with exception of *R. metallica*), daytimes or nests. This suggests that environmentally provided resources were quite stable and ant diets likely well balanced for each species. It seems to be plausible that a certain ideal nectar to protein ratio, supplemented by other items, was needed by colonies and kept up in all four species. Hence this ratio did only differ marginally among them. Such a balanced food allocation has also been described and reviewed by Blüthgen *et al.* (2010).

NECTAR ANALYSIS

A broad variety of nectar volume, colors and sucrose content was carried in by the different ant species. The significant difference in sucrose concentration between all four nectar collecting species indicates the use of different nectar sources. Since no significant differences have been found between *C. aeneopilosus*, *P. australis* and *I. rufoniger* *gp.*, potentially at least partially overlapping nectar sources are used by these three species whereas the latter two might also share sources with *T. bicarinatum*. Larger ants tended to carry greater volumes of sucrose in their crops, suggesting that the crop volume also grows with body size. Although crop loads did correlate with both body size and with sucrose concentration, a correlation between the latter two could not be found. This suggests that various sucrose concentrations

were opportunistically collected in each species according to the availability of a nectar source. The small sample size of species and the fact that *T. bicarinatum* showed unusual collection trends though delimitate the validity of this idea. Still, higher concentrated nectar was readily collected in greater volumes and hence sucrose intake maximized. A similar correlation for sucrose concentration up to about 40 % has been found by Josens *et al.* (1998) and thus represents a good example for optimal foraging in ants. Josens *et al.* (1998) further found partial crop loads for low (< 15 %) and high sucrose concentrations (\approx 70 %) with a maximum crop filling at 42.6 % for *Camponotus mus*. It is striking that the mean sucrose concentration of 44.7 % found for *C. aeneopilosus* in this assay comes very close to this optimum. This finding may further support the optimal foraging suggestion although the sampled number of ants does not allow reliable conclusions. The high variability in volume for *C. aeneopilosus* was probably related to the fact that higher concentrated nectar sources yield smaller amounts of nectar, because sucrose concentration increases with evaporation of water. The lower sucrose concentrations found in smaller ants may have partially been caused by asymmetric competition that excluded less competitive species like *T. bicarinatum* from more lucrative food sources (Blüthgen *et al.* 2004a).

PREDATORY POTENTIAL

All species were found to be omnivorous and therefore represent a potential threat to stingless bees although no direct encounters or raids were observed during the experimental period. Of the species in this thesis, *R. metallica* may represent the most likely predator due to its high demand on insect prey. Ponerine ants have further been reported to attack and kill small bees approaching extrafloral nectaries (Roubik 1989) which matches with the observation of *R. metallica* carrying stingless bee workers. *I. rufoniger* *gp.* ants that forage in large numbers and use effective trails for recruitment may also have a high potential as predators for stingless bees. Their second highest demand for protein in the monitoring experiment supports this possibility. Most notable *R. metallica* and *I. rufoniger* but also *T. bicarinatum* did readily take both bee species in the feeding choice experiment. Roubik (1989 p. 222; 233) lists the genera *Iridomyrmex*, *Camponotus* and *Polyrhachis* among those ant predators of bees that also invade nests. In this study the species of the latter two were found carrying mostly nectar and did not show aggressive behavior or high daytime foraging activity so that their potential cannot be precisely assessed. What is more, when single bee workers were presented near their nest entrances, they remained untouched or maybe unnoticed. The same unclear assessment holds true for *T. bicarinatum* where, despite their affinity to stingless bees in the feeding choice experiment, no trail recruitment was found and activity during warmer times of the day was quite low.

4.2 Trophobiotic interactions

No stingless bees were seen to visit the aphid infested *Podocarpus elatus* within the monitoring period. Hence the role of Australian stingless bees in trophobiotic interaction remains unknown. Roubik *et al.* (1986) found sugar concentrations ranging from 10-65 % in nectar collected by bees. Hence the mean sucrose concentration of 19.8 ± 4.4 % found in honeydew harvested from aphids by *I. rufoniger* *gp.* makes *P. elatus* a potential honeydew source for stingless bees although higher sucrose concentrations are preferred (Biesmeijer *et al.* 1999). Possible reasons for the fact that no bees did attend the aphids are quite numerous.

It is not known whether stingless bees attend aphids for honeydew at all. Records of apid bees attending aphids have been given by Roubik (1989) but to date observations of stingless bees attending hemipteran insects are limited to *Coccidae*, *Membracidae* and *Pseudococcidae* (Salt 1929; Castro 1975; Laroca *et al.* 1976; Schuster 1981; Figueiredo 1996; Camargo *et al.* 2002; Koch *et al.* 2011).

It is further unknown whether stingless bees do collect honeydew from this particular plant species at all. They are known to collect pollen from *Podocarpus* *sp.* (Marques-Souza 2010) but honeydew collection has not been recorded so far.

Finally the ants present may dominate and monopolize this food source (Blüthgen *et al.* 2006). They may thus outcompete and displace other visitors. Such negative correlations between the presence of ants and stingless bees have been found by Almeida-Neto *et al.* (2003) and Koch *et al.* (2011). Aggressive behavior towards small bees approaching extrafloral nectaries has been described by Roubik (1989) and defense of honeydew sources by ants was also observed by Almeida-Neto *et al.* (2003) and Figueiredo (1996). Koch *et al.* (2011) suggest that honeydew may represent an important resource in times of floral scarcity. Thus, sufficient abundance of alternative resources such as floral nectaries may not have given reason for stingless bees to forage on the honeydew sources where they would unnecessarily put themselves at risk of attack by ant defenders.

4.3 Ant repellent effects of stingless bees

All three ant species tested in choice assays did accept both of the presented bee genera as prey. For *I. rufoniger* *gp.* and *T. bicarinatum* no significant preference for either one of the two bee species was found, whereas *R. metallica* clearly preferred *Austroplebeia australis* over *Tetragonula carbonaria* workers. This preference disappeared when *T. carbonaria* workers were washed in hexane prior to testing. GCMS-analyses showed that most cuticular compounds were successfully removed by the washing procedure.

The prevalent preference for *A. australis* suggests that *R. metallica* was repelled by *T. carbonaria*. These findings agree with the results found in a similar experiment by Lehmborg *et al.* (2008). In their feeding choice experiment bees washed with solvent were significantly preferred over untreated bees. Lehmborg *et al.* (2008) suggest chemical compounds on the cuticle of the bees as possible deterrent agents. Alkanes, alkenes and esters represent compounds commonly found in cuticles of bees (Abdalla *et al.* 2003; Jungnickel *et al.* 2004; Kerr *et al.* 2004; Nunes *et al.* 2008) and many other insects (Butts *et al.* 1991; Howard 1993; Blomquist *et al.* 1998; Kaib *et al.* 2002; Martin *et al.* 2009). Terpenoid compounds in turn are exclusively found in chemical profiles of stingless bees (Leonhardt *et al.* 2009b) and were present as sesqui-, di- and triterpenes in case of *T. carbonaria* but not in *A. australis*. *Austroplebeia australis* showed a smaller diversity of chemical compounds on its cuticle and was less sticky than *Tetragonula carbonaria*. This indicates that *A. australis* either does not collect resin to the extent of *T. carbonaria* which is known to collect large amounts of resin (Wallace *et al.* 1995; Wallace *et al.* 2010) and/ or hardly (if at all) includes resinous compounds in its cuticle (Leonhardt *et al.* 2011b).

The repellent effect to ants is most likely related to these resinous compounds on bees' surfaces, given that mono-, di- and sesquiterpenes are known to act as ant repellents (Pasteels *et al.* 1983; Cane 1986; Debboun *et al.* 2007). However, the setup of the choice experiment does not allow concluding whether the repellent effect was related to particular compounds (e.g., the prominent *C. torelliana* compound or other cuticular terpenes) or to the stickiness of bees which holds the danger of gluing ants' mouth parts.

Notably, even washed *T. carbonaria* workers were less likely to be taken than workers of *A. australis* and (although not significant) strong repulsive responses towards some *T. carbonaria* workers were observed for a few ants. It is possible that hexane - soaked in different body parts of *T. carbonaria* during the washing procedure - did evaporate more slowly thereby causing an unintentional long term repellent effect of the solvent. The preference of ants for *A. australis* could also have been caused by the presence of cuticular substances that attracted ants or triggered aggressive behavior.

The lack of preferences for either one of the bee species in *I. rufoniger* *gp.* and *T. bicarinatum* indicates that these two species are either less sensitive towards resin derived substances (or cuticular compounds in general) than *R. metallica* or that the profiles of the two bee species were equally repellent/attractive to them. Since *T. bicarinatum* represents an introduced species, it is likely that its indifference is based on the fact that they are accustomed to different chemical repellents

potentially present in their native habitat. Hence chemicals used by Australian bees do not affect them. Taking this aspect into consideration it is striking that *R. metallica* – the ant species assumed to have the highest predatory potential for stingless bees – was most frequently repelled by *T. carbonaria*. This finding supports the assumption that *R. metallica* represent strong predators and that the bees may have evolved effective defensive means against them.

4.4 Efficacy of resinous compounds as ant repellents

EXTRACT COMPOSITION

Three of the four species tested did turn away significantly more often from at least one of the tested extracts in comparison with the hexane control. The intensity of responses varied between different species and extracts.

GCMS-analyses of the extracts showed that propolis from *T. carbonaria* nests was the highest concentrated and comprised the highest number of different substance classes (Figure 3.). The presence of longer chained alkanes which are commonly found in waxes of stingless bees (Blomquist *et al.* 1985; Francis *et al.* 1989; Roubik 1989; Dani *et al.* 2005) as well as the presence of different terpene classes which are main constituents of resin (Ghisalberti 1979; Langenheim 2003) shows that propolis comprises a mixture of wax and resin, also known as cerumen (Wille 1983; Roubik 2006). Extracts of propolis and *C. torelliana* show striking similarities in composition of terpenoid compounds which emphasizes the fact that a high number of Australian stingless bees collect *C. torelliana* resin (Leonhardt *et al.* 2011b) and incorporate it into their nest structure (Roubik 2006).

For *T. carbonaria* similarities in the profiles of their cuticle, propolis and *C. torelliana* resin were found (Figure 3. and Figure 3.; see also Leonhardt *et al.* (2011b)). An unknown, characteristic compound derived from *C. torelliana* was present in all three extracts but mono- and sesquiterpenes were not found on cuticles of *T. carbonaria*. This shows that bees include resinous compounds in their building material for the nest (Wille *et al.* 1973; Roubik 2006) and also supports the theory that bees do not passively accumulate chemicals on their body by contact with nest material but actively filter and include resinous compounds in their cuticles, hereby enriching their chemical profile (Leonhardt *et al.* 2011a; 2011b). Leonhardt *et al.* (2011b) further note that 32 cuticular compounds of Australian stingless bees were derived from *C. torelliana* resin. However, the great number of peaks/compounds in propolis, apart from the ones also found in *C. torelliana* resin, also indicate that *T. carbonaria* workers collect resin from other plants as well (Leonhardt *et al.* 2011b).

REPELLENT EFFICIENCY OF CUTICULAR EXTRACTS

Three of the four ant species tested, turned away from filter papers treated with *T. carbonaria* extract significantly more often than from filter papers with the hexane control (Figure 3.-8). The fact that the filter paper was less frequently approached or even accessed indicates that *T. carbonaria* extract had a repellent effect on all species except *T. bicarinatum*. These findings further suggest that the distaste for *T. carbonaria* workers in the feeding choice assay was not exclusively due to their sticky appearance but also to olfactory cues emitted by the bees' cuticles. Lehmberg *et al.* (2008) and Duangphakdee *et al.* (2009) who tested the repellent effects of bee cuticles and resinous substances, suggested resin derived terpenes to be responsible for the repellent effects.

T. bicarinatum ants did not show any significant differences in responses towards extract and hexane, thus confirming the indifferent behavior shown towards both bee species in the feeding choice assay. This result further supports the theory that the introduced species *T. bicarinatum* is not affected by cuticular chemicals of Australian bees. *P. australis* was also repelled by *T. carbonaria* extracts and showed similar responses as *R. metallica* and *I. rufoniger* *gp.* (Figure 3.). This response adds to the likelihood of *P. australis* being a predator of stingless bees but might rather just be the result of general ant repellent effects of terpenic compounds. However, a good reason against the last argument is that *T. bicarinatum* in turn was not repelled by *T. carbonaria* extract. This implies that bees may have selectively evolved a cuticular profile that is particularly effective against predators in their natural environment. A fact that needs to be further assessed by testing more extracts.

The effect of cuticular extract of *A. australis* was only tested in *R. metallica* ants. *A. australis* extract was more repellent to ants than hexane (Figure 3.) but its repellent effect was significantly lower than the one of *T. carbonaria* (Figure 3.). This difference reflects the findings of the feeding choice assay. It is highly likely that resin derived compounds (e.g. the unknown *C. torelliana* compound and many mono-, sesqui-, di- or triterpenes) mainly present on *T. carbonaria* are responsible for the repellent effect. Further candidates that were present on *T. carbonaria* but not (or only scarce) in *A. australis* and could therefore be responsible for the repellent effects are other, probably mostly endogenously produced substances such as hexadecanoic acid, hexadecanoic acid methyl ester, hexadecyl acetate, and alcohols (Leonhardt *et al.* 2011b, supplemental data).

REPELLENT EFFICIENCY OF RESIN EXTRACTS

Two of the three resinous extracts and propolis extract had significantly repellent effects on *R. metallica* in comparison with hexane (Figure 3.). The comparison of propolis with other resinous extracts (Figure 3.; Table 3.) demonstrated that it had the highest repelling effect on ants. Only a small number of ants even came close enough to the propolis filter paper to make contact and allow record of a response (personal observation). Instead most ants tended to explore the edge of the platform, staying as far away as possible from the treated filter paper. Although this extract was probably much higher concentrated which is most likely responsible for the very intense responses, the great dislike of ants indicates that the mixture of different resinous compounds with wax for nest building material has highly deterrent properties. *P. caribea*, and *C. torelliana* extracts also repelled ants (Figure 3.; Table 3.), whereas the extract of *A. bidwili* did not affect the ants' responses towards the treated filter paper. Because *A. bidwili* resin comprised monoterpenes which were not found in repellent bee extracts, it is likely that monoterpenes are not responsible for the repellent properties of resin. In turn the near absence of sesquiterpenes in *A. bidwili* resin, which were present in all other repellent extracts, suggests that these components account for deterrent effects on Australian ants. It is striking that the repellent efficacy differed significantly between *T. carbonaria* and *A. bidwili* but that no differences were found for *C. torelliana* in comparison with the two bee extracts. This implicates that the repellent effect of *C. torelliana* may range somewhere between the deterrent effects of the two cuticular extracts and that apart from compounds of *C. torelliana*, *T. carbonaria* probably includes further resinous substances in its cuticle to increase repulsive efficacy. Nevertheless it is also likely that endogenously produced substances of the bees added to the repellent effect or that dissimilar extract concentrations were responsible for the differences.

Workers of *I. rufoniger* *gp.* were repelled by resin extract of *P. caribea* but only little by *C. torelliana* (not significant after correction), whereas *A. bidwili* resin had no effect in comparison with hexane (Figure 3.). Repellency decreased from *P. caribea* which had the strongest effects (even exceeding *T. carbonaria*) over *C. torelliana* and *A. bidwili* (Figure 3.; Table 3.).

Compounds of resin from *P. caribea* and *A. bidwili* repelled workers of *I. rufoniger* *gp.* significantly more than workers of *R. metallica* although the general repellent effect of *A. bidwili* was only minuscule. Hence, *I. rufoniger* *gp.* may be more sensitive towards certain substances of resin. For extracts of *T. carbonaria* and *C. torelliana* however, no differences in the responses of the two ant species were found. When responses to hexane controls were compared, *R. metallica* showed no significant differences over the test period, whereas *I. rufoniger* *gp.* did. Workers of

I. rufoniger gp. were also more likely to turn around from the control filter paper. Although the greater number of individuals tested needs to be taken into consideration for *I. rufoniger gp.*, the significant differences in behavior towards the control extract indicate that this species is generally more variable in its responses towards the filter paper itself. A probable reason for this fact may be the size difference between both ant species. Entering the filter paper may have been a greater barrier for the smaller *I. rufoniger gp.* Besides the possibility of higher sensitivity for extract, this size factor could be a further reason for the higher number of *I. rufoniger gp.* workers turning around for both hexane and extract compared to *R. metallica*.

For all these observations, the fact that extracts were not equally concentrated needs to be kept in mind, rendering a definite ranking of the extracts by repellent efficacy impossible. Still, efficacy of about equally concentrated extracts was highest for *T. carbonaria* followed by *C. torelliana* and lowest for *A. australis*. Further *P. caribea* resin was more effective than extract of *A. bidwillii* which had no effect at all. The effect of *P. caribea* resin renders it likely that *T. carbonaria* includes further components of this resin in its cuticle, a hypothesis that needs to be confirmed by more detailed GCMS-analyses.

A last fact that is striking is the comparatively low effect of *C. torelliana* resin on *R. metallica* (Figure 3.) and especially on *I. rufoniger gp.* for which it had no effect at all (Figure 3.). Although the varying concentrations do not allow a definite conclusion, the high abundance of *C. torelliana* compounds in the profile of *T. carbonaria* could be responsible for its highly deterrent efficacy. Stingless bees are further strongly attracted by resin of *C. torelliana* (Klumpp 2007). Leonhardt *et al.* (2011b) showed that 76 % of stingless bees collected resin of this plant and included up to 32 of its compounds in their cuticle. Why they collect this particular resin in considerable quantities is not known to day. Its soft consistency probably makes it an easy to handle nest building material, but also bears the danger of collapsing nest structures at higher temperatures (Klumpp 2007). Since resin is known to possess antibiotic properties (Messer 1985; Lokvam *et al.* 1999) it is further possible that *C. torelliana* resin is collected for antimicrobial purposes. Considering the moderate repulsive responses of ants towards *C. torelliana* extract its efficacy seems to be lower than expected. It is likely that this finding was caused by the lower concentration of the extract because in comparison with hexane repellent effects were found despite its low concentration. Especially the high abundance of the unknown *C. torelliana* compound in *T. carbonaria* extract would suggest that it plays a key role in the repellent properties of its cuticle. With the present results this role cannot be clarified properly and hence needs further assessment in an assay with the isolated compound.

If bees do filter specific resin derived terpenes (Leonhardt *et al.* 2011a; 2011b) it is not clear whether bees do systematically accumulate particularly repellent compounds. It is still highly likely that *C. torelliana* resin does play a role in the repellent properties of stingless bees' cuticles but it is probably not the only resource providing repellent compounds.

CONCLUSION

Resins possess repellent properties against ants and do likely account for the repellent effects of the cuticles of stingless bees. These findings agree with the results found by Lehmborg *et al.* (2008) and Duangphakdee *et al.* (2005) and further indicate that volatile chemical compounds are more likely responsible for deterrence than stickiness. Sticky properties of resin may still play a tributary role on first contact with resin treated surfaces. However the stickiness of resin comes into use when predators need to be detained physically by gluing mouthparts or inhibiting movement at all (Pasteels *et al.* 1983; Roubik 2006; Klumpp 2007; Greco *et al.* 2010a; Halcroft *et al.* 2011). The findings of these authors further explain the increase in resin intake after ant attacks (Howard 1985; Khoo *et al.* 1987; Duangphakdee *et al.* 2009; Leonhardt *et al.* 2009a). Resin does not only help to rebuilt damaged nest structures after such attacks but also increases the defensive power of the colony. Additionally, its chemical compounds may further provide repellent effects that exceed the period of immediate danger thereby providing some long lasting protection. From the experiments performed in this study, it cannot definitely be said which particular compounds are responsible for the repellent effect but it is likely that sesquiterpenes play a key role, whereas the role of mono- and diterpenes remains unclear, although they are known to act as ant or insect repellents as well (Pasteels *et al.* 1983; Cane 1986; Debboun *et al.* 2007). It must also be stated that all extracts tested only provided a short term repellent effect (pers. obs., 2011). Most ants repelled at first contact entered the filter paper after a while or after some more approaches. Possible reasons for the change in behavior could have been the increasing concentration of trail pheromone emitted by ants that had already found the food source, or (and probably coinciding with the first fact) the process of adaptation to the smell which made it easier to transcend the smelly barrier after a while. Consequently this chemosensory based defense mechanism only provides initial but effective protection against contact with an ant predator hence adding some bonus time for escape. That bonus may further be sufficient to early warn nest mates of predatory threats and may be especially crucial for an early detection and elimination of ant scouts which might recruit further nest mates for a raid (Duangphakdee *et al.* 2009).

However stingless bees are relatively unspecialized, opportunistic resin foragers (Leonhardt *et al.* 2011a). Cuticular profiles of terpenoid compounds depend on the environmental availability of resin sources and vary more than the genetically determined composition of hydrocarbons (Leonhardt *et al.* 2011b). Sesquiterpenes were found to vary most between different tropical bee species and are also lacking in some of them (Leonhardt *et al.* 2011a).

Hence the repellent effects of sesquiterpenes are likely to be substituted or supplemented by a variety of other compounds. Moreover repellent efficacy may vary with profile composition among different habitats which leads to the conclusion that despite its important contribution to the large defensive repertory of stingless bees, resin is not solely collected to repel predators (Lehmberg *et al.* 2008; Duangphakdee *et al.* 2009). Resin has more likely evolved into a multipurpose substance for stingless bees. It serves as a sticky barrier to all kind of enemies (Duangphakdee *et al.* 2005; Roubik 2006; Duangphakdee *et al.* 2009), as building material (Roubik 2006), (especially with regard to its terpenic compounds) as antibiotic (Messer 1985) and possibly also as a source for compounds that tame aggressors (Leonhardt *et al.* 2010a) or may be used for nest mate recognition (Buchwald *et al.* 2005; Leonhardt *et al.* 2009b) by adding to their chemical diversity (Leonhardt *et al.* 2011a).

4.5 Prospect

The different aspects of interactions between stingless bees and ants brought up in this thesis provide a reasonable basis for further research. Most aspects still need to be looked into a bit further to further validate some of the assumptions made.

The food monitoring assay yielded useful and passably differentiated data about feeding habits of the five ant species observed. Next steps could comprise further analysis of prey items for size and affinities towards particular families of insect prey as well as origin of the prey items or foraging ground diversity. Sources of the nectar collected remained unknown so GCMS-analysis of nectar compounds and resources in the vicinity of the study site would help to distinguish between different nectar sources and also unfold further information about particular nectar consumption habits. A long term study throughout different times of the year could help to better understand variations in food allocation and adaptations towards seasonally varying availability of particular food sources, as well as the stability of dietary habits, for example the protein-nectar balance. Additional isotope analyses could reveal more detailed information about trophic position as well as nitrogen and carbon sources (reviewed by Blüthgen *et al.* 2010).

A more detailed observation of the ants' foraging strategies and activity would allow a clearer assessment of their predatory or nest robber potential for stingless bees.

Additionally, small, undefended dummy hives or nest parts equipped with dead bees, pollen, honey and cerumen or resin could be placed near an ant nest or trail. This could yield further information about which ant species are generally interested in raiding bees' nests, which species are able to cross the smelly resin barrier and if different species prefer specific resources stored within the bees' nest.

Further investigation on the role of stingless bees in trophobiotic interactions should comprise a higher number and variety of aphid infested plants to be monitored. Although stingless bees have been reported on honeydew sources in Africa and South-America, nothing is known about their role in Australia. If interactions are found it would be of interest to find out which species dominate the resource and which defense mechanisms are applied by bees and ants. Koch *et al.* (2011) found that the abundance of bees is negatively correlated with the abundance of ants on honeydew resources, and Almeida-Neto *et al.* (2003) recorded a reciprocal interference where bees were also able to displace ants. However nothing is known about the bees' defense mechanisms so far. Koch *et al.* (2011) further suggested that honeydew may be collected by stingless bees when floral resources are scarce. Hence an observation in these periods may be more successful.

Because *I. rufoniger gp.* and *R. metallica* (with washed bees) did show a nearly significant preference for *A. australis* in the feeding choice experiment, the findings should be validated by further trials and additional testing of washed *T. carbonaria* for the other two ant species. Future tests should also comprise further combinations of treated bees, such as tests with washed *A. australis* against untreated *T. carbonaria* or both species washed to learn more about the reasons on which the ants' decisions are based. For better assessment of the predatory potential of *P. australis* and *C. aeneopilosus* the feeding choice assay should also be conducted with these two species. Other conceivable tests could be made with extra stingless bee species combinations, different washing solvents such as chloroform or more polar ethanol and finally also other ant species. Also, with regard to the extract assay, *Oecophylla smaragdina* would be a reasonable test candidate. They are known to be arboreal predators and also represent a threat to arboreal nesting stingless bees (Duangphakdee *et al.* 2005). Similar tests concerning repellent effects of resin have already been conducted by Duangphakdee *et al.* (2005) with *Oecophylla* and the experiments used in this thesis bear potential to refine or complement their results. Yet another option could be the investigation of further introduced species to check for similar insensitivities to the cuticular defense of Australian bees' as found for *T. bicarinatum*. With regard to this fact a comparison of responses of *T. bicarinatum* between Australian and South-east Asian (its home country) stingless bees and their

cuticular profiles would provide further information and help to validate or discard the idea of region-based defensive coevolution of bee cuticles and predatory ants.

It would further be interesting to perform the extract tests with all five ant species and all six extracts. For a systematic comparison of repellent efficacy, the extracts need to be equally concentrated.

Tests with singular, isolated chemical compounds, especially the unknown chemical in *C. torelliana* resin, or compound classes would further help to determine the repellent potential of particular substances and assist in clarifying the role of *C. torelliana* in defensive applications. Finally, further resin sources could be taken into account and analyzed for their repellent potential. An additional GCMS analysis and comparison for abundance of compounds found to be effective in resin and bee cuticles would then help to find out whether bees do exploit more repellent resin sources and particularly filter repellent chemicals for their profile. It may further aid in determining, whether these compounds are accumulated to an extent exceeding the proportions found in resin and therefore help to better understand reasons for filtering of particular compounds or the role of defensive means in chemical diversity of stingless bees (Leonhardt *et al.* 2011a; 2011b).

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Appendix

Data given in the App Tables only represent excerpts of the most important facts. Fully detailed, original tables, lists, charts and statistical data for all experiments are provided on the enclosed CD.

App Table Detailed results of the food monitoring assay: Date (year 2011); Temp = Temperature, Weat = Weather (su = sunny; pa-cl = partly cloudy; sl-ovc = slightly overcast; ovc = overcast; oc-rs = occasional rain showers; rs = rain/ showers); Spec = Species (*C. ae* = *Camponotus aeneopilosus*; *I. ru* = *Iridomyrmex rufoniger* gp.; *Pa au* = *Polyrhachis australis*; *R. me* = *Rhytidoponera metallica*; *T. bi* = *Tetramorium bicarinatum*); Nr. = Nest number (c.f. Table 2.; Figure 2.); Dayt = Daytime (1 = morning; 2 = noon; 3 = evening); N = Number of Ants collected and monitored; Nec = Number of Ants carrying nectar; Pro = Number of Ants carrying protein; Pla = Number of Ants carrying plant parts; Misc = Number of Ants with miscellaneous items; Not = Number of Ants without a food item (Full table with detailed list of food items on CD: Ants-Monitoring.xlsx)

| Date | Temp | Weat | Spec | Nr. | Dayt | N | Nec | Pro | Pla | Misc | Not |
|--|---------|--------|--------------|-----|------|----|-----|-----|-----|------|-----|
| 07.03. | 25°C | oc-rs | <i>C. ae</i> | 3 | 1 | 4 | 1 | 0 | 0 | 0 | 3 |
| 22.03. | 34°C | su | <i>C. ae</i> | 3 | 1 | 10 | 8 | 1 | 0 | 0 | 2 |
| 06.04. | 26°C | pa-cl | <i>C. ae</i> | 3 | 1 | 10 | 6 | 0 | 0 | 0 | 4 |
| 28.02. | 30°C | su | <i>C. ae</i> | 3 | 2 | 5 | 0 | 1 | 0 | 0 | 4 |
| 03.03. | 32°C | su | <i>C. ae</i> | 3 | 2 | 7 | 3 | 0 | 0 | 0 | 4 |
| 15.03. | 30°C | su | <i>C. ae</i> | 3 | 2 | 10 | 6 | 0 | 0 | 0 | 4 |
| 08.03. | 27°C | sl ovc | <i>C. ae</i> | 3 | 3 | 5 | 4 | 0 | 0 | 0 | 1 |
| 15.03. | 30°C | su | <i>C. ae</i> | 3 | 3 | 10 | 7 | 0 | 0 | 0 | 3 |
| 22.03. | 34°C | su | <i>C. ae</i> | 3 | 3 | 10 | 6 | 1 | 0 | 1 | 2 |
| 24.03. | 28-34°C | su | <i>C. ae</i> | 3 | 3 | 10 | 5 | 0 | 0 | 0 | 5 |
| Species total <i>Camponotus aeneopilosus</i> | | | | | | 81 | 46 | 3 | 0 | 1 | 32 |
| 07.03. | 25°C | oc-rs | <i>I. ru</i> | 2 | 1 | 10 | 3 | 0 | 0 | 3 | 5 |
| 15.03. | 30°C | su | <i>I. ru</i> | 2 | 1 | 10 | 3 | 0 | 0 | 0 | 7 |
| 01.04. | 26°C | pa-cl | <i>I. ru</i> | 2 | 1 | 10 | 3 | 1 | 0 | 1 | 5 |
| 07.03. | 25°C | oc-rs | <i>I. ru</i> | 2 | 2 | 11 | 4 | 0 | 1 | 0 | 6 |
| 08.03. | 27°C | sl ovc | <i>I. ru</i> | 2 | 2 | 10 | 4 | 0 | 0 | 1 | 5 |
| 10.03. | 25°C | sl ovc | <i>I. ru</i> | 2 | 2 | 10 | 6 | 1 | 0 | 0 | 3 |
| 03.03. | 32°C | su | <i>I. ru</i> | 2 | 3 | 11 | 4 | 1 | 0 | 0 | 6 |
| 09.03. | 25-28°C | sl ovc | <i>I. ru</i> | 2 | 3 | 11 | 6 | 2 | 0 | 0 | 3 |
| 14.03. | 30°C | su | <i>I. ru</i> | 2 | 3 | 10 | 4 | 0 | 0 | 0 | 6 |
| 24.03. | 28-34°C | su | <i>I. ru</i> | 2 | 3 | 11 | 4 | 4 | 0 | 0 | 3 |
| 09.03. | 25-28°C | sl ovc | <i>I. ru</i> | 5 | 1 | 11 | 2 | 0 | 0 | 0 | 9 |
| 16.03. | 27°C | ovc | <i>I. ru</i> | 5 | 1 | 9 | 6 | 0 | 0 | 0 | 3 |
| 04.04. | 27°C | pa-cl | <i>I. ru</i> | 5 | 1 | 10 | 3 | 0 | 0 | 0 | 7 |
| 03.03. | 32°C | su | <i>I. ru</i> | 5 | 2 | 11 | 4 | 0 | 0 | 0 | 7 |
| 08.03. | 27°C | sl ovc | <i>I. ru</i> | 5 | 2 | 10 | 5 | 0 | 0 | 0 | 5 |
| 10.03. | 25°C | sl ovc | <i>I. ru</i> | 5 | 2 | 10 | 4 | 1 | 0 | 0 | 5 |
| 28.02. | 30°C | su | <i>I. ru</i> | 5 | 3 | 10 | 4 | 1 | 0 | 0 | 5 |
| 14.03. | 30°C | su | <i>I. ru</i> | 5 | 3 | 10 | 8 | 0 | 0 | 0 | 2 |

| Date | Temp | Weat | Spec | Nr. | Dayt | N | Nec | Pro | Pla | Misc | Not |
|---|---------|--------|-------|-----|------|-----|-----|-----|-----|------|-----|
| 28.03. | 25°C | oc-rs | I. ru | 5 | 3 | 10 | 1 | 0 | 1 | 0 | 7 |
| 01.03. | 30°C | su | I. ru | 6 | 1 | 10 | 0 | 2 | 0 | 0 | 8 |
| 24.03. | 28-34°C | su | I. ru | 6 | 1 | 10 | 0 | 0 | 0 | 0 | 10 |
| 04.04. | 27°C | pa-cl | I. ru | 6 | 1 | 10 | 1 | 0 | 0 | 0 | 9 |
| 03.03. | 32°C | su | I. ru | 6 | 2 | 10 | 1 | 1 | 0 | 0 | 8 |
| 07.03. | 25°C | oc-rs | I. ru | 6 | 2 | 10 | 3 | 1 | 0 | 0 | 7 |
| 10.03. | 25°C | sl ovc | I. ru | 6 | 2 | 11 | 5 | 2 | 0 | 0 | 5 |
| 07.03. | 25°C | oc-rs | I. ru | 6 | 3 | 10 | 2 | 1 | 0 | 0 | 7 |
| 09.03. | 25-28°C | sl ovc | I. ru | 6 | 3 | 10 | 4 | 0 | 0 | 1 | 5 |
| 21.03. | 30°C | su | I. ru | 6 | 3 | 10 | 3 | 1 | 0 | 1 | 5 |
| 28.03. | 25°C | oc-rs | I. ru | 6 | 3 | 10 | 5 | 3 | 0 | 0 | 2 |
| Species total <i>Iridomyrmex rufoniger</i> gp | | | | | | 296 | 102 | 22 | 2 | 7 | 165 |
| 07.03. | 25°C | oc-rs | P. au | 1 | 1 | 10 | 5 | 0 | 0 | 1 | 4 |
| 15.03. | 30°C | su | P. au | 1 | 1 | 10 | 6 | 0 | 0 | 0 | 4 |
| 28.03. | 25°C | oc-rs | P. au | 1 | 1 | 10 | 2 | 0 | 0 | 2 | 6 |
| 03.03. | 32°C | su | P. au | 1 | 2 | 10 | 5 | 0 | 0 | 1 | 4 |
| 08.03. | 27°C | sl ovc | P. au | 1 | 2 | 10 | 4 | 2 | 0 | 0 | 5 |
| 09.03. | 25-28°C | sl ovc | P. au | 1 | 2 | 10 | 4 | 1 | 0 | 0 | 5 |
| 28.02. | 30°C | su | P. au | 1 | 3 | 10 | 2 | 0 | 0 | 0 | 8 |
| 02.03. | 30°C | ovc | P. au | 1 | 3 | 10 | 5 | 1 | 1 | 0 | 3 |
| 10.03. | 25°C | sl ovc | P. au | 1 | 3 | 10 | 5 | 0 | 0 | 0 | 5 |
| 03.03. | 32°C | su | P. au | 4 | 1 | 10 | 3 | 3 | 0 | 0 | 4 |
| 16.03. | 27°C | ovc | P. au | 4 | 1 | 10 | 6 | 0 | 1 | 0 | 3 |
| 04.04. | 27°C | pa-cl | P. au | 4 | 1 | 10 | 5 | 0 | 0 | 0 | 5 |
| 07.03. | 25°C | oc-rs | P. au | 4 | 2 | 10 | 7 | 0 | 1 | 0 | 2 |
| 08.03. | 27°C | sl ovc | P. au | 4 | 2 | 10 | 4 | 0 | 0 | 0 | 6 |
| 09.03. | 25-28°C | sl ovc | P. au | 4 | 2 | 10 | 8 | 1 | 0 | 1 | 2 |
| 18.02. | 30°C | su | P. au | 4 | 3 | 10 | 8 | 1 | 0 | 0 | 1 |
| 10.03. | 25°C | sl ovc | P. au | 4 | 3 | 10 | 10 | 0 | 0 | 1 | 0 |
| 31.03. | 25°C | ovc | P. au | 4 | 3 | 10 | 5 | 0 | 0 | 0 | 5 |
| Species total <i>Polyrhachis australis</i> | | | | | | 180 | 94 | 9 | 3 | 6 | 72 |
| 03.03. | 32°C | su | R. me | 7 | 1 | 10 | 0 | 2 | 2 | 2 | 3 |
| 16.03. | 27°C | ovc | R. me | 7 | 1 | 10 | 0 | 4 | 1 | 0 | 5 |
| 07.04. | 24°C | pa-cl | R. me | 7 | 1 | 10 | 0 | 6 | 0 | 0 | 5 |
| 18.02. | 30°C | su | R. me | 7 | 2 | 20 | 0 | 5 | 0 | 0 | 15 |
| 07.03. | 25°C | oc-rs | R. me | 7 | 2 | 10 | 0 | 3 | 0 | 5 | 2 |
| 10.03. | 25°C | sl ovc | R. me | 7 | 2 | 10 | 1 | 5 | 0 | 0 | 4 |
| 07.03. | 25°C | oc-rs | R. me | 7 | 3 | 10 | 0 | 3 | 0 | 3 | 3 |
| 09.03. | 25-28°C | sl ovc | R. me | 7 | 3 | 10 | 0 | 4 | 0 | 2 | 4 |
| 14.03. | 30°C | su | R. me | 7 | 3 | 10 | 0 | 3 | 1 | 2 | 3 |
| 28.03. | 25°C | oc-rs | R. me | 7 | 3 | 10 | 0 | 3 | 0 | 2 | 4 |

| Date | Temp | Weat | Spec | Nr. | Dayt | N | Nec | Pro | Pla | Misc | Not |
|--|---------|--------|-------|-----|------|-----|-----|-----|-----|------|-----|
| 01.03. | 30°C | su | R. me | 8 | 1 | 10 | 0 | 3 | 0 | 0 | 7 |
| 24.03. | 28-34°C | su | R. me | 8 | 1 | 5 | 0 | 3 | 0 | 0 | 2 |
| 12.04. | 24°C | pa-cl | R. me | 8 | 1 | 10 | 0 | 2 | 0 | 0 | 8 |
| 07.03. | 25°C | oc-rs | R. me | 8 | 2 | 10 | 0 | 4 | 0 | 0 | 6 |
| 08.03. | 27°C | sl ovc | R. me | 8 | 2 | 10 | 0 | 5 | 0 | 0 | 6 |
| 10.03. | 25°C | sl ovc | R. me | 8 | 2 | 10 | 1 | 2 | 0 | 1 | 6 |
| 03.03. | 32°C | su | R. me | 8 | 3 | 10 | 1 | 3 | 0 | 2 | 5 |
| 15.03. | 30°C | su | R. me | 8 | 3 | 10 | 0 | 3 | 0 | 0 | 7 |
| 21.03. | 30°C | su | R. me | 8 | 3 | 10 | 0 | 4 | 1 | 1 | 5 |
| 31.03. | 25°C | ovc | R. me | 8 | 3 | 10 | 0 | 3 | 0 | 0 | 7 |
| 10.03. | 25°C | sl ovc | R. me | 9 | 1 | 10 | 0 | 8 | 0 | 0 | 3 |
| 21.03. | 30°C | su | R. me | 9 | 1 | 10 | 0 | 4 | 0 | 0 | 7 |
| 13.04. | 25°C | su/rs | R. me | 9 | 1 | 10 | 0 | 0 | 0 | 0 | 10 |
| 01.03. | 30°C | su | R. me | 9 | 2 | 10 | 0 | 3 | 0 | 0 | 7 |
| 03.03. | 32°C | su | R. me | 9 | 2 | 10 | 0 | 3 | 0 | 0 | 7 |
| 10.03. | 25°C | sl ovc | R. me | 9 | 2 | 10 | 0 | 4 | 0 | 0 | 6 |
| 07.03. | 25°C | oc-rs | R. me | 9 | 3 | 10 | 1 | 1 | 0 | 0 | 8 |
| 09.03. | 25-28°C | sl ovc | R. me | 9 | 3 | 10 | 0 | 4 | 0 | 0 | 7 |
| 14.03. | 30°C | su | R. me | 9 | 3 | 10 | 0 | 5 | 0 | 0 | 6 |
| 31.03. | 25°C | ovc | R. me | 9 | 3 | 10 | 0 | 3 | 0 | 0 | 7 |
| Species total <i>Rhytidoponera metallica</i> | | | | | | 305 | 4 | 105 | 5 | 20 | 175 |
| 13.04. | 25°C | su/rs | T. bi | 10 | 1 | 10 | 5 | 2 | 0 | 2 | 3 |
| 15.04. | 24°C | pa-cl | T. bi | 10 | 1 | 10 | 7 | 0 | 0 | 0 | 3 |
| 18.04. | 21°C | rs | T. bi | 10 | 1 | 10 | 3 | 0 | 0 | 0 | 7 |
| 13.04. | 25°C | su/rs | T. bi | 10 | 2 | 10 | 6 | 0 | 0 | 0 | 4 |
| 14.04. | 28°C | su | T. bi | 10 | 2 | 10 | 4 | 0 | 0 | 0 | 6 |
| 15.04. | 24°C | pa-cl | T. bi | 10 | 2 | 10 | 2 | 0 | 0 | 0 | 8 |
| 13.04. | 25°C | su/rs | T. bi | 10 | 3 | 10 | 1 | 0 | 0 | 1 | 8 |
| 15.04. | 24°C | pa-cl | T. bi | 10 | 3 | 10 | 5 | 2 | 0 | 0 | 3 |
| 18.04. | 21°C | rs | T. bi | 10 | 3 | 10 | 2 | 3 | 0 | 0 | 7 |
| Species total <i>Tetramorium bicarinatum</i> | | | | | | 90 | 35 | 7 | 0 | 3 | 49 |
| Total for all species | | | | | | 952 | 281 | 146 | 10 | 37 | 493 |

App Table Color variation in collected nectar and the number of workers found with the according item (Full table with detailed nectar items and colors on CD: Ants-Monitoring.xlsx)

| Species | Total | Clear | Opaque | Milky | Brown | Brown | Yellow | Other |
|------------------------|-------|-------|--------|-------|-------|-------|--------|-------|
| <i>C. aeneopilosus</i> | 46 | 30 | 1 | 2 | 6 | 5 | 2 | 0 |
| <i>P. australis</i> | 94 | 49 | 23 | 6 | 2 | 4 | 4 | 6 |
| <i>T. bicarinatum</i> | 35 | 35 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>I. rufoniger</i> | 102 | 96 | 1 | 0 | 5 | 0 | 0 | 0 |
| <i>R. metallica</i> | 4 | 2 | 1 | 0 | 1 | 0 | 0 | 0 |
| Pooled | 281 | 212 | 26 | 8 | 14 | 9 | 6 | 6 |

App Table Nectar collection data for the nectar assay: Date (year 2011); Spec = Species (*C. ae* = *Camponotus aeneopilosus*; *I. ru* = *Iridomyrmex rufoniger* gp.; *P au* = *Polyrhachis australis*; *T. bi* = *Tetramorium bicarinatum*); Nr. = Nest number (c.f. Table 2.; Figure 2.); Dayt = Daytime (1 = morning; 2 = noon; 3 = evening); N = Number of Ants collected and monitored; Vol = Volume; Suc = Sucrose concentration; Concentrations marked red exceeded the refractometer scale which reached to 50% (Full table on CD: Ants-Monitoring.xlsx)

| Date | Spec | Nr. | Dayt | Timeframe | Color | Vol [µl] | Suc [%] | |
|--|--------------|-----|------|-------------|-------------|----------|---------|-----|
| 22.03.2011 | <i>C. ae</i> | 3 | 1 | 9.50-10.10 | clear | 4.0 µl | 49% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 1 | 9.50-10.10 | clear | 2.5 µl | 50% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 1 | 9.50-10.10 | clear | 2.5 µl | 50% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 1 | 9.50-10.10 | milky | 0.2 µl | 16% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 1 | 9.50-10.10 | brown-milky | 0.2 µl | 9% | |
| 06.04.2011 | <i>C. ae</i> | 3 | 1 | 10.01-10.31 | clear | 2.2 µl | 60% | |
| 06.04.2011 | <i>C. ae</i> | 3 | 1 | 10.01-10.31 | brown | 0.5 µl | 53% | |
| 06.04.2011 | <i>C. ae</i> | 3 | 1 | 10.01-10.31 | brown | 0.3 µl | 64% | |
| 06.04.2011 | <i>C. ae</i> | 3 | 1 | 10.01-10.31 | brown | 1.8 µl | 73% | |
| 06.04.2011 | <i>C. ae</i> | 3 | 1 | 10.01-10.31 | brown | 0.8 µl | 74% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 3 | 14.55-15.05 | clear | 1.3 µl | 50% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 3 | 14.55-15.05 | clear | 0.7 µl | 50% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 3 | 14.55-15.05 | clear | 0.4 µl | 50% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 3 | 14.55-15.05 | brown-milky | 0.3 µl | 15% | |
| 22.03.2011 | <i>C. ae</i> | 3 | 3 | 14.55-15.05 | brown-milky | 1.0 µl | 50% | |
| 24.03.2011 | <i>C. ae</i> | 3 | 3 | 16.05-16.26 | clear | 0.1 µl | 14% | |
| 24.03.2011 | <i>C. ae</i> | 3 | 3 | 16.05-16.26 | clear | 1.4 µl | 50% | |
| 24.03.2011 | <i>C. ae</i> | 3 | 3 | 16.05-16.26 | clear | 2.0 µl | 50% | |
| 24.03.2011 | <i>C. ae</i> | 3 | 3 | 16.05-16.26 | milky | 0.2 µl | 22% | |
| Total number of ants sampled; mean vol; mean suc | | | | | | 19 | 1.2 µl | 45% |
| Standard deviation | | | | | | | 1.23 µl | 26% |
| 16.03.2011 | <i>I. ru</i> | 5 | 1 | 8.55-9.07 | clear | 0,1µl | 16% | |
| 16.03.2011 | <i>I. ru</i> | 5 | 1 | 8.55-9.07 | clear | 0.1 µl | 16% | |
| 24.03.2011 | <i>I. ru</i> | 2 | 3 | 15.20-15.35 | clear | 0.4 µl | 50% | |
| 24.03.2011 | <i>I. ru</i> | 2 | 3 | 15.20-15.35 | clear | 0.6 µl | 42% | |
| 24.03.2011 | <i>I. ru</i> | 2 | 3 | 15.20-15.35 | clear | 0.4 µl | 42% | |

| Date | Spec | Nr. | Dayt | Timeframe | Color | Vol [μ l] | Suc [%] | |
|--|-------|-----|------|-------------|--------|----------------|--------------|-----|
| 21.03.2011 | I. ru | 6 | 3 | 16.00-16.12 | clear | 1.0 μ l | 14% | |
| 21.03.2011 | I. ru | 6 | 3 | 16.00-16.12 | clear | 0.5 μ l | 27% | |
| 21.03.2011 | I. ru | 6 | 3 | 16.00-16.12 | clear | 0.1 μ l | 17% | |
| 28.03.2011 | I. ru | 6 | 3 | 16.22-16.31 | clear | 0.4 μ l | 21% | |
| 28.03.2011 | I. ru | 6 | 3 | 16.22-16.31 | clear | 0.3 μ l | 27% | |
| 28.03.2011 | I. ru | 6 | 3 | 16.22-16.31 | clear | 0.3 μ l | 27% | |
| Total number of ants sampled; mean vol; mean suc | | | | | | 11 | 0.4 μ l | 27% |
| Standard deviation | | | | | | | 0.26 μ l | 12% |
| 28.03.2011 | P. au | 1 | 1 | 10.00-10.14 | clear | 1.0 μ l | 16% | |
| 28.03.2011 | P. au | 1 | 1 | 10.00-10.14 | brown | 1.3 μ l | 13% | |
| 16.03.2011 | P. au | 4 | 1 | 10.05-10.22 | clear | 1.1 μ l | 35% | |
| 16.03.2011 | P. au | 4 | 1 | 10.05-10.22 | clear | 0.6 μ l | 20% | |
| 04.04.2011 | P. au | 4 | 1 | 10.13-10.31 | clear | 1.5 μ l | 46% | |
| 04.04.2011 | P. au | 4 | 1 | 10.13-10.31 | clear | 1.0 μ l | 42% | |
| 04.04.2011 | P. au | 4 | 1 | 10.13-10.31 | opaque | 1.0 μ l | 52% | |
| Total number of ants sampled; mean vol; mean suc | | | | | | 7 | 1.1 μ l | 32% |
| Standard deviation | | | | | | | 0.28 μ l | 16% |
| 13.04.2011 | T. bi | 10 | 1 | 10.04-10.20 | clear | 0.1 μ l | 20% | |
| 13.04.2011 | T. bi | 10 | 1 | 10.04-10.20 | clear | 0.1 μ l | 23% | |
| 15.04.2011 | T. bi | 10 | 1 | 9.51-9.56 | clear | 0.1 μ l | 14% | |
| 15.04.2011 | T. bi | 10 | 1 | 9.51-9.56 | clear | 0.1 μ l | 15% | |
| 13.04.2011 | T. bi | 10 | 2 | 13.10-13.16 | clear | 0.1 μ l | 16% | |
| 13.04.2011 | T. bi | 10 | 2 | 13.10-13.16 | clear | 0.1 μ l | 20% | |
| 13.04.2011 | T. bi | 10 | 2 | 13.10-13.16 | clear | 0.1 μ l | 17% | |
| 13.04.2011 | T. bi | 10 | 2 | 13.10-13.16 | clear | 0.2 μ l | 12% | |
| 13.04.2011 | T. bi | 10 | 3 | 16.07-16.11 | clear | 0.2 μ l | 15% | |
| 15.04.2011 | T. bi | 10 | 3 | 16.09-16.15 | clear | 0.1 μ l | 14% | |
| 15.04.2011 | T. bi | 10 | 3 | 16.09-16.15 | clear | 0.1 μ l | 16% | |
| Total number of ants sampled; mean vol; mean suc | | | | | | 11 | 0.12 μ l | 17% |
| Standard deviation | | | | | | | 0.04 μ l | 3% |

App Table Overview of the nectar sampling for different species, mean values (\pm SD) of volume and sucrose content are provided. N gives the number of nectar samples per species. Due to the limited refractometer scale, values above 50 % were counted as 50 % (Full table on CD: Ants-Monitoring.xlsx)

| Species | Body size | N | $\bar{\text{O}}$ Volume | $\bar{\text{O}}$ Sucrose [%] |
|-------------------------|-----------|----|-------------------------|------------------------------|
| <i>C. aeneopilosus</i> | 5-9 | 19 | 1.18 \pm 1.07 | 45 \pm 20 |
| <i>P. australis</i> | 4-6 | 7 | 1.07 \pm 0.28 | 32 \pm 16 |
| <i>I. rufoniger</i> gp. | 2-3.5 | 11 | 0.38 \pm 0.26 | 27 \pm 12 |
| <i>T. bicarinatum</i> | 3.5-4.5 | 11 | 0.12 \pm 0.04 | 17 \pm 3 |

App Table Wilcoxon rank sum test for differences in nectar volume among the different species (significant p values are indicated in bold)

| Species | <i>C. aeneopilosus</i> | <i>I. rufoniger gp.</i> | <i>P. australis</i> |
|-------------------------|------------------------|-------------------------|---------------------|
| <i>I. rufoniger gp.</i> | 0.08 | - | - |
| <i>P. australis</i> | 0.64 | 0.005 | - |
| <i>T. bicarinatum</i> | 0.0002 | 0.01 | 0.001 |

App Table Wilcoxon rank sum test for differences in nectar concentration among the different species (significant p values are indicated in bold)

| Species | <i>C. aeneopilosus</i> | <i>I. rufoniger gp.</i> | <i>P. australis</i> |
|-------------------------|------------------------|-------------------------|---------------------|
| <i>I. rufoniger gp.</i> | 0.087 | - | - |
| <i>P. australis</i> | 0.189 | 0.649 | - |
| <i>T. bicarinatum</i> | 0.012 | 0.079 | 0.168 |

App Table Aphid tree monitoring on Podocarpus elatus (Coordinates: S 26°41'01.5" E 153°00'52.3"); Observation period: 10 minutes; Dayt = Daytime (1 = morning; 2 = noon; 3 = evening); N = Number of bees approaching the tree (Full table on CD: Ants-Monitoring.xlsx)

| Date | Start time | Daytime | N | Other flying Insects (number and specification) |
|------------|------------|---------|---|--|
| 12.04.2011 | 10:23 | 1 | 0 | 0 - |
| 13.04.2011 | 10:21 | 1 | 0 | 6 3x Syrphid fly, Wasp, Moth, Shield bug |
| 15.04.2011 | 10:00 | 1 | 0 | 4 Wasp, Syrphid fly, 2x Fly, (Polyrhachis daemeli) |
| 15.03.2011 | 13:20 | 2 | 0 | 0 - |
| 11.04.2011 | 11:50 | 2 | 0 | 2 Syrphid fly, Wasp |
| 13.04.2011 | 13:43 | 2 | 0 | 4 2x Ladybug, 2x Fly |
| 11.04.2011 | 15:12 | 3 | 0 | 0 - |
| 13.04.2011 | 16:15 | 3 | 0 | 1 Moth |
| 15.04.2011 | 16:18 | 3 | 0 | 1 Syrphid fly |

App Table Pearson's Chi-squared test results for differences in behavioral responses towards extracts and hexane for *R. metallica*; N_{Hex} and N_{Ext} show the numbers of Ants monitored for hexane and extract treated filter paper. N_{Tot} gives the total number of ants observed (significant p values are indicated in bold).

| Extract | N _{Hex} | N _{Ext} | N _{Tot} | χ^2 | df | p |
|-------------------------------|------------------|------------------|------------------|----------|----|-----------------|
| Propolis from Nest | 101 | 75 | 176 | 55.20 | 2 | < 0.0001 |
| <i>T. carbonaria</i> | 111 | 98 | 209 | 43.88 | 2 | < 0.0001 |
| <i>A. australis</i> | 104 | 112 | 216 | 9.83 | 2 | 0.007 |
| <i>C. torelliana</i> capsules | 92 | 80 | 172 | 29.46 | 2 | < 0.0001 |
| <i>P. caribea</i> | 88 | 100 | 188 | 37.04 | 2 | < 0.0001 |
| <i>A. bidwili</i> | 78 | 82 | 160 | 4.14 | 2 | 0.13 |

App Table Pearson's Chi-squared test results for differences in behavioral responses towards extracts and hexane for *I. rufoniger* gp.; N_{Hex} and N_{Ext} show the numbers of Ants monitored for hexane and extract treated filter paper. N_{Tot} gives the total number of ants observed (significant p values are indicated in bold)

| Extract | N _{Hex} | N _{Ext} | N _{Tot} | χ^2 | df | <i>p</i> |
|-------------------------------|------------------|------------------|------------------|----------|----|-----------------|
| <i>T. carbonaria</i> | 312 | 366 | 678 | 51.72 | 2 | < 0.0001 |
| <i>C. torelliana</i> capsules | 269 | 304 | 573 | 7.71 | 2 | 0.02 |
| <i>A. bidwili</i> | 296 | 341 | 637 | 3.65 | 2 | 0.16 |
| <i>P. caribea</i> | 331 | 274 | 605 | 138.22 | 2 | < 0.0001 |

App Table Pearson's Chi-squared test results for differences in behavioral responses towards hexane and *T. carbonaria* extract for two ant species (significant p values are indicated in bold)

| Species | N _{Hex} | N _{Ext} | N _{Tot} | χ^2 | df | <i>p</i> |
|-----------------------|------------------|------------------|------------------|----------|----|-----------------|
| <i>P. australis</i> | 121 | 130 | 251 | 29.23 | 2 | < 0.0001 |
| <i>T. bicarinatum</i> | 101 | 91 | 192 | 2.59 | 2 | 0.27 |

App Table Pearson's Chi-squared test results for differences between four different ant species in behavioral responses towards the same extract; N gives the number of species compared (p-values significant after correction for multiple testing are indicated in bold)

| Extract | N | χ^2 | df | <i>p</i> |
|-------------------------------|---|----------|----|-----------------|
| <i>T. carbonaria</i> | 4 | 37.44 | 6 | < 0.0001 |
| <i>C. torelliana</i> capsules | 2 | 8.66 | 2 | 0.01 |
| <i>P. caribea</i> | 2 | 23.19 | 2 | < 0.0001 |
| <i>A. bidwili</i> | 2 | 22.87 | 2 | < 0.0001 |

Declaration / Erklärung

I hereby declare that my thesis in its entirety is the result of my own work. I did not use any other sources and / or materials than listed and specified in the thesis. All charts, diagrams and pictures were – unless marked otherwise – prepared by myself.

Hiermit versichere ich, dass ich diese Arbeit in allen Teilen selbstständig gefertigt und keine anderen als die in der Arbeit angegebenen Hilfsmittel benutzt habe. Die Zeichnungen, Diagramme und bildlichen Darstellungen habe ich – soweit nicht anders vermerkt – selbst gefertigt.

Würzburg, den 29.09.2011