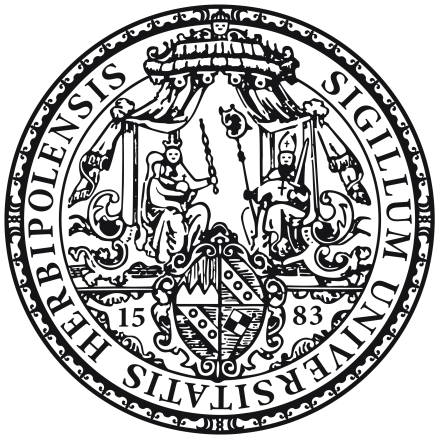
Scents as Floral Defence:

Impact on Species and Communities, Mechanisms and Ecological Consequences.



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Die toten, getrockneten Pflanzen- und Tierleiber zogen mich weniger an, als das tausendfach ineinander greifende Leben der Geschöpfe. Ich strebte immer danach das Ganze zu erfassen, und, wäre es bloss auf einem beschränkten Raum, das Zusammenspiel von Pflanze und Tier zu belauschen. Dieser Drang führte mich zum Studium der Insekten […] in ihren Beziehungen zur Pflanzenwelt. Das ist mein Grenzgebiet, das ich […] so sehr liebe, und das ich immer wieder begehen muss, weil es so unendlich reichhaltig und unerschöpflich an Überraschungen ist.

1946, Dr. Rob. Stäger

Erklärung

gemäß §4 Abs. 3 Ziff. 3, 5 und 8

der Promotionsordnung der Julius-Maximilians-Universität Würzburg

Hiermit erkläre ich, Robert R. Junker, ehrenwörtlich, dass ich die vorliegende Dissertation selbständig angefertigt habe und keine weiteren als die angegebenen Quellen und Hilfsmittel verwendet habe. Die Dissertation wurde bisher weder vollständig noch teilweise einer anderen Hochschule mit dem Ziel der Erlangung eines akademischen Grades vorgelegt.

Am 11.01.2007 hat mir die Universität Würzburg den akademischen Grad des „Diplom-Biologen Univ." verliehen. Weitere akademische Grade habe ich weder erworben noch versucht zu erwerben.

Würzburg, den 07.07.2010

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Robert R. Junker

Thesis is based on the following manuscripts:

Junker RR, Heidinger IMM, Blüthgen N (2010) Floral scent terpenoids deter the facultative florivore *Metrioptera bicolor* (Ensifera, Tettigoniidae, Decticinae). ***Journal of Orthoptera Research*** in press

Junker RR, Bretscher S, Dötterl S, Blüthgen N (submitted) Phytochemical cues affect hunting-site choices of a nursery web spider (*Pisaura mirabilis*) but not of a crab spider (*Misumena vatia*).

Junker RR, Loewel C, Gross R, Dötterl S, Keller A, Blüthgen N (in prep) Composition of epiphytic bacterial communities differs on flowers and leaves.

Junker RR, Daehler CC, Dötterl S, Keller A, Blüthgen N (submitted) Ant-flower networks in Hawai'i: native plants are exploited, introduced plants defended.

Junker RR, Höcherl N & Blüthgen N (2010) Responses to olfactory signals reflect network structure of flower-visitor interactions. ***Journal of Animal Ecology*** 79: 818-823

Junker RR & Blüthgen N (2010) Floral scents repel facultative flower visitors, but attract obligate ones. ***Annals of Botany*** 105: 777-782

Junker RR, Blüthgen (2010) Dependency on floral resources determines the animals' responses to floral scents. ***Plant Signaling and Behavior*** 5

Junker RR, Bleil R, Daehler CC, Blüthgen N (submitted) Intrafloral resource partitioning between endemic and invasive flower visitors: consequences for pollinator effectiveness.

Bleil R, Blüthgen N, Junker RR (submitted) Invasive ants reduce flower parasitism of the endemic Hawaiian shrub *Vaccinium reticulatum* (Ericaceae).

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I. Summary

Floral scents are compositions of diverse volatile substances. Despite the chemical complexity, the interpretation of their ecological relevance was mostly confined to the attractive function facilitating interactions with pollinators. However, the negative impact on plants' reproduction by non-pollinating flower visitors is pronounced and demands floral adaptations that exclude antagonists. The aim of this dissertation was to explore the defensive properties of floral odours and to imbed them into ecological contexts. The thesis covered four scopes: the scents' impact on individual species and on flower-visitor communities, the mechanisms that explain the dual function of floral volatiles (attraction and defence), and the ecological consequences of missing defences for plants and pollinators.

The most important floral antagonists that are known to reduce the reproductive fitness of plants were identified and their responses towards floral scents were examined. We found that representatives of non-pollinating florivores (bush crickets), predators that lure for pollinators (spiders), and microorganisms that potentially colonize petals were repelled, deterred or inhibited in their growth by floral secondary metabolites. An earlier study revealed the same effect on nectar thieving ants. These experimental studies clearly demonstrate that scents universally serve as floral defences that have the potential to reduce or even prevent the visitation and exploitation of flowers by these antagonists.

Within diverse communities, we tested whether species-specific responses to odours reflect the structure of naturally occurring flower-visitor interactions in order to examine the ecological importance of defensive floral scents. On three Hawaiian Islands, ant-flower interactions involving co-occurring native and introduced plants were observed. Ants were historically absent from the geographically isolated Hawaiian archipelago. Thus, we hypothesized that native Hawaiian plants lack floral features that exclude ants and therefore would be heavily exploited by introduced, invasive ants. We quantified the residual interaction strength of each pair of ant/plant species as the deviation of the observed interaction frequency from a null-model prediction based on available nectar sugar in a local plant community and local ant activity at sugar baits. As predicted, flowers of plants that are endemic or indigenous to Hawaii were stronger exploited by ants than flowers of co-occurring introduced plants, which share an evolutionary history with ants. We showed experimentally that the absence of ants on flowers of most introduced and few native plants species was due to morphological barriers and/or repellent floral scents, examined in a mobile olfactometer. Analysis of floral volatiles, however, revealed no consistent ant-

repellent “syndrome”, probably due to the high chemical variability within the floral scent bouquets. On a fallow land in Germany, we linked the responses of receivers (flower visitors) towards signals (flower scent) with the structure of a highly diverse natural flower-insect network. For each interaction, we defined link temperature – a newly developed metric – as the deviation of the observed interaction strength from neutrality, assuming that animals randomly interact with flowers. Link temperature was positively correlated to the specific visitors' responses to floral scents. Thus, communication between plants and consumers via phytochemical signals reflects a significant part of the microstructure in a complex network. Negative as well as positive responses towards floral scents contributed to these results, where individual experience was important, apart from innate behaviour. The demonstration of the contrasting functions of floral scents that control the visitor spectrum of flowers represents the first evidence that floral scents act as filters allowing access to some flower visitors but simultaneously exclude others.

These findings raise the central question of this thesis: what evolutionary mechanism explains the dual function of floral scents? The view of flower visitors as mutualistic and antagonistic agents considers primarily the interest of plants. A classification emphasizing the consumer’s point of view, however, may be more useful when considering adaptations of animals to flower visits. Therefore, we introduced a novel classification that acknowledges the consumers’ interest in the interaction: some animals evolved an obligate dependence on floral resources, others use nectar and pollen as supplement to their diet and are thus regarded as facultative flower visitors. In a meta-analysis covering 18 studies on the responses of animals to floral scents, we assigned the animals to the categories of obligate or facultative flower visitors. Their responses to floral scents were compared. On average, obligate flower visitors, often corresponding to pollinators, were attracted to floral scent compounds. In contrast, facultative and mainly antagonistic visitors were strongly repelled by flower odours. The findings confirm that floral scents have a dual function both as attractive and defensive cues. Whether an animal depends on floral resources determines its response to these signals, suggesting that obligate flower visitors evolved a tolerance against primarily defensive compounds. These findings were confirmed in an experimental study.

We conclude that floral scents protect flowers against visitors that would otherwise reduce the reproductive success of plants. In Hawaii, where flowers do not have defensive means against ants, we studied the impact of ants on the pollination effectiveness of endemic and introduced bees and on the fruit set of an endemic tree *Metrosideros polymorpha* (Myrtaceae). Ants were dominant nectar-consumers that mostly depleted the

nectar of visited inflorescences. Accordingly, the visitation frequency, duration, and consequently the pollinator effectiveness of nectar-foraging bees strongly decreased on ant-visited flowers, whereas pollen-collecting bees remained largely unaffected by ants. Overall, endemic bees (*Hylaeus* spp.) were much poorer pollinators than introduced honeybees (*Apis mellifera*). The average net effect of ants on pollination of *M. polymorpha* was neutral, corresponding to a similar fruit set of ant-visited and ant-free inflorescences. A second Hawaiian plant species, *Vaccinium reticulatum* (Ericaceae), was visited by the caterpillars of an introduced plume moth (*Stenoptilodes littoralis*) that destroyed buds and flowers of this species. The ants' presence on flowers strongly reduced flower parasitism by the caterpillars and consequently decreased the loss of flowers and buds. This is, to our knowledge, the first documented mutualism between invasive ants and an endemic plant species in Hawaii. Thus, ants that have been shown to be detrimental flower visitors elsewhere, had neutral (*M. polymorpha*) or even positive (*V. reticulatum*) effects on endemic Hawaiian plants. However, their overall negative effect on the Hawaiian flora and fauna should not be disregarded.

II. Zusammenfassung

Blütendüfte sind aus vielen flüchtigen Einzelsubstanzen zusammengesetzt. Trotz ihrer chemischen Komplexität wurde das Anlocken von Bestäubern als nahezu alleinige ökologische Funktion angesehen. Viele Blütenbesucher haben allerdings schädliche Einflüsse auf die Fortpflanzung von Pflanzen, die davon profitieren würden, wenn sie diese antagonistischen Organismen vom Blütenbesuch ausschließen könnten. Das Ziel dieser Arbeit war es, die defensiven Funktionen von Blütendüften zu untersuchen und in einen ökologischen Kontext zu stellen. Hierbei wurden vier Aspekte genauer betrachtet: Wir untersuchten die Wirkung von defensiven Blütendüften auf einzelne antagonistische Arten und deren Einfluss auf die Strukturierung von diversen Gemeinschaften bestehend aus Blütenpflanzen und Insekten. Weiterhin haben wir Mechanismen beschrieben, die mögliche Lösungsansätze für die Frage liefern können, wie Blütendüfte in der Lage sind, eine zweifache Funktion zu erfüllen, nämlich Anlocken und Abschrecken. Die ökologischen Konsequenzen von fehlender Blütenabwehr bildeten den letzten Schwerpunkt der Arbeit.

Nektardiebe, Florivore, die Blütengewebe konsumieren, aber dabei nicht bestäuben, Räuber, die Bestäuber fressen, und Mikroorganismen stellen die vier bedeutenden Organismengruppen dar, die Blüten potentiell Schaden zufügen. In experimentellen Studien konnten wir zeigen, dass Blütendüfte repellente, deterrente und wachstumsinhibierende Wirkungen haben und somit die Blüten gegen Vertreter jeder der genannten Gruppen verteidigen. Die repellente Wirkung von Blütendüften auf Ameisen, die häufig Nektardiebe darstellen, wurde in früheren Studien nachgewiesen, die nicht Teil dieser Arbeit sind. Diese Ergebnisse zeigen deutlich, dass Blütendüfte universelle Abwehrstoffe darstellen, die den Besuch von antagonistischen Blütenbesuchern reduzieren oder ganz verhindern können.

Die strukturierende Rolle defensiver Blütendüfte wurde innerhalb zweier Blütenbesucher Gemeinschaften untersucht. Erstens haben wir in Hawaii die Interaktionen zwischen Blüten und Ameisen untersucht. Hawaii stellt das größte Ökosystem dar, das in seiner Entstehungsgeschichte nicht mit Ameisen konfrontiert war, die in anderen Regionen bedeutenden Nektardiebe sind. Durch anthropogene Einflüsse konnten sich die Ameisen allerdings auf allen Hauptinseln der Inselgruppe ausbreiten und sind heute in den meisten Habitaten die dominanten Arthropoden. Aufgrund der historischen Abwesenheit von Ameisen, vermuteten wir, dass hawaiianische Pflanzen keinerlei Abwehrmechanismen besitzen, die Ameisen von den Blüten fernhalten könnten. Innerhalb kleiner Habitate haben wir Besuchsmuster von Ameisen auf Blüten aufgenommen und diese mit generierten Mustern verglichen, die auf Nullmodellen basierten. Die Nullmodelle berücksichtigten die

Aktivität der Ameisen an Zuckerködern, sowie die gesamte Zuckermenge, die die einzelnen im Gebiet vorhandenen Pflanzen in Form von Blütennektar anboten. Wir konnten feststellen, dass heimische hawaiianische Blütenpflanzenarten stärker von Ameisen besucht wurden, als auf Grund des Nullmodells erwartet wurde. Dahingegen wurden eingeführte Pflanzenarten, die an Ameisen angepasst sind, deutlich weniger als erwartet aufgesucht. Verantwortlich für dieses Ergebnis waren morphologische Barrieren der Blüten und repellente Blütendüfte, wobei beide Mechanismen häufiger bei den eingeführten Pflanzenarten zu finden waren.

Die zweite Studie, die sich mit den Effekten von Blütendüften auf Blüten-Besucher Gemeinschaften beschäftigte, wurde auf einer brachliegenden Wiese in Deutschland durchgeführt. Auch hier wurde die Abweichung der beobachteten Verteilung der Insekten auf Blüten von einer zufälligen Verteilung (Nullmodell) gemessen und untersucht, ob Reaktionen auf Blütendüfte diese Abweichungen erklären können. Wir konnten zeigen, dass Interaktionen zwischen Blütenpflanzenarten und Insekten verschiedener Ordnungen, die selten oder nie auftraten, oft mit negativen Verhaltensantworten einhergehen, häufige Interaktionen dagegen mit positiven Reaktionen. Die Ergebnisse geben Anlass zu der Feststellung, dass die anlockenden und abschreckenden Eigenschaften von Blütendüften stark zu der Strukturierung von Blüten-Besucher Netzwerken beitragen. Weiterhin ist diese Studie der erste direkte Beweis, dass Blütendüfte Filter darstellen, die den Besuch einiger Tiere zulassen, während andere vom Konsum von Nektar und Pollen abgehalten werden.

Diese doppelte Funktion von Blütendüften führt zu der zentralen Fragestellung der vorliegenden Arbeit: Wie ist es evolutionär zu erklären, dass einige Tiere positive und andere negative Reaktionen auf die gleichen Düfte zeigen. Bislang wurden Blütenbesucher nach ihrer Auswirkung auf die Pflanzenreproduktion entweder in Mutualisten oder in Antagonisten eingeteilt. Diese Einteilung erscheint allerdings nicht geeignet zu sein, um die verschiedenen Reaktionen zu erklären, weil sie das tierische Interesse, den Konsum von Blütenressourcen, nicht berücksichtigt. Daher haben wir eine neue Einteilung vorgeschlagen, die die Konsumentensicht berücksichtigt: Einige Tiere sind obligat abhängig von Nektar oder Pollen, während andere diese Ressourcen nur nutzen, um ihr breiteres Nahrungsspektrum zu ergänzen. Das Ergebnis einer von uns durchgeführten Metaanalyse bestätigt die Relevanz dieser Dichotomie: Obligate Blütenbesucher waren zumeist von Blütendüften angelockt, während fakultative Besucher stark negative Reaktionen aufwiesen. Spezielle Mundwerkzeuge von Insekten, zum Beispiel, stellen eine Anpassung von obligaten Blütenbesuchern dar, die ihnen erlaubt die Blütenressourcen effektiv zu nutzen. Eine Toleranz gegenüber anderweitig repellenten und/oder giftigen Sekundärmetaboliten

könnte eine weitere wichtige Anpassung sein. Die Ergebnisse der Metaanalyse konnten auch in einer experimentellen Arbeit bestätigt werden, bei der Ameisen (fakultative Blütenbesucher) von vielen Substanzen abgeschreckt waren, von denen sich Hummeln (obligate Blütenbesucher) anlocken ließen.

Die bisherigen Ergebnisse zeigen eindeutig, dass defensive Blütendüfte genutzt werden, um antagonistische Organismen von Blüten fernzuhalten. An hawaiianischen Blüten, denen Abwehrmechanismen gegen Ameisen fehlen und die dementsprechend stark von Ameisen aufgesucht werden, haben wir untersucht, welchen Einfluss Ameisen auf die Reproduktion zweier endemischer Pflanzen und das Verhalten von Bestäubern haben. Invasive Ameisen und Honigbienen (*Apis mellifera*) und endemische Bienen (*Hylaeus* spp.) waren die dominierenden Besucher auf Blüten der endemischen Baumart *Metrosideros polymorpha* (Myrtaceae). Wir konnten feststellen, dass die Bestäubereffektivität der Bienen stark von der Bienenart, der Anwesenheit von Ameisen und der gesammelten Ressource (Nektar oder Pollen) abhängig war. Insgesamt betrachtet hatten Ameisen aber keinen Einfluss auf den Fruchtansatz der Pflanze. Die Blüten und Knospen einer zweiten endemische Art (*Vaccinium reticulatum*, Ericaceae) wurden von den Raupen einer eingeführten Federmotte (*Stenoptilodes littoralis*) gefressen und abgetötet. Auf Blüten, die stark von Ameisen frequentiert wurden, war der schädliche Einfluss der Raupen stark abgemildert. Dieses tritrophische System stellt den ersten dokumentierten Fall dar, bei dem eine endemische hawaiianische Pflanzenart von der Anwesenheit invasiver Ameisen profitiert. Diese neutralen und positiven Effekte von Ameisen auf edemische hawaiianische Pflanzen sollte aber nicht darüber hinweg täuschen, dass Ameisen die wohl schädlichsten Neozoen auf Hawaii darstellen.

III. General introduction

Organisms are confronted with competitors, enemies, (living) food items, mating partners or mutualists with whom they interact and communicate. Animals and plants utilize signals to facilitate positive interactions or to avoid negative encounters prior to physical contact. Communication via signals requires a sender that displays visual, acoustical or olfactory cues and an actor that receives the message and responds to it. Depending on the current receiver, the senders may flexibly choose from their behavioural or physiological repertoire to adapt their signals to the situation. Male birds, for example, often produce short and simple calls during male competition, while they court for females with longer and more elaborate songs (Catchpole 1987). More subtle alterations of the same signals may also change the meaning of a signal: short bouts of leg raising in male wolf spiders are displayed during male-male interactions, while slightly longer bouts of the same behaviour are correlated to mating success (Delaney *et al.* 2007). In other species exactly the same signals such as a colourful plumage are both a warning to potential intruders and an advertisement of the own mating-quality (Berglund *et al.* 1996). The attractive effect of a colourful plumage on females and its function as a warning to male competitors is based on the same massage of this phenotype: the more colourful the male the stronger and fitter it is, which promises healthy offspring to mating partners but also serious injuries during encounters with conspecifics of the same sex. In macaques the pregnancy colouration of females' faces triggers an attentive behaviour in males but is also a warning to both sexes (Gerald *et al.* 2009). Likewise, herbivore attacks on plants induce the emission of volatile signals that serve as direct and indirect defences by repelling the enemies and attracting parasitoids of the herbivores (Unsicker *et al.* 2009). These examples indicate that the appearance, content and effect of a signal are conditioned by the situation of the sender as well as the identity of the receiver and that communication via signals is an evolutionary response to both the presence of mutualists and antagonists such as competitors or enemies.

Immobile plants advertise their edible floral resources with visual and olfactory cues in order to employ and pay visitors for the transport of male gametes. Thus, floral signals are regarded as synomones as both partners profit from the interaction. However, animals try to maximise the gain of nutrients while minimizing their effort, which is often associated with detrimental effects for the plants if costs exceed the benefits (Morris *et al.* 2010). Often, plants do not receive any benefits by some flower visitors that consume resources but do not pollinate in return (Inouye 1980). In natural ecosystems, the presence of both

mutualistic *and* antagonistic visitors forces flowers to respond with adaptations that invite the former but exclude the latter. Nectar, palatable for mutualists but toxic for antagonists, for example, could represent a solution for the conflicting tasks (Janzen 1977, Adler 2000, Johnson *et al.* 2006), but signals with a dual message would pre-empt conflicts even before antagonistic agents enter flowers. The aim of this dissertation was to explore the defensive function of floral scents and to investigate the dual role of these signals in pollinator attraction and antagonist repellence.

Pollination and exploitation of flowers

Mutualism

Over two hundred years ago, Christian Konrad Sprengel (1793) was the first who noted that floral nectar serves as resource for insects that in turn pollinate the flowers. Thus, he founded the pollination biology that is nowadays a multifaceted discipline in ecology. Ever since, pollination biologists were fascinated by the diversity of interactions between flowers and their pollinators and the adaptations of both trophic levels to either improve reproductive fitness or nutrient intake, respectively. The adaptations of flowers and pollinators led to a continuum from highly specialized interactions where species pairs coevolved, to largely generalized systems where each species interacts with multiple partners. Effective pollen transfer – the ultimate factor shaping floral traits – can be achieved either by high efficiency (accuracy) or high frequency of interactions. Accurate pollen transfer is most likely in specialised systems where a plant species is solely visited by one pollinator that is – in turn – specialised on that plant species. Orchids and their pollinators may be the best examples of such a co-adaptation were traits of flowers and animals represent a perfect match between the actors (Nilsson 1992). For instance, extremely long nectar tubes of some orchids allow only moths with equally long tongues to visit and pollinate the flowers (Darwin 1862). Even more specifically are deceptive orchids that exploit the sensory system of their pollinators by mimicking sex-pheromones in order to attract males in search for mating partners (Peakall *et al.* 1987, Ayasse *et al.* 2003).

Effective pollen transfer in more generalized systems, however, requires other and more flexible mechanisms. The classical pollination syndromes (Knuth 1908, Vogel 1954, Faegri and Pijl 1979) hypothesise suites of floral features that are assumed to be evolved as adaptation to certain visitor groups. According to these syndromes, pleasant and lightly scented, red to purple flowers with fair amounts of nectar in tubes, for example, tend to be visited by butterflies, while beetle-pollinated flowers are often dull-coloured, heavily

scented, open and produce large amounts of pollen (Faegri and Pijl 1979). The syndromes imply that the assembly of floral features can predict the most relevant and most frequent pollinators of plants and that their visitor spectrum therefore is taxonomically restricted. This notion, however, has been criticized (Waser *et al.* 1996) and multivariate statistical approaches revealed that the syndromes are not suited to determine the visitor community of flowers (Ollerton *et al.* 2009) and that flowers that share traits are not visited by the same visitor spectrum in different habitats (Lazaro *et al.* 2008). Overall, these and data from several studies analysing flower-visitor networks (e.g. Olesen *et al.* 2008, Petanidou *et al.* 2008) show that many flowering plant species are visited and potentially pollinated by diverse animal species from different genera, families, orders and even classes and that the identities of the interacting partners are highly variable over time (Waser *et al.* 1996).

Like the plants, the visitors also interact with multiple partners at the same time (e.g. Olesen *et al.* 2008, Petanidou *et al.* 2008), which would, assumed that animals visited different plant species in random sequences, lead to a high percentage of lost pollen that is not deposited on stigmas of conspecific plants. However, even though an animal species appears to be highly generalized within a community, individuals often have a long- or short- term specialization to flowers of one plant species and thus exhibit a high degree of flower fidelity (Heinrich 1976, Wright and Schiestl 2009). Flower fidelity is necessary but not sufficient for an effective pollination, which additionally requires certain behavioural and physiological characteristics of the animal visitors. Efficient pollinators need to touch anthers and stigmas while visiting the flowers and a body surface where pollen grains can be deposited and transported from flower to flower. Therefore, pollinator effectiveness is a product of efficiency per visit and visitation frequency. Lower efficiency in pollen removal and deposition may be compensated by higher visitation frequency and *vice versa* (Sahli and Conner 2007, Madjidian *et al.* 2008). Different animals that visit the same flowering plant species strongly vary in these properties and their pollinator effectiveness for that plant species thus also varies (Kandori 2002, Reynolds and Fenster 2008). Furthermore, species that effectively pollinate one plant species may be largely unable to pollinate other plant species they visit, which often results in pollen or nectar theft (Inouye 1980, Hargreaves *et al.* 2009). Pollen and nectar thieves are thus antagonistic agents since these consumers of floral resources do not contribute to pollination in these cases.

Antagonism

Almost one hundred years after Sprengel (1793) described floral adaptations to pollinators, Anton Kerner von Marilaun (1879) also emphasized the existence of non-

pollinating flower visitors that exploit floral resources and thus may have detrimental effects on plants' reproduction. Kerner (1879) viewed morphological features of flowers, in contrast to Sprengel (1793), as adaptations to prevent antagonistic animals from illegitimately feeding on their rewards. The detrimental effects for the plants may range from the destruction of whole flowers, interference with pollinators and the exploitation or spoiling of rewards to more subtle effects where floral signals may be altered leading to a reduced attractiveness of the flowers. The four major groups of antagonistic flower visitors are nectar thieves or robbers, herbivores feeding on flowers, predators of pollinators dwelling on flowers and microorganisms.

Nectar thieves and robbers

Nectar thieves exploit floral nectar but do not contribute to the pollination of the flowers they visit. Nectar theft often results from a morphological mismatch between flowers and visitors that do not touch anthers or stigmas. Various taxa are potential nectar thieves of some plant species (Irwin *et al.* 2001), e.g. bees (Johnson *et al.* 2006), moths (Sazima *et al.* 1994), and birds (Johnson *et al.* 2006); but the most prominent among them are ants that require large amounts of carbohydrates – the dominant ingredient of nectar in form of sugar – for their colonies' nutrition (Blüthgen and Feldhaar 2010). Despite the fact that ants are frequent flower visitors of some plant species, they mostly fail to efficiently transfer pollen between flowers. Ants are often too small to touch the reproductive plant parts (Pijl 1955), or their body surfaces are too smooth for pollen attachment or is covered with metapleural secretions that inhibit the germination of pollen (Beattie *et al.* 1984, Beattie *et al.* 1985). Furthermore, their central-place foraging behaviour pre-empts long distance pollen dispersal (Hölldobler and Wilson 1990).

Known effects of nectar thieves (mostly ants) on flowers cover the full range from negative to positive (Irwin *et al.* 2001). Negative outcomes may comprise direct and indirect effects. Effects that directly lead to a reduction in seed set (Galen 1999) appear when larceny of nectar is accompanied by the destruction of reproductive structures of flowers (Galen 1983, Galen and Geib 2007) or if the pollen viability is reduced due to the presence of antagonists (Galen and Butchart 2003). Competition with pollinators may alter the frequency of visits or the behaviour of pollinators and thus indirectly decrease seed set. Nectar thieving ants often outcompete pollinators due to their vast abundance leaving no or little nectar for other flower visitors (Lach 2005, 2008b). Furthermore, the aggressive behaviour of ants often deters pollinators from flowers (Ness 2006) that consequently reduce their visitation frequency and time (Tsuji *et al.* 2004, Junker *et al.* 2007) which may

also translate into detrimental effects on the reproduction of plants (Blancafort and Gomez 2005). In other cases, flower visitors that spend less time on individual flowers due to the aggression by ants move more frequently between flowers and may thus be more efficient in transferring pollen (Altshuler 1999).

Morphological barriers of flowers often prevent ants and other nectar *thieves* from stealing nectar (Herrera *et al.* 1984, Galen and Cuba 2001), but these obstructions are not absolutely safe: nectar *robbers*, as opposed to *thieves*, bite holes in the perianth of flowers in order to reach the sugary solution that would not be reachable for these nectar consumers in intact flowers (Inouye 1980). The most common nectar robbers are birds and bees (Maloof and Inouye 2000) that have, similar to nectar thieves, effects with variable outcome on the reproduction of the plants they rob. However, positive effects are more common in this type of larceny than in nectar theft since many nectar robbers also pollinate the robbed flowers (Maloof and Inouye 2000). Negative effects result from reduced visitation frequency by pollinators and decreased long-distance pollen flow (Irwin 2003).

Non-pollinating florivores

Florivory, the consumption of floral tissues, pollen and nectar, usually is the requisite for pollination (Frame 2003). Florivorous animals always visit flowers in search for food, which may result in a mutualism if the animal also pollinates the flowers but may also result in antagonism if the pollination service is lacking. Non-pollinating florivores usually are common herbivores such as beetles, orthopterans and caterpillars (McCall and Irwin 2006). Petals serve as advertisement of flowers as they attract pollinators with shape, colour and scent and are thus not a reward themselves. Nonetheless, petals of most plant species suffer from damage by flower-feeding herbivores (Breadmore and Kirk 1998). The proportion of flowers within a population that is affected by florivory is neglectable in some species (Breadmore and Kirk 1998) but high in others (Schuster 1974). Even minor damages of the petals lead to a reduction in floral attractiveness for pollinators and consequently to a reduced visitation frequency and thus reduced seed set (Krupnick *et al.* 1999). More severe are damages to the reproductive structures of flowers, i.e. stigmas or styles, that immediately destroy the functionality of flowers (Kerner 1879).

Predators of pollinators

Predators of pollinators dwell on flowers and lure for flower visiting insects. The predacious animals often take advantage of the attractive appeal of flowers to potential prey and thus exploit phytochemical signals. Crab spiders are the most prominent and abundant

representatives of flower dwelling predators. Some crab spider species are able to match their body colour to the flowers' colour and their UV reflecting properties may even increase the visitation frequency of pollinators due to an enhanced perceived contrast within the flower (Heiling *et al.* 2003). Interestingly, spiders and pollinators often share their preferences for flowers during their anthesis when they offer the most rewards (Fritz and Morse 1981, Chien and Morse 1998, Morse 2000a). These adaptations to flowers as hunting site by crab spiders is also reflected in the taxonomically restricted prey spectrum that solely includes common flower visiting insects (Nentwig 1986). The presence of predators on flowers has strong impacts on plants' reproduction and pollinator behaviour. Predacious flower visitors may have positive effects in some cases when they also deter herbivores from flowering plant individuals (Romero and Vasconcellos-Neto 2004), but their presence is mostly associated with detrimental effects. Flowers occupied by predators are less frequently visited by pollinators (Dukas and Morse 2003, Goncalves-Souza *et al.* 2008), which often translates into reduced seed set and reduced fruit biomass (Goncalves-Souza *et al.* 2008, Brechbühl *et al.* 2010). Additionally, pollinators that were attacked by a spider more carefully evaluate subsequent flowers (Ings and Chittka 2008) or even avoid those flowers (Dukas and Morse 2003).

Microorganisms

Microorganisms are ubiquitous and colonize nearly all surfaces in nature including flowers. Several specific interactions between microorganisms and flowers have been intensively studied, for example those between yeast and nectar (Kevan *et al.* 1988, Herrera *et al.* 2008), fungi that alter flower traits or induce pseudo-flowers (Raguso and Roy 1998, Dötterl *et al.* 2009) and floral pathogens (Johnson and Stockwell 1998). Despite the great diversity and abundance of microorganisms and their manifold biochemical abilities, much less is known about bacteria colonizing flowers in more generalised systems. However, studies on bacteria and their interactions with insects and flowers suggest the following potential effects of bacteria on the plants' reproduction and pollinator behaviour.

(a) The majority of studies dealing with microorganisms and flowers focused on pathogens of economically important crops such as *Erwinia amylovora*, the causal agent of fire blight occurring in apples and other Rosaceae (Johnson and Stockwell 1998). However, floral infections also reduce the fecundity of flowers in natural ecosystems and thereby may affect population dynamics (Gilbert 2002).

(b) Instead of destroying whole flowers, microorganisms also spoil nectar or pollen and alter the nutritional composition, which may make the resource less suitable for

pollinators (Herrera *et al.* 2008). Next to the alteration of resources, secondary metabolites of microorganisms may accumulate in nectar such as ethanol that is toxic for pollinators (Ehlers and Olesen 1997).

(c) The scents emitted by microorganisms correspond often to those that are emitted by flowers (Knudsen *et al.* 2006, Schulz and Dickschat 2007) but also include unknown substances (Kai *et al.* 2008). Alterations of the original bouquet (e.g. due to microorganisms colonising flowers) may lead to an isolation of flowers with modified scents (for artifically modified floral scent bouquets see Waelti *et al.* 2008). Similar effects are conceivable for floral colours since microorganisms colonising leaf and flower surfaces are often strongly pigmented as an adaptation to the intense ultraviolet radiation in these surfaces (Sundin and Jacobs 1999).

Floral filters

In their natural habitat, flowers are exposed to both mutualistic and antagonistic agents that benefit from floral resources or signals but have either positive or negative net effects on the plants they visit. Overall, antagonistic flower visitors often have stronger (negative) effects in magnitude than mutualistic ones (Morris *et al.* 2007). Thus, plants would greatly benefit from excluding the former but inviting the latter. Traits that selectively promote the visitation of some animals while simultaneously prevent the visitation of others – floral filters – provide a solution for this dilemma. Morphological barriers in form of narrow nectar tubes, for example, facilitate resource partitioning between potential flower visitors within one guild, e.g. granting long-tongued bumblebees access to nectar while short-tongued are excluded (Inouye 1978, Graham and Jones 1996). Morphological barriers also prevent antagonists from exploiting nectar, e.g. ants may not fit in nectar tubes while long mouthparts of pollinators can reach the nectar (Herrera *et al.* 1984, Galen and Cuba 2001, Galen and Geib 2007). Next to morphological features, secondary metabolites produced by flowers and accumulated in nectar and pollen may serve as floral filters. Nectar is basically composed of water and the primary metabolites sugar and – to a lesser extent – amino acids (Baker *et al.* 1978). Secondary metabolites are also solved in this liquid including alkaloids, phenolics, iridoid glycosides and terpenoids and add a defensive character to the otherwise attractive nectar (Adler 2000, Kessler and Baldwin 2006, Hansen *et al.* 2007). Johnson *et al.* (2006), for example, explored the function of phenolic compounds in the floral nectar of *Aloe vryheidensis* and documented both the deterrent effect on detrimental flower visitors and the attractive effect on beneficial ones.

Similar effects were proposed for secondary metabolites in pollen (Dobson *et al.* 1996), but have not been tested so far.

Volatiles emitted by flowers are well known attractants for diverse flower visitors (Dobson 2006) but common compounds in floral scent bouquets are also known to have deterrent, repellent and toxic functions to many organisms (Harrewijn *et al.* 1995, Gershenzon and Dudareva 2007) suggesting that floral scents may also serve as floral filter (Raguso 2008b). However, the defensive properties of floral scents are underrepresented in the literature.

Floral scents

Composition of floral scents

Floral scents are one of the most diverse and complex signals in plant-animal interactions and consist of products of the primary and mainly the secondary metabolism. Volatiles emitted by flowers are blends of few up to more than one hundred different substances mostly with a molecular mass less than 300 facilitating the volatility (Knudsen and Gershenzon 2006). The molecules derive from several different biosynthetic pathways, the most common chemical classes are mono- and sesquiterpenes, benzenoids, phenylpropanoids and fatty acid derivates, less common are diterpenes, irregular terpenes, nitrogen or sulfur containing compounds and miscellaneous cyclic compounds (Knudsen *et al.* 2006). All of these substance classes may comprise oxygenated substances, i.e. hydrocarbons with functional groups. The most common functional groups are alcohols, aldehydes, esthers, ethers and ketones (Knudsen *et al.* 2006). CO_2 as the sole product of the plants' primary metabolism described as floral volatile is certainly omnipresent in flowers and its ecological function has recently been demonstrated (Guerenstein *et al.* 2004, Goyret *et al.* 2008), but it will not be covered in the following text. Some individual compounds are emitted by a high percentage of flowering plant species, e.g. some monoterpenes occur in more than 70% of all floral scent bouquets analysed so far (Knudsen *et al.* 2006). Nonetheless, the interspecific variability of the composition of floral scent compounds is enormous due to the numerous combinatorial possibilities of frequently emitted substances and the presence of compounds unique to one or few plant species (Raguso 2008b). Additionally, the intra-specific variability is also pronounced: male and female flowers in dioecious plants have distinct scent profiles (Ashman 2009) and the scent emission of individual flowers is not constant in quantity and quality over time (Schiestl *et al.* 1997, Irwin and Dorsett 2002).

Biosynthetic pathways of floral scent compounds

Floral scents are either produced in epidermis cells of floral tissues or in osmophores, glandular trichomes and hairs that are specialised organs for scent production and emission, the latter ones being restricted to few (highly evolved) taxa (Effmert *et al.* 2006). The three major classes of floral volatiles, terpenoids, phenylpropanoids/benzenoids and fatty acid derivates each derive from distinct precursors and their high diversity of floral scent compounds is owed to the ability of the involved enzymes to form multiple products from a single substrate (Knudsen and Gershenzon 2006).

Biosynthesis of terpenoids

Terpenes are built out of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). In the cytosol, IPP and DMAPP are the product of the condensation of acetyl-CoA in the mevalonic acid pathway. In the plastids, the methylerythritol phosphate pathway forms IPP and DMAPP with pyruvate and glyceraldehyde as substrates (Dudareva and Pichersky 2006b). IPP as product of any of these pathways may afterwards be transported through the membrane of the plastids into the cytosol where sesquiterpenes are synthesised and to a lesser extent from the cytosol into the plastids where monoterpenes are synthesised (Dudareva *et al.* 2005, Schie *et al.* 2006). Farnesyl diphosphate synthase, located in the cytosol, transforms two IPP molecules and one DMAPP to farnesyl diphosphate, the direct substrate for several terpene synthases that built the great variety of sesquiterpenoids (Dudareva and Pichersky 2006a). In the plastids, the geranyl diphosphate synthase condensates IPP and DMAPP to geranyl diphosphate the substrate for monoterpenoids (Dudareva and Pichersky 2006a). Many terpenes are either direct products of the terpene synthases, others are modified by hydroxylation, dehydrogenation, acetylation or further types of chemical reactions (Dudareva and Pichersky 2006a). Some of the most common monterpenoids are limonene, α- and β-pinene, linalool and ocimene, frequently occurring sesquiterpenoids are β- caryophyllene, farnesol and nerolidol.

Biosynthesis of phenylpropanoids/benzenoids

The universal precursor of phenylpropanoids/benzenoids is the amino acid phenylalanine from which the aromatic benzene ring is preserved. Phenylalanine is a product of the Shikimate pathway located in the plastids with chorismate as intermediate product (Schie *et al.* 2006). The benzene ring is complemented by oxidation, acylation or methylation in the following biosynthetic steps leading to the volatile compounds (Schie *et al.* 2006). Several enzymatic processes are involved in the conversion of phenylalanine to phenylpropanoids and benzenoids (Dudareva and Pichersky 2000, Dudareva and Pichersky 2006b). Common representatives of this compound class are benzaldehyde, eugenol and benzyl alcohol.

Biosynthesis of fatty acid derivates

Volatile fatty acid derivates originate in membrane lipids that are transformed in the lipoxygenase pathway where mostly C_{18} fatty acids are used as precursors. C_{18} units are transformed by a lipoxigenase prior to a cleavage to C_{12} and C_6 units by a hydroperoxide lyase (Dudareva and Pichersky 2006b). The common green leaf volatiles 3-cis-hexenal and hexanal are the first products of the hydroperoxide lyases, which may afterwards be modified into further volatile compounds (Dudareva and Pichersky 2006b). Pentadecane and hexanol are frequently occurring fatty acid derivates in floral scent compositions.

Ecological function of scents in floral biology

Floral scents as synomones

Synomones are signals that mediate interactions that are beneficial for both the sender and the receiver. Olfactory traits are, next to visual traits and resource characteristics, important factors shaping flower-pollinator interactions. The continuum from specialisation to generalisation in mutualistic flower-visitor interactions is paralleled in the specificity or generality of the floral scents utilized as signals received by the visitors, respectively. In highly specialised systems where plants rely on a single pollinator, “private channels” are often utilized for the species-specific communication and the targeted attraction. The substances serving as private channel range from compounds that are also used by the visitor for intra-specific communication to those that are common floral scent compounds but elicit behavioural responses of the desired addressee only. For instance, deceptive orchids often emit sex-pheromones of female insects that attract mating partners

(Peakall *et al.* 1987, Ayasse *et al.* 2003). In these cases, however, the scents need to be regarded as allomone, as it is beneficial to the sender but detrimental for the receiver. Another and also deceptive strategy of orchids is to emit green-leaf volatiles that are usually emitted by vegetative plant parts after herbivory to attract the enemies of the herbivores (Brodmann *et al.* 2008). The tight associations between figs and its nursery pollinating fig-wasps, for example, are mediated via olfactory signals that are emitted by the flowers but are not intra-specifically used by the wasps. The scent bouquets of figs are often composed of common floral scent volatiles (Grison-Pige *et al.* 2002a, Grison-Pige *et al.* 2002b) but the key attractant may be less common: benzenoid 4-methylanisole is the attractive component of the scent bouquet of *Ficus semicordata* that specifically attracts the obligate pollinator *Ceratosolen gravelyi* but is otherwise seldom found to be emitted by figs (Chen *et al.* 2009). However, such one-to one mutualisms may also be mediated via very common floral volatiles that dominate the bouquets and are attractive to the insect-partner (Svensson *et al.* 2010).

Dobson (2006) made an attempt to complement the pollination syndromes (see above) with individual volatile compounds that are proposed to be characteristic to groups of pollinators. However, most individual substances were attributed to several groups, thus the study does not convincingly demonstrate the existence of "scent-syndromes". Nonetheless, flowers that share the same type of pollinators often also share rough scent-features, which is more pronounced in systems were vertebrates are involved as pollinators: bird pollinated flowers often lack any scent or produce it only in trace amounts (Raguso and Pichersky 1995, Knudsen *et al.* 2004) and bat pollinated flowers mostly contain sulphur compounds in their odours (Bestmann *et al.* 1997). For insects, the supposed characteristic volatiles often overlap: butterfly (Andersson *et al.* 2002), moth (Raguso and Pichersky 1995), wasp (Shuttleworth and Johnson 2010), bee (Dobson 2006) and fly pollinated flowers (Shuttleworth and Johnson 2010) emit linalool (monoterpene alcohol) in considerable amounts, for example.

Individual-based, short-term specificity of insects (see above) is often mediated via common scent compounds (Wright and Schiestl 2009), which emphasises the flexible cognition abilities of the animals. The learning of individual scent compounds or compositions associated with rewards is a key process in non-specialised flower-insect interactions (Smith *et al.* 2006). Moths, for example, have the ability to use various substances as learned signal to locate nectar bearing flowers (Cunningham *et al.* 2004). Thus, the insects use scents to efficiently find and consume floral resources of plants to which they do not have co-evolved links. However, moths are more attracted to the floral

scents of plants to which they have innate preferences than to floral scents that have been associatively learned (Riffell *et al.* 2008). While naïve responses to odour blends are often stronger than responses to individual compounds (Stringer *et al.* 2008), learned responses mainly base on "key odorants" within bouquets that are required to recognise a reinforced multi-component signal (Laloi *et al.* 2000, Dötterl *et al.* 2006, Riffell *et al.* 2009, Reinhard *et al.* 2010).

Similar to other floral traits, scents have been mostly viewed as attractive signals that promote mutualistic relationships between flowers and their pollinators (Pichersky and Gershenzon 2002). However, individual substances attract pollinators at low, but repel them at high concentrations (Terry *et al.* 2007), and the concentration threshold where a substance has a positive or negative quality may vary across species. Thus, the emission of scents in certain concentrations function as defence if the antagonistic agents have a lower tolerance-threshold than the mutualist.

Floral scents as allomones

Allomones are beneficial signals for the sender but negatively affect the receivers. In different contexts than pollination biology, many substances that occur in floral scent bouquets also serve as defences against herbivores, parasites and pathogens (Harrewijn *et al.* 1995, Gershenzon and Dudareva 2007). Although non-volatile secondary metabolites in floral tissues or resources were occasionally demonstrated to be defensive (e.g. Kessler and Baldwin 2006, Johnson *et al.* 2008, Kessler *et al.* 2008, Hanley *et al.* 2009), the repellent effects of volatile secondary metabolites on antagonistic flower visitors has been rarely investigated (but see Junker and Blüthgen 2008, Willmer *et al.* 2009). In my dissertation, we aimed to fill that gap. We explored the defensive function of floral scent on the species and community level and searched for mechanisms behind the dual function of floral scents (attraction and defence). Additionally, we studied the consequences of the lack of floral defences and associated nectar theft by ants on the reproduction of plants and behaviour of pollinators.

IV. Defensive floral scents: impact on species and communities, mechanisms and ecological consequences – a synopsis

Despite the high complexity of floral scents that are composed of diverse chemical substances from several biosynthetic pathways, the way of looking at the ecological functions has been astonishingly one-dimensional, mostly focussing on the attractive properties. However, the plants' necessity to exclude antagonistic flower visitors (Kerner 1879, Brown 2002) and the well known defensive qualities of many substances occurring in floral scent bouquets (Gershenzon and Dudareva 2007) suggest that volatiles emitted by flowers are also utilized as defensive traits. The goal of this thesis was to explore the repellent, toxic, and growth-inhibiting effects of floral scents and imbed them into this ecological context. We investigated how defensive floral volatiles affect individual species and how this acts upon the structure of multi-species flower-visitor communities. The contrasting responses (attraction *and* defence) towards individual scent bouquets or compounds by different animals demanded the exploration of the mechanisms explaining the dual function of floral scents. We provided a conceptual solution for this phenomenon and supported it with meta-analytical and empirical data. Furthermore, we investigated consequences of antagonistic flower visitors in absence of floral defences on the behaviour of other flower visitors and the plants' reproduction.

Impact on species

Individuals in search for food locate and evaluate the resources by signals prior to the contact with the food item. Flower-visitors may use scents in order to find their host plants (Raguso 2008a) or to choose between rewarding and non-rewarding flowers (Howell and Alarcon 2007). The same signals may also prevent other animals from consuming floral resources, as shown for ants that are repelled by the scent bouquets of several plant species of diverse families and individual floral scent compounds (Junker and Blüthgen 2008, Willmer *et al.* 2009). One aim of this thesis was to investigate whether representatives of all the four major groups of floral antagonists (nectar thieves, non-pollinating florivores, predators of pollinators and microorganisms; see above) negatively respond to flower odours, as do ants representing nectar thieves.

We studied a bush cricket (*Metrioptera bicolor*) with a generalised diet that is known to occasionally feed on petals to test whether floral scents have the potential to deter or repel non-pollinating florivores. We found two out of five tested floral scent compounds to be strong antifeedants against this bush cricket, a result that was mirrored in feeding trials with fresh plant material (**Chapter V**).

In experiments with predators of pollinators, we compared the responses to floral scents of crab spiders (*Misumena vatia*) that predominantly hunt on flowers as sit-and-wait-predator with those of nursery web spiders (*Pisaura mirabilis*) that are exclusively found in the vegetation. According to their natural hunting-site choices, *M. vatia* tolerated floral scent compounds that were strongly avoided by *P. mirabilis* spiders. The preferences for the respective plant organs were also evident in trials with fresh plant material (**Chapter VI**).

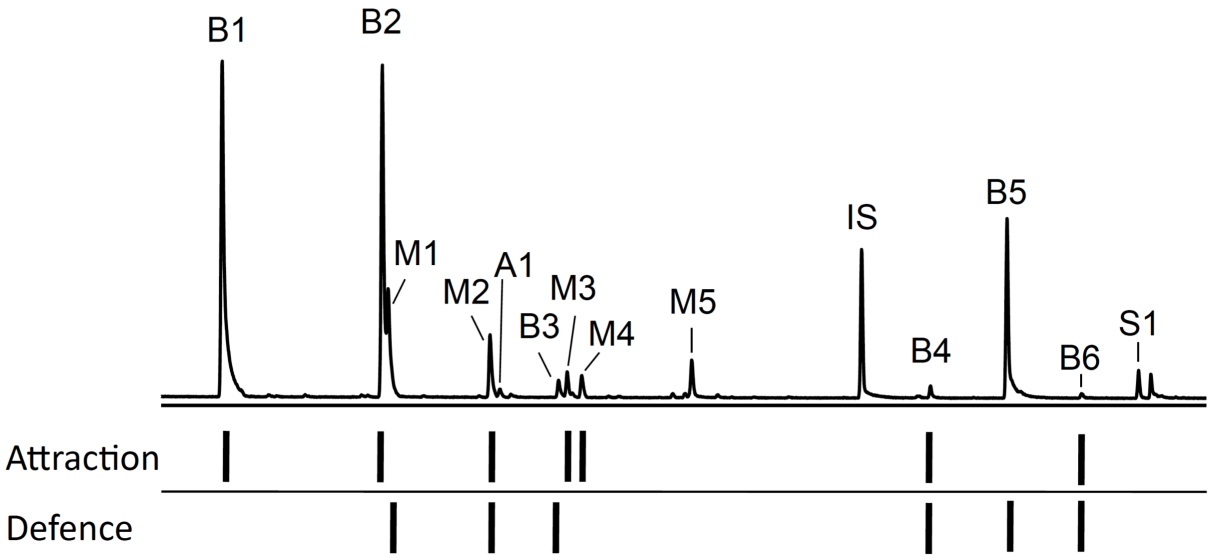
We compared the bacterial communities colonizing the leaves and flowers of two naturally growing plant species (*Saponaria officinalis*, Caryophyllaceae and *Lotus corniculatus*, Fabaceae) and investigated the growth-inhibiting effect of floral scent compounds with agar-diffusion assays using different bacterial strains. Generally, we found the same bacterial families on leaves and flowers, but their composition was different. While Pseudomonadaceae and Microbacteriaceae were the most abundant families on leaves, Enterobacteriaceae dominated floral communities. In the agar diffusion assays, bacteria from the genus *Serratia* (Enterobacteriaceae) were least affected by floral scent compounds that were emitted by the flowers colonized by these bacteria. Bacteria from other families that were mostly found on the leaves of this plant species, were much stronger inhibited in their growth by floral scent compounds than by volatiles emitted by leaves (**Chapter VII**).

These results demonstrate that volatile secondary metabolites produced by flowers have repellent, deterrent and growth inhibiting effect on various antagonistic taxa from different kingdoms. This suggests that defensive features of floral scents have strong ecological impacts: flowers that opportunistically attract all kinds of potential flower visitors would inevitably risk the plants' reproductive outcome, given the great diversity of potential flower visitors with negative net effects on the plants' fitness. However, while defending floral resources against antagonists (**Chapter V-VII**, Junker and Blüthgen 2008), floral scents synchronously attract mutualists (see above) – emphasising their important role as floral filters. The negative responses to floral scents were often a result from the presence of repellent, deterrent, toxic or growth-inhibiting individual substances within the bouquet. Similarly, the attractive function of scent bouquets can often be attributed to individual volatiles (see above). Thus, the contrasting functions may be mapped onto a floral

scent composition where individual floral scent compounds bear the one and/or the other function (Fig. 1).

Impact on communities

The multifunctionality of a scent profile caused by individual substances that affect multiple taxa (Fig. 1) suggests that flower odours shape the diverse interactions found in a flower-visitor community. In two studies, we tested whether responses to floral scents correspond to interaction patterns within complex flower-visitor networks.



Chemical class	Substance		Function	Interaction partner	Citation
Benzenoids	B1	Benzaldehyde	Attraction	bee (*Apis mellifera*)	Theis (2006) J Chem Ecol 32: 917-927
	B2	Benzene acetaldehyde	Attraction	ant (Formicidae)	Theis (2006) J Chem Ecol 32: 917-927
			Attraction	bee (*Apis mellifera*)	Theis (2006) J Chem Ecol 32: 917-927
			Attraction	moth (*Pseudoplusia includens*)	Meagher (2002) Entomol Ex et App 103: 219-226
	B3	Benzeneacetonitrile	Growth inhibition	bacteria (diverse bacteria)	Chapter 3
	B4	Eugenol	Attraction	bumblebee (*Bombus terrestris*)	Chapter 7
			Attraction	thrips (*Thrips flavus*)	Kirk (1985) J Chem Ecol 11: 35-43
			Repellence	ant (*Lasius niger*)	Chapter 7
	B5	Methyleugenol	Repellence	ant (*Linepithema humile*)	Shorey *et al.* (1996) Environ Entomol 25: 114-119
	B6	Methylisoeugenol	Attraction	fly (*Psila rosae*)	Guerin (1983) J Chem Ecol 9: 843-861
			Growth inhibition	bacteria (*Campylobacter jejuni*)	Rossi *et al.* (2007) J Agric Food Chem 55: 7332-7336
Monoterpenoids	M1	Ocimene	Repellence	moth (*Manduca sexta*)	Kessler and Baldwin (2006) Plant J 49: 840-856
	M2	Linalool	Antifeedant	bush cricket (*Metrioptera bicolor*)	Chapter 1
			Attraction	bee (*Apis mellifera*)	Theis (2006) J Chem Ecol 32: 917-927
			Repellence	ant (*Camponotus floridanus*)	Junker and Blüthgen (2008) Evol Ecol Res 10:295-308
			Repellence	ant (*Lasius fuliginosus*)	Junker and Blüthgen (2008) Evol Ecol Res 10:295-308
			Repellence	ant (*Lasius niger*)	Chapter 7
			Repellence	ant (*Monomorium floricola*)	Junker and Blüthgen (2008) Evol Ecol Res 10:295-308
			Repellence	fly (*Musca domestica*)	Lee *et al.* (2003) J Strored Prod Res 39: 77-88
	M3 + M4	Lilac aldehyde A + B	Attraction	moth (*Hadena bicruris*)	Dötterl *et al.* (2006) New Phytologist 169: 707-718
	M5	Lilac alcohol	NA		
Sesquiterpenoids	S1	Germacrene D	NA		
Aliphatics	A1	Nonanal	NA		

Fig. 1 Multifunctional floral scent bouquet of *Phlox paniculata* (Polemoniaceae). Scent mediated responses of a hypothetical flower visitor community with flowers of *P. paniculata* are shown. To most of the scent compounds, an attractive and/or defensive function was assigned either based on studies that are part of this dissertation (highlighted in grey) or from the literature. Bars underneath the peaks of the gas-chromatogram illustrate whether the compounds have attractive and/or defensive functions, which are outlined in the table below. IS = internal standard.

Hawaii represents the largest landmass worldwide that evolved in the absence of social hymenopterans, including ants. Therefore, we assumed that endemic Hawaiian plant species do not possess any traits associated to ants. Correspondingly, only a low proportion of Hawaiian plants possesses extrafloral nectaries, an ant-related plant trait (Keeler 1985). We tested the hypothesis that the ecological dominance of ants in most terrestrial habitats shaped floral traits that aim to exclude ants from flowers, and that these traits did not evolve in Hawaii where ants were missing. We observed ant-flower interactions and compared their interaction strengths with expected values that were calculated by a null-model based on the ant-species composition within the habitat and the proportional amount of sugar in form of nectar offered by each of the plant species. We found that the flowers of plant species endemic and indigenous to the Hawaiian Islands were more often and more abundantly visited by ants than flowers of introduced plant species. This pattern was the result of repellent floral scents, morphological barriers and unpalatable nectar – floral features that were more pronounced in non-native plant species (**Chapter VIII**). Thus, the status of the plants, whether they are endemic, indigenous or introduced, determined the presence or absence of floral features that prevent ants from consuming nectar. In contrast, these features were neither related to the phylogeny of involved plant species nor to the main pollinators (bird vs. insect) of the plants. Therefore, it is likely that ants are important agents in the evolution of floral morphology and scents (**Chapter VIII**).

The second study involved a flower-visitor community in Germany comprising insects from several orders and plants from different families. Flower-visitor interactions usually do not take place in isolation; they are rather part of diverse communities where multiple plant and animal species interrelate at the same time and location. Flower-visiting insects are usually not randomly distributed among the floral resources but display their species-specific preferences for, and aversions against certain plant species. Therefore, these communities show a relatively high degree of specialisation (Blüthgen *et al.* 2007). These non-random patterns may be the result of floral traits including colour, shape, quality and quantity of resources and also floral scents. The match or mismatch between the morphology of flowers and the insects' mouthparts has been analysed in detail and was shown to be an important factor shaping flower-visitor webs (Stang *et al.* 2006, 2007). We investigated the influence of floral scents on the interaction strength between flowers and their visitors. In this multi-species approach we were able to test whether floral scent bouquets act as floral filters within one system, which would require qualitatively different responses to the same scents by different insect species. We defined link temperature as the observed deviation from the expected random distribution in absence of any preferences,

aversions and constrains (**Chapter IX**). This new metric ranges from -1 to 1 and expresses negative and positive deviations from neutrality, respectively. Secondly, we tested the responses of animals directly caught from the field to floral scents of unpicked, naturally growing plants. Link temperatures strongly correlated to the responses (also ranging from -1 to 1, indicating repellence and attraction) indicating that floral scents strongly influence the microstructure of flower-visitor webs. Furthermore, this study represents the first direct evidence that volatiles function as floral filters that repel some flower visitors but attract others (**Chapter IX**).

Both of these studies dealing with the effects of repellent (and attractive) flower odours on community structure demonstrated that these signals have profound impacts on multi-species interactions and that not only mutualists are selecting forces on floral traits but also antagonists.

Mechanisms explaining the dual function of floral scents

So far, it became evident that the defensive function of floral scents is ecologically important in protecting flowers against antagonistic visitors (**Chapters V – IX**) and complements the well-known attractive function. Beyond this, **chapter IX** points out that the same scent bouquets have a dual role depending on the receiver of these signals (see also Fig. 1). This apparent paradox raises the central question of this thesis: What is the evolutionary mechanism behind the contrasting effects of volatile floral signals?

Flower visitors are traditionally assigned to either mutualistic or antagonistic agents (see above), thus emphasizing the net effect for the plant. This phytocentric point of view is helpful if the plant's reproduction is in focus and if the interaction between a specific species pair is under consideration. However, it is not possible to unequivocally attribute one of the categories to a flower visiting species in a context where several plant species are involved. Morphological (mis-) matches of insects and flowers or the insects' behaviour while collecting floral resources may determine whether the interaction is mutualistic or antagonistic. For example, bees are very efficient pollinators of many plants but are severe pollen thieves (Hargreaves *et al.* 2009) or nectar robbers (Maloof and Inouye 2000) in the interaction with other species. On the other side, ants usually are nectar thieves but few plant species rely on ants as pollinators (Gomez and Zamora 1992, de Vega *et al.* 2009). Even an undescribed orthopteran species, an insect order that usually is not associated with pollination, was recently found to be the exclusive pollinator for an orchid in Mauritius and

Reunion (Micheneau *et al.* 2010). The second disadvantage of this phytocentric categorisation is that it is unsuited to explain insects' adaptations to flower visits.

Animals visit flowers as consumers, not as pollinators, thus pollen transfer is merely a by-product of nectar- or pollen consumption (Frame 2003). The adaptations of animals that are specialized to floral resources are, for example, mouthparts to reach nectar in deep nectar tubes (Labandeira and Sepkoski 1993), visual abilities like trichromacy (Chittka 1996), but may also include the ability to tolerate repellent or toxic secondary metabolites produced by flowers and even use them as host finding signal (see **Chapter IX**). Adaptations to tolerate plant defences are commonly found and described in the context of herbivory where consumers of certain plant species, genera or families are "immune" against specific defensive substances and often use them to locate their hosts (Cornell and Hawkins 2003, Smallegange *et al.* 2007). These examples exclusively relate to highly specialised consumers that are obligately dependent on their host-plants that are well defended against generalists. Analogously, flower visitors are either obligately dependent on floral resources, e.g. bees exclusively feed on nectar and pollen, or are facultative flower visitors that otherwise live on non-floral resources. Following this line of reasoning, we hypothesized that the dependency on floral resources determines the response to floral scents, and thus provided a conceptual framework that helps to understand the dual function of floral scents (**Chapter X**). In a meta-analysis comprising studies on the responses of diverse animals to many floral scent compounds, we compared the quality of responses of obligate and facultative flower visitors. Overall, obligate flower visitors usually were attracted to floral scent compounds, while facultative flower visitors were generally repelled by them (**Chapter X**). In an experimental study, we were able to confirm these results using ants as facultative and bumblebees as obligate flowers visitors. As predicted by the meta-analysis, the former were repelled by, the latter attracted to the same synthetic floral volatiles (**Chapter XI**). Both studies suggest that terpenoids have a rather defensive function within floral scent bouquets, while benzenoids have rather attractive effects (see also Schiestl 2010).

Chapters X and XI imply that the defensive function of floral scents is the primary one, to which obligate flower visitors have adapted to. In contrast, the volatiles retained their repellent function for unadapted facultative flower visitors. Most importantly, the dichotomy of obligate and facultative flower visitors provides a feasible explanation to the question how floral scents can serve as attractants and as repellents.

Ecological consequences of lacking floral defences

The impact of all of the four major groups of antagonistic flower visitors on plant reproduction and interactions with pollinators is well documented (above). In my thesis, we complemented the existing literature by two further studies investigating the effects of flower-visiting ants on the behaviour of pollinators and on resulting fruit set. In Hawaii, where most endemic flowers are not defended against ants (**Chapter VIII**), negative effects may be particularly detrimental. In many Hawaiian habitats, ants dominate the arthropod fauna and are the most frequent flower visitors (Lach 2005, 2008b). Accordingly, native insect and bird pollinator populations declined in recent years (Benning *et al.* 2002, Magnacca 2007). Besides ants, invasive honeybees (*Apis mellifera*) that are known to pollinate many native plants in invaded areas (Dick 2001, Goulson 2003) frequently visit flowers of many endemic Hawaiian plants. In our study sites, the flowers of an endemic Hawaiian tree *Metrosideros polymorpha* (Myrtaceae) were visited by invasive ants and honeybees (*Apis mellifera*) and by endemic *Hylaeus* spp. bees. We examined the bees' pollinator effectiveness as a function of the presence of ants and the resource collected by the bees. Pollinator effectiveness was defined as the product of visitation frequency, duration of visits, the stigma contacts per visit and number of pollen deposited on the stigmas per contact. All of these observed factors were influenced by the bees' identity, the resource they collected and/or the presence or absence of ants (**Chapter XII**) resulting in a pronounced conditionality of the pollinator effectiveness. Overall, ants did not negatively affect mean net pollinator effectiveness, although they had a negative impact on specific functions such as the visitation frequency and duration of nectar collecting honeybees. Surprisingly, invasive honeybees were much more efficient pollinators than endemic *Hylaeus* bees, which was even more pronounced when ants and honeybees shared the floral resources (**Chapter XII**).

The flowers of a second endemic plants species, *Vaccinium reticulatum* (Ericaceae), were strongly parasitized by the caterpillars of an introduced plume moth (*Stenoptilodes littoralis*) leading to the abortion of the flowers and fruits. Besides the caterpillars, ants were the only flowers visitors observed, and they depleted the nectar of the flowers and thereby protected flowers against plume moths. This tri-trophic interaction represents, to our knowledge, the first documented case where invasive ants positively affect a native Hawaiian plant (**Chapter XIII**).

These results suggest that invasive ants may have neutral (**Chapter XII**) or even beneficial effects (**Chapter XIII**) on the reproduction of endemic plants in Hawaii. However,

these results need to be evaluated with caution. Albeit non-negative effects emerged in these studies, ant invasions usually have devastating consequences for the Hawaiian biota (Medeiros *et al.* 1986, Holway *et al.* 2002, Krushelnycky *et al.* 2005) and these studies should not be used to absolve ants from being one of the most serious threats for the native ecosystems.

Conclusions and future directions

The main conclusion of this thesis is that defence is an important function of floral scents; which, in combination with the attractive function, renders these volatile signals as potent floral filters. We were able to attribute defensive properties to common floral scent compounds that were so far exclusively viewed as attractants. These results along with the high diversity of potential floral antagonists suggest that organisms damaging flowers and reducing the plants' reproductive success are selective agents that shape floral traits to a similar degree as mutualistic interaction partners.

For a more complete understanding of the ecological role of floral scent bouquets and their multifunctionality, future studies should address the following issues and avoid shortcomings of the present thesis:

- As indicated in Fig. 1, the functions of individual components of complex floral scent compositions may be examined with focus on one (or few) plant species but several potential mutualistic, commensalistic and antagonistic interaction-partners. Experimental approaches with natural scent bouquets and synthetic substances may help to get insights into the evolutionary processes that shaped the complexity of floral scents.
- Some physiological effects of terpenoids that are potentially lethal to insects are summarised by Gershenzon and Dudareva (2007). However, so far, the (neuro-) physiologic mechanisms behind repellence and toxicity by floral scents are not fully understood. Furthermore, pilot studies demonstrated that obligate flower visitors are able to tolerate floral scents that are toxic to facultatively flower-visiting animals (results not shown). Like the lethal effects, the physiological processes that enable obligate flower visitors to tolerate these toxic substances remain unknown.
- In this thesis, we usually classified flower visitors either as mutualistic or antagonistic, as obligately or facultatively dependent on floral resources and their responses as either positive (attraction) or negative (repellence). This clear-cut categorisation is

useful in highlighting the full range of the flower visitors' effects on the plants' reproduction, their "specialisation" to floral resources or their responses towards scents; but it underestimates the flexibility of the involved species and the conditionality of the interactions' outcome and the animals' responses. For example, (a) animals may be attracted to floral scents either innately or after associative learning (**Chapter IX**), likewise repellence may be caused by the toxicity or other negative effects of certain substances, or may also result from the experience of absent rewards. (b) The rule of thumb that obligate flower visitors are attracted and facultative visitors are repelled by floral scents does not apply to all interactions (**Chapter X**) and exceptions should be linked to ecological outcomes and selective (dis-) advantages. For instance, ants are pollinator of some pant species that obviously do not repel these visitors. (c) The pollinator effectiveness is highly conditional and may depend on several factors including the presence of other flower visitors (**Chapter XII**) and other plant species. Thus, the net effect for both partners of a relationship may be highly variable over space and time. These complex interactions between different flower visitor species and their effectiveness in pollination should be addressed in future studies.

- Pollinators display a complex behaviour during individual flower visits, which may be modulated by floral scents. For example, *Osmia* bees are able to detect nectar-rewarding flowers within inflorescences by their scent (Howell and Alarcon 2007). Furthermore, the concentration of repellent floral scent compounds may regulate the visitation frequency and duration of floral visits of individual visitors (c.f. Terry *et al.* 2007). Scent augmentation or reduction experiments may be suited to explore the role of scents in fine-tuning pollinator behaviour.
- Scents emitted by flowers are variable over the life span of individual flowers (Schiestl *et al.* 1997, Irwin and Dorsett 2002) and may also change as response to biotic influences. Herbivory (Theis *et al.* 2009) and florivory (Zangerl and Berenbaum 2009) both induce a quantitative change in floral scents. In both studies, the emission of terpenoids increased after damage of leaves or flowers, which implies that flowers respond with the emission of defensive compounds to these intruders and thus are better protected against upcoming antagonists. The induction of defences is well documented in the context of herbivory but the ecological importance in flower defence is still in its infancy.
- Our study on the epiphytic bacteria colonizing petals is pioneering the research on these kinds of interactions; some potential effects are outlined in the general

introduction. The alteration of floral signals by bacteria and the resulting consequences for pollinator behaviour and plants' reproduction may be a promising field for future studies.

V. Floral scents deter facultative florivores

This chapter has been published as:

Junker RR, Heidinger IMM and Nico Blüthgen (2010) Floral scent terpenoids deter the facultative florivore *Metrioptera bicolor* (Ensifera, Tettigoniidae, Decticinae). ***Journal of Orthoptera Research*** 19: 99-104

Summary

Non-pollinating florivores (animals feeding on floral resources) represent severe antagonists that have negative impacts on the plants' reproduction. Plants would thus benefit from excluding them from their flowers. In this study, we tested whether floral scent compounds that are attractive to many pollinators also have the potential to prevent facultatively flower-feeding herbivores from consuming flowers. For feeding trials, we chose the bush cricket *Metrioptera bicolor* that mainly consumes grasses but occasionally also feeds on flowers. Linalool and β-caryophyllene (mono- and sesquiterpenoid, respectively) turned out to be effective antifeedants, while other floral scent compounds had no effect. Furthermore, bush crickets completely rejected flowers of *Convolvulus arvensis* (Convolvulaceae) and *Melilotus alba* (Fabaceae), but preferred flowers of *Echium vulgare* (Boraginaceae) over leaves. Additionally to feeding experiments, excrements of bush crickets and other orthopterans were searched for pollen. Most individual bush crickets had pollen in their faeces, largely from Poaceae and Gymnosperms, suggesting an accidentally ingestion of wind-dispersed pollen rather than targeted consumption of floral pollen. Our results support the hypothesis of a dual role of floral scents in attraction and defence.

Introduction

Recently, it was demonstrated that an undescribed orthopteran species of the family Gryllacrididae functions as an effective and probably exclusive pollinator of the orchid *Angraecum cadetii* on the islands of Mauritius and Reunion (Micheneau *et al.* 2010). This tight mutualism represents a specialised system probably unique to this interaction. Usually, orthopterans do not contribute to pollination in more generalized systems although they are frequent flower visitors to some plants but rather have negative effects on these (Schuster 1974, Ingrisch 1976, Ingrisch and Köhler 1998). The consumption of flowers by herbivores

can have detrimental effects on the plants' reproduction (McCall and Irwin 2006) either by the destruction of anthers and stigmas (Kerner 1879) or by reducing the attractivity of flowers by feeding on petals (Krupnick and Weis 1999, Krupnick *et al.* 1999).

It was hypothesized that the reproductive fitness of plants increases if floral traits are both attractive for mutualists and simultaneously defensive against antagonists (Brown 2002, Irwin *et al.* 2004, Junker and Blüthgen 2010b). Accordingly, floral resources are often toxic, unpalatable or unreachable for exploiters that would otherwise consume nectar, pollen or petals without transferring pollen from one plant individual to the other (Dobson and Bergström 2000, Galen and Cuba 2001, Johnson *et al.* 2008). Different mechanisms involving floral scents that have the potential to exclude certain taxa from florivory have been proposed. (1) Euler and Baldwin (1996) and Kessler *et al.* (2008) demonstrated that certain floral secondary metabolites are produced at different locations in *Nicotiana attenuata* (Solanaceae) that interact with different types of flower visitors: the defensive nicotine at a basal part and the attractive benzyl acetone at the outer corolla. Thus, different info-chemicals affect flying and crawling flower visitors. (2) It has been proposed that diurnal scent emission rhythms correspond to activity patterns of mutualists but not antagonists (Theis *et al.* 2007). The emission of floral scents that would attract both pollinators and antagonists may be reduced at times when the latter are most active (Euler and Baldwin 1996, Theis *et al.* 2007). (3) The same floral scent compounds may serve both functions together: attract pollinators and repel antagonists. For instance, linalool attracts bees (Harrewijn *et al.* 1995) and butterflies (Andersson *et al.* 2002), but also efficiently repels ants from stealing nectar (Junker and Blüthgen 2008), suggesting a dual function of this floral scent compound (Junker *et al.* 2010). Junker and Blüthgen (2010b) proposed that the dependency on floral resources determines whether an animal is attracted or repelled by floral scents. Obligate flower visitors that depend on floral resources usually are attracted to floral scents, while facultative flower visitors that have a broad dietary spectrum are often repelled or deterred by secondary metabolites produced by flowers and thus may predominantly feed on non-floral resources (Junker and Blüthgen 2010b, a). In this study, we tested whether floral scent compounds that are attractive to common pollinators have the potential to prevent an orthopteran herbivore from consuming petals, pollen or nectar. We choose *Metrioptera bicolor* (Ensifera, Tettigoniidae, Decticinae) that feeds on grass, various herbaceous plants and small insects but also occasionally on the flowers of some species (Ingrisch and Köhler 1998). Despite this highly generalistic diet, *M. bicolor* does not fully develop without grass as principal food (Ingrisch 1976) and thus represents a facultative consumer of floral resources. This bush cricket was furthermore

allowed to choose between leaves and flowers of three plant species to reveal potential preferences. In addition, by searching for pollen in excrements of different species of bush crickets and grasshoppers including *M. bicolor*, we quantified the natural utilisation of floral resources by orthoptera.

Materials and Methods

Organisms

For laboratory trials, we used the two-coloured bush cricket *Metrioptera bicolor* (Philippi 1830) a medium-sized (body length: 15 - 18 mm), thermo- and xerophilic bush cricket (Harz 1969, Bellmann 2006). *M. bicolor* mainly inhabits semiarid grassland but can also be found on juniper heath or poor and sandy grasslands (Detzel 1998). Individuals of *M. bicolor* were sampled at four sites in semi-arid grasslands in Northern Bavaria, Germany (nature reserve "Hohe Wann", and in Würzburg) and kept separately in gauze cages (20x20x29 cm). All bush crickets were kept in a climate chamber under long day conditions (day / night: 14 h / 10 h, 26 °C / 19 °C) with a constant humidity at 50%. Water was sprayed two times daily at one side of the cages to provide drinkable water for the animals.

Dual Choice Tests

We explored the responses of *M. bicolor* to five different floral scent compounds. Selected substances represent widespread and dominant floral scent compounds from a broad spectrum of plant species, e.g. linalool occurs in 70 % of all plant species sampled so far (Knudsen *et al.* 2006). Each component was offered on an artificial food as substrate (wafers: Hoch Oblaten-Bäckerei, Miltenberg, Germany, ø = 44 mm; ingredients: wheat flour, starch) and compared against an untreated wafer in a dual choice test. For this purpose, substances were diluted in acetone (p.a.) and 666 µl of the solution were applied on a wafer (~330 mg) for the treatment, pure acetone was applied for the control. After acetone entirely evaporated, a section of the treatment and control wafer (1/4 of the circumference) was placed in the cages in an upright position. Substances were applied in different concentrations ranging from 1 to 100 mMol kg^{-1} wafer. For linalool, for example, this means that 1285.4 to 12.9 ng were offered per quarter of a wafer, which is within the range of mean hourly production of this substance by individual flowers (see e.g. Andersson *et al.* 2002). Additionally to the floral scent compounds we also tested the effect of a flavonoid (Quercetin dihydrate), a floral pigment, on the feeding behaviour of the bush crickets.

In a second test series, three insect pollinated plant species were used that occur in the same habitat as the bush crickets: *Convolvulus arvensis* L. (Convolvulaceae), *Echium vulgare* L. (Boraginaceae) and *Melilotus alba* Desr. (Fabaceae). All of these species are visited and pollinated by several insect taxa (Waddington 1976, Rademaker *et al.* 1999; unpublished observations). Approximately equal amounts of leaves and flowers were offered to *M. bicolor* in a dual choice test in order to compare the bush crickets' consumption of vegetative and reproductive plant parts of the plant species. Each individual was fed with leaves and flowers from the same plant individual. Leaves and flowers were provided in an upright position in wet foam blocks to maintain their moisture during the trial.

In a third experiment, extracts of flowers and leaves provided on wafers were also offered in dual choice tests. Extracts were prepared and applied in the following way: oven-dried plant material (60°C for at least 3 days) was ground in a mortar into a fine powder. This powder was extracted with hexane and subsequently with acetone for 24 h each (2 ml solvent 100 mg^{-1} powder) and shaken several times. Supernatants were stored in a freezer (-18°C) until use. Hexane and acetone fractions were concentrated to 8 ml using an air stream and were both applied subsequently to the same wafer. The amount of extract applied to each wafer was chosen to represent the natural concentration of substances in leaves and flowers, respectively (i.e. the mass of plant material extracted corresponded to the wafer mass offered). After both solvents entirely evaporated, a section of each wafer (1/8 of the circumference) was placed in the cages in an upright position.

Each experiment lasted for 24 hrs. Most individuals were used in several consecutive trials, but not repeatedly for the same treatment. Between subsequent trials, bush crickets were fed with grass seeds, fish food and fresh plant material for at least 24 h. Before and after each trial, plant material or wafer pieces were scanned digitally to acquire respective areas (pixels) and consumed area was obtained by subtraction from the original area. Dry mass consumption was calculated from consumed area using specific dry weight (mg $pixel^{-1}$) for which 10 - 20 leaves, flowers and wafers were oven-dried at 60°C for at least 3 days and weighted. Individuals that did not feed at all in an experiment were excluded from the statistical analysis of a choice test.

Statistical Analysis of Dual Choice Tests

Proportions of the consumed biomass of the treated wafers or flower of the total consumption (treatment + control wafer or flower + leaf) were calculated for each replicate. Therefore, values larger than 0.5 indicate a preference for flowers or treatment, and values below 0.5 a preference for leaves or control. We used generalized linear models (GLM with binomial error distribution) with these proportions as response variable. For the trials with chemical compounds, we used substance, concentration of substance (mMol) and sex of bush crickets as explanatory variables. In the second analysis, plant species, treatment (fresh plant material or wafers treated with extracts) and sex of bush crickets were chosen as explanatory variables.

Beginning with the full model containing all explanatory variables, the models were reduced stepwise and each reduced model was compared with the previous one with a chi^2-test (Crawley 2005). Additionally, proportions of flower or treatment consumption were individually tested against the null hypothesis (assuming equal consumption of flower and leaves / treatment and control, i.e. proportion = 0.5) with a Wilcoxon test. Significant values were corrected for multiple tests by false discovery rate (FDR, Benjamini and Hochberg 1995). All statistical analyses were performed using R 2.4.0 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

Excrement Analysis

In order to quantify the importance of floral resources for othopterans in their natural habitat, excrements of 40 *M. bicolor* individuals and 16 other orthopterans from 5 species (*Chorthippus biguttulus* L., 6 samples; *Chorthippus dorsatus* Zetterstedt, 5; *Conocephalus discolor* Thunberg, 1; *Gomphocerippus rufus* L., 1; *Phaneroptera falcata* Poda, 3) were scanned for pollen. Animals were caught on a flower-rich fallow field in Würzburg (*M. bicolor* in May / June 2008; the others in July 2007), and placed individually in containers. After two days, excrements were collected and stored in a freezer (-18 °C) until inspection for pollen. Two faecal pellets per orthopteran were pooled and solved in 100 µl water using ultrasound. One aliquot of this solution was placed on an object slide and amount of pollen was estimated using a light optical microscope.

Results

Dual Choice Tests.

Linalool as well as *β*-caryophyllene significantly deterred the bush crickets from feeding the wafers, while all other substances tested (*α*-pinene, 1,8-cineol, hexanol and quercetin dihydrate) did not evoke any significant deterrence (Fig. 1a). The different substances affected the bush crickets' food choice, while the other factors, including the concentration of substances (mMol), had no effect on feeding decision (Table 1a).

Tab. 1 Results from generalized linear model (GLM with binomial error distribution) analysis on data of the proportional consumption of wafers treated with individual substances (a) and on consumption of flowers or wafers treated with extracts (b) by Metrioptera bicolor. Starting with the full model containing all explanatory parameters, each reduced model was compared with the previous one with a Chi2 test resulting in deviance, degree of freedom (df) and significance (p) for each parameter.

a) Parameter	*Deviance*	*df*	*p*
Substance × mMol × Sex	16.89	11	0.11
Sex	0.34	1	0.56
mMol × Substance	4.23	5	0.52
mMol	1.64	1	0.2
Substance	13.91	5	**0.02**
Residual error	124.95		
Total	161.95		

b) Parameter	*Deviance*	*df*	*p*
Plant × Treatment × Sex	7.39	5	0.193
Sex	0.02	1	0.876
Plant × Treatment	12.45	2	**0.002**
Treatment	0.51	1	0.477
Plant	44.65	2	**<0.001**
Residual error	25.84		
Total	90.85		

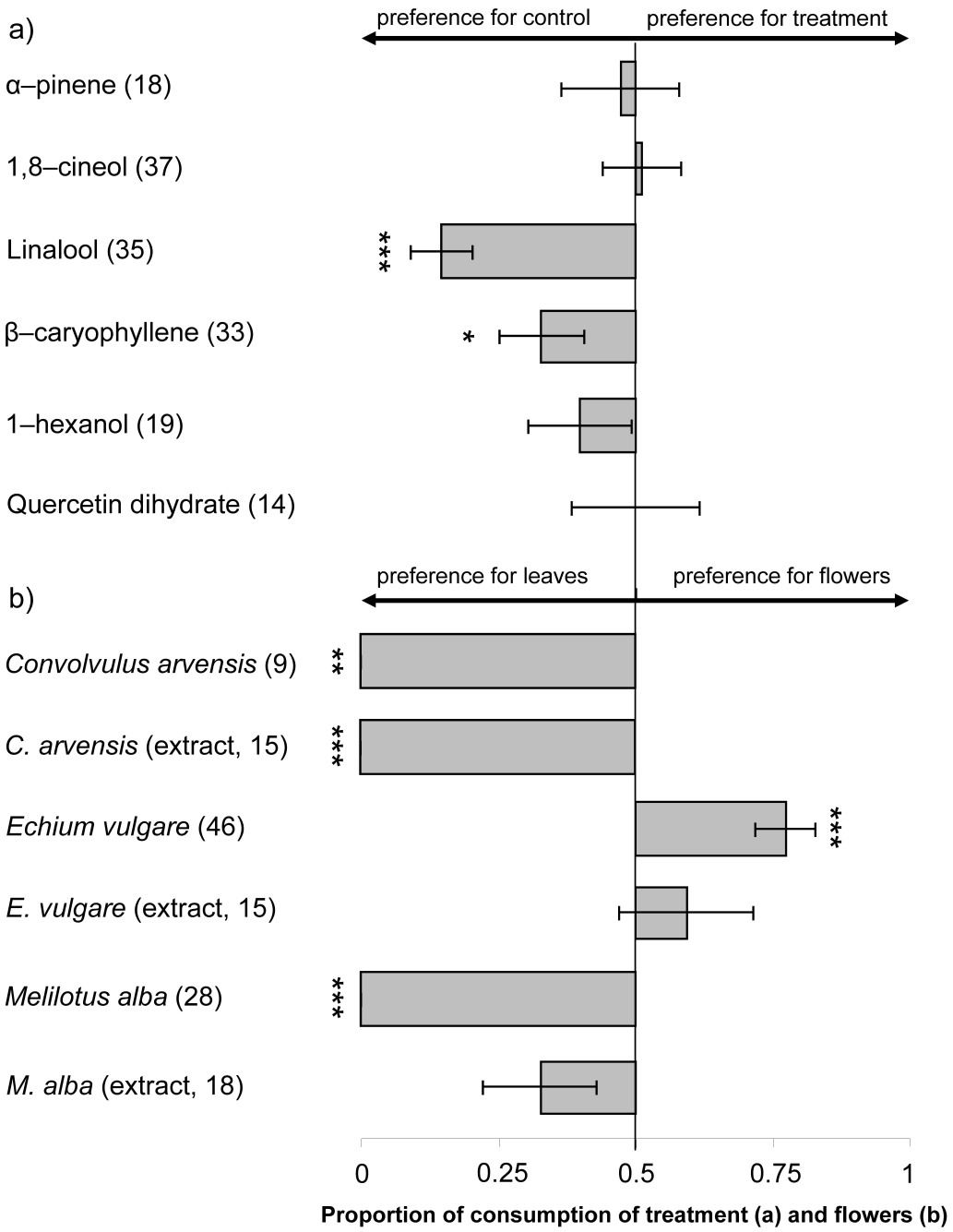


Fig. 1 Feeding preferences of bush crickets in dual choice tests. Preferences were measured as the proportion of dry mass consumption of flowers of the total consumption (flowers plus leaves), or wafers treated with flower extracts and compounds, respectively. In the upper part (a), effects of single floral scent compounds on consumption by *M. bicolor* are shown. Floral scent compounds and one flavonoid (quercetin dehydrate) were tested against untreated wafers. Below (b), preferences between fresh flowers and leaves and flower extracts versus leaf extracts are shown. Significant deviation from an equal consumption of flowers versus leaves, or treatment versus control indicated by asterisks according to paired Wilcoxon rank sum test (proportion was tested against 0.5, FDR corrected) are given (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Overall, in a high proportion (43.1 %) of all trials, bush crickets completely rejected to feed on the food items offered. In trials were wafers were offered, rejection rate did not significantly vary between the different substance treatments (mean ± *SE* = 29.2 ± 5.1 %, Chi^2 = 3.3, p = 0.65) and extracts (mean ± *SE* = 67.7 ± 3.4 %, Chi^2 = 0.5, p = 0.77). In the trials were fresh leaves and flowers were offered, the rejection was highest in trials with *Convolvulus arvensis* (88.75%) followed by *Mellilotus alba* (50%) and *Echium vulgare* (0 %; $Chi^2$2 = 27.0, $p < 0.001$). However, bush crickets that fed on food items showed a strong and highly significant preference for leaves over flowers in *C. arvensis* and *M. alba*, while they significantly preferred flowers over leaves in *E. vulgare* (Fig. 1b). In trials with extracts of *C. arvensis* the preference for leaves was confirmed. Wafers treated with flower extracts from *E.vulgare* and *M. alba* were not significantly more or less consumed than wafers treated with leaf extracts (Fig. 1b). Plant species and the interaction term plant · treatment had a significant influence on the feeding behaviour, while sex, treatment and the other interaction term did not influence the choices of the bush crickets (Table 1b). Sample sizes and mean consumption [mg dry weight] in all trials are shown in Table 2.

Excrement Analysis

Pollen was found in 85 % of all faecal samples from *M. bicolor* in various amounts. More than the half (58 %) of the samples contained pollen from Poaceae, 28 % from gymnosperms, 20 % angiosperms (including Asteraceae, *Galium* sp., *Knautia* sp. and *Plantago lanceolata*; percentages add to more than 100% because some samples contained two different types of pollen). Half of the faecal samples from the other orthopterans (eight out of 16 individuals) did not contain pollen (three of six *Chorthippus biguttulus* specimen and all five *Chorthippus dorsatus*). Samples from four individuals (three *C. biguttulus* and one *Gomphocerippus rufus*) contained low amounts of pollen, suggesting that they accidentally ingested pollen rather than specifically feed on flowers. Only four samples (all three *Phaneroptera falcata* and one *Conocephalus discolor*) contained numerous pollen. Two individuals of *P. falcata* were caught on flowers (*Daucus carota*, Apiaceae and *Picris hieracioides*, Asteraceae) while consuming pollen or other flower tissues. Excrements of two *P. falcata* contained pollen from Asteraceae, the other individual contained a mixture of Asteraceae and Apiaceae pollen. Faeces of *C. discolor* contained pollen from Apiaceae.

Tab. 2 Mean consumption [mg dry weight] and standard deviation of (a) wafers treated with six substances[1] and untreated wafers or (b) flowers and leaves of three plant species[2]. Concentrations [mMol kg^{-1}] of applied substances are given in brackets. Note that concentrations did not influence the decision made by the bush crickets (Tab. 1b). n = sample size of each trial.

b) Substance	n	treatment	control
α – pinene (1, 20, 50)	18	4.0 ± 7.0	4.2 ± 5.2
1,8 – cineol (1, 20, 50)	37	5.1 ± 5.9	4.1 ± 4.7
Linalool (1, 10, 50, 100)	35	1.3 ± 4.3	6.2 ± 5.0
β – caryophyllene 1, 5, 10, 20, 50)	33	2.8 ± 4.6	6.2 ± 5.4
1 – hexanol (1, 10, 20)	19	5.7 ± 6.5	7.7 ± 5.8
Quercetin dihydrate (1, 20, 50)	14	4.0 ± 4.5	5.4 ± 6.3

a) Plant species	n	flowers	leaves
Convolvulus arvensis	9	0 ± 0	2.0 ± 2.0
Convolvulus arvensis (extract)	15	0 ± 0	1.3 ± 0.9
Echium vulgare	24	3.9 ± 2.3	2.3 ± 5.1
Echium vulgare (extract)	7	5.8 ± 6.1	4.3 ± 4.8
Melilotus alba	12	0 ± 0	3.8 ± 1.7
Melilotus alba (extract)	9	7.2 ± 7.8	5.1 ± 5.1

[1] Substances belong to four chemical classes: Monoterpenoids (α – pinene, 1,8 – cineol and linalool), sesquiterpenoid (β – caryophyllene), aliphate (1-hexanol), flavonoid (quercetin dehydrate).
[2] Plant species belong to three plant families: Convolvulaceae (*C. arvensis*), Boraginaceae (*E. vulgare*), Fabaceae (*M. alba*).

Discussion

Most orthopterans mainly feed on grasses, herbaceous plants and insects and occasionally on flowers (Schuster 1974, Ingrisch and Köhler 1998). Nymphs and adults of *Phaneroptera falcata* can be occasionally observed feeding on some Asteraceae, Apiaceae and Ranunculaceae flowers (own observation). Florivory by the orthopterans seems to be restricted to a narrow taxonomic range as suggested by the pollen analysis in faecal pellets, the feeding trials of this study and also by data from the literature (Schuster 1974, Ingrisch and Köhler 1998). Although faecal samples of *Metrioptera bicolor* often contained large amounts of pollen from grasses and gymnosperms, this species cannot be regarded as palynivorous. Pollen from such wind-pollinated plants is often scattered on leaf surfaces and is unlikely to be harvested directly from flowers. These kinds of pollen may have been either accidentally ingested while consuming leaves or perhaps grazed from the leaf surface, also suggested by the fact that no gymnosperms were present close by the field site the orthopterans were caught. Pollen from insect-pollinated angiosperms was rare in *M. bicolor* faeces, but more frequent in some other orthopterans.

The taxonomically restricted occurrence of florivory suggests that flowers of other taxa are either less palatable to orthopterans or defended against them. Floral adaptations as protection against non-pollinating florivores have been proposed by some authors (e.g. Dobson and Bergström 2000, Frame 2003), but examined in a few case studies only. It has been suggested that the function of floral scents is not restricted to pollinator attraction (Raguso *et al.* 2003, Raguso 2008b, Junker and Blüthgen 2010b), but also includes herbivore repellence (Pellmyr *et al.* 1991, De Moraes *et al.* 2001) which may have been the primary function during the early diversification of angiosperms in the Cretaceous (Frame 2003). In feeding trials with wafers treated with floral scent compounds we tested the hypothesis that pollinator attracting substances have also deterrent / repellent effects on *M. bicolor* that may be detrimental for the plants when feeding on flowers with no pollination service (Kerner 1879, Schuster 1974). We chose floral scent compounds that are produced by a large number of flowering species (Knudsen *et al.* 2006) and/or are assumed to attract pollinators. For example, *α*-pinene attracts moths (Cunningham *et al.* 2004), 1,8-cineol moths and euglossine bees (Raguso and Light 1998, Schiestl and Roubik 2003), linalool bees and butterflies (Laloi *et al.* 2000, Andersson *et al.* 2002), *β*-caryophyllene butterflies (Andersson *et al.* 2002) and 1-hexanol which is also an green leaf volatile attracts herbivores (Reinecke *et al.* 2002). Terpenoids are often the dominant chemical class in floral scent compositions (Knudsen *et al.* 2006) and are known to have toxic, deterrent and antimicrobial functions in plant defences (Gershenzon and Dudareva 2007). Our results demonstrate that monoterpenoids (linalool) as well as sesquiterpenoids (*β*-caryophyllene) may serve as an antifeedant against herbivorous insects. Two other monoterpenoids (*α*-pinene and 1,8-cineol) and one aliphate (1-hexanol) did not affect the consumption of the bush crickets. However, this outcome may be explained by the different volatility of floral scent compounds used for the tests. Since dual choice tests ran for 24 hours, substances with a relatively high boiling point (linalool and β-caryophyllene) were more likely to retain their effective dose during the whole period of time. On the other hand, the applied quantity of substances did not affect the choice of *M. bicolor*, suggesting that even very low amounts (i.e. 1 mMol kg^{-1}) of certain floral scent compounds have deterrent / repellent properties. Thus, floral resources may be unpalatable for orthopterans due to the emission of floral scents that are either adaptation to attract pollinators or as defence against antagonists. In contrast, the Gryllacrididae species that pollinates the orchid *Angraecum cadetii* feeds on the floral nectar but does not destroy reproductive plant parts. Sequentially, the pollinia of the orchid are attached on the mouthparts of the cricket and the animals reliably transport the pollen to conspecific orchids indicated by the high percentage of fruit set (Micheneau *et al.*

2010). The authors of this study also report that these orchids emit a monoterpene-dominated bouquet but in low quantities only (Micheneau *et al.* 2010).

Few studies compared the palatability of flowers and leaves for phytophagous animals. For instance, it was shown that *Pieris brassicae* (Lepidoptera) caterpillars, representing specialised consumers of Brassicaceae, preferred flowers of *Brassica nigra* over leaves of the same species (Smallegange *et al.* 2007). On flowers, these caterpillars achieved a higher growth rate although flowers contained more defensive glucosinolates than leaves (Smallegange *et al.* 2007). However, results from such a highly specialised herbivore may not reflect the outcome in more generalised systems such as the one in our study. We focused on bush crickets (*M. bicolor*) with a generalized diet, which is known to occasionally feed on flowers besides vegetative plant parts (Ingrisch and Köhler 1998). This species represents a potential, i.e. unspecialised and facultative, consumer of floral tissues. The trials in which herbivores had the choice between flowers and leaves showed conflicting results: *M. bicolor* completely rejected flowers (fresh material) of *Convolvulus arvensis* and *Melilotus alba*. Extracts of flowers used in this bioassay are likely to contain both floral volatiles and other substances solved from floral tissues. The chemical defence of flowers may thus include various kinds of deterrents, and some highly volatile components may have been missing from the extracts due to the oven-dried plant material. This may also explain why *M. alba* had repellent flowers, but the extract had no effect on the feeding choice. Flowers of *Echium vulgare* (largely scentless, S. Dötterl, unpublished data) were preferred over leaves of the same plant species. In this case, mechanical defences may have played a major role in the decision of the bush crickets between leaves and flowers. Leaves of *E. vulgare* possess rigid trichomes, which are known as protective structures against herbivores in general (Valverde *et al.* 2001). The density of such trichomes was much higher on leaves than on flowers (Fig. 2). The other plant species used in this study do not feature any potentially defensive structures like trichomes.

Overall, our results add evidence to hypothesis that secondary floral metabolites serve as defensive traits against herbivorous animals that would otherwise have negative impacts on the plants' reproduction and not only as attractive signals to pollinators.

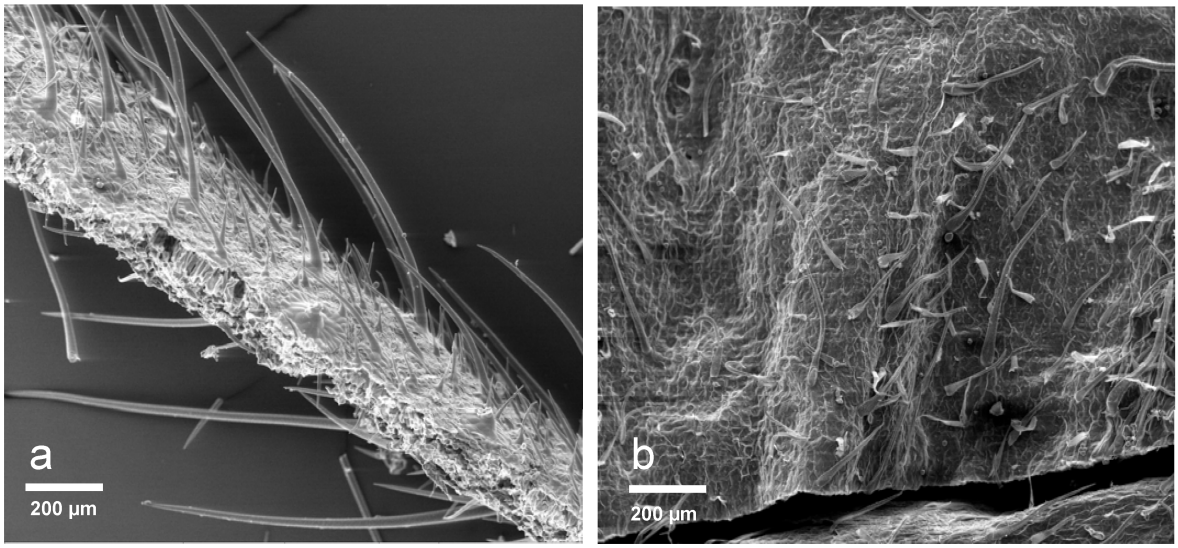


Fig. 2 Scanning electron microscope photographes of leaves (a) and petals (b) of E. vulgare. Leaves and flowers where oven dried (30°C, 2 d) prior to preparation for SEM.

VI. Contrasting responses to floral scents by flower and leaf dwelling spiders

This chapter has been submitted for publication as:

Junker RR, Bretscher S, Dötterl S and Blüthgen N (submitted) Phytochemical cues affect hunting-site choices of a nursery web spider (*Pisaura mirabilis*) but not of a crab spider (*Misumena vatia*).

Summary

Predacious arthropods such as spiders benefit from choosing hunting-sites to which they are best adapted and where their hunting success is greatest. We investigated the responses of two spiders to phytochemical cues that they could experience while hunting on leaves or flowers, and how this could influence their decisions where to forage. We compared the behaviour of *Pisaura mirabilis* that commonly lures for prey in the vegetation and *Misumena vatia* that predominantly hunts on flowers as sit-and-wait predator. In choice tests, *P. mirabilis* frequently preferred leaves and leaf extracts over flowers and floral extracts and avoided substrates treated with the sesquiterpenes β-caryophyllene and nerolidol in natural concentrations. In contrast, *M. vatia* did not show any preferences for any of the substrates offered. The lack of a positive stimulus contrasts with earlier studies on crab spiders that use phytochemical cues as guide to rewarding flowers. The deterrent effect of floral metabolites on *P. mirabilis* may suggest that these substances serve as defensive traits against such generalised predators that potentially decrease the visitation and pollination success of a plant.

Introduction

Optimal foraging theory predicts that animals are behaviourally, morphologically and physiologically adapted to maximize their net rate of energy intake (Schoener 1971, Cowie 1977). A behavioural adaptation of predacious animals is to choose foraging patches that are frequently visited by prey or to which the animals are best adapted (Krebs *et al.* 1974, Shafir and Roughgarden 1998). Some crab spiders, for example, show adaptations as sit-and-wait predators on flowers: they are able to change colour for camouflage and enhance the attractiveness of flowers for pollinators due to their ultraviolet contrast against

petals (Heiling *et al.* 2003). The high specialisation on flowers by crab spiders is associated with a relatively narrow prey-spectrum which is limited to common flower visitor taxa (Nentwig 1986). Other non-webbuilding spiders hunt or ambush predominantly in the vegetation and thus utilize a broader spectrum of prey taxa (Nentwig 1986). To benefit from their adaptations to different plant structures (vegetative versus reproductive), spiders need to perceive and thus recognize those structures. For crab spiders (*Thomisus spectabilis*) it was shown that they use visual and olfactory flower cues for patch choice (Heiling *et al.* 2004).

We experimentally compared the substrate choices of two non-webbuilding spiders based on phytochemical cues: the crab spider *Misumena vatia* (Thomisidae) which typically lures on flowers to catch flower visitors, and the nursery web spider *Pisaura mirabilis* (Pisauridae) which hunts in the vegetation. In concordance with their lifestyle, we expected that *M. vatia* is attracted to flower cues, while *P. mirabils* may prefer leaves.

Material and Methods

On several days between June and August 2008, *M. vatia* and *P. mirabilis* were caught on fallow lands in Würzburg, Germany. Fifty-eight individuals of *M. vatia* were collected on flowers of *Achillea millefolium*, *Aegopodium podagraria*, *Leucanthemum vulgare*, *Saponaria officinalis*, *Solidago canadensis*, *Trifolium pratense*, *Tripleurospermum maritimum*, while nearly all individuals of *P. mirabilis* (40 of 41) were collected from the vegetation (one individual was collected from an *Achillea millefolium* flower). Spiders were individually kept in small plastic containers and were fed with flies twice a week; water was continuously provided. Plants used for the laboratory experiments were picked in the same area.

In pair-wise choice tests, spiders were allowed to choose between different substrates including flowers vs. leaves, filter papers with extracts of flowers vs. extracts of leaves, and scented vs. unscented filter papers. The principal setup of these tests was the same: individual spiders were placed on pieces of cork representing "islands" (*ca* 30 cm^2) in water filled bowls preventing spiders from escaping. On each of these islands, two wooden sticks (height = 140 mm, Ø = 3 mm) were attached in an upright position. The different substrates used in the tests were attached to the tip of these sticks. The distance between the substrates (*ca* 1 cm) was chosen close enough that the spiders could freely change between the substrates without descending to the islands but large enough that spiders were enforced to make a choice. Neon lamps from above illuminated the whole setup. After spiders were placed on the islands, they were observed for 1 h, recording their position on

either substrate every 3 min. Individual spiders were used for several tests but not repeatedly for the same treatment.

Freshly picked flowers and leaves from *Achillea millefolium*, *Centaurea cyanus*, *Tanacetum vulgare* (all Asteraceae), *Medicago sativa* (Fabaceae) and *Saponaria officinalis* (Caryophyllaceae) were placed in small water filled vases. The vases were 1.5 ml standard microcaps that were attached on top of the wooden sticks. In each pair wise test, the number of leaves and flowers or inflorescences was adjusted so that both substrates represented approximately the same area, providing sufficient space for spiders to sit on.

The same five plant species were used to prepare leaf and flower extracts. Freshly hackled plant material was placed into an extraction thimble and was continuously extracted with 50 ml *n*-hexane in a Soxhlet apparatus for three hours at a temperature of 85°C. Solvent was removed under vacuum and the extract was resolved in acetone. Volume of acetone was determined as 0.75 · g dry weight · 200µl acetone. Aliquots of the extract (200µl) were applied on round filter papers (Ø = 35 mm) that were attached on top of the wooden sticks. Thus the extract was applied to filter papers with a mass of 75% of the plant dry weight to account for losses of the extract during the process. Flower and leaf extracts of each plant species were again tested pair wise. Extracts were analysed using a Varian 3800 gas chromatography fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane; length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 µm; Phenomenex) and a Varian Saturn 2000 mass spectrometer. 1 µl of the samples was placed into a quartz vial in the injector port of the GC by means of the ChromatoProbe kit (Amirav and Dagan 1997). The injector split vent was opened, and the injector was heated at 40°C to flush any air from the system. After 2 min, the split vent was closed and the injector heated at 200°C min^{-1}, then held at 260°C until the end of the run. The split vent was opened after 4.5 min again. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.0 ml min^{-1}). The GC oven temperature was held for 4.5 min at 40°C, then increased by 6°C min^{-1} to 300°C, and held for 15 min at this temperature. Mass spectra were taken at 70 eV with a scanning speed of one scan per second from m/z 30 to 650. Analysis of the data was performed as described elsewhere (Dötterl *et al.* 2009), and an internal standard (3-chloro-4-methoxytoluene) was used for quantification.

Since we expected that the phytochemical cues to which spiders respond are not specific to certain plant species, we used these analyses to determine those compounds in the extracts that frequently occur in flower and leaf scents, and these compounds were subsequently used in biotests. Among the compounds identified in the samples, we selected benzaldehyde (benzenoid), 1-hexanol, *cis*-3-Hexen-1-ol, *cis*-3-hexen-1-yl acetate (all

aliphatics), limonene, linalool (monoterpenoids), β-caryophyllene and nerolidol (mixture of cis- and trans-isomers, sesquiterpenoids), because these compounds represent more common and widespread floral scent compounds (Knudsen *et al.* 2006) than other compounds found in the extracts (unpublished data). 1-Hexanol, *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate are also common green leaf volatiles (Pare and Tumlinson 1999); *cis*-3-Hexen-1-ol and *cis*-3-hexen-1-yl acetate were tested with *P. mirabilis* only. Substances were dissolved in acetone and applied in different amounts starting with 0.01 mMol filter paper^{-1}. In cases where a substance affected the choice of one of the spider species in this initial concentration, the amount was subsequently reduced (0.005, 0.0025 and 0.00125 mMol filter paper^{-1}) in order to control for concentration-dependent effects. Scented filter papers and filter papers only treated with acetone as controls were attached on top of the wooden sticks. Tests started after solvent was evaporated after approximately 10 min.

Each trial (1-h period) yielded up to 20 observations from which the proportion of choices for one of the two substrates was obtained, disregarding observations where the spider was not present on one of the substrates. Some spiders spent time on the islands others did not leave it during the entire time; these rare events were not included in the calculation of the proportion. Generalized linear models (GLM) with quasibinomial error distribution were performed in order to explore the parameters influencing the spiders' choice. Tests with fresh plant material and extracts were analysed in one GLM with the proportion of choices for flowers or flower extracts as response variable and spider species, plant species and treatment (i.e. fresh plant material or extracts) as explanatory variables. In the GLM for tests with floral scent compounds spider species, substance and concentration (mMol) were used as explanatory variables. Beginning with the full model, models were stepwise reduced and compared with the previous one with a *chi^2 test* (Crawley 2005). Prior to the stepwise statistical analysis, the full model was compared to a null model (model with no explanatory variables) to validate the overall effect of the combined parameters. Only if the full model had significantly more explanatory power than the null model, individual parameters were tested (see Mundry and Nunn 2009). Additionally, proportions were individually tested against the null hypothesis (assuming equal visitation of both treatment and control, i.e. proportion = 0.5) with a Wilcoxon test. All statistical analyses were performed using R 2.4.0 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

Results

In 93.3 and 97.5 % of all trials with *M. vatia* and *P. mirabilis*, respectively, the spider chose one of the substrates within the first eight minutes. Once a spider climbed up a wooden stick, they rarely descended to islands again. While *M. vatia* often changed the substrates during the trial (3.0 ± 0.2 times, mean ± SE), *P. mirabilis* was more constant in its choices with only 0.8 ± 0.2 changes of the substrate per trial. The responses to fresh plant material were usually consistent with responses to extracts of the same plant species, but the spiders' choices between leaves and flowers differed strongly between plants (Tab. 1a). *P. mirabilis* strongly preferred leaves over flowers (and their extracts) in three plant species, whereas *M. vatia* did not show any preferences (Tab. 1a and Fig. 1a).

In trials where spiders were allowed to choose between filter paper treated with scent compounds and scentless filter paper, the choices depended on the particular substance and spider species. Overall, the concentration of the compounds did not affect the spiders' choices (Tab. 1b). Similar to the previous tests, *M. vatia* was less selective than *P. mirabilis* (Tab. 1b and Fig. 1b). *M. vatia* avoided filter paper treated with nerolidol, and P. mirabilis avoided both nerolidol and β-caryophyllene (Fig. 1b). *P. mirabils* was not affected by the green leaf volatiles cis-3-Hexen-1-ol and cis-3-hexen-1-yl acetate either (Wilcoxon-test: $V \leq 50.5$, $p \geq 0.37$). Large amounts of nerolidol occurred in floral extracts of S. officinalis, and β-caryophyllene in *A. millefolium*. These substances may have triggered the preference of *P. mirabilis* for leaves and leaf extracts in *S. officinalis*, and for leaf extracts of *A. millefolium* over the respective flowers or floral extracts (Fig. 1). Living flowers of *A. millefolium* were not avoided by *P. mirabilis*, suggesting that some substances are included in the extracts that are not or in a lesser amount present in fresh plant material.

Tab. 1 Generalized linear models (GLM with quasibinomial error distribution) of the choices in Pisaura mirabilis and Misumena vatia: (a) trials using fresh plant material and extracts (treatments), and (b) trials using synthetic floral scent compounds. Starting with the full model containing all explanatory parameters, each reduced model was compared with the previous one with a Chi2 test resulting in deviance, degree of freedom (df) and significance (p) for each parameter.

Parameter	***Deviance***	***df***	***p***
a)			
Spider species × plant species × treatment	4.53	9, 288	0.58
Treatment	0.00	1, 297	0.99
Spider species × plant species	5.48	4, 298	0.053
Plant species	7.95	4, 302	**< 0.01**
Spider species	14.25	1, 306	**< 0.001**
Residual error	199.85		
Total	232.06		
b)			
Spider species × substance × concentration	2.54	3, 226	0.27
Concentration	0.08	1, 227	0.73
Spider species × substance	5.41	5, 232	0.14
Substance	8.94	5, 237	**0.014**
Spider species	4.37	1, 238	**< 0.01**
Residual error	163.61		
Total	184.94		

Discussion

The results of our study imply that *P. mirabils* perceive phytochemical cues and use them to decide where to ambush for prey. In *M. vatia*, behavioural responses to these cues were much less pronounced, and the crab spiders only weakly responded to the sesquiterpene nerolidol. We had expected that *M. vatia* would prefer flowers and their extracts over leaves and their extracts, since other crab spiders (*Thomisus spectabilis*) positively responded to floral odours (Heiling *et al.* 2004). Crab spiders including *M. vatia* were shown to prefer flowers during their anthesis over senescent ones (Chien and Morse 1998, Heiling and Herberstein 2004a) and therefore have the same preferences as pollinators and use olfactory besides visual cues (Heiling *et al.* 2004). It may thus be surprising that we could not confirm positive responses to floral odours or compounds thereof; but results from Greco and Kevan (1994, 2001) also reported no discrimination between leaves and flowers by the same spider species. It was shown that *M. vatia* remains

longer on flowers that are frequented by pollinators (Chien and Morse 1998, Morse 2000a) and on flowers that they have experienced before (Morse 2000b). We used picked flowers (i.e. not the preferred state of the flowers) that were not visited by insects, which may contribute to a lack of

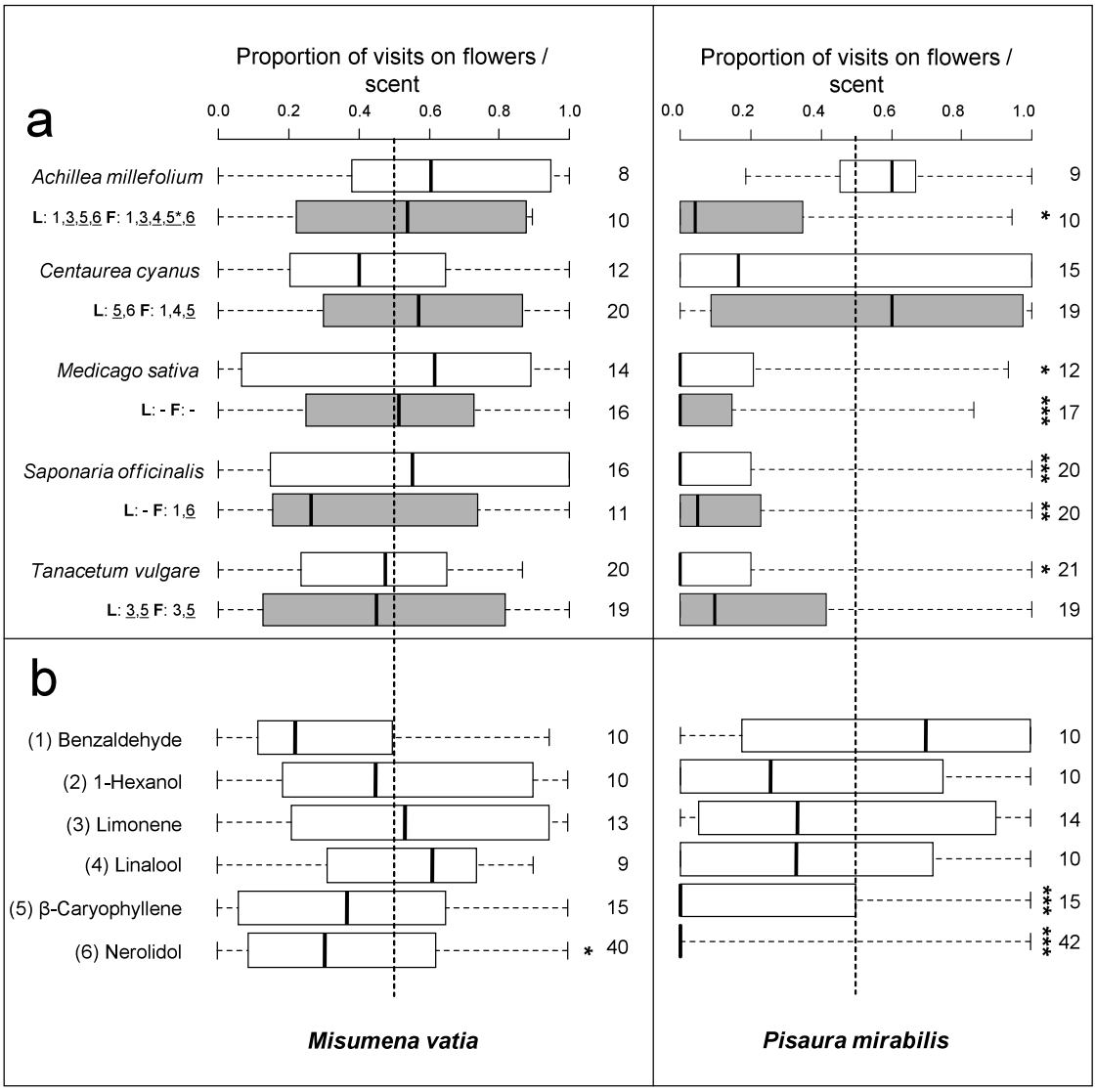


Fig. 1 Dual choices of *Pisaura mirabilis* and *Misumena vatia* between flowers and leaves, extracts or compounds thereof. Choices were measured as proportion of time spend on flowers, flower extracts or scents of the total time on both treatments. Significant deviation from an equal time spent on flowers and leaves, or scent and control (i.e., proportion = 0.5) is indicated by asterisks according to paired Wilcoxon rank sum test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Sample sizes are given next to each boxplot. (a) White boxes show trials with fresh plant material, grey boxes flower vs. leaf extracts. Leaf (L) and flower (F) extracts often contained one or more substances used in the biotest, which are listed below each species name. Numbers correspond to the substance code below (b). Concentrations of substances are labelled as follows: plain numbers: $1 \cdot 10^{-5}$ – 0.01 mMol g^{-1} dry weight; underlined numbers: 0.011 - 10 mMol g^{-1}; underlined numbers marked with an asterisk: > 10.1 mMol g^{-1}. (b) Results of trials using synthetic floral scent compounds.

preferences. The preference for leaves over flowers in *P. mirabils* may either result from an attraction to leaves or from a deterrent effect of flower secondary metabolites. The trials with individual substances are consistent with the latter, and suggest that floral scents or perhaps other non-volatile metabolites have a deterrent effect on this spider. Plant volatiles emitted by flowers and leaves were shown to repel or deter various arthropods (Pichersky and Gershenzon 2002, Gershenzon and Dudareva 2007, Junker and Blüthgen 2008, Kant *et al.* 2009, Unsicker *et al.* 2009, Willmer *et al.* 2009, Junker and Blüthgen 2010b). Therefore, it is likely that the floral repellence found for this spider represents a typical response of a broad spectrum of generalised predators and other taxa that are not specifically adapted to flowers.

Crab spiders are predators that exploit the mutualism between flowers and pollinators and thereby have detrimental effects on pollination and consequently reproduction of plants (Dukas 2001, Dukas and Morse 2003, Heiling and Herberstein 2004b, Reader *et al.* 2006, Goncalves-Souza *et al.* 2008, Ings and Chittka 2008, Brechbühl *et al.* 2010). Chemical floral cues that simultaneously prevented predators such as spiders and other floral antagonists from visiting flowers and attract pollinators would maximize the plants' reproductive success (Brown 2002, Irwin *et al.* 2004, Junker and Blüthgen 2008). It was shown that animals that depend on floral resources (obligate flower visitors) are able to tolerate defensive floral scent compounds and even use them as host finding cue, while facultative flower visitors are not (Junker and Blüthgen 2010b). Thus, the adaptations on flower visits that have been evolved by *M. vatia* may include a tolerance against otherwise defensive floral compounds. In contrast, *P. mirabilis* that is adapted to use the vegetative plant parts as hunting site may not have been subjected to a selective pressure to tolerate the same compounds.

VII. Floral scents contribute to the establishment of flower- and leaf-specific bacterial communities

This chapter is in preparation for publication as:

Junker RR, Loewel C, Gross R, Dötterl S, Keller A and Blüthgen N (in preparation) Composition of epiphytic bacterial communities differs on flowers and leaves

Summary

The epiphytic bacterial communities colonizing roots and leaves have been described for many plant species. The ephemeral floral surfaces of naturally growing plants have rarely been considered by microbiologists. We identified bacteria isolated from petals and leaves of two plant species, *Saponaria officinalis* (Caryophyllaceae) and *Lotus corniculatus* (Fabaceae). The bacterial diversity was much lower on flowers than on leaves and the compositions on the plant organs were different: while Pseudomonadaceae and Microbacteriaceae were the most abundant families on leaves, Enterobacteriaceae dominated floral communities. We hypothesize that antibacterial floral volatiles trigger the low diversity on petals, which is supported by agar diffusion assays using substances emitted by flowers and leaves of *S. officinalis*. These results suggest that bacteria should be included in the interpretation of floral traits and bacterial effects on pollination biology are proposed and discussed.

Introduction

Above ground plant surfaces provide diverse habitats for bacterial colonists. Environmental factors and specific features of the plant organs determine the character of these surfaces and thus may affect the composition of the bacterial communities (Andrews and Harris 2000). The establishment and the growth of bacteria strongly depends on the availability of nutrients that may be variable on a macroscopic level (different plant parts) and on a microscopic level where nutrients are heterogeneously distributed within small areas (Andrews and Harris 2000, Mercier and Lindow 2000). Next to nutrients, the emission or secretion of secondary metabolites that either inhibit or facilitate bacterial growth may have an impact on the distribution of bacteria on different plant parts (Bednarek and Osbourn 2009). This notion is supported by numerous studies that investigated the

antibacterial properties of essential oils (Harrewijn *et al.* 1995, Lokvam and Braddock 1999, Velickovic *et al.* 2003, Gershenzon and Dudareva 2007, Tomczykowa *et al.* 2008).

Besides roots that may be the best examined plant part regarding its associated bacteria (Andrews and Harris 2000), leaves were often the target of microbiologists that isolated and identified the microbial taxa dwelling on them. The most common bacteria found on leaves are representatives of the families Enterobacteriaceae, Pseudomonadaceae and Microbacteriaceae (Ercolani 1991, Thompson *et al.* 1993, Lindow and Brandl 2003, Krimm *et al.* 2005) that build diverse communities. Several studies have focused on the distribution of specific groups or species of bacteria across plant species (Corpe and Rheem 1989, Brighigna *et al.* 1992). These studies revealed non species-specific distribution of the investigated taxa (but see Yang *et al.* 2001) leading to the conclusion that these bacteria may be well adapted to the phyllosphere irrespective species-specific properties of leaf surfaces (Hirano and Upper 2000). A recent study by Östman *et al.* (2010) also indicates that habitat-specific microbial communities have a high degree of similarity across sites within a large spatial scale.

Similar to leaves, petals offer colonizable surfaces, but received much less attention. Due to the severe economic and social impacts caused by pathogenic microorganisms, previous work on flower dwelling bacteria focused on crop diseases (e.g. Windels 2000) such as the bacterium *Erwinia amylovora* that causes fire blight (Buban *et al.* 2003). Much less is known about bacteria growing on flowers of uncultured plants or about those with no obvious detrimental effect on the plants' reproduction. However, nectar and exudates of stigma and pollen offer excellent growing media for microorganisms (Brysch-Herzberg 2004, Stockwell 2005) and the visitation by pollinators or other dissemination mechanisms of pollen provide ideal dispersal conditions for microorganisms (Giles *et al.* 2005). Nonetheless, a study by Krimm *et al.* (2005) indicates that the diversity of bacteria is lower on flowers than on leaves.

In this study we compared the bacterial communities on flowers and leaves of two naturally growing plants species. Within the flowers we excluded stigmas, nectar and pollen from our investigation and restricted it to petals in order to ensure a better comparability to leaves. Additionally, we examined the role of plant volatiles in structuring the bacterial communities.

Material and Methods

Isolation and identification of epiphytic bacteria

At different sites in Würzburg and Reichenberg, Germany, we sampled young leaves and flowers from *Lotus corniculatus* (Fabaceae) and *Saponaria officinalis* (Caryophyllaceae) from spatially separated patches. Several leaves and flowers per sample were placed in 30 ml phosphate buffered saline (PBS) and were sonificated for 7 min to separate bacteria from plant material. 100 µl of different dilutions of PBS were plated on LB agar plates. After incubation at 30 °C for 48 h colony forming units (cfu) were counted and density of bacteria on plant parts were estimated by calculation of the surface area of all leaves and flowers in each sample [cfu cm^{-2}]. Three colonies per distinct morphotype were cultivated on a separate LB agar plate at the same conditions as described above.

From isolated bacterial strains one colony was picked and DNA was isolated as template for polymerase chain reaction using the primer pair 27f and 1492r targeting the 16S rRNA gene. Purified DNA was sent to SeqLab (Sequence Laboratories, Göttingen, Germany) for sequencing. For methodological details see Appendix A.

Sequences were matched with sequences at GenBank nucleotide database (accessed 23. March 2010) (Benson *et al.* 2009). We decided to integrate ribosomal secondary structure information additionally to sequence information into the phylogenetic reconstructions, as a recent simulation study confirmed the benefit regarding accuracy and robustness (Keller *et al.* 2010). Thus, we used according to the workflow published for ITS2 sequence and structure phylogenetics 4SALE alignments (Seibel *et al.* 2008) and Profile Neighbor-Joining (Wolf *et al.* 2008) for our 16S data with a general time reversible model and 100 bootstrap replicates. Sequences of the genus *Deinococcus* (GI:219846824, GI:222083990 and GI:110277976) were used as outgroup. The resulting tree was displayed with iToL (Letunic and Bork 2007). Taxonomy information was added according to the most often occurring taxonomic annotation (genus and family) within the best 25 BLAST hits with minimal manual corrections for recently split genera. For methodological details see Appendix A.

Volatile collection

Scents of leaves and flowers of both plant species were sampled in mixture (1:1) of Tenax-TA (mesh 60-80) and Carbotrap (mesh 20-40) and samples were analysed in a Varian

Saturn 2000 system that was equipped with a ChromatoProbe kit. For further details see Dötterl *et al.* (2005) and Appendix A.

Agar diffusion assay

In the agar diffusion assay, the potential effect of volatile compounds on the growth of bacteria that were isolated from leaves or flowers of *S. officinalis* was examined. We used two volatiles that were predominately emitted by leaves and three that were predominately emitted by flowers (see Fig. 2 and Appendix B). Bacterial strains used for the tests were either isolated from leaves or flowers of *S. officinalis* (see Fig. 2 and Appendix A). 100 µl of a bacterial suspension was mixed with 5ml top agar and poured upon dried LB agar plates. 0.06 or 0.04 mMol of the substances dissolved in acetone were applied on sterile cellulose discs (Ø 6 mm, Oxoid, Hampshire, United Kingdom) and cellulose discs were placed on agar plates with bacterial suspensions. Pure acetone was used to control for potential growth inhibitory effects of the solvent. The control did never inhibit growth of any bacterial strain and was thus removed from statistical analysis. After incubation for 48 h, the diameter of inhibition zones was measured.

Statistical analysis

We used random forest, a machine-learning algorithm (Breiman 2001), to assign individual bacterial communities and scent compositions to specific groups (leaves and flowers of *L. corniculatus* and *S. officinalis*) and to estimate the variable importance (bacterial genus and scent compound) for the correctness of the assignment. Recently, this statistical classification tool was established for the interpretation of ecological multivariate data and its utility and advantages (i.e. it calculates the importance of each variable for a right classification independently of the others but also considers multivariate interactions with the others) were demonstrated (Prasad *et al.* 2006, Ranganathan and Borges 2009). For each analysis n_{tree} = 100,000 bootstrap samples were drawn with m_{try} = 2 variables randomly selected at each node. For each bacterial family or scent compound with a variable importance > 0, we used a t-test for bacteria or an ANOVA for scent compounds as post-hoc test to validate the results of random forest.

Results

Bacterial communities

In total, we identified 130 bacterial strains from 10 families and 25 genera (Fig. 1). Density of bacteria on plant surfaces [bacteria cm^{-2}] and diversity of bacteria families and genera differed between flowers and leaves of *S. officinalis* and *L. corniculatus* (Tab. 1). In general, diversity of bacteria colonizing flowers was much lower than those colonizing leaves, both on family (Wilcoxon rank sum test: $W = 1$, $n = 10$, $p < 0.001$) and genus level ($W = 17$, $n = 10$, $p = 0.012$). We did neither find differences between the communities colonizing flowers of *S. officinalis* and *L. corniculatus* nor between the leaves of these species as the flowers and the leaves, regardless of the plant species, were each assigned to one group only by random forest analysis (result not shown). Thus, we repeated the random forest analysis considering only the plant part, not the plant species. On the family level, flower-communities were all correctly assigned to flowers, 9 out of 10 leaf-communities to leaves (Tab. 2a). On the genus level, flower communities were correctly assigned, but half of the leaf-communities were also assigned to flowers (Tab. 2b). Bacterial communities on flowers were dominated by representatives of genera belonging to the Enterobacteriaceae, but *Pseudomonas* was the most common bacterial genus colonizing leaves (Tab. 2 a and b).

Volatile compositions

Scent compositions from flowers and leaves of *S. officinalis* and *L. corniculatus* were distinct from each other, except for one floral scent composition of *S. officinalis* which was assigned to leaf scents of the same species (see Appendix B). Leaves and flowers of *L. corniculatus* emitted the same volatiles but in different proportions. Leaves and flowers of *S. officinalis* shared some compounds but some were exclusively emitted by flowers or in much higher amounts (Appendix B).

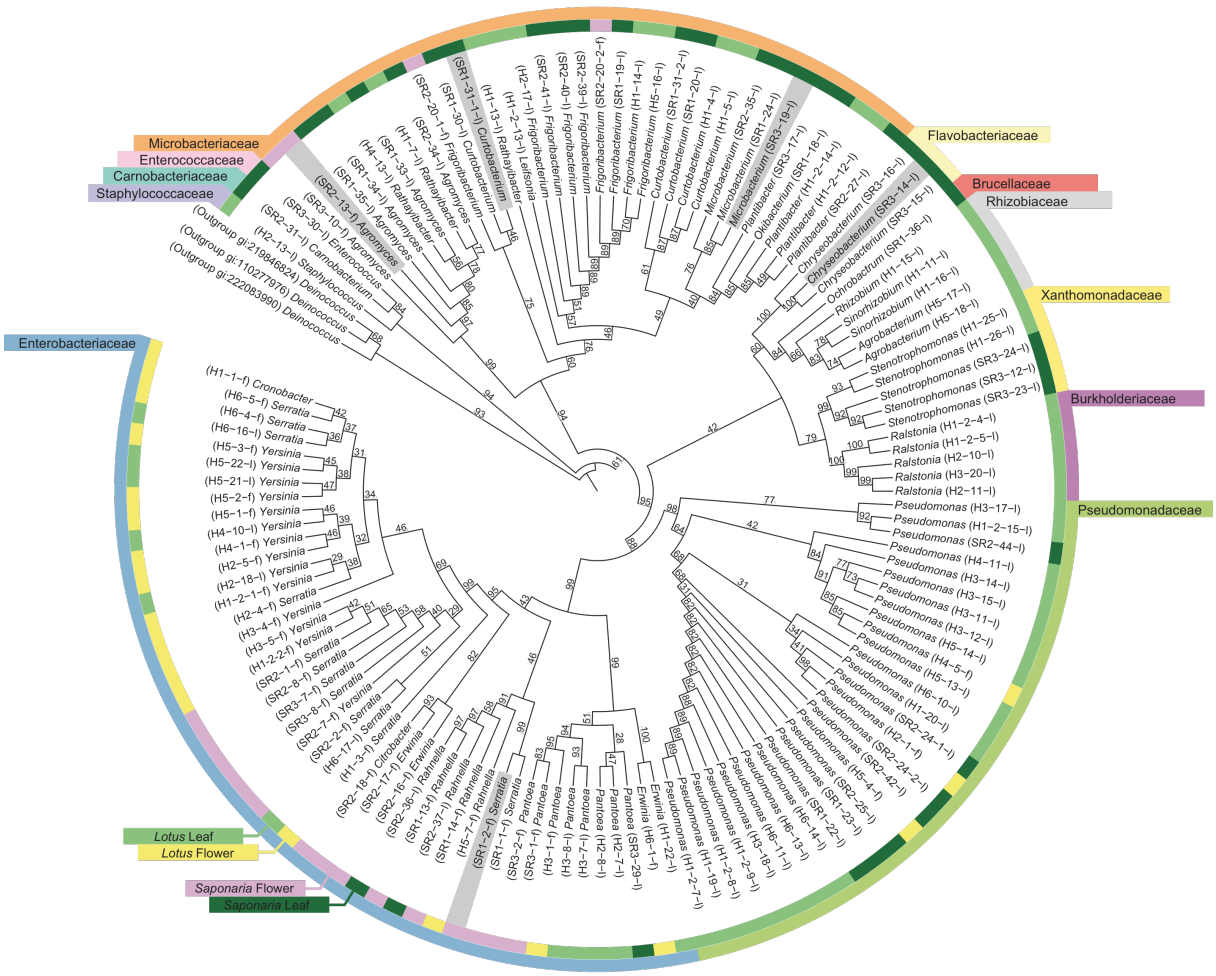


Fig. 1 Phylogenetic Profile Neighbor Joining tree representing evolutionary relationships between all sampled specimen. Bootstrap values were determined with 1000 pseudoreplicates. Specimens were assigned to genera according to the majority of the first 20 BLAST hits against the GenBank database. Voucher identifiers are displayed in parenthesis. Sample communities are indicated by the inner ring, whereas the outer ring represents current family classifications. Three Deinococcus species were added as the outgroup. Strains used for the agar diffusion assay are highlighted in gray.

Tab. 1 Density and diversity (Simpsons) of bacteria colonizing flowers and leaves of *Saponaria officinalis* and *Lotus corniculatus*. Shown are Median and interquartile range.

	Saponaria officinalis		*Lotus corniculatus*	
	fower	leaf	fower	leaf
N	3	3	7	7
Bacteria cm^{-2}	3759 (3192 - 7999)	7166 (6998 - 7297)	146247 (43823 - 449895)	700 (454 - 1360)
Diversity (family)	1.02 (1.01 - 1.14)	1.47 (1.28 - 2.18)	1.00 (1.00 - 1.06)	1.74 (1.68 - 2.39)
Diversity (genus)	1.67 (1.47 - 1.71)	3.54 (2.65 - 4.07)	1.17 (1.09 - 1.65)	1.74 (1.69 - 2.39)

Tab. 2 Classification of the bacterial communities colonizing flowers and leaves of *Saponaria officinalis* and *Lotus corniculatus* based on bacterial families (a) or genera (b) using random forest. Confusion matrix shows number of correctly assigned communities and proportional class error. Families (a) or genera (b) that were important in the classification (i.e. variable importance $E > 0$) are listed in decreasing order. Additionally, number of samples from which each family or genus was isolated is given and in parenthesis the proportion of colony forming units that belong to it in the samples were the family or genus occurred. Flower and leaf samples were compared with *t*-tests, asterisks indicate significance level with *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

a) Confusion matrix

	Flower	Leaf	Class error
Flower	10	0	0
Leaf	1	9	0.1

Variable importance

Family	*E*	Flower	Leaf	*t*
Enterobacteriaceae	75.78	10 (0.98 ± 0.01)	8 (0.29 ± 0.07)	9.66 ***
Pseudomonadaceae	64.34	3 (0.03 ± 0.00)	9 (0.40 ± 0.11)	3.42 **
Microbacteriaceae	34.34	2 (0.06 ± 0.05)	8 (0.37 ± 0.13)	2.53 *
Burkholderiaceae	19.20	0	3 (0.06 ± 0.04)	1.44
Xanthomonadaceae	14.85	0	2 (0.15 ± 0.04)	1.44
Rhizobiaceae	10.61	0	2 (0.08 ± 0.07)	1.09

b) Confusion matrix

	Flower	Leaf	Class error
Flower	10	0	0
Leaf	5	5	0.5

Variable importance

Genus	*E*	Flower	Leaf	*t*
Pseudomonas	53.76	3 (0.03 ± 0.00)	9 (0.40 ± 0.11)	3.42 **
Serratia	28.69	6 (0.58 ± 0.12)	1 (0.37)	2.50 *
Yersinia	22.68	6 (0.72 ± 0.15)	3 (0.37 ± 0.20)	1.94
Plantibacter	20.69	0	3 (0.13 ± 0.06)	1.56
Ralstonia	20.51	0	3 (0.06 ± 0.04)	1.44
Microbacterium	20.16	0	3 (0.09 ± 0.08)	1.12
Frigoribacterium	19.96	1 (0.01)	5 (0.24 ± 0.14)	1.58
Rathayibacter	16.27	0	2 (0.20 ± 0.05)	1.46
Stenotrophomonas	13.45	0	2 (0.15 ± 0.04)	1.44
Curtobacterium	8.90	0	2 (0.03 ± 0.01)	1.31
Rahnella	2.21	2 (0.46 ± 0.42)	1 (0.01)	1.04

Agar diffusion assay

In total, we performed 450 agar diffusions assays with five bacterial strains; two scent compounds that were predominantly emitted by leaves of *S. officinalis* and three floral volatiles of the same species. The diameter of the inhibition zones was affected by the bacterial strain, the scent compound used and interaction of both (multiple ANOVA: bacterial strain: $F_4 = 43.5$, $p < 0.001$; scent: $F_4 = 131.5$, $p < 0.001$; bacterial strain · scent: $F_{16} = 9.9$, $p < 0.001$; residuals = 425). Benzyl nitrile and 2-Phenylethylalcohol, both floral scent compounds, had the strongest growth-inhibitory effect on most bacterial strains, while Methyl-benzoate and the green leaf volatiles only slightly affected the growth of the bacteria (Fig. 2). *Serratia* sp. (Enterobacteriaceae, strain: SR1-2-f) was least inhibited in its growth by the floral scents compounds (Fig. 2). The different concentrations of substances applied in the assay (0.06 and 0.04 mMol) did not affect the diameter of the inhibition zones (Welch corrected *t*-test: $t_{422.02} = 1.58$, $p = 0.11$). Both concentrations are well beyond the daily emission [ng d^{-1} dry weight $[g]^{-1}$] of the substances (e.g. 50 times more in the case of Methyl benzoate) suggesting that the maximal inhibition is reached. Thus, the extent of inhibition may not reflect natural conditions but the comparison between substances remains valid.

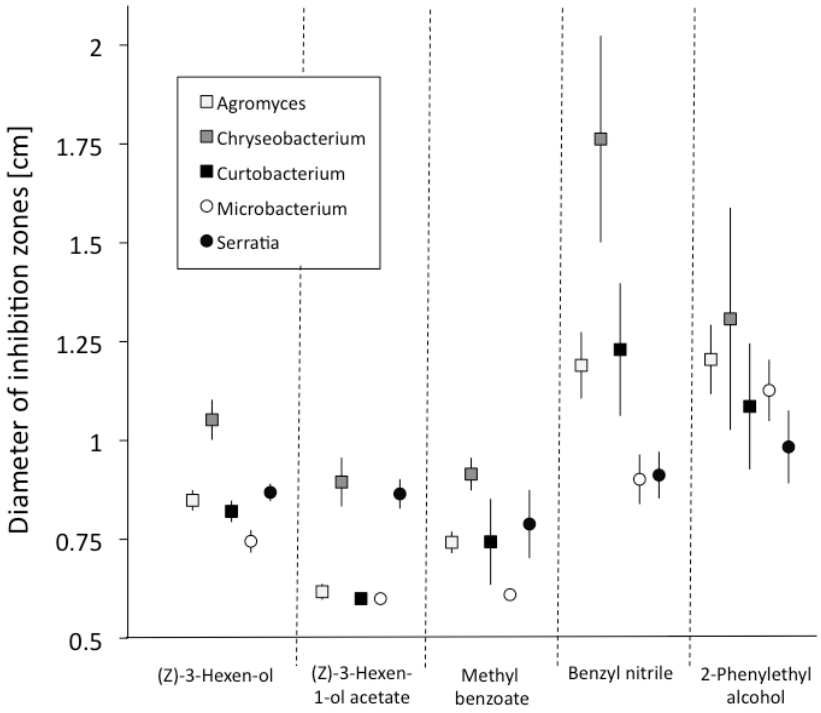


Fig. 2 Results of agar diffusion assay. Mean and 95% confidence intervals are given; significant differences in the growth inhibition are indicated if confidence intervals do not overlap. (Z)-3-Hexen-ol and (Z)-3-Hexen-1-ol acetate were predominantly emitted by leaves, the others predominantly from the flowers of *Saponaria officinalis*.

Discussion

On the family and genus level, we found the bacteria that colonized leaves of *Lotus corniculatus* and *Saponaria officinalis* to be consistent with those found on leaves of other plant species (*cf*. Ercolani 1991, Thompson *et al.* 1993, Krimm *et al.* 2005). The bacteria that colonized the flowers of these plant-species where generally from the same families as those found on leaves but their composition was fundamentally different. The communities on flowers were less diverse than those on leaves – as also suggested by data from Krimm *et al.* (2005) – and were dominated by bacteria of the family Enterobacteriaceae. Overall, the bacterial compositions were differed between the plant parts but not between the plant-species, which suggests that flowers and leaves to a certain extend have their distinct communities. In the agar diffusion assay we explored one out of several causes that may be responsible for the flower-specific and taxonomically restricted bacterial communities. The antimicrobial function of substances that are also frequently produced by flowers including terpenoids (Velickovic *et al.* 2003, Gershenzon and Dudareva 2007) and benzenoids (Karapinar and Aktug 1987) is well known. Correspondingly, with the exception of *Serratia* sp. (Enterobacteriaceae, isolated from *S. officinalis* flowers) scent compounds emitted by flowers of *S. officinalis* had a stronger antibacterial effect on most bacteria tested than those emitted by leaves. Thus, floral scents may contribute to the relatively low diversity of bacteria colonizing petals. These results may suggest that floral volatiles serve as defenses against microorganisms that potentially could be pathogenetic or otherwise detrimental for the reproduction of the plants. This hypothesis may contribute to the recent discussion about alternative functions of floral scents besides pollinator attraction (Raguso 2008b) and the notion that defensive properties of floral volatiles are crucial for the fitness of plants (Junker and Blüthgen 2010b).

In pollination biology, the presence of microorganisms and their potential impact on floral signals, rewards, and consequently on pollinator behavior and plants' reproduction was mostly neglected. Exceptions from this gap are the interactions between yeast and nectar (Kevan *et al.* 1988, Herrera *et al.* 2008), fungi altering flower traits or induce pseudo-flowers (Raguso and Roy 1998, Dötterl *et al.* 2009) and floral pathogens (Johnson and Stockwell 1998). The omnipresence of bacteria and their virtually endless biochemical abilities as well as insights into floral pathogens presume that bacteria may have additional profound impacts on ecological processes related to flowers and pollination. These potential bacterial impacts may include effects on floral rewards and signals and the bacterial communities may in turn be affected by the visitation pattern of flower visiting insects. (1)

Bacteria colonizing flower surfaces may spoil nectar or pollen e.g. by the activity of pollinators, which may severely affect the nutritional composition, an effect that was recently demonstrated for yeasts dwelling in nectar (Herrera *et al.* 2008). Next to the alteration of resources, bacterial metabolites such as ethanol may accumulate in nectar and thereby make it toxic to pollinators (Ehlers and Olesen 1997). (2) The scents emitted by bacteria include many of those that are also emitted by flowers (Knudsen *et al.* 2006, Schulz and Dickschat 2007) but also include unknown substances (Kai *et al.* 2008). Floral scents mediate several mutualistic and antagonistic interactions (Junker and Blüthgen 2010b, Junker *et al.* 2010) and the complementation of floral volatile compositions by bacterial odors may interfere with those interactions. For instance, alternations of the original bouquet (e.g. due to bacteria) may lead to an reproductive isolation of flowers with modified scents (Waelti *et al.* 2008). (3) The ability of different bee species to spread antagonistic bacteria of plant pathogens has been demonstrated in several studies (Johnson *et al.* 1993, Maccagnani *et al.* 2009) suggesting that naturally occurring bacteria may be dispersed similarly. Therefore, the taxonomically relatively restricted visitor spectrum of flowers (Blüthgen *et al.* 2007) may contribute to the establishment of floral bacterial communities.

VIII. Historical absence of antagonistic ants led to an absence of specific floral defences in Hawaii including repellent scents

This chapter has been submitted for publication as:

Junker RR, Daehler CC, Dötterl S, Keller A and Blüthgen N (submitted) Ant-flower networks in Hawai'i: native plants are exploited, introduced plants defended

Summary

Ants are omnipresent in most terrestrial ecosystems, and many plants responded to their dominance by evolving traits that either facilitate positive interactions with ants or reduce negative ones. Because ants are generally poor pollinators, plants often protect their floral nectar against ants. Ants were historically absent from the geographically isolated Hawaiian archipelago, the flora of which harbours one of the highest rates of endemism in the world. We hypothesized that native Hawaiian plants lack floral features that exclude ants and therefore would be heavily exploited by introduced, invasive ants. To test this hypothesis, ant-flower interactions involving co-occurring native and introduced plants were observed in ten sites on three Hawaiian Islands. We quantified the residual interaction strength of each pair of ant/plant species as the deviation of the observed interaction frequency from a null-model prediction based on available nectar sugar in a local plant community and local ant activity at sugar baits. As predicted, flowers of plants that are endemic or indigenous to Hawaii were more strongly exploited by ants than flowers of co-occurring introduced plants, which shared an evolutionary history with ants. We also found that the percentage of plant species with ant-visited flowers was much higher in Hawaii than in other continental and island systems, even reaching 100 % in habitats dominated by endemic species. We showed experimentally that the absence of ants on flowers of most introduced and few native plants species was due to unpalatable nectar, morphological barriers and/or repellent floral scents. Analysis of floral volatiles, however, revealed no consistent ant-repellent "syndrome" attributable to negative responses by ants, probably due to the high chemical variability within the floral scent bouquets. Results from a molecular phylogeny imply that floral defences against ants were convergently lost in native Hawaiian plants. Exploitation of floral nectar by ants may be an important threat to

Hawaiian ecosystems, reducing nectar resources available to native flower visitors and potentially reducing the reproductive success of the endangered endemic flora.

Introduction

Biological invasions, along with other anthropogenic modifications of the environment, are severe threats for ecosystems and biodiversity (Mooney *et al.* 2005). Low species diversity (Denslow 2003) and functional group diversity (Tilman 1997, Symstad 2000), disharmonic floras and faunas (Denslow 2003) and isolation from source habitats (Lonsdale 1999) in combination with the human capacity to transport biological material over long distances (Mooney 2005) make oceanic islands highly susceptible to invasions. The Hawaiian archipelago, one of the most isolated island groups worldwide, is a paramount example of an island system threatened by biological invasions by non-native plant and animal species, and it features many characteristics that suggest high susceptibility to invasions.

Fifteen percent of the native plant genera and 89 % of the native plant species are endemic to these islands. Today, however, nearly half of the plant species naturally occurring in Hawaii were introduced during the last two centuries (Wagner *et al.* 1990). Similarly, some insect taxa show high degrees of endemism, e.g. *Drosophila* flies and *Hylaeus* bees (Daly and Magnacca 2003, Magnacca and Danforth 2007) while others had been absent from the islands prior to their human introduction. It is widely accepted that ants are among those previously missing components in the Hawaiian ecosystems (Keeler 1985, Krushelnycky *et al.* 2005) although it has been suggested that some rather inconspicuous and subterranean ant species could be indigenous to these islands (Wheeler 1934, Medeiros *et al.* 1986). The vulnerability of the native Hawaiian arthropod fauna to invasive ants (Medeiros *et al.* 1986, Wetterer 1998, Krushelnycky and Gillespie 2008) suggests, however, that if ants had been indigenous to the Hawaiian Islands, they were not nearly as ecologically important as ants in most other terrestrial ecosystems.

A number of studies in Hawaii and elsewhere focussed on the impact of alien plants on native plants (Stone and Scott 1985, Stone *et al.* 1992, Allison and Vitousek 2004), or on the impact of ants on native arthropods (Medeiros *et al.* 1986, Holway *et al.* 2002, Krushelnycky *et al.* 2005, Krushelnycky and Gillespie 2008). Comparably little is known about the interactions between introduced ants and native and / or introduced plants. Hawaii offers a unique opportunity to study those interactions, where plants that shared an evolutionary history with ants (introduced plants) co-occur with plants that had evolved in

habitats only recently invaded by ants (native plants). Many plant traits are adaptations to interactions with ants (Heil and McKey 2003, Lach *et al.* 2010), and Hawaiian plants may be expected to lack many of these traits. Correspondingly, very few endemic Hawaiian plant species possess extrafloral nectaries (EFNs) (Keeler 1985), a trait that is assumed to be ant-related, whereas EFN-bearing plants constitute an important part of tropical floras where ants are common (Blüthgen and Reifenrath 2003).

While the presence of ants on vegetative structures is often beneficial for plants (Rico-Gray and Oliveira 2007), flower visiting ants are – in most cases – detrimental to plant reproduction: they are poor pollinators (Pijl 1955, Beattie *et al.* 1984, Beattie *et al.* 1985), nectar thieves (Galen 1983, Galen and Butchart 2003) and negatively interfere with pollinators (Tsuji *et al.* 2004, Junker *et al.* 2007); but see e.g. Beattie (2006), Gomez *et al.* (1992, 1996, 2000) and de Vega *et al.* (2009). In order to avoid conflicts with ants on their valuable reproductive structures, plants display various mechanisms to reduce or prevent flower visitation by ants (see below). In Hawaii, floral nectar may be an important carbohydrate source since EFNs (Keeler 1985) and ant-attended honeydew-producing hemipterans are uncommon in many habitats (RRJ and NB, personal observation). Accordingly, it was reported that the flowers of a common Hawaiian plant species *Metrosideros polymorpha* (Myrtaceae) are heavily exploited by various introduced ant species (Lach 2005, 2008b), suggesting that this resource is not well protected against ants.

From the consumer's (i.e. the ant's) perspective, four distinct but non-exclusive barriers need to be overcome before nectar from a given plant can be consumed (Fig. 1). We regard them as a hierarchical sequence. This conceptual framework, although developed for ants, may be adapted to any type of flower visitor.

When ants and nectar-bearing flowers co-occur in space and time (Fig. 1A), floral scents and the flowers' morphology represent the first barriers (B_1 and B_2). Whether morphological barriers or floral scents act first or second may depend on the morphology of the flowers. Floral scents (B_1) are important defensive traits against facultative flower visitors (Junker and Blüthgen 2010b) and have been recently shown to effectively prevent ants from consuming nectar in a wide spectrum of flowering plants (Junker and Blüthgen 2008, Willmer *et al.* 2009), see also Willmer and Stone (1997) and Ghazoul (2001). Mechanical or morphological barriers (B_2) comprise either narrow nectar tubes (Herrera *et al.* 1984, Galen 1999, Galen and Cuba 2001, Galen and Geib 2007) or special features like sticky or greasy poles (Harley 1991) or stems or calyxes with dense trichomes that can not be passed by ants and other crawling arthropods (Kerner 1879). Unpalatable or even toxic nectar (C_1) was suggested to be the major reason for the conspicuous absence of ants on

flowers observed in many regions of the world (Janzen 1977) resulting from secondary metabolites dissolved in the sugary solution (Adler 2000, Raguso 2004, Kessler and Baldwin 2006). Summarizing several studies that tested the acceptance of nectar offered outside flowers to ants of different species (Feinsinger and Swarm 1978, Guerrant and Fiedler 1981, Haber *et al.* 1981, Kessler and Baldwin 2006, Junker and Blüthgen 2008), we conclude that unpalatable / toxic nectar has the potential to prevent floral ant visits in a few cases, but its general importance in a large number of plant species is questionable (Junker and Blüthgen 2008). The quality of floral nectar (C_2) may reduce the visitation if ants rate the resource as unfavourable. Blüthgen and Fiedler (2004b) support this assumption by showing a strong preference and a more intense recruiting behaviour to more concentrated sugar and amino acid solutions by several ant species. Given that an ant species succeeded in reaching palatable and nutrient-rich nectar, it may still encounter a dominant ant species that monopolises the resource (D) and therefore prevents visitation of the first species (Blüthgen *et al.* 2004b).

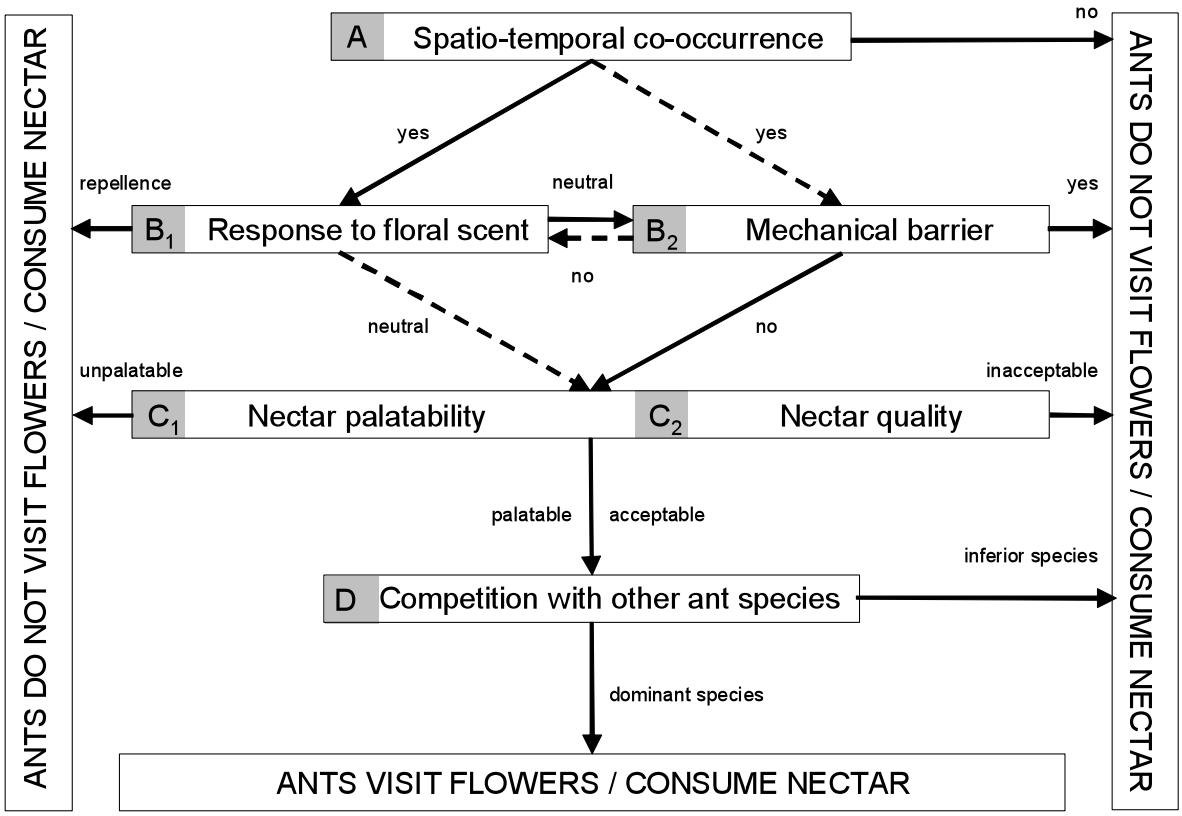


Fig. 1 Hierarchical framework that summarizes prerequisites and potential barriers for exploitation of floral nectar by ants. Steps A – D are sequentially encountered by ants approaching floral nectar. The order of B_1 and B_2 depends on the morphology of the flower, thus either the solid or the dashed path can be followed from A to C. C_1 and C_2 operate simultaneously.

In our study, we observed ant-flower interactions within communities and quantified the interactions between invasive ants and flowers of native and introduced plants, considering both resource quality and the ant species' proportional abundance. We combined the hierarchical framework (Fig. 1), quantitative observations, phylogenetic analysis of the plant species and experimental approaches to test the following hypotheses regarding the visitation pattern found in Hawaii's ant-plant communities: (1) The flowers of plant species that are endemic or indigenous to the Hawaiian Islands are more regularly and strongly exploited by ants than those of introduced plant species after accounting for the nectar quantity and quality. (2) This pattern is due to more effective defensive mechanisms by the introduced plant species. (3) As suggested by Willmer *et al.* (2009), we expect that flowers posses mainly one type of defence (morphological barriers or repellent scent, Fig. 1) as a result from a trade-off between these. (4) The combination of floral features either allowing or preventing strong nectar exploitation by ants is the result of independent evolutionary processes, which may have been triggered by the absence / presence of nectar thieving ants, respectively. Potential implications regarding the evolution and the conservation of the flora and fauna in Hawaii are critically discussed.

Materials and Methods

Study sites

The study was conducted on the islands of Hawaii, Oahu and Kauai in natural habitats and garden settings. Sites were selected due to their accessibility, the availability of flowers and the presence of ants. Names, location, altitude and number of ant and plant species are given in Table 1. The ten study sites featured a varying degree of endemism of the plants ranging between 0 – 100 % endemic plant species. The study was conducted between March and June 2009.

Ant-flower networks

In each study site, on two consecutive days (6 am – 10 am) all flowers within a small area (0.01 – 0.1 ha) were individually checked for presence of ants. Samples of ants were taken for identification. The total number of ant workers momentarily visiting flowers of a certain species (abundance) was recorded once per plant individual per day. Since ants are social insects, these counts do not represent independent decisions, but provide a suitable surrogate for the interaction strength (here the nectar consumption rate) between both trophic levels.

Tab. 1 Study sites on the Hawaiian Islands. Names and locations of the study sites, the altitude [m above sea level] and number of ant and plant species are given.

#	Island	Location	GPS	Altitude	Ant species	Plant species
1	Big Island	Amy B.H. Greenwell Ethnobotanical Garden	N19°29.5 W155°54.7	461	7	10
2	Big Island	Hawaii Volcanoes National Park	N19°17.5 W155°08.7	96	4	3
3	Big Island	Hawaii Volcanoes National Park	N19°26.2 W155°17.9	1240	1	2
4	Big Island	Hawaii Volcanoes National Park	N19°20.7 W155°12.7	901	3	2
5	Big Island	Hawaii Volcanoes National Park	N19°19.9 W155°16.7	901	1	3
6	Big Island	Hawaii Volcanoes National Park	N19°17.6 W155°05.9	17	2	5
7	Kauai	McBryde Garden	N21°54.3 W159°30.5	61	4	9
8	Oahu	Sandy Beach	N21°17.5 W157°39.7	20	5	6
9	Oahu	University of Hawaii at Manoa	N21°18.1 W157°48.9	71	1	10
10	Oahu	Lyon Arboretum	N21°19.9 W157°48.2	177	3	6

Number of flowers of each species present in the habitat was counted in small plants or estimated in larger shrubs or trees by multiplying the number of inflorescences with mean number of flowers per inflorescence. Nectar samples of 2 – 90 flowers per species were taken with micro-capillaries (5 µl) to quantify the amount [µl] and the sugar content [% w/w] using a handheld refractometer (Eclipse, Bellingham + Stanley, UK). Total volume of sugar provided by each plant species (standing crop) was calculated by multiplying number of flowers with mean amount of nectar [µl] and with mean sugar content [% w/w]. Plant species were assigned to the three categories: endemic, indigenous and introduced, following Wagner *et al.* (1990). Since bird pollination plays an important role on the Hawaiian Islands, plant species were classified as bird-pollinated or non-bird pollinated based on literature reports and/or floral syndrome. A complete list of plant species used in this study and information on their origin (endemic, indigenous or introduced) and their typical pollinators is given in Appendix C.

In order to determine the species pool of ants in the area, underneath every plant or, in dense clusters of plants, every 5 m, pieces of cardboard were laid out baited with sucrose solution (50 % w/w). After approximately one hour, all baits were checked and number and species of ants on each of the baits were recorded. In cases where two or more ant species shared bait, interactions between these species were noted (i.e. which species defended the resource against another species).

Quantification of residual interaction strength

Because we were interested in traits that promote or prevent interactions between ants and flowers, we focused our analysis to the residual interaction strength (i.e. the degree to which ants interact more or less often than expected with flowers from particular plant species) after accounting for a null model. We generated the null model prediction based on

two assumptions: (1) In the absence of mechanical or chemical barriers, preferences and constraints, ants distribute themselves proportionally to the sugar supply of the different plant species they encounter in a given habitat (optimal foraging, (Taylor 1977, Bonser *et al.* 1998). (2) Ant species composition on sugar baits reflects their potential composition on flowers. This is supported by a study in an Australian tropical rain forest where nearly the same ant species composition was found on baits (Blüthgen and Fiedler 2004b) as on naturally occurring sugar sources like honeydew, EFNs and floral nectar (Blüthgen *et al.* 2004b).

In the interaction matrix, each link defines the interaction between an ant species i and a plant species j, and the total number of potential links in a site is $I \times J$, with I being the total number of ant species and J the total number of plant species. The expected relative proportion E_{ij} of each link between ant species i and plant species j of the total number of interactions would be $E_{ij} = A_i \cdot P_j$, with A_i as the proportional number of workers of species i among all I ant species visiting the sugar baits, and P_j as proportional amount of sugar offered by plant species j of all J plants at the site. Thus, at sites with one ant species i and several plant species j, $E_{ij} = P_j$. The deviation of the observed from the expected proportion of a given interaction was expressed as the residual $R_{ij} = O_{ij} - E_{ij}$, with O_{ij} as observed proportion of ant species i on plant species j of the total number of ants visiting flowers in the focal interaction network. R_{ij} thus ranges from -1 to 1, and $\sum_{i}^{I}\sum_{j}^{J} R_{ij} = 0$. Negative R_{ij} indicate that interactions occurred less frequently than expected, positive R_{ij} unexpectedly frequent interactions. Whether each R_{ij} significantly deviated from zero was tested by Monte Carlo statistics: We randomly assigned the same total number of ant individuals that were actually found on flowers in a given network to all possible links $I \times J$ one million times, with E_{ij} as the probability that each link ij is occupied. The randomly assigned values were compared to the observed numbers of ants in each link ij. When the observed number of ants in link ij overlapped with less than 5 % of the simulated values, it was regarded as significant. For each of the randomisations, we calculated the residuals in the same way as described above. Additionally, we calculated the variance of the observed and randomized residuals $var(R_{ij})$ which expresses the overall deviation from the expected distribution of ants on flowers and thus the degree of specialisation within the habitat. Commands for R software (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) are available in Appendix D. In addition to R_{ij}, $R_i = \sum_{j}^{J} R_{ij}$ and $R_j = \sum_{i}^{I} R_{ij}$ were calculated which are the row and column totals of each ant

species *i* and plant species *j*, respectively, and denote the total deviance from the expected contribution of the species within the other species in the same trophic level in each network. We compared R_i of the three different ant subfamilies (Dolichoderinae, Formicinae and Myrmicinae) and R_j of plants that are endemic, indigenous or introduced to the Hawaiian Islands using an ANOVA. Prior to statistical analysis, values were transformed to meet requirement of normality: $R'_i = s_i \cdot \log(|R_i|+1)$ where s_i maintains the original sign of R_i, thus $s_i = +1$ if $R_i > 0$ and $s_i = -1$ if $R_i < 0$. The same applies to R_j. We performed the ANOVA including data only from networks with at least two subfamilies of ants (for R_i) or plants with at least two different origins (for R_j). Furthermore, we tested the influence of the "pollination syndrome" (bird vs. insect pollination) on the residuals R'_j of plant species with a *t*-test. Note that flowers assigned as bird-pollinated are additionally visited and potentially pollinated by insects.

Comparison to other oceanic islands and continents

The proportion of ant-visited flowering plant species within each of the Hawaiian networks was compared to other flower-visitor networks that included ants elsewhere. Additional to published networks known to the authors, further datasets were found online using appropriate search terms or were provided by colleagues. For each network, the proportion of plant species that were visited by ants was quantified. Studies without ants where omitted from the analysis. Prior to statistical analysis, percentage data were arcsin-transformed in order to stabilize the variance.

Olfactometer trials

Ants' responses to floral scents were examined in a mobile olfactometer which allowed behavioural assays in the field with unpicked flowers and free living ants (Junker *et al.* 2010). A battery driven electric pump (Thomas Gardner Denver, G 24/08 30W) produced an airstream of filtered air that was cleaned and humidified in charcoal and distilled water. The airstream supplied four flowmeters (Analyt-MTC, 112-08SA) that led the airstream to spiral Teflon tubes (PKMSA, CH) and regulated it to 100 ml min^{-1}. Flower stems were swathed with Teflon tape (PTFE) and one side of an oven bag (Toppits, Melitta Haushaltsprodukte GmbH & Co. KG, Minden, Germany) was tightly affixed at the Teflon tape using masking tape. The other open end of the oven bag was then pushed through a cut top-part of a washing flask and a Teflon washing flask topping was pressed into the overlapping oven bag resulting in a tight connection between the Teflon tubes coming from the flowmeter and the oven bag which thereupon inflated itself. The whole assemblage was held

in place by a post and a laboratory clamp. Another Teflon tube attached to the washing flask topping supplied a four-field arena with scented air. Usually, two separate flowers / inflorescences of an individual plant where used as the scent source for the two scented air-fields within the arena. In exceptional cases the scent of one flower / inflorescence was split into two Teflon tubes. Scented air was pumped in two opponent fields of the arena, the remaining two fields where supplied with neutral, unscented air. The four-pointed star-shaped arena (length: 200 mm from tip to tip, depth: 10mm) was modified after Petterson (1970) and Vet (1983) and was similarly used by Junker *et al.* (2008, 2010). The arena allowed creation of four distinct odour fields and was manufactured from a single Teflon block. Air left the arena at a central hole. The whole olfactometer setup was fitted in a wheeled aluminium box for its application in the field (Appendix E). For the tests, six ants were caught on sugar baits and were placed in the arena. After 60, 90, 120 and 150 s number of ants in scented and neutral fields were counted. 150 s intervals were repeated twelve times with each ant / plant combination and with different sets of ants and data from each interval were condensed to a mean number of ants in the scented fields. These values were used to calculate a response index $Q_{ij} = \frac{2(N_{obs} - N_{\exp})}{N_{total}}$, with N_{obs} = number of ants in scented fields; N_{exp} = expected number of ants in each field assuming random choices, i.e. 50 % of tested animals; and N_{total} = total number of ants tested. Like R_{ij}, Q_{ij} varies between -1 (repellence) and 1 (attraction). Scented and neutral fields were altered after each 150 s interval to compensate for potential side preferences. All parts of the olfactometer that had contact to floral scents and ants were thoroughly cleaned with hexane and acetone. Oven bags were used only once. Olfactometer tests were performed for selected ant-plant pairs (*ij*), including the most common ant- and plant species. For several plant species, the response of two or more ant species was examined. For statistical analysis of the ants' responses, Q_{ij} values were transformed in the same way as described above. In order to compare responses to plants of different origins, the mean value of response indices Q_{ij} of different ant species to each plant species was taken for the ANOVA. In cases where ant species *i* encountered plant species *j* in two or more different communities, we tested the interaction only once but used Q_{ij} in all communities for analysis. In four different habitats, we tested the responses of *Linepithema humile* and *Pheidole megacephala* to the floral scent of *Metrosideros polymorpha* in order to compare different populations. The responses Q_{ij} were similar and did not change signs: -0.16 ± 0.06 for *L. humile* (mean ± standard error, ANOVA: $F_{3,56} = 1.4$, $p = 0.26$) and -0.07 ± 0.01 for *P. megacephala* ($F_{3,56} = 0.09$, $p = 0.96$).

Volatile collection and analysis

Scent samples were taken from the same flower individuals as used in the olfactometer trials. Additionally, further plant species that were not included in the ant-flower networks were used for additional olfactometer trials with *Pheidole megacephala* workers and scent sampling. After each olfactometer trial, the oven bag was closed with masking tape and scent was either immediately sucked through a volatile trap or scent first accumulated in the oven bag and was than sucked through a volatile trap using a battery driven pump (Method and sampling time is given in Appendix F). Scent traps consisted of microvials (Varian, Darmstadt, Germany) from which the bottoms were removed and which were filled with a mixture of 1.5 mg Tenax-TA (mesh 60-80) and 1.5 mg Carbotrap (mesh 20-40). Microvials with trapped scents were frozen at -20°C as soon as possible and stored in glass vials until further use.

Scent samples were analysed using a Varian 3800 gas chromatography fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane; length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 µm; Phenomenex) and a Varian Saturn 2000 mass spectrometer. Scent traps were placed into the injector port of the GC by means of the ChromatoProbe kit (Amirav and Dagan 1997, Dötterl *et al.* 2005). The injector split vent was opened, and the injector was heated at 40°C to flush any air from the system. After 2 min the split vent was closed and the injector heated at 200 °C min^{-1}, then held at 200 °C for 4.2 min, after which the split vent was opened (1/20) and the injector cooled down. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.8 ml min^{-1}). The GC oven temperature was held for 7 min at 40 °C, then increased by 6 °C min^{-1} to 260 °C and held for 1 min at this temperature. The mass spectra were taken at 70 eV with a scanning speed of 1 scan s^{-1} from m/z 30 to 350. To identify the floral scent compounds of the GC-MS spectra, the data bases NIST 08, Wiley 7, Adams (2007), and MassFinder 3 were used, and identifications were confirmed by comparison of retention times with published data (Adams 2007). Identification of some compounds was also confirmed by comparison of mass spectra and retention times with those of authentic standards. We estimated total scent emission (absolute amount) by injecting known amounts of monoterpenoids, benzenoids, and fatty acid derivatives. The mean response of these compounds (mean peak area) was used to determine the total amount of each compound available in the samples (Dötterl *et al.* 2009).

For statistical analysis, mean amounts of individual substances were taken in cases of repeated scent sampling of plant species and emission was standardized to one hour. We tested three alternative hypotheses in search for patterns explaining ant-repellence. (a)

Firstly, we tested whether the total hourly emission of the flowers was correlated to the mean values $\overline{Q}_{ij}$ of several ant species or Q_{ij} of *Pheidole megacephala* ants that were used for tests with most plant species. (b) We secondly tested whether response index Q_{ij} correlates with the amount [ng h^{-1}] of individual substances within floral scent bouquets. For correlations we used individual substances only if they were emitted by at least seven plant species. For responses, we either used the mean values $\overline{Q}_{ij}$ of several ant species or Q_{ij} of *Pheidole megacephala* ants. (c) We thirdly tested whether the floral scent composition of plant species that share certain features are separated from groups of plants with different features. The features we tested included the significant repellence against at least one ant species, the presence of mechanical barriers and the origin of the plant species, i.e. whether they are endemic, indigenous or introduced to the Hawaiian Islands. Most individual substances were emitted by one or few plant species only, thus we grouped the compounds according to their biosynthetic pathways and the presence or absence of a functional group: benzenoids (B), fatty acid derivates (FAD), monoterpenes (MT), oxidized monoterpenes (MTO), sesquiterpenes (ST), oxidized sesquiterpenes (STO) and others. We performed two non-metric multidimensional scalings (NMDS) based on Bray-Curtis distances, the first with quantitative data and the second with proportional data. Environmental vectors were fitted into the plots indicating the most rapid change in the indicated scent group (direction of vector) and strength of the gradient (length of vector). Environmental vectors were only included if the significance was $p < 0.1$.

Nectar accessibility and palatability

Using a micrometer, we measured the width of the nectar holder tube from three to 15 flowers per plant species and the width of the head capsules of 10 individuals of each ant species to the nearest 0.01 mm. The mean width of each flower was compared to the mean width of the head capsules of the ant species present in the respective habitat in order to assess the accessibility of nectar for the ants. In the di- or polymorphic species *Pheidole megacephala* and *Solenopsis* spp. we measured the width of the head capsule of the smallest caste. We never observed cases of nectar robbing, i.e. cases where ants bit holes in the perianth in order to access the nectar. In addition to narrow nectar tubes, we also checked for further mechanical barriers that could prevent the nectar consumption by ants. Furthermore, the nectar of some plant species was extracted with micro capillaries and small amounts were offered to the ants next to the sugar baits in order to test the palatability.

Phylogenetic Analysis

For the phylogenetic analysis of the plant species encountered in our study, we used internal transcribed spacer 2 (ITS2) sequences of the ribosomal cistron. Secondary structures of the ITS2 were included in the analysis to receive support for a broad range of taxonomic relationships (Keller *et al.* 2010). Sequences were obtained from GenBank (Benson *et al.* 2009) and delimited at the ITS2 database (Keller *et al.* 2009). For several plant species no ITS2 sequences were obtainable from GenBank. For these, we chose representatives of close relatives for a complete taxon sampling. The amount of sequences per species was dependent of the availability of complete sequences at the database. GenBank accession numbers and representative taxa are listed in Appendix G. Data analysis followed the method described in Schultz and Wolf (2009) and Keller *et al.* (2008) for secondary structure phylogenetics with the ITS2.

The ITS2 secondary structures of *Armeria villosa* (Caryophyllales), *Musa velutina* (Liliopsida), *Myoporum parvifolium* (Asterids) and *Sida fallax* (Rosids) were predicted with RNA structure 4.6 (Mathews *et al.* 2004). These structures served as templates for homology modelling for the remaining sequences of the respective taxonomic groups at the ITS2 database (Koetschan *et al.* 2009). Thus, each sequence in the data set was complemented with an individual secondary structure.

Sequences and secondary structures were automatically and synchronously aligned with 4SALE 1.5 (Seibel *et al.* 2008). 4SALE translates sequence-structure tuple information prior to alignment into pseudo-proteins. Pseudo-proteins were coded such that each of the four nucleotides may be present in three different states: unpaired, opening base pair and closing base pair. Thus, an ITS2 specific 12x12 scoring matrix was used for calculation of the alignment (Seibel *et al.* 2008).

To determine evolutionary distances between plant species simultaneously on sequences and secondary structures we used Profile Neighbor Joining (PNJ) as implemented in ProfDistS 0.98 (Wolf *et al.* 2008). The tree-reconstructing algorithm works similarly to the alignment method on a 12-letter alphabet with an ITS2-specific general time reversible substitution model. Profiles were automatically built for nodes with bootstrap support values (1000 replicates) above 70% or with at least 95% nucleotide identities. A profile is regarded as a sequence, although it is composed of probability distribution vectors instead of characters. PNJ was iterated until no more profiles can be defined. The resulting tree was displayed with FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and further refined with Adobe Illustrator CS4 (Adobe Corporation, San Jose, CA).

The resulting Neighor-Joining distance matrix was compared with distances of species-specific mean nectar volume [µl], mean nectar sugar concentrations [% w/w], nectar holder tube width and mean residual R_j values. Distances based on a single variable were standardized between 0 and 1 as $D = |x_i - x_j| / (x_{max} - x_{min})$, where x_i and x_j represent the value for species *i* and *j*, x_{max} and x_{min} the maximum and minimum value of all species, respectively. Mean evolutionary distances were used for taxa with multiple sequences in the analysis. Matrices were compared using a Mantel test (Spearman rank correlation, 10000 randomisations). Distances were additionally calculated for the ants' responses to floral scents and amount of total hourly emission per dry weight [ng h^{-1} g^{-1}]. Bray-Curtis distances were calculated for floral scent composition both with quantitative data and proportional data. Since this information was not available for all plant species, we compared this matrix with a subset of the evolutionary distance matrix including only those plant species used for olfactometer trials.

Results

Ant-flower networks and residuals

In total, we screened 21,940 flowers of 39 species in ten habitats for ant visits. The flowers of 24 species were visited by a total of 1,635 ants from 12 species; on the remaining 15 plant species we never observed any ant. Five additional ant species visited sugar baits, but we did not observe them on flowers. Twelve plant species were endemic to the Hawaiian Islands (10 of them visited by ants), ten were indigenous (7 visited by ants) and 17 were introduced (7 visited by ants). The introduced species are native to continental regions where they have shared an evolutionary history with rich ant faunas. Forty-four of 194 potential interactions between plants and ants were recorded (Appendix H).

The proportion of plant species with ant-visited flowers varied between 33.3 and 100 % in the ten communities and was strongly and positively correlated with the proportion of endemic plant species in a habitat (see below). The total deviation from the expected visitation pattern expressed by the variance of the residuals *var* (R_{ij}) was less pronounced in habitats with a high proportion of endemic plant species (exponential regression: $R^2 = 0.79$, $df = 9$, $p < 0.01$) indicating that ants distributed more disproportional to the available resources in habitats dominated by introduced plant species. The variance of the randomised residuals, however, was independent of the plant species composition in the habitats ($R^2 < 0.001$, $df = 9$, $p = 0.49$). Thirty-three percent of all residuals R_{ij} deviated significantly from zero (i.e. number of observed ants in link *ij* overlapped with less than 5%

of the simulated ones): 14.4 % of all potential interactions occurred significantly more frequently than expected, 18.6 % occurred less frequently (Appendix H). In two of the observed sites, ants were distributed proportional to the resources offered by the plant species, i.e. all residuals R_{ij} did not significantly deviate from zero. In both sites only *Linepithema humile* ants and plants native to the Hawaiian Islands were present (Tab. 1, #3 and #5). On average, R_j values of endemic and indigenous plants were positive, while those of introduced plants were negative in sites where plants of at least two origins were present (ANOVA: $F_{2,43}$ = 3.7, p = 0.03, Fig. 2), indicating that flowers of endemic and indigenous plants are favoured over flowers of introduced plants. Across ant subfamilies, R_i values of Dolichoderinae were positive (mean ± SE R_i = 0.38 ± 0.12), while those of Formicinae (-0.01 ± 0.12) and Myrmicinae (-0.18 ± 0.07) were negative (ANOVA: $F_{2,25}$ = 7.3, p = 0.003), i.e. dolichoderines used floral nectar as resource more intensely than expected by their relative abundance in each habitat.

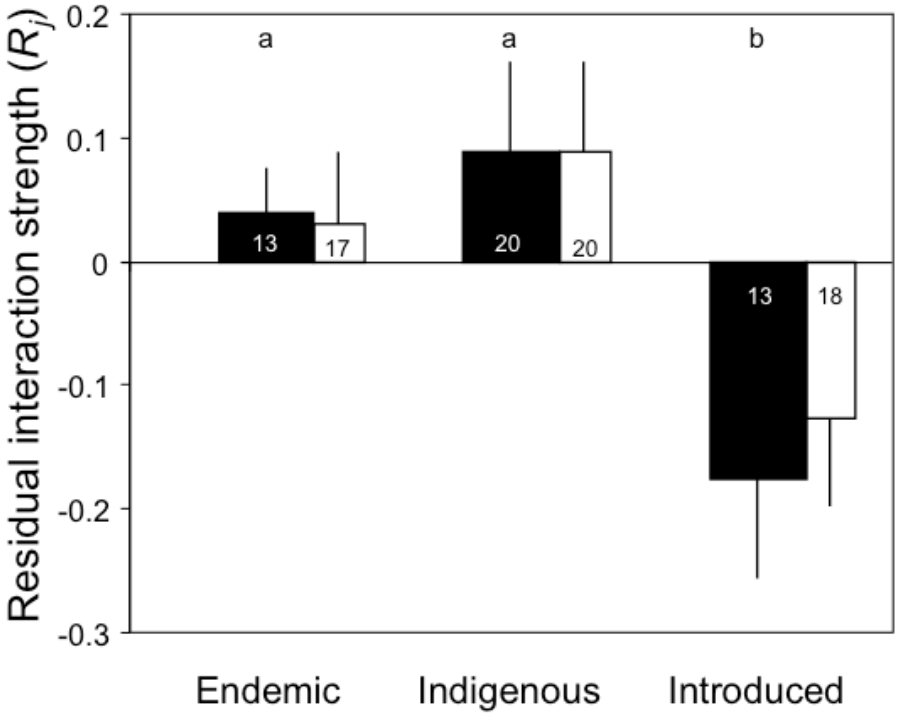


Fig. 2 Residuals R_j of plant species that are endemic, indigenous and introduced to the Hawaiian Islands. Shown are mean and 95 % confidence intervals. Black bars denote R_j from plant species that were sympatric to at least one species of a different origin (i.e. endemic, indigenous or introduced). Letters indicate significant differences according to pairwise *t*-tests. Difference between indigenous and introduced plants remained significant after *p*-values were Bonferroni-corrected. White bars denote R_j from all plants in our study regardless the plant community. Sample size of each group is given in bars.

The "pollination syndrome", i.e. bird-pollinated plants (13 species, R_j values = mean ± *SE*: -0.09 ± 0.06) or insect-pollinated plants (26, 0.05 ± 0.05), did not significantly influence R_j values (Welch two sample *t*-test: $t_{36.3} = 1.6$, $p = 0.11$).

We rarely observed interactions between ant species although baits were occasionally shared by two ant species. *Solenopsis geminata* displayed aggression against *Ochetellus glaber*, *Paratrechina vaga* against *Technomyrmex albipes*, *Tetramorium tonganum* against *P. vaga* and *Pheidole megacephala* against *Plagiolepis alluaudi*. However, we never saw similar interactions on flowers. Thus it is unlikely that inter-specific aggressions on flowers influenced immediate foraging decisions, but they may still have long-term effects on the network pattern.

Comparison to other oceanic islands and continents

We compared the Hawaiian ant-flower networks to ten flower-visitor networks from continents and 15 from other islands (Appendix I). On average, the proportion of ant-visited flowering plant species within each network was lower on continents and other islands than in Hawaii (ANOVA: $F_{2,32} = 6.9$, $p < 0.01$, Fig. 3). Within the Hawaiian networks, those with no or only a low proportion of endemic plant species had a similarly low proportion of ant-visited flowering plants as networks on islands in other parts of the world (Fig. 3). The proportion of ant-visited plants increased linearly with the proportion of endemic plant species occurring in the networks (Pearson's $R^2 = 0.93$, $df = 8$, $p < 0.001$, Fig. 3). The proportion of ant-visited flowering plants was independent on the size of the network, i.e., product of ant and plant species (Pearson's $R^2 = 0.06$, $df = 33$, $p = 0.17$, Fig. 3)

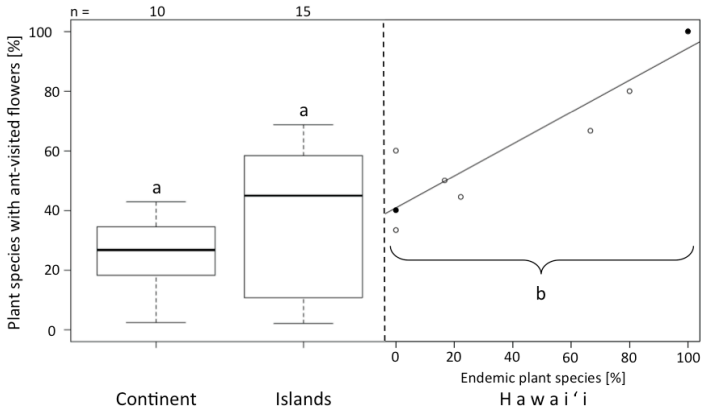


Fig. 3 Proportion of plant species with ant-visited flowers in Hawaii and other on islands or continents. Letters indicate significant differences according to pair wise *t*-tests with arcsin transformed data. Differences remained the same after Bonferroni-correction. In the Hawaiian networks, proportion of plant species with ant-visited flowers is shown as a function of the proportion of endemic plant species within the networks. Closed circles denote to two overlapping points. Sample size is given above box plots.

Olfactometer trials

In total, we performed 46 olfactometer trials (n = 771 individual trials with 6 ants each) where we tested the response of nine ant species to floral scents of nine endemic, eight indigenous and eight introduced plant species (Appendix J). Ants showed 34 negative (10 of them significantly) and 12 positive (none of them significantly) responses (Q_{ij}). On average, introduced plant species emitted volatiles that were stronger ant repellent than those of endemic and indigenous species (ANOVA: $F_{2,21}$ = 4.0, p = 0.034, Fig. 4). *Pheidole megacephala* was the most abundant ant species observed; hence we tested their responses to 16 plant species. In these trials, we found the same distinction between plant origins albeit only marginally significant (ANOVA: $F_{2,13}$ = 3.1, p = 0.08, Fig. 4). Residuals R_{ij} were positively correlated with the response index Q_{ij} (Pearson's R^2 = 0.13, df = 53, p < 0.01), suggesting that olfactory cues influence foraging decisions of ants.

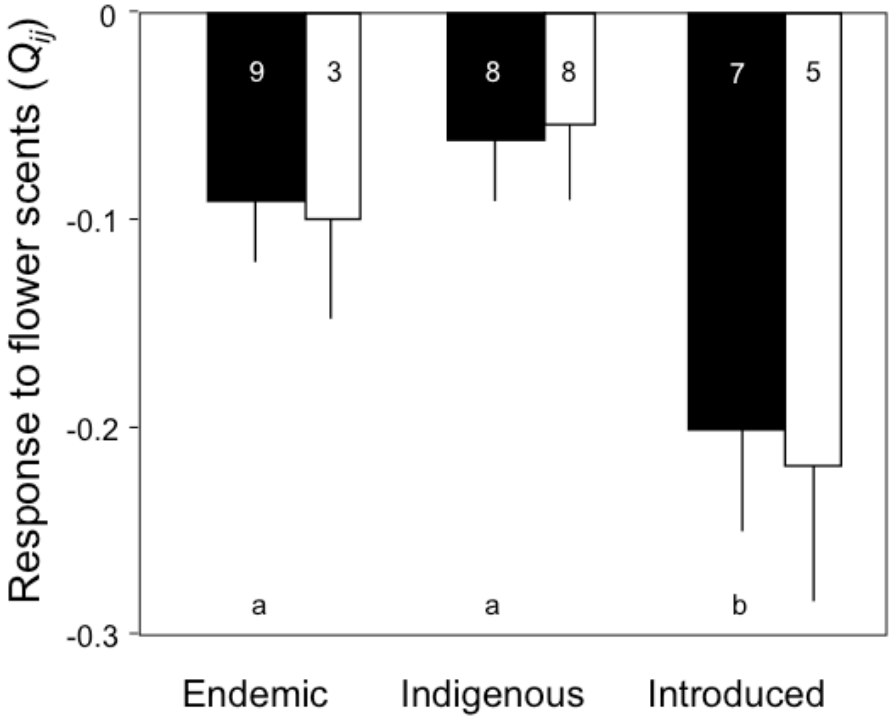


Fig. 4 Response indices Q_{ij} of ants toward the floral scent of plant species that are endemic, indigenous and introduced to the Hawaiian Islands. Shown are mean and 95 % confidence intervals. Black bars denote response indices from olfactometer trials with various ant species. In cases where plant species were tested with two ore more ant species, the mean value of Q_{ij} was taken (total number of olfactometer trials: 46). Letters indicate significant differences according to pair wise t-tests. Differences between indigenous and introduced plants remained significant after p-values were Bonferroni-corrected. White bars denote response indices from trials with *Pheidole megacephala* only. Sample size of each group is given in bars.

Volatile collection and analysis

In 29 analysed floral scent bouquets, we found a total of 222 different substances, most of them were emitted by one plant species only (median = 1), 30 substances occurred in seven or more floral scent bouquets. We did not find a consistent pattern that explained the qualitatively different responses towards scents by ants: (a) Total amount of hourly emission did not influence the ants' average responses $\overline{Q}_{ij}$ or the responses Q_{ij} of *Pheidole megacephala* (Pearsons $R^2 \leq 0.05$, $df = 27, 22$, $p \geq 0.27$). (b) We did not find an individual floral scent compound that explained the variance of Q_{ij} in *P. megacephala* and only one out of 30 that was correlated to the mean ant species' responses $\overline{Q}_{ij}$: the amount of an unidentified sesquiterpene was negatively correlated to $\overline{Q}_{ij}$ (Pearsons $R^2 = 0.70$, $df = 6$, $p = 0.0097$, but note the Bonferroni-corrected $\alpha = 0.05/30 = 0.0017$). (c) After grouping the scent compounds to their biochemical pathway and the presence or absence of functional groups, the compositions of floral scent bouquets of plants that repelled ants were not different from those that did not, the same is true for plant species with mechanical barriers and for plants endemic, indigenous and introduced to the Hawaiian Islands: non-metric multidimensional scaling revealed no patterns, neither for the quantitative data (Fig. 5 a) nor for the proportional data (Fig. 5 b). Total hourly emission and emission of substances from different classes of compounds are given in Appendix F.

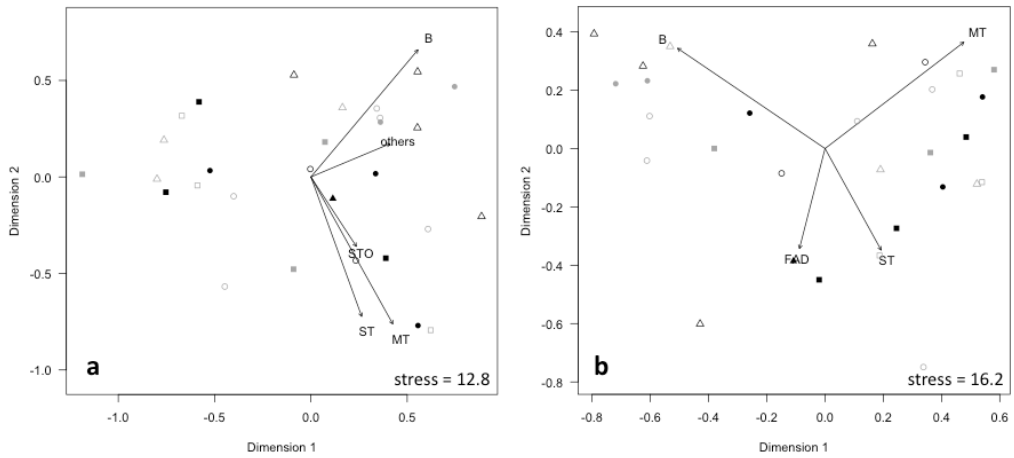


Fig. 5 Non-metric multidimensional scaling of floral scent bouquets based on quantities of compounds originated from different biosynthetical pathways (a) and their proportion within the composition (b). Circles denote for scent compositions of endemic, triangles for indigenous and squares for introduced plant species. Closed symbols represent floral scent bouquets that significantly repelled at least one ant species; open symbols those that did not. Grey symbols denote plant species with morphological barriers (or those with an unclear morphology), black symbols represent plant species without morphological barriers. Environmental vectors are included for cases with $p < 0.1$: B = benzenoids, FAD = fatty acid derivates, MT = monoterpenes, ST = sesquiterpenes, STO = oxidized sesquitepenes.

Nectar accessibility and palatability

Narrow nectar tubes prevented nectar access in 32.2 % of all possible interactions, i.e. the head capsules were broader than the tubular width. While most flowers of endemic (78.9 %) and indigenous (72.6 %) plant species granted access to ants by broad nectar tubes, the floral nectar of only 42.2 % of introduced plant species was available to ants that occurred in the same habitats (χ^2 = 18.5, df = 2, p < 0.001, Fig 6, Appendix J). Apart from narrow nectar tubes, we found three cases of rather unusual mechanical barriers: (1) The calyx of *Plumbago zeylanica* (Plumbaginaceae) possessed very sticky glandular hairs that effectively function as barrier for crawling insects. (2) The calyx of *Abutilon eremitopetalum* (Malvaceae) was covered with dense, fine hairs that prevented ants from reaching the nectaries of these flowers. However, stamens, stigmas and petals of these flowers were often connected with leaves of the same plant or other parts of the surrounding vegetation, resulting in ant visits and associated nectar theft. (3) The inflorescence stalk of *Russelia equisetiformis* (Scrophulariaceae) deterred / repelled ants: *Pheidole megacephala* ants avoided walking on these stems (median = 0 ants min^{-1}) while they readily climbed control sticks (median = 1 ants min^{-1}) in a bioassay (Wilcoxon signed rank test: V = 21, n = 7, p = 0.02). The presence of mechanical barriers (including narrow nectar tubes and the three mechanisms described) effectively suppressed ant visits to floral nectar. Correspondingly, on average, links between ants and flowers where the head capsules were broader than the width of the nectar tube received negative residuals R_{ij} (-0.035 ± 0.009, mean ± se) while the others received positive ones (0.017 ± 0.016, t-test: t_{175} = 3.1, p < 0.01). In five cases, however, one or few ants were recorded on flowers despite a predicted mechanical barrier but it remains unclear whether they reached the nectaries.

Endemic, indigenous and introduced plant species strongly varied in volume and sugar quality of the floral nectar and in the average number of ants per flower (Tab. 2). However, the difference in ant visits on flowers could not be explained by any of the nectar features (Tab. 2; Spearman rank correlations: $R \leq 0.23$, n = 55, $p \geq 0.1$). In contrast to olfactory and mechanical mechanisms that may effectively exclude ants from flowers, unpalatable nectar explained only in five out of 43 cases tested negative residuals R_{ij}. The nectar of one endemic (*Gardenia brighamii*, Rubiaceae), two indigenous (*Myoporum sandwicense*, Scrophulariaceae and *Osteomeles anthyllidifolia*, Rosaceae) and one introduced (*Saraca asoca*, Fabaceae) plant species was not consumed by the ant species the nectar was offered to (Appendix J).

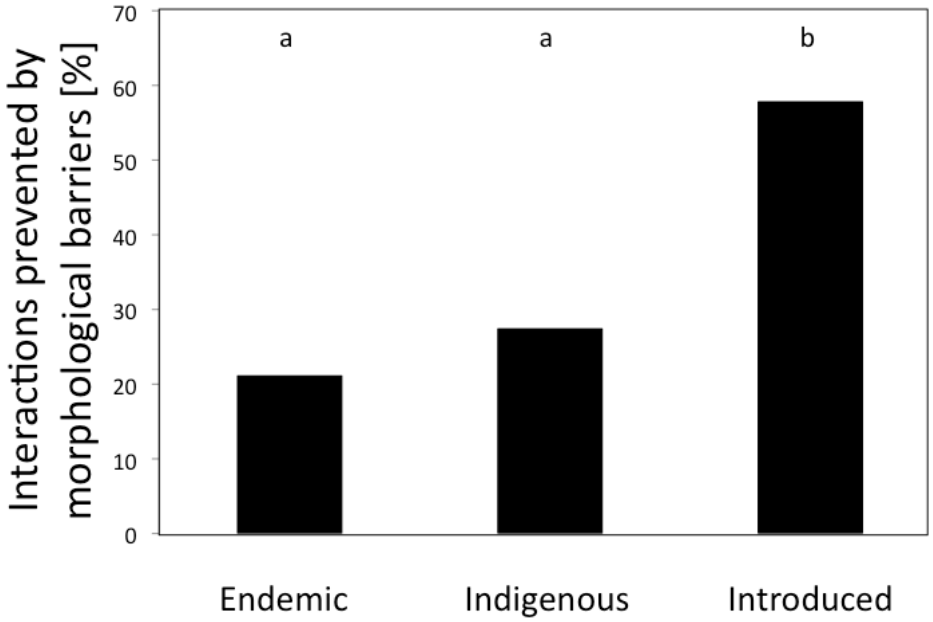


Fig. 6 Proportion of links between ants and flowers of plants that are endemic, indigenous or introduced to the Hawaiian Islands that are prevented by mechanical barriers. Letters indicate significant differences according to pair wise *Chi²* tests.

Table 2 Nectar features and average number of ants per flower of endemic, indigenous and introduced plant species. Median and interquartile range and results of Kruskal – Wallis ANOVA are shown. Significant values are bold.

	Volume [µl]	**Mass % w/w**	**Volume * Mass %**	**Ants flower^{-1}**
Endemic	8.3, 13.9	17.2, 8.8	1.1, 2.6	0.12, 1
Indigenous	0.6, 0.9	23.6, 22.7	0.1, 0.2	0.03, 0.1
Introduced	2.0, 5.1	18.2, 8.3	0.3, 1.1	0, 0.3
χ^2	13.5	5.6	8.4	6.9
p	**< 0.01**	0.06	**0.015**	**0.032**

Trade off between repellent floral scents and morphological barriers

We found evidence for a trade-off between repellent floral scents and morphological barriers across introduced plant species (logistic regression: $R^2 = 0.45$, $df = 13$, $p < 0.01$, Fig. 7), i.e. many flowers possess either one or the other defensive mechanism. Among flowers of indigenous plants, we did not find such a trade-off ($R^2 = 0.014$, $df = 13$, $p = 0.34$, Fig. 7). For endemic species, we even found a highly significant opposite trend ($R^2 = 0.56$, $df = 17$, $p < 0.001$, Fig. 7). However, this result was strongly influenced by *Hibiscus brackenridgei* subsp. *brackenridgei* (Malvaceae) with both repellent scent and morphological barrier. Apart from this species, only *Nama sandwicensis* (Hydrophyllaceae) possessed morphological barriers among the endemic plants in our study.

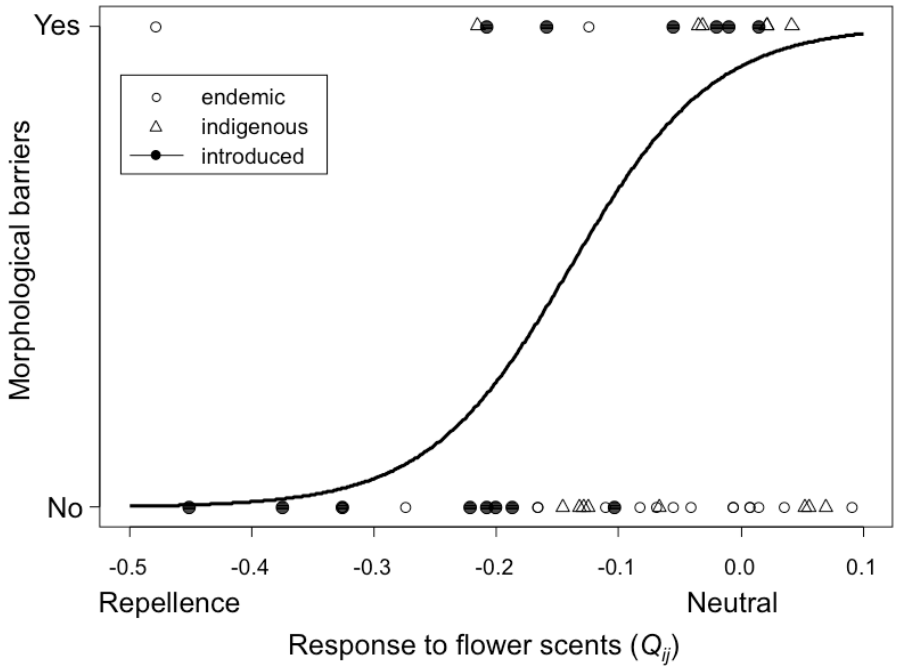


Fig. 7 Trade off between repellent floral scents and morphological barriers. Negative Q_{ij} values indicate repellence, positive attraction. Morphological barriers are either present (1) or absent (0) from the flowers. Trait combination for endemic (open circles), indigenous (open triangles) and introduced plant species (filled circles) and logistic regression for introduced plant species is shown.

Phylogenetic Analysis

The phylogenetic analyses resulted in a Neighbor-Joining tree (Fig. 8) that is comparable to the current classification presented at the NCBI taxonomy database (Sayers *et al.* 2009). Major clades were clearly separated, supported by high bootstrap values. Evolutionary relationships between orders were in some cases not well resolved so that inter-order relationships should be regarded with caution (e.g. the clustering of the three clades Ericales, Asterales and Lamiales).

The phylogenetic analysis shows that the endemic, indigenous and introduced plant species in our study are polyphyletic groups, which was also true for the floral features that may or may not promote ant visits (Fig. 8). Distances of mean sugar concentrations in the nectar [% w/w] and nectar volume per flower correlated with the evolutionary distances of the plant species (Mantel statistic $R \geq 0.12$; $p \leq 0.04$). Traits related to protection against ants featured by the plants included in our study (including R_j values, nectar holder tube width and responses to floral scents Q_{ij}) were independent of the evolutionary signal ($R \leq 0.05$; $p \geq 0.23$). Total hourly floral scent emission and the distances of the scent composition (quantitatively and proportionally) did not correlate with phylogenetic distances, too ($R \leq 0.1$; $p \geq 0.54$).

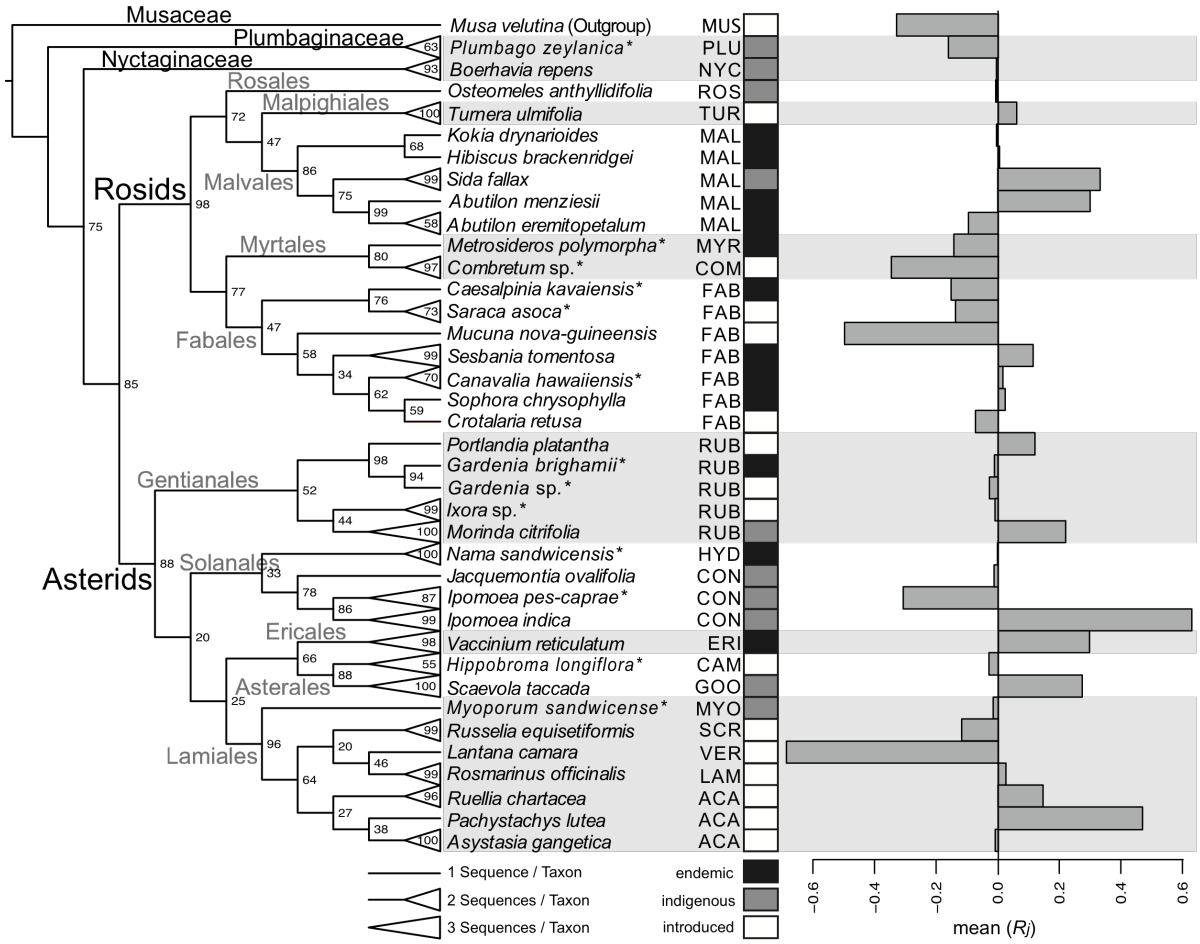


Fig. 8 Evolutionary relationship of plants species encountered in the habitats studied as revealed by Profile Neighbor Joining with ITS2-RNA sequences and structures. Given are bootstrap values (1000 replicates), order (according to NCBI taxonomy (Sayers *et al.* 2009)), family[1] and origin (endemic, indigenous and introduced) of each plant species. Additionally, mean residuals R_j are given. Plant species that were substituted by representatives for the phylogenetic analysis are marked with asterisks (s. Appendix G). Species with more than one sequence in the analysis were collapsed in the tree for clarity.
ACA = Acanthaceae; CAM = Campanulaceae; COM = Combretaceae; CON = Convolvulaceae; ERI = Ericaceae; FAB = Fabaceae; GOO = Goodeniaceae; HYD = Hydrophyllaceae; LAM = Lamiaceae; MAL = Malvaceae; MUS = Musaceae; MYO = Myoporaceae; MYR = Myrtaceae; NYC = Nyctaginaceae; PLU = Plumbaginaceae; ROS = Rosaceae; RUB = Rubiaceae; SCR = Scrophulariaceae; TUR = Turneraceae; VER = Verbenaceae.

Greater rates of ant visitation in endemic species than in introduced species was observed within the Fabaceae, the only plant family were representatives of both endemic and introduced species were available in sufficient replication: The residuals R_{ij} of endemic Fabaceae-species were 0.00 ± 0.01 (mean ± se, n = 18), those of introduced species -0.06 ± 0.02 (n = 11, *t*-test: $t = 3.0$, $p < 0.01$).

Discussion

Our four hypotheses about ant-flower interactions in Hawaii and the underlying mechanisms were confirmed: (1) Ants visited flowers of plant species endemic or indigenous to the Hawaiian Islands more frequently than those of introduced plant species. This was evident on the link and community level: Introduced plants were visited by no or few ants per flower and received negative residuals R_j, while flowers of indigenous and endemic plants were visited by more ants and therefore received positive residuals R_j. Furthermore, in communities with a higher proportion of endemic species, a higher proportion of all plants within the community had ant- visited flowers and the variance of the residuals $var(R_{ij})$ was close to zero in these communities, where ants were distributed across the flowers as expected by the amount of nectar and the ants' abundances. The proportion of plant species with ant-visited flowers in communities that were dominated by endemic plant species was not only exceptionally high compared to other Hawaiian habitats with few or no endemic plant species but also compared to other ecosystems on other oceanic islands or on continents. (2) The poor visitation of introduced plants was the result of more efficient defence mechanisms. Repellent floral scents, morphological barriers and, to a lesser extent, unpalatable nectar each explained negative residuals R_{ij} of ant-flower interactions. (3) We confirmed a trade-off between different floral defence mechanisms as suggested by Willmer *et al.* (2009) for introduced plant species that often prevented ants from visiting their flowers either by repellent floral scents or by morphological barriers. We did not find such a relationship in endemic and indigenous plant species, which overall showed little evidence of floral defences. (4) The distribution of plants whose flowers were heavily exploited by ants (positive R_{ij}) among the taxa was found to be independent of the phylogenetic classifications. Therefore, the different susceptibility to floral ant visits of native and introduced plant species was not a result of an inadvertent selection of a phylogenetically narrow or isolated group of study species in this study. Floral defences against ants are more likely convergently lost in response to prior absence of ants in native Hawaiian ecosystems. In contrast, nectar features (volume and sugar concentration) correlated with the phylogenetic signal.

Ants are dominant components of many ecosystems and interact with other organisms of all trophic levels with varying net effects (Lach *et al.* 2010). Many of those interaction partners adapted to the ecological importance of ants, either by evolving traits that intensify mutualistic interactions (Heil and McKey 2003) or by traits that reduce or even prevent interactions where ants have negative effects (Rico-Gray and Oliveira 2007).

Myrmecophytic Acacias for example have an ambivalent relationship with ants: they benefit from ants patrolling on their foliage, flower buds and fruits but also profit from keeping ants away from flowers (Willmer and Stone 1997). They succeed in both tasks by offering food and housing to ants (Heil and McKey 2003) and by repelling them from flowers during anthesis (Willmer and Stone 1997, Ghazoul 2001, Willmer *et al.* 2009). Ant repellent floral scents were documented, or at least suggested, for many plant species from many different regions on nearly all continents (Willmer and Stone 1997, Ghazoul 2001, Junker *et al.* 2007, Junker and Blüthgen 2008, Willmer *et al.* 2009). The examples involve different plant life forms, not only myrmecophytes or other plants with a tight relationship to ants. Our result that ants heavily exploit nectar of Hawaiian plant species, while introduced plants that share an evolutionary history with ants are not as affected by these antagonists, suggests that the presence of ants had selected for floral traits that protect this valuable resource (including morphology, scent and nectar features). Accordingly, the ants' selective influence on floral morphology has been suggested in several studies (Herrera *et al.* 1984, Galen 1999, Galen and Cuba 2001, Galen and Geib 2007). Galen and Cuba (2001), for example, showed that flowers of *Polemonium viscosum* in populations with high densities of nectar-thieving ants are morphologically better defended against these antagonists than flowers in populations with low densities of ants. A similar relationship was suggested for scent-morphs of the same plant species (Galen 1983).

The historic lack of ants in Hawaii is thus likely to be a possible evolutionary cause of ant accessible flowers. However, the fauna of Hawaii lacked – next to ants – also other social hymenopterans and other groups of insects that are common flower visitors in other parts of the worls and their absence may have also contributed to the lack of certain floral features. Furthermore, the vacant functional niche of insect-pollinators was often filled by nectarivorous birds (Lammers and Freeman 1986, Gardener and Daehler 2006). A shift from insect to bird pollination may result in changes of floral traits such as scent (Raguso and Pichersky 1995) or morphology (Wilson *et al.* 2004). In our study, however, bird pollinated flowers were not more heavily exploited by ants than insect pollinated flowers, suggesting that the unusual commonness of bird-pollination in Hawaii did not bias our conclusions.

Our study clearly confirms the findings of Junker and Blüthgen (2008) and Willmer *et al.* (2009) that floral odours often repel ants. However, our methodology using unpicked flowers as scent source, a controlled and constant air stream and the distribution of ants in scented and neutral fields within an olfactometer-arena to measure repellence instead of aggression against manually confronted odour sources (see Willmer *et al.* 2009) may be

even better suited to unequivocally reveal the repellent effect of naturally emitted floral scents. We furthermore demonstrated the ecological significance of the defensive function of floral scents by combining the olfactometer results in a community network analysis. We found, however, no evidence for ant attraction by floral scents. Despite the ants' qualitatively broad spectrum of responses to floral scents, we were not able to detect features of floral scent that are shared by ant-repellent bouquets: ant-repellence could not be attributed to the total hourly emission, the presence of individual volatiles or the composition of substances deriving from different biochemical pathways with and without functional groups. We did not find a consistent ant-repellent "syndrome" as the composition of ant-repellent floral scent bouquets did not stand out against non-repellent scents suggesting that the composition is not crucial for defensive functions and. Similarly, the attractive function of floral scent compositions is often elicited by one or few substances within complex bouquets, especially learned responses often base on "key odorants" that are required to recognise a reinforced multi-component signal (Laloi *et al.* 2000, Dötterl *et al.* 2006, Riffell *et al.* 2009, Reinhard *et al.* 2010); but note that naïve responses may be more pronounced towards blends of volatiles instead of individual substances (Stringer *et al.* 2008). Several studies demonstrated that several individual substances strongly repel ants (Cane 1986, Kessler and Baldwin 2006, Junker and Blüthgen 2008, Junker and Blüthgen 2010a). Therefore, the presence of one ant-repellent substance in a relevant concentration within a complex bouquet may thus determine the ants' responses. These specific compounds may therefore be hard to determine within highly diverse compositions in a multivariate or correlative approach where most substances are emitted by one of few plants only. Potential additive or synergistic effects (Junker and Blüthgen 2008) may even more complicate the quest for patterns.

Altogether, this study emphasises that protection is an important function of floral traits (Kerner 1879, Irwin *et al.* 2004, Junker and Blüthgen 2008, Gomez *et al.* 2009, Hanley *et al.* 2009, Junker and Blüthgen 2010b, Junker *et al.* 2010), and that floral traits operate as filters allowing only a selection of floral visitors to access the rewards (Johnson *et al.* 2006, Stang *et al.* 2006, 2007, Raguso 2008a, 2008b). Defences may also involve expenses in terms of energetic costs of synthesizing chemical substances and forming special floral structures, or costs in terms of loosing potential pollinators that are also negatively affected by these traits. Thus, the trade-off between chemical and mechanical defence mechanisms observed in introduced plant species but not in endemic and indigenous species is also consistent with the hypothesis that ants are selective forces on floral traits.

It has been shown that introduced plants and flower visiting animals are well integrated in native interaction networks and often even outnumber the native competitors (Kato *et al.* 1999, Memmott and Waser 2002, Morales and Aizen 2006, Lopezaraiza-Mikel *et al.* 2007, Vila *et al.* 2009). The consideration of interactions between native and introduced flowering plants and antagonists (ants) that have not been present prior to their recent introduction provides novel insights in invasional processes. Flower visiting invasive ants can have devastating effects on the reproduction of native plants and their pollinators (Holway *et al.* 2002, Lach 2005, 2007, 2008a, b), suggesting that plants endemic or indigenous to the Hawaiian Islands are negatively affected by nectar feeding ants, while introduced plants remain largely unaffected. The success of an introduced species often depends on other non-indigenous species that promote their establishment (Simberloff and Von Holle 1999, Ricciardi 2005), e.g. introduced plants often rely on introduced pollinators (Richardson *et al.* 2000). In the Hawaiian scenario, the introduced plants may be indirectly facilitated by introduced ants due to their negative impact on the reproduction of native plants. Thus, the defensive traits featured by the flowers of introduced plants along with the introduction of ants in the same habitat may be disadvantageous for the heavily exploited natives. While the detrimental effects of ants on the Hawaiian arthropod community are well documented (Medeiros *et al.* 1986, Krushelnycky and Gillespie 2008), their effect on plant communities and their pollinators still needs to be assessed. However, the studies of Lach (2005, 2008b) in combination with our results on the visitation pattern of ants on flowers imply that ant impacts on the Hawaiian flora may be similarly detrimental.

IX. Repellent and attractive properties of floral scents influence microstructure of flower-visitor networks

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Summary

Network analyses provide insights into the diversity and complexity of ecological interactions and have motivated conclusions about community stability and coevolution. However, biological traits and mechanisms such as chemical signals regulating the interactions between individual species – the microstructure of a network – are poorly understood. We linked the responses of receivers (flower visitors) towards signals (flower scent) to the structure of a highly diverse natural flower-insect network. For each interaction, we define link temperature – a newly developed metric – as the deviation of the observed interaction strength from neutrality, assuming that animals randomly interact with flowers. Link temperature was positively correlated to the specific visitors' responses to floral scents, experimentally examined in a mobile olfactometer. Thus, communication between plants and consumers via phytochemical signals reflects a significant part of the microstructure in a complex network. Negative as well as positive responses towards floral scents contributed to these results, where individual experience was important apart from innate behaviour. Our results indicate that (1) biological mechanisms have a profound impact on the microstructure of complex networks that underlies the outcome of aggregate statistics and (2) that floral scents act as a filter, promoting the visitation of some flower visitors, but also inhibiting the visitation of others.

Introduction

Network patterns such as nestedness, connectance, and degree distribution have been described for numerous ecological networks (Bascompte and Jordano 2007). They serve as aggregate statistics for the entire web, i.e. reducing the diversity of species and interactions within a community to a single value or formula. Variation across networks in these metrics may result from the species' abundances, body sizes, phenology and other

factors (Vazquez *et al.* 2009a, Vazquez *et al.* 2009b) or reflects variation in the number of observations per species (Blüthgen *et al.* 2008) The mechanisms influencing each individual interaction between the species within a network, however, remain largely unexplained (Vazquez *et al.* 2009b). For a more thorough understanding of network topology, mechanisms affecting the link-level (interactions between species pairs) need to be considered, focusing on the question how biological traits can influence the interaction strength.

In a qualitative flower-visitor web, Stang, Klinkhamer and Meijden (2006) demonstrated that morphological mismatches represent a feasible mechanistic explanation of the absence of certain interactions related to biological traits. These size-related traits form a clear-cut threshold which divides the potential nectar consumers in those that are able and unable to reach the nectar with their mouthparts (Stang *et al.* 2007). However, visitation by pollen consumers and the remaining variation within the 'allowed' nectar-mediated interactions in an observed quantitative flower – visitor network must be due to other factors than morphology. Such factors may include primary metabolites, e.g. sugars and amino acids, in nectar (Gardener and Gillman 2002, Petanidou *et al.* 2006) and pollen (Roulston and Cane 2000), floral display size and form (Glaettli and Barrett 2008), and colour (Haslett 1989, Chittka and Menzel 1992, Whitney *et al.* 2009). Secondary metabolites in nectar or pollen may increase or decrease certain interactions (Adler 2000, Dobson and Bergström 2000, Raguso 2004, Kessler and Baldwin 2006), and scents are known to play an important role in attraction and repellence either innately or following associative learning (Knudsen *et al.* 2006, Junker and Blüthgen 2008, Raguso 2008b, Wright and Schiestl 2009, Junker and Blüthgen 2010b). In this study, we focused on the interplay between floral scents and their impact in the animals' behaviour as an explanation for the observed patterns, integrating biological signals and responses of receivers into network analysis to understand the interactions between trophic levels in a diverse community. Additionally, we used data from the literature to explore the role of floral colour and morphology.

Plant-animal interaction networks comprise a continuum from highly generalised to highly specialised assemblages. Flower-visitor networks are on average more specialised than plant-frugivore or nectar-mediated plant-ant relationships (Blüthgen *et al.* 2007) showing a high degree of species complementarity (flower partitioning) between visitor species. Flower fidelity shown by species or individuals of pollinators either by a high specialisation or by short-term specificity is crucial for a successful pollen transfer and may thus be promoted by natural selection (Blüthgen *et al.* 2007, Wright and Schiestl 2009). Different proximate mechanisms (floral filters) may enhance the visitation of some species

and / or prevent the visitation of others and thus lead to high flower partitioning and specialisation. For instance, it was shown that secondary metabolites in the nectar of *Aloe vryheidensis* prevent consumption by non-pollinating honeybees and sunbirds, but do not affect the visitation of pollinating birds (Johnson *et al.* 2006). Moreover, Raguso (2008b) hypothesised that olfactory cues may act as floral filter, but evidence is scant so far. It has been shown that floral scents serve as defence next to their well-known function as innate attractants or recognition cues for pollinators (e.g. Junker and Blüthgen 2008, Willmer *et al.* 2009, Junker and Blüthgen 2010b). The distribution of nectar-thieving ants on flowers could be explained by volatiles. Scents from ant-visited flowers did not affect the ants' behaviour in an olfactometer, but scent from flowers that were not visited by ants were repellent (Junker and Blüthgen 2008). The defensive and attractive properties of floral volatiles, along with the flowers' necessity to filter out the least beneficial visitors suggest that floral scents are important traits structuring interaction networks. In order to understand the mechanisms that influence the network structure, we used a new metric (link temperature) to quantify the interaction strength of each link – representing the network microstructure – and explored whether it is reflected by the animals' responses towards olfactory cues.

Material and Methods

Study site and organisms

We studied two temporally separated quantitative flower-visitor networks near the University campus of Würzburg, Germany (Fig. 1, Appendix K). The first survey (early June 2008) covered 41 hours, the second one (early July 2008) covered 26 hours where several people helped to collect flower-visitors within an area of 0.1 ha on a flower-rich fallow land. Two separate networks were established (one for each month). During random walks, all individual insects that visited flowers were recorded as well as the flower species on which they were found. Several individuals per known species and all unknown specimen were collected, sorted and identified to species level where possible with the help of experts (see Acknowledgements).

Network metric

Since we defined a network as a spatiotemporal entity, summarizing all interactions between insects and flowers recorded in a small area within a short time period, we can assume that all species can potentially interact. This allows us to focus on traits that shape the interactions apart from space and time. We defined a new metric (link temperature T_{ij}) to quantify the deviation of the observed interaction frequency between each species pair from an expected value predicted by a neutral model. This metric extends the network- and species-level indices recently established (H_2' and d_i', see (Blüthgen *et al.* 2006). All three metrics focus on the residual deviation of the interaction frequency from the neutral expectation (Blüthgen *et al.* 2008). If all species interacted randomly with the other trophic level in absence of any constraints, preferences or aversions (neutral model), the expected interaction strength would be $e_{ij} = \frac{A_i \cdot A_j}{m}$, where A_i and A_j are the total number of records of visitor species i and plant species j, respectively and m is the grand total of recorded interactions for all species (Blüthgen *et al.* 2008). For each possible link, the observed deviation (henceforth termed link temperature T_{ij}) from the neutral model was defined as $T_{ij} = \frac{a_{ij} - e_{ij}}{A_i}$, with a_{ij} as the observed number of visits of animal species i on plant j. T_{ij} ranges between -1 and 1; negative values correspond to cold links (i.e. fewer observations than expected by the neutral model) and positive ones to hot links. T_{ij} is thus defined from the animal's perspective using A_i as denominator. Whether link temperatures were significantly hot or cold, was defined using Monte-Carlo statistics by comparing each observed a_{ij} with the distribution of interaction frequencies between i and j (α'_{ij}) obtained from the Patefield algorithm (Patefield 1981, Blüthgen *et al.* 2006) generating 1000 random matrices with a fixed distribution of marginal totals A_i and A_j. The mean α'_{ij} across all randomised networks approximates e_{ij}.

Olfactometer trials

Links between relatively common species were haphazardly selected for olfactometer experiments, including significantly hot and cold links. We used a mobile olfactometer to test whether the animals' responses to floral scents reflect the variation in link temperatures. The system allowed us to conduct bioassays in the field with scents from naturally growing, unpicked flowers and flower-foraging insects that did not live in captivity. Most of the insects were caught during their foraging activities on flowers, most of

them (94 %) on flower species that were visited more often by this insect species than expected (hot links). In two different types of arenas, these visitors were confronted with floral scents and neutral air (filtered and humidified in charcoal and distilled water). For crawling insects like ants and beetles, a four field arena was used (similar to the one described in detail in Junker and Blüthgen (2008)). For flying insects such as bees, bumblebees and hoverflies, a Y-shaped arena was used. Glass plates covered the arenas. The whole apparatus was fitted into an aluminium box making it mobile for an operation in the field. Technical details, dimensions of the arenas, protocol used for the tests and a list of conducted biotests are provided in Appendices L and M in Supporting Information. Next to an innate foraging behaviour elicited by floral scents, these signals are also often associated with rewards and are therefore learnt by the animals (Cunningham *et al.* 2004, Bruce *et al.* 2005, Cunningham *et al.* 2006). In order to account for the learning ability, in a third of the trials we compared the responses from (1) individuals that were caught from flowers of the focal species used as scent source in the olfactometer to (2) conspecific individuals that were caught while visiting a flower from a different plant species. The animals' responses were expressed by the index $R_{ij} = \frac{2(N_{obs} - N_{\exp})}{N_{total}}$, with N_{obs} = choices for, or time spent in scented fields; N_{exp} = expected value for each field assuming random choices, i.e. 50 % of tested animals or total time; and N_{total} = total number of animals tested or total time span. Like T_{ij}, R_{ij} varies between -1 (avoidance) and 1 (attraction).

Colour and morphology

In order to estimate the influence of floral colour and morphology on link temperatures, we obtained data on basic colours and morphological flower types after Kugler (Kugler 1970) for each plant species in our webs from the internet database "BiolFlor" (www.ufz.de/biolflor/index.jsp; Klotz *et al.* 2002). We performed Kruskal-Wallis rank sum tests with pooled link temperatures of ants, bees, bumblebees, beetles, hoverflies and butterflies as response variables and colours (Appendix N) or flower types (Appendix O) as explanatory variables. This approach enabled us to search for preferences of insect groups regarding certain colours or morphologies. However, note that the resolution of these data is not specific to each link unlike our olfactometer approach. Furthermore, many features are neglected in these categories: the colours neither include information on UV-reflectance nor on nor subtle differences in the reflectance spectrum. The morphological categories bear limited information on potential barriers such as nectar tube length or other features that prevent certain animals from consuming nectar (see Stang *et al.* 2006, 2007).

Results

Network metric

In the first network, 303 links between 35 plant and 164 insect species were recorded (connectance C = 0.053, 2251 individual interactions) and 170 links between 23 plant and 64 insect species in the second (C = 0.115, 1080 individual interactions). Specialisation and complementarity (flower partitioning) of visitor species was pronounced (H_2' = 0.47 and 0.52, respectively), similar to other flower-visitor networks recorded so far (Blüthgen *et al.* 2007). Correspondingly, many links deviated strongly from neutrality: 47.5 % and 47.7 % of the realised links were significantly hot, and 20.8 % and 31.8 % were significantly cold, respectively (Appendix K, Fig. 1). We observed a strong species turnover between both dates where we recorded the interactions (Appendix K). However, link temperature of those links present in both networks were strongly correlated between the two dates (Pearson's R^2 = 0.40, df = 78, P < 0.001).

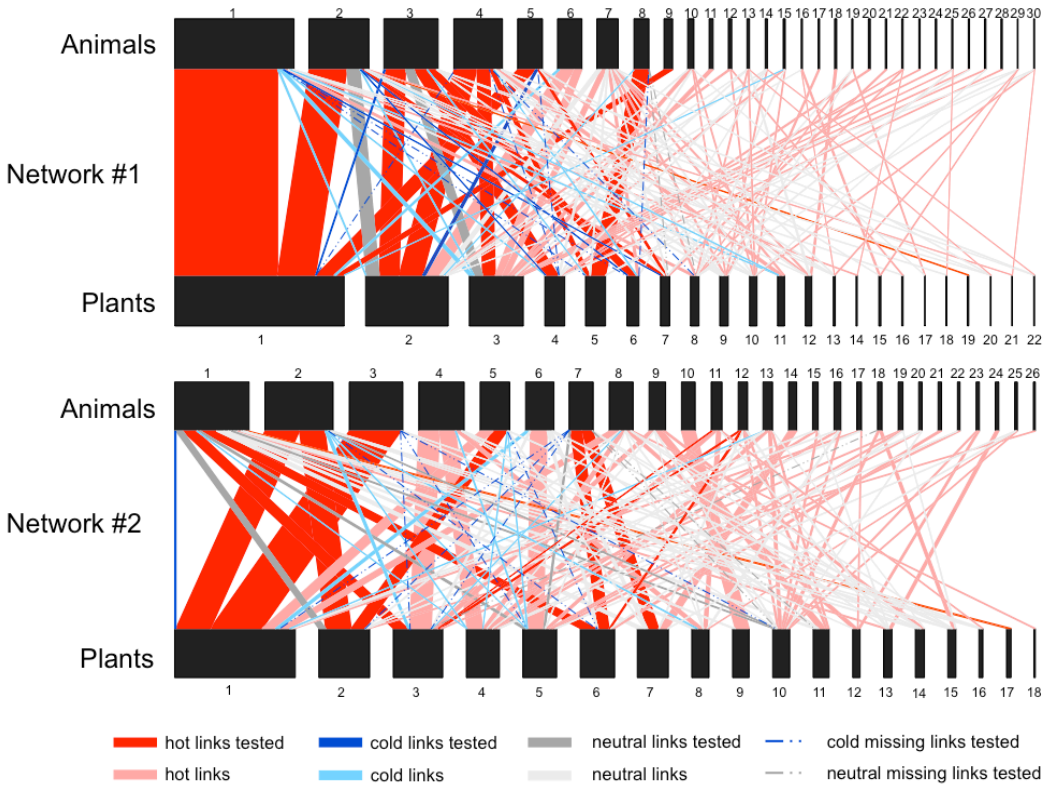

Fig. 1. Interaction networks used in this study. Numbers denote animal and plant species (names are shown in Appendix K). Widths of nodes correspond to the total number of observed interactions of each species, width of links to the number of interactions between species pairs. Missing links are only shown as dashed lines if they were tested in the olfactometer trials.

Olfactometer trials

We performed a total of 59 olfactometer trials including floral scents of 18 plant species and 10 animal species (total n =1557). On average, hot links received positive and cold links negative response indices, whereas neutral links had intermediate values (ANOVA: $F_{2,56} = 9.3$, $P < 0.001$, Fig. 2). Animals that were caught from different flowers than the focal ones responded similarly ($F_{2,49} = 3.3$, $P = 0.04$, Fig. 2). Individuals caught from the focal species used for the olfactometer trials received mostly (17 of 20 tested) higher R_{ij} values than conspecifics caught from other flowers (paired t-test: $t_{19} = 3.8$, $P < 0.001$; Fig. 3). However, because cold links are *per se* rarely or never realised in a community, we were able to test animals only from three cold links. Four cases of significant attraction corresponded to significantly hot links, nine cases of significant repellence to significantly cold links (Appendix M in Supporting Information). For instance, *Bombus pascuorum* Scop. interacted significantly more often than expected with *Ballota nigra* L. ($T_{ij} = 0.37$, $P < 0.001$) but never with *Crepis vesicaria* L. ($T_{ij} = -0.25$, $P < 0.001$). This hot link corresponded to significant attraction ($R_{ij} = 0.21$, $P = 0.035$), the cold link to avoidance ($R_{ij} = -0.56$, $P = 0.018$). The same flower species often triggers different responses to different visitors. Overall, we found a highly significant correlation between the animals' response R_{ij} and the link temperature T_{ij} (Pearson's $R^2 = 0.17$, $df = 57$, $P < 0.001$; Fig. 3). For this correlation, we used data from all animal individuals regardless of their experience in order to represent the responses of the whole flower visitor population. The positive relationship between animals' response R_{ij} and the link temperature T_{ij} was confirmed within each animal taxon used in the olfactometer trials, namely ants, bees, bumblebees, beetles and hoverflies, albeit significant for beetles only (Appendix P).

Colour and morphology

Overall, floral colour and morphology had a significant influence on link temperatures (Kruskal-Wallis rank sum test: $X^2 \geq 28.95$, $df = 7$ and 5 for colour and morphology, respectively, $P < 0.001$). The link temperatures T_{ij} of bees, beetles and butterflies differed across colours (Appendix N), the link temperatures T_{ij} of all groups of insect groups across flower types (Appendix O). However, differences were largely confined to a few colours or flower types, while most were associated with similar responses.

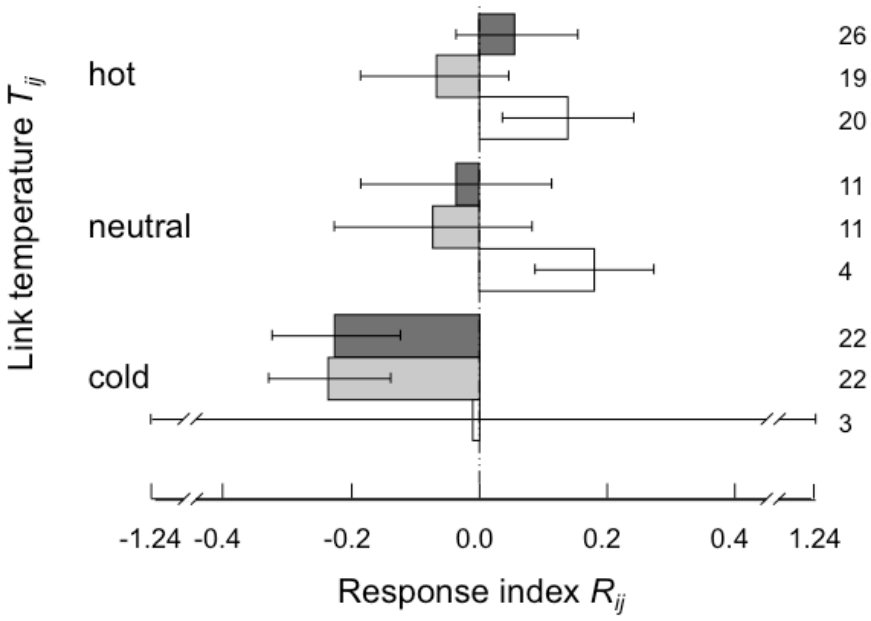

Fig. 2. Animals' responses R_{ij} towards floral scents originating from significant hot, neutral and cold link temperatures T_{ij}. Dark grey bars denote to all tests, light grey bars to tests with animals caught from other flowers than from focal ones, white bars denote tests with animals caught from focal flowers. Mean R_{ij} values and 95% confidence intervals (CI) shown, with sample sizes (n) next to each bar. CI for the final bar cover the entire possible range of R_{ij} (-1 to 1).

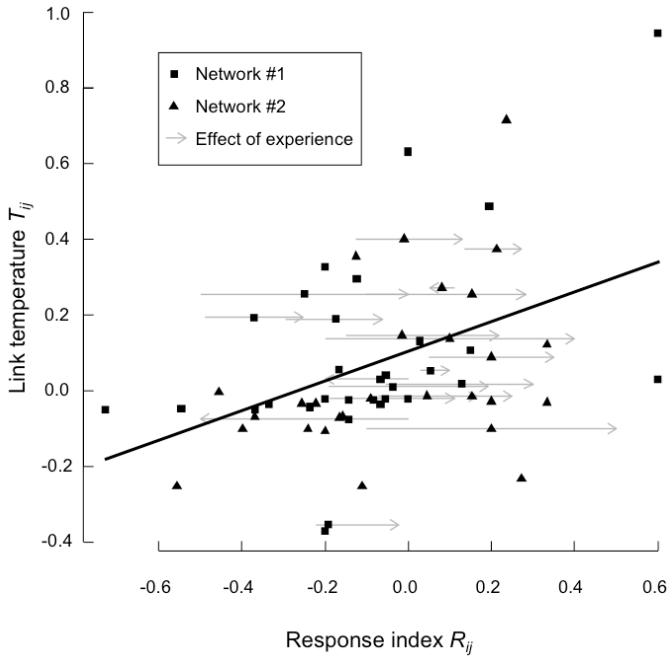

Fig. 3. Correlation between response index R_{ij} and link temperature T_{ij} in each network. For the regression between R_{ij} and T_{ij}, we used mean R_{ij} values of all individuals of a given species irrespective of their previous experience. Arrows denote the effect of immediate experience on the animals' response in floral scents, pointing from the response of animals not caught from target flowers to those caught from the same flower species used in the trial.

Discussion

Network analyses complement the pair-wise treatment and interpretation of biological interactions and draw attention to their broader context in multi-species communities (Proulx *et al.* 2005, Ings *et al.* 2009). Network analyses were often applied to flower-visitor interactions and revealed valuable insights to the structure of these communities (e.g. Olesen *et al.* 2008, Ings *et al.* 2009). The common term "pollination network" or "mutualistic network", however, simplifies the variation in the quality of interactions: pollinators are not equally beneficial to each plant species (Stanton 2003, Johnson *et al.* 2006, Reynolds and Fenster 2008), and a considerable proportion of all visitors are not mutualistic, e.g. exploiting floral resources without pollen transfer (e.g. Brody *et al.* 2008). The mutualistic service provided by a flower visitor is not only dependent on the flower visitors' identity but also on the focal link (Junker and Blüthgen 2010b), an information that is not available for most interactions.

The presence of both mutualists and antagonists forces flowers to display attractive as well as defensive traits (Brown 2002, Irwin *et al.* 2004). Therefore, floral filters – individual traits that invite mutualists but keep antagonists at bay – are needed that may include certain characteristics of nectar (Johnson *et al.* 2006, Hansen *et al.* 2007), morphological structures (Galen 1999, Galen and Cuba 2001, Stang *et al.* 2006, More *et al.* 2007), and scent (Dobson and Bergström 2000, Junker and Blüthgen 2008, Junker and Blüthgen 2010b). Therefore, floral filters that affect several species can not be detected in pair-wise interactions where suites of traits of both partners were viewed as an outcome of co-evolution but in studies where whole communities are considered which is facilitated by network analysis (Stanton 2003, Raguso 2008a).

Our results demonstrate that the animals' responses to floral scents reflect a considerable proportion of the network structure after neutral effects were accounted for. We found both positive and negative responses towards floral scents, suggesting that next to attraction, repellence may shape the visitation and contribute to the reproductive success of plants (Junker, 2010). In our study, repellent functions of floral scents did not only involve antagonists (Junker and Blüthgen 2008, Kessler *et al.* 2008), but also affected the visitation by typical pollinators. For instance, the cold links between *Bombus terrestris* L. and *Apis mellifera* L. and *Achillea millefolium* L. corresponded to negative significant responses R_{ij} (see Appendix M). Furthermore, our results suggest that the insects' ability to associate rewards with specific floral scents also contribute to the regulation of the microstructure of the network. The trials accounting for the insects' immediate experience

confirm that appetitive learning plays an important role for foraging insects (Raguso 2008b) implying that floral scents often function as recognition traits in addition to their function as innate attractants (Cunningham *et al.* 2004, Cunningham *et al.* 2006). Our experiments are unable to unequivocally differentiate between innate and learned responses. Innate responses (attraction and repellence) would imply that scents are a regulating force of the microstructure of networks, while learned responses (positively or negatively) would represent a reinforcement of decisions based on other factors than scent, e.g. proximately other floral traits like colour or morphology or ultimately the resource quality. However, in both cases volatile cues would influence the network structure. Floral colour and particularly morphology (see Stang *et al.* 2006, 2007) are also involved, since link temperatures are affected by both factors. For example, nectar-mediated visits by ants and hoverflies where infrequent in lip flowers (i.e. negative link temperatures), where nectar is not accessible to these animals with short mouthparts. However, to compare the importance of different traits on network microstructure, visual, structural and nutritional features should be evaluated for each link in a similar manner as in the present study.

The outcome of the olfactometer trials may be strongly influenced by the selection of insects, i.e. whether they are caught from focal plant species or from non-focal ones. However, the virtual absence of "experienced" animals from cold links and the overrepresentation of "experienced" animals from hot links are an important part of the realised distribution of visitors on flowers in natural communities. Our finding that animals with an immediately prior experience to a floral odour had significantly more positive responses than those without, emphasizes the importance of associative learning and short-term specificity. Long-term experience and flower fidelity may have an even stronger influence on the outcome of olfactometer trials; thus, effects of experience and conditional learning may be underestimated in our approach.

Both individual substances as well as the proportional composition of substances within floral bouquets play a role in flower recognition (Bruce *et al.* 2005). In the field, Waelti *et al.* (2008) clearly demonstrated that floral scents of two closely related *Silene* species strongly contribute to maintain the reproductive isolation of these species due to their role in promoting floral constancy of pollinators. Pollinators, in return, may benefit from flower fidelity by minimizing their handling time (Stanton 2003).

Immobile flowers may use scents in concert with other signals to influence their visitor spectrum, explaining the non-random associations observed in these communities. Although we ignored the morphology of flowers and insects' mouthparts (see Stang *et al.* 2006, 2007, Stang *et al.* 2009) and additional floral signals such as colour, shape, size and

quality of the rewards, we found a correlation between the animals behaviour elicited by scent (expressed as R_{ij}) and the interaction strength (expressed as T_{ij}). The congruence of positive or negative link temperatures and responses, respectively, additionally accentuates the importance of scents in these communities. Thus, floral scents are important biological signals that may regulate network structure due to attraction (Knudsen *et al.* 2006, Junker and Blüthgen 2010b), associative learning (Cunningham *et al.* 2006) and repellence (Junker and Blüthgen 2008, Junker and Blüthgen 2010b). The inter- and intraspecific partitioning of visitors on flowers (Palmer *et al.* 2003) shaped by biological filters may facilitate the coexistence of the large number of flower visitors that compete for the floral resources. These mechanisms determine which partners do and do not interact with each other and thereby also a large proportion of network structure.

X. How to attract and defend? Dependency on floral resources determines the animals' responses to floral scents

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Summary

Biological mutualisms rely on communication between partners, but also require protective measures against exploitation. Animal-pollinated flowers need to attract pollinators but also to avoid conflicts with antagonistic consumers. The view of flower visitors as mutualistic and antagonistic agents considers primarily the plants' interest. A classification emphasizing the consumer's point of view, however, may be more useful when considering animal's adaptations to flower visits which may include a tolerance against defensive floral scent compounds. In a meta-analysis covering 18 studies on the responses of animals to floral scents, we assigned the animals to the categories of obligate and facultative flower visitors which considers their dependency on floral resources. Their responses on floral scents were compared. On average, obligate flower visitors, often corresponding to pollinators, were attracted to floral scent compounds. In contrast, facultative and mainly antagonistic visitors were strongly repelled by floral scents. The findings confirms that floral scents have a dual function both as attractive and defensive cues. Whether an animal depends on floral resources determines its response to these signals, suggesting that obligate flower visitors evolved a tolerance against primarily defensive compounds. Therefore, floral scent bouquets in conjunction with nutritious rewards may solve the conflicting tasks of attracting mutualists while repelling antagonists.

Introduction

Plant volatiles serve as cues that mediate various interactions with animals. Scents emitted by vegetative plant parts effectively function as allelopathic agents, herbivore deterrents or as attractants for the herbivores natural enemies, and flower volatiles are traditionally regarded as pollinator attracting signals (Pichersky and Gershenzon 2002, Raguso 2008b, Unsicker *et al.* 2009). The notion that floral traits such as colour, shape, nutritious rewards and scent are adaptations for efficient pollination by animals dates back to Sprengel's (1793) and Darwin's (1862) classical work and has stimulated numerous detailed investigations since then (Pellmyr 2002, Dudareva and Pichersky 2006a). Non-pollinating visitors from several taxa potentially exploit rewards like nectar and pollen (Inouye 1980), disturb true pollinators (Tsuji *et al.* 2004, Junker *et al.* 2007) or consume floral tissues (McCall and Irwin 2006). The negative impact of such antagonists on plant reproduction may even exceed the benefits from mutualists (Herrera 2000, Morris *et al.* 2007). Accordingly, defensive properties of flowers have recently received attention. Defensive traits include floral scents (Kessler and Baldwin 2006, Junker and Blüthgen 2008, Kessler *et al.* 2008, Raguso 2008b, Willmer *et al.* 2009), unpalatable or even toxic nectar (Stephenson 1981, 1982, Adler 2000, Johnson *et al.* 2006, Liu *et al.* 2007) and non-volatile secondary metabolites (Johnson *et al.* 2008, Hanley *et al.* 2009). Here we focus on floral scents and explore their role as attractants and as repellents. Floral scent bouquets are complex blends composed of volatile substances from various biosynthetical pathways (most commonly mono- and sesquiterpenoids, benzenoids and aliphatics) and often with diverse functional groups (e.g. alcohols, aldehydes, esters, ethers and ketones) (Dudareva and Pichersky 2006a, Knudsen *et al.* 2006).

The dual role of floral traits (Irwin *et al.* 2004) may enable flowers to select their visitor spectrum in order to optimize pollination success. For instance, some floral scent compounds (e.g. linalool) commonly occurring in floral scent bouquets were shown to attract pollinators (Laloi *et al.* 2000, Andersson *et al.* 2002) but also repelled nectar thieves (Junker and Blüthgen 2008). Similarly, iridoid glycosides in nectar of *Catalpa speciosa* (Stephenson 1982) and phenolics in nectar of *Aloe vryheidensis* (Johnson *et al.* 2006) deterred antagonistic flower visitors while pollinators remained unaffected. Furthermore, Hanley *et al.* (2009) reported a trade-off between chemical (cyanogens) and morphological defences that exclude potential florivores.

Classifying flower visitors as mutualistic and antagonistic agents emphasizes the plants' need to maximize reproductive success. However, this classification may not be able

to explain the diverse responses to floral traits by different flower visitors. Flower visitation by pollinators is driven by their interest as consumers, not necessarily as mutualists – their mutualistic service is merely a by-product of searching for resources or mating opportunities (Frame 2003). To understand the behaviour of flower visitors towards floral traits, we propose a different classification that emphasises the consumers' perspective. We distinguish *obligate* from *facultative* consumers of floral resources in contradiction to *mutualistic* and *antagonistic* agents. Obligate flower visitors are defined as those that require floral resources for at least part of their life-cycle, while facultative ones occasionally consume floral resources but are not obviously dependent on them. Given a reasonable knowledge of the animal species' natural history, the dependency on floral resources can usually be unequivocally assessed. In contrast, whether the relation between an animal and a plant is mutualistic, commensalistic or antagonistic depends not only on the involved species, but also on the focal interaction. For example, ants are most often floral antagonists (e.g. Galen and Butchart 2003) but in some comparatively rare cases they act as pollinating mutualists (e.g. Gomez and Zamora 1992, Gomez *et al.* 1996), while their facultative use of floral resources is undisputed: they are omnivorous and feed on various resources, not just flower nectar (Blüthgen and Feldhaar 2010). Furthermore, the positive or negative net effect of interactions between species may be variable over space and time (Bronstein 1994), which makes clear classifications of flower visitors even more difficult.

Our meta-analysis examines whether the dependency of animals on floral resources determines their response to floral scents and we hypothesize that obligate flower visitors are better adapted to potential floral defences. Possible consequences to flowers with respect to the dilemma to attract mutualists but repel antagonists are discussed.

Material and methods

Apart from studies that we knew, we queried Zoological Record, BIOSIS Previews and Google Scholar using relevant search terms (combinations of the terms attraction, deterrence, floral scent / odor, herbivory, pollinator, repellence, terpenoids, volatiles) to find appropriate articles. All studies that contrasted the effect of floral volatiles to a scentless control and which gave information on the variance of experimental and control were included.

The classification of animal species as obligate and facultative flower visitors was either provided in the original studies, or was assigned based on the literature and/or information from entomologists by considering the resource spectrum of the animals

(Appendix Q). Animals with an unclear status were excluded from our analysis. In studies that did not specifically deal with flower ecology, substances used in the tests were assigned as flower volatiles if they were listed in the extensive compilation by Knudsen *et al.* (2006). Scent was applied in natural concentrations in most studies, although this was not evident for all data. Information on the volume, mass or emission rate of scent compounds used in the bioassays was mostly provided in the literature (Appendix Q). Koschier *et al.* (2000) applied several concentrations of their test substances; in this case, we used the results from the lowest concentration that are the most natural ones (e.g. 8700 ng linalool).

The effect size of an animal's response to a scent was extracted from the original study as log response ratio $L = \ln(\overline{X}_E / \overline{X}_C)$, with $\overline{X}_E$ and $\overline{X}_C$ as the mean response of the focal organism to the experimental scent treatment and scentless control, respectively (Hedges *et al.* 1999). We chose log response ratio *L* to measure effect size because, unlike the commonly used Hedges' *d*, it assumes that a scent or bouquet has a proportional effect on the visitors' response, which is more appropriate when the response cannot be negative (Hedges *et al.* 1999, Morris *et al.* 2007). In the rare event that either $\overline{X}_E = 0$ or $\overline{X}_C = 0$, zero was replaced by 0.01 in order to calculate *L*. Using the standard deviation *SD* and the sample size *n* of treatment and control, we computed the variances *v* of all *L* using MetaWin 2.0 and used v^{-1} as weight for the subsequent analysis of variance (ANOVA). Firstly, a one-factorial weighted ANOVA was conducted with the complete dataset with *L* as response variable and the dependency of the animals on flower visits as explanatory variable. Secondly, in order to compare the magnitude of positive and negative responses by obligate and facultative flower visitors, the absolute values |*L*| of *L* (excluding cases where $L = 0$) were used in a weighted two-factorial ANOVA as response variable with the dependency of the animals and the sign of the response as first and second explanatory variable, respectively. Thirdly, reduced data sets comprising data on either the effects of aliphatics, benzenoids, mono- and sesquiterpenes or the effects of alcohols, aldehydes, esters, ethers, hydrocarbons and ketones were used to perform two-factorial weighted ANOVAs with the dependency and the biosynthetical origin or functional group as explanatory variables. Here, groups of compounds from different biosynthetical origins or with different functional groups where only included if sufficient data from both obligate and facultative flower visitors were available. The meta-analysis may have its limitations in the independency of data, since several observations were extracted from individual studies. In order to address this concern, the data set was reduced to a single value (*mean L*) for each study prior to another ANOVA with *mean L* as response and the dependency as explanatory variables. In cases

where a given study reported results from both obligate and facultative flower visitors, we separately calculated the mean for both groups.

Additionally, 17 studies from which not all required data could be extracted to estimate *v* (*mean*, *SD* and/or *n* of control and experimental) were subjected to a second ANOVA based on unweighted data (Appendices R and S). All statistical analyses were performed using R (Version 2.7.0, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria).

Results

Our meta-analysis included 18 publications that tested the effect of floral scent bouquets or individual synthetic substances that are common in flower odour blends on a broad spectrum of animals. In total, 425 observations (83 substances from 7 chemical classes and bouquets from 31 plant species) were included in the analysis (Appendix Q). Our designation of 24 obligate and 16 facultative visitor species was often consistent with their putative role as mutualists and antagonists, respectively. Most obligate visitors in this study were represented by putative pollinator taxa (butterflies, moths, bees, hoverflies and hummingbirds), but also included thrips for which it is unknown whether they are mutualistic, commensalistic or even antagonistic. Facultative flower visitors were represented by omnivorous ants that often act as non-pollinating nectar-thieves (Galen 1983), herbivores such as Tettigoniidae, certain beetles belonging to the families Cerambycidae, Chrysomelidae, Mordellidae, Scarabaeidae, and bugs occasionally feeding on flowers, and generalist dipterans that use a variety of non-floral resources. For example, the study of Andrews *et al.* (2007) involved the facultative flower visitor *Acalymma vittatum*, a chrysomelid beetle that feeds on cucurbit leaves, fruits and flowers, and the obligate flower visitor *Peponapis pruinosa*, a squash bee that exclusively feeds on pollen and nectar of cucurbit flowers. A full list of the species involved, their dependency on flower visits (obligate and facultative), and assumed typical effect on flowers (mutualistic and antagonistic) is provided in (Appendix Q).

The meta-analysis revealed significantly different responses of obligate and facultative flower visitors to floral scents (Table 1a). Obligate flower visitors were attracted to the majority of floral scents, whereas facultative flower visitors were negatively affected (Figure 1a). The magnitude of negative responses by facultative flower visitors was about four times stronger than the remaining responses (Table 1b, Figure 1b). Flower visitors' responses differed between floral scent compounds from four pathways of plant secondary

metabolism and between compounds with different functional groups (Table 1c, d). The strongly negative responses of facultative flower visitors towards monoterpenes and alcohols, ethers and ketones (Figure 2 and 3) are particularly remarkable. Their negative response was even found for linalool alone, the most common substance in our analysis: facultative flower visitors negatively responded to this substance (95% confidence interval [CI] based on the weighted data =-5.14 – -0.17), while obligate ones responded mainly positively ([CI] = -0.55 – 0.96) (weighted ANOVA: $F_{1,20} = 3.3$, $p = 0.085$). However, results of these subsets examining the effects of linalool and different compound classes should be viewed with caution because of small sample sizes of some study groups.

Tab 1. Effect of the animals' dependency on floral resources on their responses to floral scents. Results of the weighted ANOVA for (a) effects of floral scents on animals with different dependencies in floral resources (complete data set); (b) differences in the magnitude of positive and negative responses ($|L|$) on obligate and facultative flower visitors, complete dataset excluding cases where $L = 0$; (c) the effects of floral scents from different biosynthetical pathways (aliphatics, benzenoids, mono- and sesquiterpenes) on obligate and facultative flower visitors (using a subset of the data with these chemical classes only); (d) the effects of floral scents with different functional groups (alcohols, aldehydes, esters, ethers, hydrocarbons and ketones) on obligate and facultative flower visitors (using a subset of the data with these functional groups only).

a) Meta-analysis - Parameter	*df*	*F*	*p*
Dependency	1	52.6	**< 0.001**
Residuals	423		

b)	*df*	*F*	*p*
Dependency × Sign of *L*	1	7.5	**<0.01**
Dependency	1	20.2	**< 0.001**
Sign of *L*	1	8.1	**<0.01**
Residuals	364		

c)	*df*	*F*	*p*
Dependency × Pathway	3	16.6	**< 0.001**
Pathway	3	31.2	**< 0.001**
Dependency	1	130.2	**< 0.001**
Residuals	343		

d)	*df*	*F*	*p*
Dependency × Functional group	5	4.4	**< 0.001**
Functional group	6	33	**< 0.001**
Dependency	1	134.6	**< 0.001**
Residuals	298		

Overall, the results were confirmed when the analysis was repeated with an extended dataset including 17 additional studies from which no variance of the response ratios could be additionally extracted (Appendices R and S). Observations on obligate flower visitors were dominated by thrips (eleven species), facultative visitors by ants (four species). However, the log response ratios L of thrips ([CI] = 0.32 – 0.57) were slightly higher than those of the other obligate flower visitors ([CI] = 0.04 – 0.43) but they were not significantly different from each other (weighted ANOVA: $F_{1,243} = 45.3$, $p = 0.095$). Negative effects of floral scents on ants ([CI] = -0.33 – -0.17) were less pronounced than on other facultative flower visitors ([CI] = -5.1 – -3.18) (weighted ANOVA: $F_{1,178} = 82.3$, $p < 0.001$). Therefore, the over-representation of thrips and ants did not bias the general conclusions. Different, often complex, methods were applied in the original studies in an effort to reveal the influence of floral odours on the study animals: 62 % of all observations derive from studies using scented traps, 22 % were based on olfactometer assays and 10 % on bait assays. The remaining observations were taken from toxicity- (4 %) and food-choice-tests (1 %) besides miscellaneous other experimental setups (1 %). Study design had a strong influence on the animals' responses (weighted ANOVA: $F_{6, 417} = 777.3$, $p < 0.001$). However, the only significant differences in a *post hoc* comparison were found between toxicity tests *versus* all other designs and traps versus olfactometer tests (weighted pair wise *t*-tests $t \geq 4.6$, Bonferroni-corrected $p \leq 0.02$).

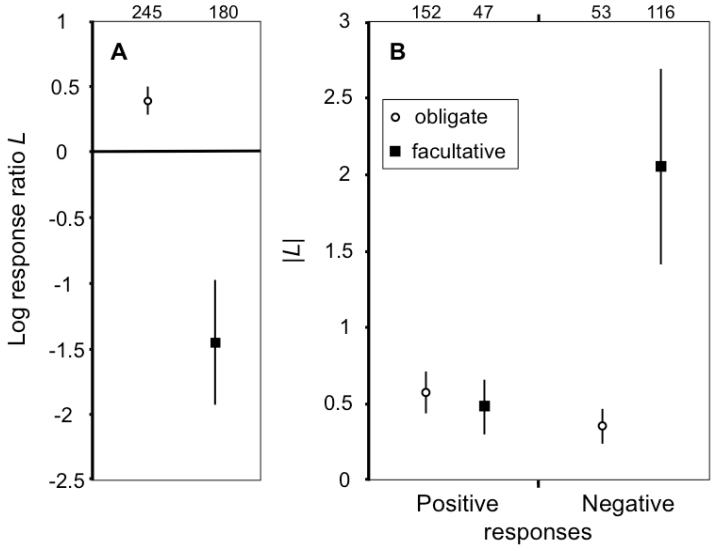

Fig. 1. Effects of floral scents on obligate (open circles) and facultative flower visitors (black squares). Weighted mean and 95% confidence intervals of the log response ratio L are shown. L describes the proportional difference between the mean effect of the scent treatment and the control. Sample sizes are given at the top of the figure. (A) Results of all data obtained from the literature. $L > 0$ indicates an attractive effect; $L < 0$ a repellent effect. A significant effect in either direction is indicated in cases where the confidence intervals do not include zero. (B) Magnitude of positive and negative responses by obligate and facultative flower visitors. Absolute values of log response ratios $|L|$ are shown (excluding $L = 0$).

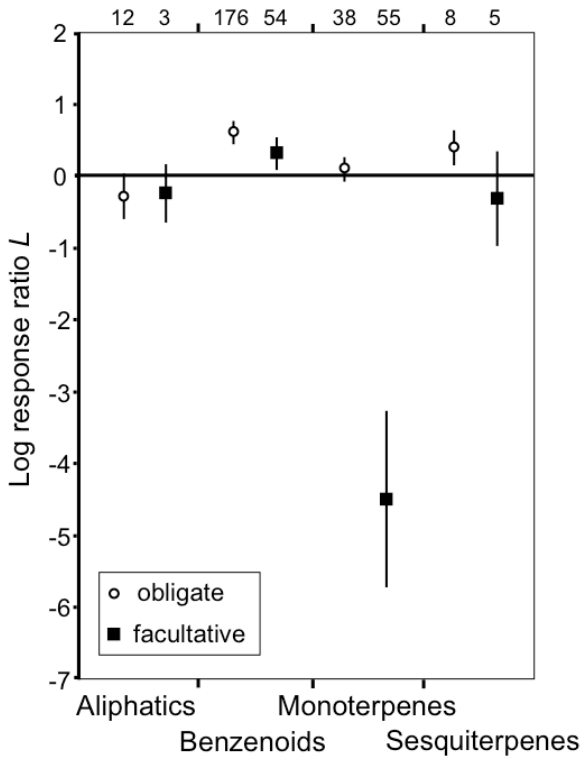

Fig. 2. Effects of individual floral scent compounds derived from different biosynthetical pathways. Weighted mean and 95% confidence intervals of the log response ratio *L* are shown. Effects of chemical classes where included if sufficient data for both obligate and facultative flower visitors where available. A significant effect in either direction is indicated in cases where the confidence intervals do not include zero.

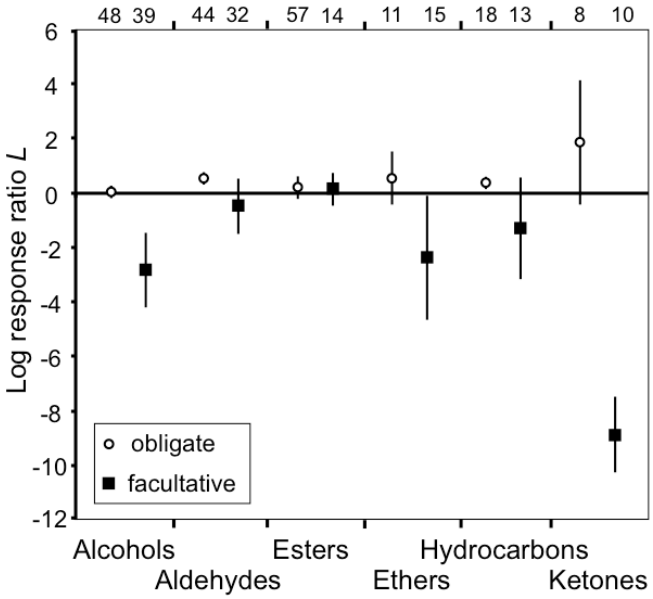

Fig. 3. Effects of individual floral scent compounds with different functional groups. Weighted mean and 95% confidence intervals of the log response ratio *L* are shown. Effects of substances with certain functional groups where included if sufficient data for both obligate and facultative flower visitors where available. A significant effect in either direction is indicated in cases where the confidence intervals do not include zero.

When data derived from toxicity tests were removed from the analysis (they include only tests with facultative flower visitors), the effect of the dependency was consistent to the complete dataset (weighted ANOVA: $F_{1, 406} = 62.7$, $p < 0.001$). Hence, the toxicity tests did not bias our general finding. The contrast between obligate and facultative visitors was also consistent when the dataset was restricted to trap experiments (weighted ANOVA: $F_{1, 260} = 10.9$, $p = 0.001$). The difference between responses by facultative and obligate visitors remained significant (weighted ANOVA: $F_{1, 18} = 4.9$, $p = 0.04$) in the analysis where the data set was reduced to a single value (*mean L*) for each study ($n = 13$ and 7 for obligate and facultative flower visitors, respectively).

Discussion

We found that floral scents may act as filters (see Raguso 2008b, Raguso 2008a) that attract obligate flower visitors but repel facultative ones. Our results suggest that our proposed dichotomy of dependencies on floral resources (obligate versus facultative) can explain responses to floral signals. In turn, such a framework allows us to suggest hypotheses about the ecology and evolution of flower- animal interactions: if the animals' and plants' interests meet, this may lead to coevolutionary trajectories wherein the attractive function serves as a mutualistic signal and the repellent function as an effective defence. This is true if obligate flower visitors often serve as mutualists, while facultative flower visitors typically represent antagonists. This dichotomy is tentatively supported in our dataset (Appendix Q), but may require further investigations across species. The correspondence between dependence, net effect and response may provide a solution for the plants' dilemma of attracting pollinators while defending antagonists.

It has been hypothesised that attractive cues may have evolved from floral herbivore deterrents that occurred in early angiosperms (Pellmyr and Thien 1986) and perhaps in other non-angiosperm taxa that may have been the first zoophilous plants during the Mesozoic (Ren *et al.* 2009). Some floral volatiles are emitted by archaic taxa as well as in modern angiosperms (Pellmyr *et al.* 1991, Goodrich and Raguso 2009), hence it is probable that floral scents maintained this primary function. In a different context, terpenoids were shown to have harmful effects on various organisms (Gershenzon and Dudareva 2007). The defensive function of individual floral scent compounds dissolved in nectar and of entire floral scent bouquets was recently shown for *Nicotiana attenuata* flowers (Kessler and Baldwin 2006) and for a larger set of flowers repelling facultative nectar-thieving ants (Junker and Blüthgen 2008, Willmer *et al.* 2009). As a consequence, obligate consumers of

floral resources may have secondarily evolved a tolerance against repellent, deterrent or even toxic substances. This notion might be reflected in our finding that the magnitude of negative responses by facultative flower visitors is by far the largest compared to negative responses by obligate flower visitors and positive responses of both groups. Along with the view that pollination may represent a special case of phytophagy (Frame 2003), one may thus hypothesize that plant-pollinator-mutualism is the consequence of, rather than the prerequisite for, obligate flower visits.

Obligate flower visitors are believed to optimize their utilisation of floral resources. This can be achieved by evolving accordant abilities like specialised mouth parts (Labandeira and Sepkoski 1993), the capability to digest pollen (Roulston and Cane 2000) and visual abilities like trichromacy (Chittka 1996), but may also involve mechanisms to overcome flower defences or even the use of primarily defensive volatiles as host finding cues reinforced by learned associated rewards (Carlsson and Hansson 2006, Riffell *et al.* 2008). In contrast, the selective pressure on facultative flower visitors to adapt to flowers is expected to be low or absent. Similarly, variable responses to defensive secondary metabolites are a common scenario in plant vegetative defences, where generalised herbivores are deterred, but specialised leaf-feeding herbivores tolerate and even utilize such defensive compounds as host finding signals (Smallegange *et al.* 2007). While generalists may use alternative plants, specialists represent obligate consumers of their specific host, in analogy to obligate flower visitors. However, in the herbivory context, adaptations that enable specialist folivores to cope with defences of their host plants are usually species-specific results of co-evolution (Cornell and Hawkins 2003). In our definition of obligate flower visitors, we do not distinguish between specialists that visit only flowers of a narrow taxonomic range and generalists with a wide host spectrum. Obligate flower visitors are animals that depend on the resources offered by flowers in general. In our dataset, only few specialist flower visitors are included where we would expect an innate attraction to scents, which may be the reason for the low magnitude of positive responses of obligate flower visitors. Andrews *et al.* (2007) tested the responses of *Peponapis pruinosa*, the specialist pollinator of *Cucurbita moschata* (Cucurbitaceae) to three floral scent compounds of this plant species and found a strong and significant attraction to two of these scents. We assume that specialised obligate flower visitors should be more attracted by the floral scents of its host plants (probably due to innate preferences) than generalists that may use these scents as learnt cues associated with rewards (Cunningham *et al.* 2004, Cunningham *et al.* 2006). Colour and shape may also facilitate the association of

rewards with certain plant species, but in contrast to scent these visual traits may not explain any aversions to a rewarded flower.

Our finding that benzenoids are attractive while monoterpenes, alcohols, ethers and ketones are particularly repellent for facultative flower visitors may suggest that these compounds have been evolved for these respective functions. This idea is supported by the fact that defensive terpenoids are commonly produced by vegetative tissues, whereas the benzenoids and phenylpropanoids have direct biosynthetic links to floral colours (Pichersky and Gershenzon 2002, Schie *et al.* 2006). The adaptive functions of benzenoids (attraction) and monoterpenes (defence) within floral scent bouquets are also suggested by Schiestl (Schiestl 2010). However, there are also examples for defensive benzenoids and attractive terpenoids, alcohols, ethers and ketones within our study and in the literature (e.g. Kessler and Baldwin 2006). Thus, phytochemicals that share certain chemical properties cannot be regarded as ecologically uniform, and the average trends of substances from a given biosynthetical pathway or with a certain functional group may not apply to all substances of a given group.

For an improved understanding of trait-mediated flower-visitor interactions, interests of both parties – plants and animals – need to be considered: Obligate outcrossing, animal pollinated flowers depend on pollen transferring mutualists, but may also attract commensalists and antagonists. Consumers visit flowers in search for resources, for which some of them evolved an obligate dependence and others are facultative consumers of flowers and floral rewards. Therefore, from the animals' perspective, flower visitors can be divided into obligate and facultative ones. Our meta-analysis revealed that the dependency on floral resources is a good predictor of the animals' response to floral signals.

XI. Adding empirical evidence: Responses of obligate and facultative flower visitors to floral scent compounds

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Summary

Animal-pollinated angiosperms either depend on cross-pollination or may also reproduce after self-pollination – the former are thus obligately, the latter facultatively dependent on the service of animal-pollinators. Analogously, flower visitors either solely feed on floral resources or complement their diet with these, and are hence dependent or not on the flowers they visit. We assume that obligate flower visitors evolved abilities that enable them to effectively forage on flowers including mechanisms to bypass or tolerate floral defences such as morphological barriers and repellent / deterrent secondary metabolites. Facultative flower visitors, in contrast, are supposed to lack these adaptations and are often prevented to consume floral resources by defence mechanisms. In cases where obligate flower visitors are mutualists and facultative ones are antagonists, this dichotomy provides a solution for the plants' dilemma to attract pollinators and simultaneously repel exploiters. In a meta-analysis, we recently supported this hypothesis: obligate flower visitors are attracted to floral scents, while facultative ones are repelled. Here, we add empirical evidence to these results: bumblebees and ants, obligate and facultative flower visitors, respectively, responded as predicted by the results of the meta-analysis to synthetic floral scent compounds.

Introduction

The mutualism between flowers and their pollinators is often exploited by cheaters that consume floral rewards but do not contribute to or even reduce the reproductive success of plants (Bronstein 2001). The classification into mutualistic and antagonistic flower visitors represents a phytocentric point of view and only considers the interaction's net effect for the plant. However, the outcome of each plant-flower visitor interaction may be highly conditional and variable over time (Bronstein 1994) and thus constitutes a

continuum between beneficial and detrimental, and it may not be unequivocally assigned to be either positive or negative. Furthermore, many flower visitor species that function as effective pollinators of some plant species represent severe antagonists to other plant species (Hargreaves *et al.* 2009). Thus, except for highly specialised systems, it is difficult to predict whether an interaction is mutualistic, commensalistic or antagonistic. We proposed a different classification of flower visitors based on the animals' interest in flower visits (Junker and Blüthgen 2010b). Animals visit flowers primarily in search for food; pollination is just a secondary effect (Frame 2003). For some taxa nectar and pollen are the sole nutrient supply, others only supplement their more generalistic diets with floral resources. These different dependencies on floral resources can often be unequivocally assigned to each animal species. Bees, for example, strongly depend on pollen and nectar and are thus obligate flower visitors. In contrast, ants are omnivores and thus facultative flower visitors that consume large amounts of floral nectar of some plant species but obtain most of the nutrients required by the colony from non-floral resources (Blüthgen and Feldhaar 2010).

Optimal foraging theory predicts that animals evolve physiological and behavioural features that allow them to exploit their resources as effectively as possible (Schoener 1971, Cowie 1977). Therefore, a classification considering the animals' dependencies on floral resources (obligate *versus* facultative) may be better suited to explain adaptations to flower visits than their effect on plants' reproduction (mutualistic *versus* antagonsitic). One very important adaptation to the consumption of floral resources is the ability to tolerate or overcome floral defences that are employed by the flowers to reduce the visitation frequency of detrimental flower visitors (Brown 2002). Floral scents are innate attractants or reinforce floral visits due to associative learning but do also serve as effective repellents against antagonists (Junker *et al.* 2010). In a meta-analysis, we recently demonstrated that the dependency on floral resources determines the responses to floral scents (Junker and Blüthgen 2010b). In the bioassay presented here, using bumblebees (*Bombus terrestris*) and ants (*Lasius niger*), we empirically tested the predictions deduced from the meta-analysis. We expected that bumblebees – as obligate flower visitors – are attracted to floral scent compounds, while ants – as facultative flower visitors – are repelled.

Material an methods

Bumblebees (*B. terrestris*, one colony provided by a commercial supplier) were reared in a climate chamber (day / night: 12 h / 12 h, 24 °C / 19 °C). Ants (*L. niger*, two colonies studied *in situ*) were studied in a fallow land near the university campus in Würzburg, Germany. Workers of both species were allowed to choose between sugar-baits (15 % sucrose solution) surrounded by different scents. The sugar solution was presented in 1.5 ml microcaps and dispensed by a wick that was inserted through a hole in the lid. Thirty baits were placed on a wooden board with drilled holes. Each bait was surrounded by a filter paper (Ø = 55mm) treated with substances from 4 chemical classes (aliphates, benzenoids, mono- and sesquiterpenes) and a control. Six replicates were used per treatment. Treatments were arranged on the board in a randomized block design. Substances were solved in acetone p.A. and 200 µl of this solution containing 0.01 or 0.005 mMol of the respective substance was applied on the filter paper. Pure acetone p.A. was used as control. Three different combinations of scents were used: 1.) 1-hexanol (Roth, >98%), benzaldehyde (Fluka, >99%), linalool (Merck, >97%) and nerolidol (Merck, >95%); 2.) n-pentadecane (Roth, >99%), eugenol (Merck, >99%), limonene (Roth, >95%) and β-caryophyllene (Roth, >95%); 3.) n-pentadecane, eugenolmethylether (Roth, >98%), citronellol (Roth, >90%) and trans-farnsesol (Aldrich, >96%). Trials with each substance combination were repeated 2-3 times per animal species (at least one time with each concentration per compound). The board with the scented baits was placed in the climate chamber and number of bumblebees visiting the baits within 1 hr was counted. For ants, the board was placed in the vicinity of a nest, after 30 minutes the filter papers were treated with scents and number of ants at baits was counted for 30 minutes in 5-minute intervals. Log response ratios $L = \ln\left(\overline{X}_E / \overline{X}_C\right)$ were calculated with $\overline{X}_E$ = number of insects visiting each scented bait and $\overline{X}_C$ = mean number of animals on the six control baits per trial (*L* was also used in Junker and Blüthgen (Junker and Blüthgen 2010b) to which results can be compared). Positive values of *L* indicate attraction, negative values repellence. A three-factorial ANOVA was conducted to reveal the effects of the substances in different concentrations on the two species.

Results

Ants negatively responded to all floral scent compounds except for aliphatics (1-hexanol and n-pentadecane). In contrast, foraging bumblebees were unaffected by individual floral scent compounds (Fig. 1). *L* was strongly affected by the insects and the substances tested, but not by the different concentrations used in the bioassay (Tab. 1). Overall, results of the bioassay presented here are consistent with those of the meta-analysis (Junker and Blüthgen 2010b): facultative flower visitors are repelled by most floral scent compounds, while obligate flower visitors are rather attracted by the same substances, albeit not significant in the bioassay. Furthermore, within the substances that derive from the same biosynthetical pathway (benzenoids, mono- and sesquiterpenoids) those compounds possessing functional groups were stronger ant-repellent than those without functional groups. Although the meta-analysis suggests that benzenoids have no defensive properties, eugenol and eugenolmethylether had the strongest ant-repellent effect, which may be explained by the functional groups of the compounds.

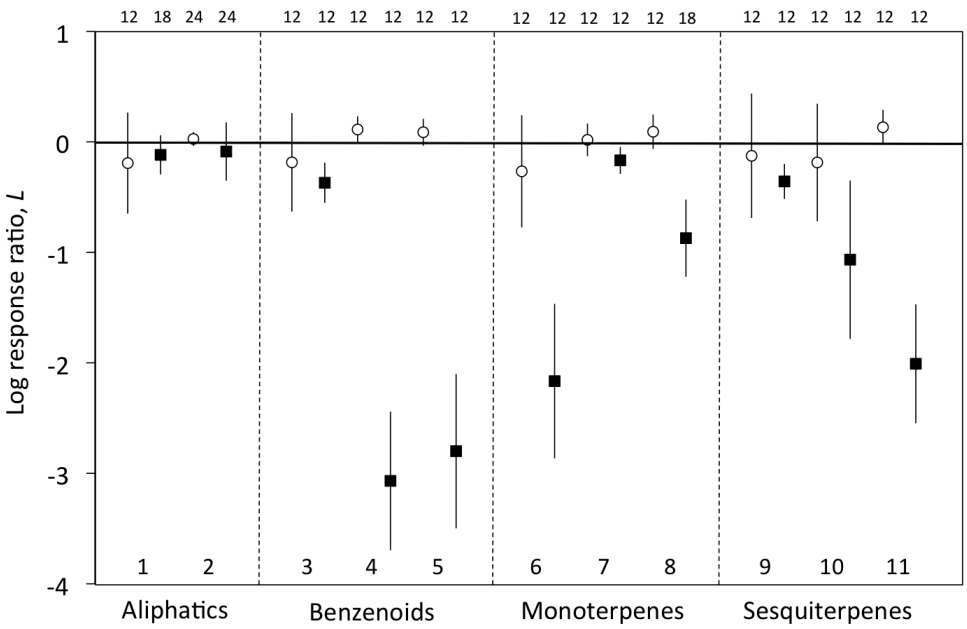

Fig. 1. Effects of floral scent compounds on bumblebees (open circles) and ants (black squares). Data are mean and 95% confidence interval of the log response ratio *L*. Since different concentrations did not affect the animal's decision, they were pooled in this figure. Sample sizes are indicated above each point. A significant deviation from neutrality or other trials is indicated in cases were confidence intervals do not overlap zero or other confidence intervals. Numbers below each pair of a circle and a square are: (1) 1-hexanol; (2) n-pentadecane; (3) benzaldehyde; (4) eugenol; (5) eugenolmethylether; (6) citronellol; (7) limonene; (8) linalool; (9) β-caryophyllene; (10) trans-farnesol; (11) nerolidol.

Tab. 1 Results of the ANOVA for the bioassay with ants and bumblebees. Significant results are highlighted in bold.

Parameter	*d.f.*	*F*	*P*
Insect × Substance × Concentration	4	0.16	0.96
Insect × Substance	10	20.44	**< 0.001**
Insect × Concentration	1	1.02	0.31
Substance × Concentration	4	0.80	0.52
Insect	1	175.65	**< 0.001**
Substance	10	18.97	**< 0.001**
Concentration	1	0.29	0.59
Residuals	280		

Discussion

The stability of mutualisms is ensured if the costs for each of the partners are compensated by the benefits they gain from the partnership. Immobile flowers may utilise defensive secondary metabolites in combination with morphological barriers to protect themselves against an excessive exploitation by both mutualists and antagonists. Well-adapted, obligate flower visitors may have evolved the ability to cope with these defences, analogously to specialised herbivores that tolerate the specific defences of their host-plants (Cornell and Hawkins 2003). In contrast to herbivory, the obligate use of floral resources is often mutually beneficial for animals and plants. This mutualism is the result of a long co-evolution, the earliest interactions between flowers and animals may have been generally detrimental for the plants' reproduction (Pellmyr and Thien 1986, Frame 2003). Thus, floral scents may have primarily served as defensive traits and were secondarily used as host-finding signals (Pellmyr and Thien 1986). Some floral volatiles are emitted by archaic and modern as well as by insect-pollinated and non-insect pollinated angiosperms (Pellmyr *et al.* 1991, Knudsen *et al.* 2006, Goodrich and Raguso 2009) which supports the defensive origin of floral scents. Our results suggest that attractive as well as defensive properties of floral volatiles are equally important in shaping the floral visitor spectrum of angiosperms.

XII. Impact of resource partitioning between pollinating and non-pollinating insects on the reproductive success of an endemic Hawaiian plant

This chapter has been submitted for publication as:

Junker RR, Bleil R, Daehler DC, Blüthgen N (submitted) Intrafloral resource partitioning between endemic and invasive flower visitors: consequences for pollinator effectiveness

Summary

Sympatric flower visitor species often partition nectar and pollen and thus affect each other's foraging pattern. Consequently, their pollination service may also be influenced by the presence of competitors. Ants are solely interested in nectar and frequent flower visitors of some plant species but usually poor pollinators. Obligate flower visitors such as bees depend on both nectar and pollen and are often more effective pollinators. In Hawaii, we studied the complex interactions between flowers of the endemic tree *Metrosideros polymorpha* (Myrtaceae) and both, endemic and introduced flower-visiting insects. We tested whether the pollinator effectiveness of endemic and invasive bees was conditional in regard to the type of resource collected and the presence of ants. Ants were dominant nectar-consumers that mostly depleted the nectar of visited inflorescences. Accordingly, the visitation frequency, duration, and consequently the pollinator effectiveness of nectar-foraging bees strongly decreased on ant-visited flowers, whereas pollen-collecting bees remained largely unaffected by ants. Overall, endemic bees (*Hylaeus* spp.) were much poorer pollinators than introduced honeybees. The average net effect of ants on pollination of *M. polymorpha* was neutral, corresponding to a similar fruit set of ant-visited and ant-free inflorescences. Our results suggest that invasive social hymenopterans that often have negative impacts on the Hawaiian flora and fauna may occasionally provide neutral (ants) or even beneficial net effects (honeybees).

Introduction

Resource partitioning in response to interspecific competition stabilizes the coexistence of species that occupy a similar niche (Arthur 1986). Interactions between

sympatric flower visitors have been frequently studied and it has been shown that the presence or absence of one species affects the resource utilisation of other species, either influencing the plant species visited (Inouye 1978, Nagamitsu and Inoue 1997) or the temporal (Stone *et al.* 1996) and spatial (Morse 1982) pattern of visitation to individual plants. Many invasive alien species (e.g. *Apis mellifera*) are particularly dominant competitors (Traveset and Richardson 2006), displace native pollinators (Kato *et al.* 1999) and thereby potentially have negative effects on the reproduction of native plants (Traveset and Richardson 2006) when their pollinator effectiveness is lower than the effectiveness of the native, displaced pollinators (see Madjidian *et al.* 2008). Pollinator effectiveness is the product of a floral visitor's quantity and quality of actions leading to pollination (Herrera 1989, Kandori 2002, Reynolds and Fenster 2008). The visitation rate, the duration of visits, the frequency of contacts to stigmas and the amount of pollen deposited on stigmas per contact are surrogates to evaluate effectiveness. The fruit or seed set after visits of individual species represent more direct measures of effectiveness. Several studies demonstrated that pollinator effectiveness differs between different flower visitors of the same plant species (Kandori 2002, Madjidian *et al.* 2008, Reynolds and Fenster 2008). Only few studies, however, have examined how nectar- or pollen collection potentially affects effectiveness (Free 1966, McIntosh 2005), and the presence of other flower visitors has been neglected in this respect.

Ants tend to monopolize and aggressively defend valuable resources such as nectar or other carbohydrate sources against inferior ants (Blüthgen and Fiedler 2004a) or other animals (Madden and Young 1992, Lach 2008a). Although many plant species defend their flowers against ants (Junker *et al.* 2007, Junker and Blüthgen 2008, Willmer *et al.* 2009), they are common flower visitors in some plant species and may even contribute to the plants' reproduction (Gomez and Zamora 1992). Usually, however, ants are non-pollinating, nectar-thieving flower visitors (Galen 1983, Galen and Butchart 2003, Beattie 2006, Nicklen and Wagner 2006) that may even reduce pollen viability (Beattie *et al.* 1984). Besides the reduction of floral nectar, the ants' territorial and aggressive behaviour against pollinators may also influence the plant's reproduction. Several studies reported that ants reduce the visitation frequency and visitation length of pollinators on flowers with some positive but mostly negative effects (Altshuler 1999, Tsuji *et al.* 2004, Lach 2008a).

Ants and bees visit flowers in search for a different set of resources: ants as facultative flower visitors (Junker and Blüthgen 2010b) do not depend on floral resources and are solely interested in sugar-rich but usually amino acid-poor nectar. Bees as obligate flower visitors (Junker and Blüthgen 2010b) collect both nectar and amino acid-rich pollen,

which cannot be digested by most ants with a few exceptions (Urbani and deAndrade 1997, Blüthgen and Feldhaar 2010). Thus, regarding pollen, ants and bees segregate the resource, while with respect to nectar, the consumption of this resource may be influenced by interspecific competition. These contrasting interests may lead to complex interactions between ants and bees on flowers where both resources (nectar and pollen) are spatially separated within one flower as in *Metrosideros polymorpha* Gaudich. (Myrtaceae).

On the Hawaiian Islands where no ants had been present prior to their human introduction, the flowers of the endemic tree *M. polymorpha* are numerously visited by invasive ants (Lach 2005, 2008b). Lach (2005) observed that invasive ants deplete and defend nectar of visited flowers and thereby reduce the visitation frequency of endemic *Hylaeus* spp. bees in cases of flowers that were visited by *Pheidole megacephala* Fabricius ants (2008b). We examined how invasive flower visiting ants interfere with invasive honeybees (*Apis mellifera* L.) and endemic *Hylaeus* spp. bees on flowers of *M. polymorpha* and how these flower visitors partition the resources. Additionally, we assessed how the bees' pollinator effectiveness is influenced by the resources collected and the presence or absence of ants.

Material and methods

Study sites and organisms

This study was performed on the island of Hawai'i in Hawai'i Volcanoes National Park (HAVO) from March to May 2009. We selected two sites within HAVO where *Metrosideros polymorpha* (Myrtaceae) trees dominated the vegetation and provided the most floral resources within the area. Alanui Kahiko (188 m a.s.l.; N 19° 18.18', W 155° 08.93') is an old lava flow with sparse ground-vegetation and was invaded by *Anoplolepis gracilipes* F. Smith and *Plagiolepis alluaudi* Emery ants. The second site, the Broomsedge Burn Area (1230 m a.s.l.; N 19° 26.23', W 155° 17.97'), was densely covered with the naturalized grasses *Andropogon virginicus* and *Schizachyrium condensatum* (Poaceae) and was inhabited by *Linepithema humile* Mayr ants.

M. polymorpha is found from sea level to tree line (0 – 2600 m a.s.l.) in a variety of habitats and successional stages and occurs in various growth forms from small shrubs to large trees (Wagner *et al.* 1990). The brush-like inflorescences are composed of 10 to 30 flowers that open sequentially either within a long period (up to 40 days) or a short time span (Carpenter 1976). The open flowers are cup-like with a central style and numerous filaments encircling the nectar cup. Length of style and filaments was up to 3.5 cm in our

study trees but may be much shorter in other populations (Wagner *et al.* 1990). The proportional fruit set by the partially self-incompatible flowers is usually much higher after pollination (Carpenter 1976), but see Burton (1982). The flowers are regarded as bird pollinated (Carpenter 1976) and were historically visited by native honeycreepers (Drepanididae), but today also by introduced bird species (e.g. *Zosterops japonicus*) (Carpenter 1976). Only occasionally we observed birds (e.g. *Himatione sanguinea*) foraging on *M. polymorpha* flowers at both sites. Several species of hymenopterans also frequently visit the flowers and some of them also contribute to pollination (Carpenter 1976, Lach 2008b). The flowers in both study sites where visited by invasive honeybees *Apis mellifera,* and the flowers in Alanui Kahiko where also visited by endemic bee species of the genus *Hylaeus*. These bees are much smaller and less abundant than honeybees. The flowers in Alanui Kahiko where visited by *Anoplolepis gracilipes* ants (body size ~5 mm), those in the Broomsedge Burn Area by *Linepithema humile* ants (body size ~2.5 mm).

Treatment of inflorescences

In each of the study sites, ten trees were selected that featured sufficient flower buds. Several inflorescences on each tree were treated in one of the following ways. (*1*) *Ant exclusion*: a large branch, often bearing several inflorescences, was covered with a sticky barrier (Raupenleim, Schacht, Germany) at its base, and ants currently visiting the branches were removed. Thus, only flying animals had access to the flowers. (*2*) *Control*: untreated branches – inflorescences were accessible to all visitors. (*3*) *Ants only*: one inflorescence per tree was haphazardly selected and encased with a plastic cup (250 ml) with the bottom facing to the base of the branch. We cut a hole (one third of the circumference) in the bottom to allow ants to crawl in and a second notch for the branch. The top was covered with a pollen-tight mesh to exclude flying insects and wind drifted pollen. A strip of the plastic cup was cut and likewise covered with pollen-tight mesh in order to reduce the "green-house effect" inside the cups. We used cups instead of mesh bags in order to prevent the mesh touching the reproductive structures and thereby contribute to pollination. (*4*) *Complete exclusion*: one inflorescence of the ant exclusion branch (*1*) was haphazardly selected and encased with a cut plastic cup similar to (*3*). Places of treatments were chosen on a comparable height within each tree and facing towards the same compass direction in order to reduce variability caused by abiotic parameters.

Visitation rate

Between 06:30 and 12:00, from a distance of 1-2 meters we observed all ant-visited and ant-free inflorescences (treatments *1* and *2*) of a tree and noted the flower visitor species (i.e. *Apis mellifera* or *Hylaeus* spp. that were the only frequent visitors next to ants), duration of visits, presence / absence of ants, resource used by flower visitor (pollen or nectar) and the total observation time. At the end of each observation period the number of ants inflorescence^{-1} and flowers inflorescence^{-1} was noted.

Nectar availability

On eight days between 07:30 and 14:00 the standing crop of nectar [µl] in flowers of ant-visited (n = 92) and ant-free branches (n = 92) was determined using micro-capillaries (5 µl). In Broomsedge Burn Area additional five trees were treated with a sticky barrier for nectar measurements in order to evaluate the amount of nectar in ant-free inflorescences.

Ant-bee interactions

In another test series, we carefully approached the inflorescences after bees had landed on ant-visited branches (treatment *2*) and observed interactions between bees and ants. Interactions were assigned to aggressive interactions (bees left the inflorescences after contact or ant approaching) and non-aggressive interactions (ants and bees shared the inflorescence).

Stigma contacts and pollen deposition

The number of stigma contacts during flower visits [contacts min^{-1}] of *A. mellifera* (n = 48 visits) and *Hylaeus* spp. (n = 26) was counted and it was noted which resource the bees collected. Furthermore, 27 inflorescences were enveloped with a wire frame covered with pollen mesh prior to anthesis in order to prevent contamination of stigmas with pollen by animals or wind. During anthesis the wire frame was removed and three stigmas were immediately removed using forceps and were prepared for microscopy: stigmas were placed between an object slide and a cover slip and were gently squeezed. Afterwards, inflorescences with the remaining stigmas were free to be visited by bees and contacted stigmas were also prepared for microscopy. The preparations were stained with basic fuchsine-solution (see Kearns and Inouye 1993) and pollen grains were counted approximately ten minutes after staining, when the staining caused a pink colouration of the stigma-tissue while the pollen grains not yet absorbed the staining and remained yellow.

Pollinator effectiveness

Pollinator effectiveness was individually calculated by bootstrapping for nectar and pollen collecting honeybees and *Hylaeus* bees on ant-free and ant-visited flowers and defined as follows:

$$E = f \cdot t \cdot c \cdot d$$

with E as pollinator effectiveness [pollen h^{-1}], f as visitation frequency [visits h^{-1}], t as visitation time [h $visit^{-1}$], c as stigma contacts [contacts h^{-1}] and d as pollen deposition [pollen $contact^{-1}$]. We used bootstrapping to calculate the mean pollinator effectiveness and its standard error for both bee taxa as a function of resource collected and presence/absence of ants. A combination of the specific data-sets f, t, c and d for nectar- and pollen-collecting honeybees and *Hylaeus* spp. bees on flowers with and without ants were separately resampled (with replacement) for n = min (n_f, n_t, n_c, n_d) times and we calculated the mean of their products. We repeated this procedure 1000 times and used the mean and the standard error of the means of the products as pollinator effectiveness. Bootstrapping values were compared with *t*-tests.

Fruit set

On all treated trees, number of flower buds and flowers of three inflorescences of treatments *1* and *2* and the inflorescences of *3* and *4* were counted before or during their anthesis and compared to the number of developing fruits a few weeks later.

Results

Visitation rate

Linepithema humile ants visited the flowers (treatment *2*) of *Metrosideros polymorpha* in the Broomsedge Burn Area in high densities (mean ± *SE*: 1.0 ± 0.16 ants $flower^{-1}$), while *Anoplolepis gracilipes* ants visited the flowers in Alanui Kahiko in lower densities (0.13 ± 0.02). Honeybees (*Apis mellifera*) collected nectar and pollen of *M. polymorpha* flowers at both sites, while we observed *Hylaeus* spp. bees only in Alanui Kahiko, collecting pollen. Overall, ant-free inflorescences were more frequently visited by bees (mean ± *SE*: 0.25 ± 0.09 bees $flower^{-1}$ h^{-1}) than ant-visited ones (0.07 ± 0.02), albeit this was only marginally significant (paired *t*-test: t_{25} = 2.01, p = 0.055). With an average of 15.6 ± 1.4 (mean ± *SE*) flowers per inflorescence, the visitation rate amounts to 3.9 bees per ant-free inflorescence per hour. Nectar-foraging honeybees strongly preferred ant-free flowers

over ant-visited ones (paired *t*-test: t_{14} = 2.2, p = 0.049; Fig. 1), whereas pollen-collecting honeybees and *Hylaeus* did not discriminate either of the flowers ($t \leq 0.79$, $p \geq 0.45$) (Fig. 1). The same is true for the time spent on inflorescences: Pollen collecting honeybees and *Hylaeus* remained similarly long on inflorescences with and without ants (Welch corrected *t*-test: $t \leq 0.503$, $p \geq 0.62$) (Fig. 1). Nectar collecting honeybees, however, remained twice as long on ant-free inflorescences than on ant-visited ones ($t_{44.1}$ = 3.3, $p < 0.01$, Fig. 1).

The proportion of nectar to pollen collecting honeybees (proportion = nectar collectors / pollen collectors) was typically lower on ant-visited (1.00 ± 0.02) inflorescences than on ant-free ones (1.25 ± 0.11). Thus, the relative frequency of nectar foragers significantly increased on ant-free inflorescences while the relative frequency of pollen foragers decreased compared to ant-visited inflorescences and *vice versa* (paired *t*-test: t_{20} = 2.48, p = 0.022). This resource shift was independent of the density of ants visiting the flowers (Pearson's R^2 = 0.08, df = 19, p = 0.23). Since *A. gracilipes* ants visited the flowers in lower densities than *L. humile* ants, the effects of ant species and density cannot be separated; we thus pooled results from both sites.

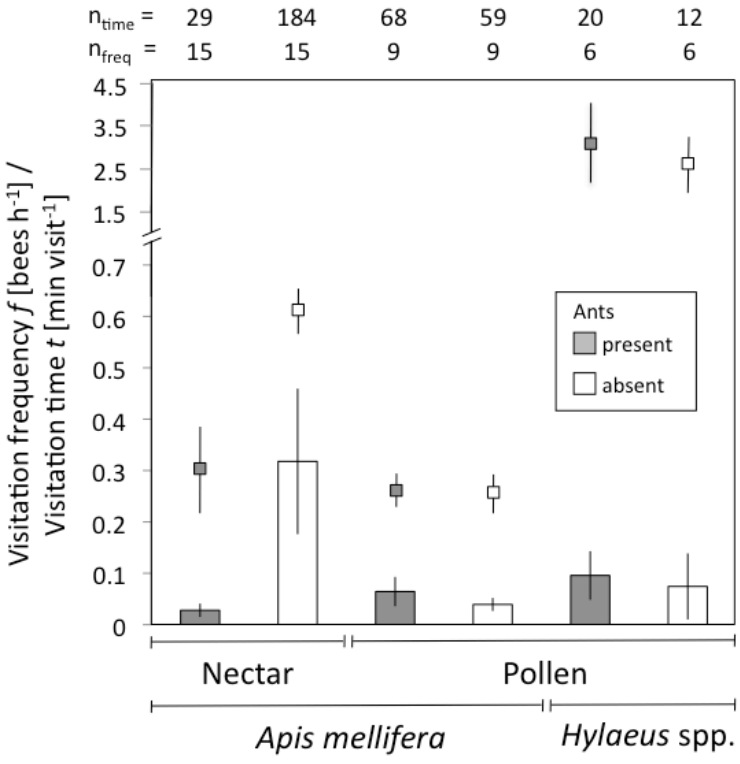

Fig. 1 Visitation frequency (bars) and time (squares) of *Apis mellifera* and *Hylaeus* spp. in flowers of *Metrosideros polymorpha*. Bees are distinguished by the kind of resource collected (nectar or pollen), and flowers are distinguished by the presence of ants. Mean and standard error are shown. Sample sizes are given above the bars and squares.

Nectar availability

Ants dramatically reduced available nectar: on average, flowers of inflorescences where ants were excluded (treatment *1*) provided eleven times more nectar (mean ± SE: 6.11 ± 0.97 µl flower^{-1}) than flowers where ants had access (treatment *2*, 0.55 ± 0.35 µl flower^{-1}) (paired *t* - test: t_{22} = 5.3, p < 0.001). This effect was equally pronounced in both sites ($t \geq 2.89$, $p \leq 0.023$). Average sugar concentration of nectar was 32.1 ± 2.6 % w/w (mean ± SE) measured with a handheld refractometer (Eclipse, Bellingham + Stanley, UK).

Ant-bee interactions

In total, we observed 98 cases where ants and bees shared an inflorescence; in only 13.3 % of all cases ants displayed aggressive behaviour and displaced bees. Bees and ants often collected resources, respectively, on the same or an adjacent flower without an interaction. However, most of the observations (91.8 %) involved pollen-collecting bees since nectar-foragers on ant-visited flowers were rare. One third of interactions that involved nectar-collecting bees were aggressive, but note the small sample size (n = 6).

Stigma contacts and pollen deposition

Number of stigma contacts was highly dependent on the bee species and the resource the bees collected (ANOVA: $F_{2,71}$ = 88.6, p < 0.001) (Fig. 2): nectar collecting honeybees and pollen-collecting *Hylaeus* rarely touched any stigmas whereas pollen-collecting honeybees had a high frequency of stigma contacts. Furthermore, honeybees deposited significantly more pollen per stigma contact on the receptive structure than *Hylaeus*. After contacts by *Hylaeus*, stigmas did not contain more pollen grains than control stigmas ($F_{2,44}$ = 5.6, p < 0.01, Fig. 3). Ants very rarely climbed up the styles or filaments and thus almost never had contact to the stigmas and thus most probably do not contribute to pollination.

Pollinator effectiveness

The presence/absence of ants and the resource collected (nectar or pollen) by bees strongly influenced the effectiveness *E* of the pollinators (Fig. 4). Honeybees that collected nectar were much more effective on ant-free flowers than on ant-visited flowers (Welch two sample *t*-test: $t_{1003.9}$ = 14.5, p < 0.001) – a result caused by a reduced visitation frequency *f* and time *t* spent on flowers (Fig. 1). Pollen-collecting honeybees on ant-visited flowers, however, were more effective than those on ant-free flowers ($t_{1535.0}$ = 11.2, p < 0.001). This

is the result from a slightly (but not significantly) higher visitation frequency of pollen-collecting honeybees on ant-visited flowers. *E* of pollen-collecting *Hylaeus* was not affected by the presence of ants in inflorescences ($t_{1964.0}$ = 1.5, p = 0.12). Overall, the pollination effectiveness of both bee taxa, regardless whether nectar or pollen was collected, differed only slightly between ant-visited (mean ± SE: *E* = 20.2 ± 0.24) and ant-free flowers (22.7 ± 0.34) ($t_{1784.9}$ = 5.88, p < 0.001). The average pollinator effectiveness of honeybees (28.7 ± 0.23) was much higher than that of *Hylaeus* (7.6 ± 0.29) ($t_{1900.3}$ = 57.7, p < 0.001).

Fruit set

Fruit set strongly varied between treatments (ANOVA: $F_{3,116}$ = 22.8, p < 0.001) (Fig. 5): it was highest in inflorescences to which all visitors had access (control, *2*) and where ants were excluded but flying insects were allowed to visit (treatment, *3*). Inflorescences from which all visitors were excluded (complete exclusion, *4*) and inflorescences where only ants had access (ants only, 3) had the lowest fruit set.

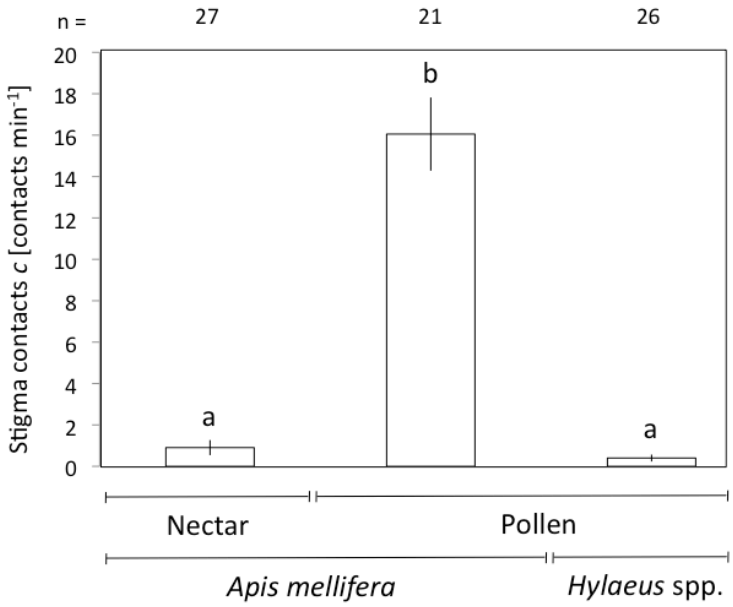

Fig. 2 Number of stigma contacts of nectar- and pollen collecting *Apis mellifera* and pollen-collecting *Hylaeus* bees. Mean and standard error are shown. Different letters correspond to differences according to Tukey's post hoc comparisons. Sample sizes are given above the bars.

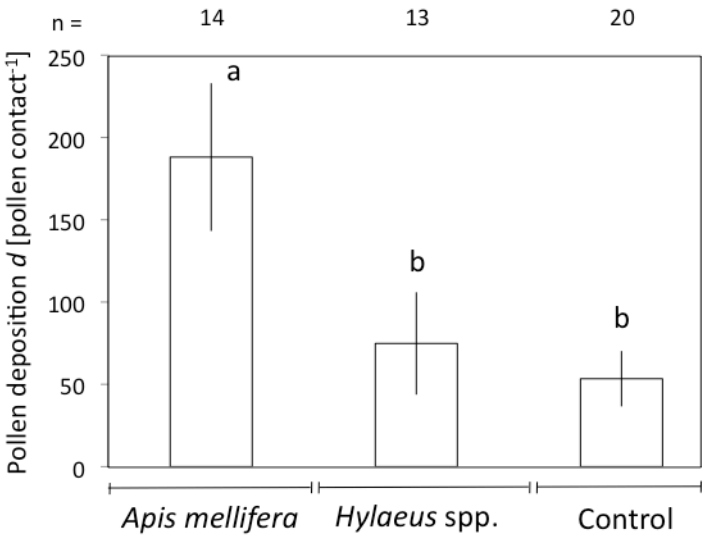

Fig. 3 Number of pollen deposited on a stigma by *Apis mellifera* and *Hylaeus* spp. per contact. Mean and standard error are shown. Different letters correspond to differences according to Tukey's multiple comparisons of means. Sample sizes are given above the bars.

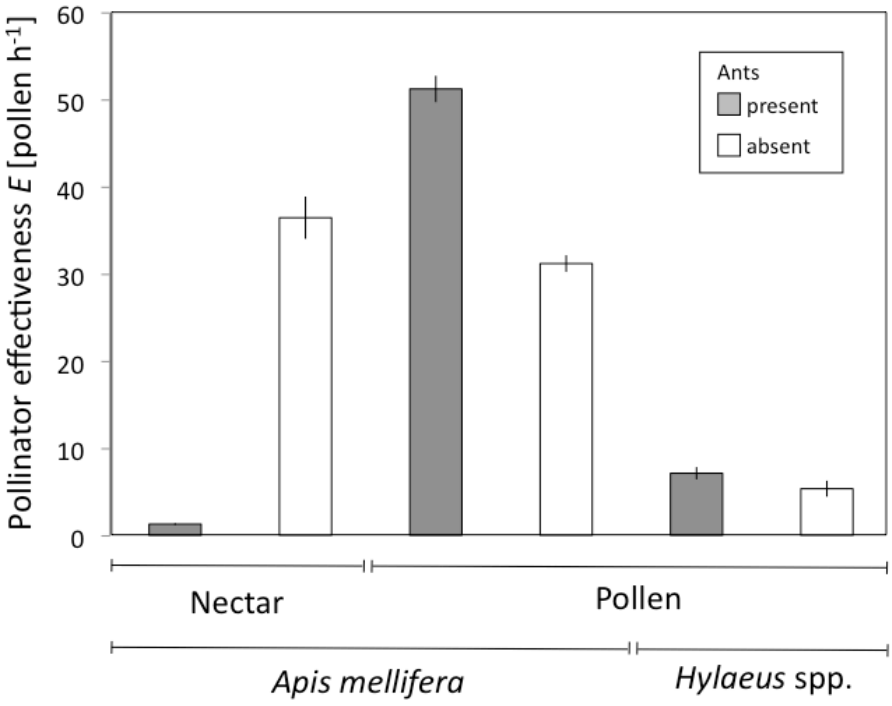

Fig. 4 Pollinator effectiveness of *Apis mellifera* and *Hylaeus* spp. dependent on the presence and absence of ants and the resource collected in flowers of *Metrosideros polymorpha*. Mean and standard error of 1000 bootstrapping results are shown.

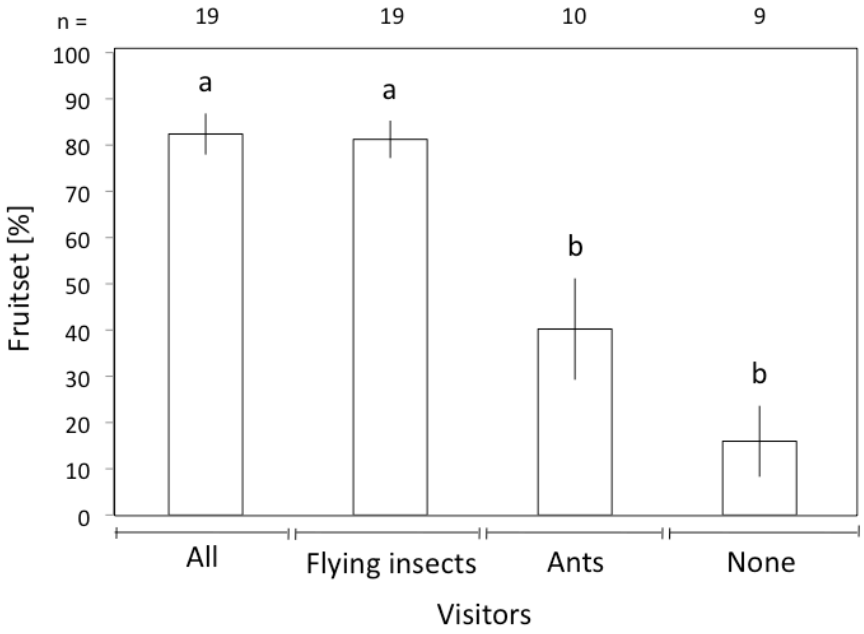

Fig. 5 Proportional fruit set of inflorescences from *Metrosideros polymorpha* with different flower visitor spectra. Mean and standard error are shown. Different letters correspond to differences according to Tukey's multiple comparisons of means. Sample sizes are given above the bars.

Discussion

Ants were competitively dominant nectar-foragers on *Metrosideros polymorpha*, where they almost completely depleted the nectar of flowers and sometimes actively defended this resource against bees. Thus, exploitation and, to a lesser extent, interference competition led to intrafloral resource partitioning: the frequency of nectar foraging honeybees strongly increased on ant-free flowers while ants did not strongly affect the frequency of pollen-collectors. This dichotomy can be explained by the ants' interest in nectar but not pollen, and by the floral morphology of *M. polymorpha* where both resources are spatially separated from each other. Thus, ants stayed only in the cup-like structures that bear the nectaries and where nectar accumulates, while the anthers and the stigmas remain disregarded by the ants. The absence of nectar-collecting *Hylaeus* bees remains unexplained since they are known to collect nectar from other plant species (Magnacca 2007). Our results partly support results from Lach (2005, 2008b) who also observed the effects of *Anoplolepis gracilipes* and *Linepithema humile* ants on bee visitation on *M. polymorpha* flowers. However, *Hylaeus* bees were shown to strongly reduce their visitation frequency on flowers visited by *Pheidole megacephala* (Lach 2008b) although they also collected pollen only (Lori Lach, pers. comm.). Honeybees were not affected by ant-visits in

terms of their visitation frequency or length of visitation but no information on the resource collected by honeybees is given (Lach 2008b).

Furthermore, while Lach (2005) found that *A. gracilipes* strongly outcompetes other nectarivorous flower visitors by interference and exploitation, and *L. humile* mainly by interference, our data suggest a strong exploitation and weak interference competition for both species. The extent to which ants deter bees from flowers may be dependent on the ant-species present mainly due to different aggression levels (Ness 2006). The two ant species observed in our study occupied flowers in different densities, thus we were not able to separate effects of density and species. However, results do not indicate effects of different densities and/or species. Furthermore, since the main effect of ants was the exploitation of the nectar reward where both ant species succeeded equally well, no ant species-effects may be expected in this case.

The identity of the bees, the ants' presence or absence, and the utilisation of either nectar or pollen, led to a conditionality of the pollinator effectiveness. The smaller *Hylaeus* bees rarely touched stigmas while the larger honeybees had inevitably multiple contacts to stigmas while collecting pollen. Flowers of *M. polymorpha* are typically bird pollinated (Carpenter 1976) and thus the flowers' morphology is not adapted to the *Hylaeus* bees' small body size. The structural mismatch between flower visitors and flowers often leads to pollen theft (Hargreaves *et al.* 2009), which may be pronounced in this specific interaction. Furthermore, the longer duration of *Hylaeus* visits on individual inflorescences (and often even on individual anthers) may severely reduce the amount of pollen from other plant individuals deposited on stigmas. Invasive, feral honeybees are assumed to have multiple negative effects on native ecosystems (Goulson 2003) but are also known as long-distance dispersers of pollen in plants for which native pollinators are rare or absent (Dick 2001). In this study, honeybees pollinated the flowers of *M. polymorpha* much better than endemic *Hylaeus* bees in terms of pollen deposition per time.

Although ants are effective pollinators for some plant species (Gomez and Zamora 1992), they do not contribute to pollination in *M. polymorpha* since they never or only rarely contact anthers and stigmas of these flowers and the fruit set of inflorescences that were visited by ants only was low. Unexpectedly, when invasive ants consume nectar and invasive honeybees collected pollen on the same inflorescence, the flowers were most effectively pollinated. However, note that this was a multiplied effect of a slightly higher (but not significantly) visitation frequency on ant-visited flowers by pollen-collecting honeybees, thus this result may be viewed with caution. Since bird populations have declined in Hawaii (Benning *et al.* 2002), the presence of honeybees may ensure the pollination of this endemic

species. Furthermore, the ants' effect on overall pollination effectiveness by the bee taxa may be ignored. On average, ant-visited flowers were only slightly less effectively pollinated than ant-free flowers. This result was reflected in the fruit set of flowers from which ants have been experimentally excluded over the whole period from flower maturation to fruit set, but to which flying insects had access. Due to a partial self-incompatibility of red flower morphs, which were present in our study sites, maximal fruit set occurs only after cross-pollination (Carpenter 1976). The high proportion in fruit set in bee-visited flowers suggests that bees deposited not only pollen from the same but also from other individuals. Flower visiting birds were very rare in our study sites suggesting a low contribution to the pollination of our study-trees.

Madjidian *et al.* (2008) identified some restrictions regarding estimates on pollinator effectiveness that also apply to our study. The most important restrictions in our systems may be: (1) our measurements of parameters incorporated in the pollinator effectiveness were "snapshots" and it is unclear whether visitation frequencies and times remain constant over the whole receptive period of flowers. (2) The estimate of the effectiveness is only meaningful if pollen is limited, which is likely for *M. polymorpha* since a high percentage of the seeds are non-viable (Drake 1992). (3) The effectiveness of *Hylaeus* bees may be overestimated since number of pollen grains deposited on stigmas was not significantly higher than on control stigmas. (4) It is unclear whether duration of visit is positively or negatively correlated to pollinator effectiveness. We assumed a positive relationship, which is supported by some studies (Ivey *et al.* 2003) but the opposite is suggested in others (Gomez and Zamora 1999). Since *Hylaeus* bees spent comparatively long times on individual flowers or anthers, the proportion of pollen deposited in stigmas from other tree individuals may decrease over the time and consequently the contribution to pollination as well.

Our study reveals a strong conditionality of pollinator effectiveness that is caused by an intrafloral resource partitioning between endemic and invasive flower visitors. We did not find any direct negative effects of invasive ants and bees on endemic flower visitors; but negative effects resulting from competition for nesting sites or resources and predation are demonstrated in other studies (Gross 2001, Magnacca 2007). These results suggest that invasive social hymenopterans that have devastating effects on the native Hawaiian flora and fauna (Medeiros *et al.* 1986, Holway *et al.* 2002, Goulson 2003, Krushelnycky *et al.* 2005, Krushelnycky and Gillespie 2008, Lach 2008b) may also have neutral (ants) or even positive effects (honeybees) for endemic plant species.

XIII. Beneficial effect of flower-visiting invasive ants on an endemic Hawaiian plant?

This chapter has been published as:

Bleil R, Blüthgen N, Junker RR (submitted) Invasive ants reduce flower parasitism of the endemic Hawaiian shrub *Vaccinium reticulatum* (Ericaceae)

Summary

Ants had been absent from the Hawaiian Islands prior to their human introduction. Today they cause severe alterations of ecosystems and displace native biota. Due to their strong demand on carbohydrate-rich resources, they often exploit floral nectar of native Hawaiian plant species with largely unknown consequences for the plants' reproduction. We examined the effects of flower-visiting invasive ants on the reproduction of the endemic shrub *Vaccinium reticulatum* (Ericaceae) in Hawai'i Volcanoes National Park. Ant densities in flowers were high and floral nectar was excessively exploited, which may lead to a reduced visitation rate of pollinators. However, the ants' presence on flowers strongly reduced flower parasitism by caterpillars of the introduced plume moth *Stenoptilodes littoralis* and thus decreased the loss of flowers and buds. This is, to our knowledge, the first documented mutualism between invasive ants and an endemic plant species in Hawai'i. The developed fruits of this partly self-incompatible plant, however, bore relatively low proportions of viable seeds, irrespective of the experimentally controlled visitor spectrum of the flowers. This may indicate that ants do not function as pollinators and that effective pollinators (probably *Hylaeus* bees) are scant or absent. Correspondingly, we never observed any other flower visitor apart from ants and caterpillars of *S. littoralis*. Ants are known to displace native arthropods including *Hylaeus* bees. Together with their competition with pollinators, this suggests that the overall negative effects of ants may outweigh the documented positive effect.

Introduction

Invasive ants are generally characterized as widespread, abundant, aggressive and omnivorous (Holway *et al.* 2002, Lach *et al.* 2010). They often cause severe alterations of ecosystems by displacing native species and thereby breaking up mutualistic interactions

and ecological key functions such as nutrient cycling, seed dispersal, or pollination (Holway *et al.* 2002). Island ecosystems are particularly vulnerable to invasions due to disharmonic floras and faunas and their isolation from other terrestrial habitats (Mooney *et al.* 2005). With a distance of 3900 km to the closest continent, the Hawaiian Islands are among the most isolated archipelagos of the world. They bear a high percentage (90 %) of endemic species (Wagner *et al.* 1990), more than any region of similar size on earth. Only recently, ants have been introduced to Hawai'i where plants and animals have evolved in the absence of any social hymenopterans and their spreading has had devastating consequences to native ecosystems (Krushelnycky and Gillespie 2008).

Ants require carbohydrate-rich resources to fuel their colony's nutrition. Sources of carbohydrates may be honeydew (sugary exudates of insects), as well as extrafloral and floral nectar (Blüthgen and Fiedler 2004a, Blüthgen *et al.* 2004a). However, honeydew-producing insects are rare in many Hawaiian habitats (authors' own observation) and only few endemic plants possess extrafloral nectaries (Keeler 1985). Thus, floral nectar may be an important sugar-supply for ants in Hawai'i and can be heavily exploited (Lach 2005, 2008b). Flower visiting ants are detrimental to the reproduction of many plant species as they usually are poor pollinators (Hölldobler and Wilson 1990) due to their relatively low mobility, central-place foraging, often hairless cuticles and glandular secretions that can reduce pollen fertility (Beattie *et al.* 1984, Beattie *et al.* 1985, Galen and Butchart 2003). Thus, ants are generally referred to as nectar thieves (Inouye 1980) that often drive off pollinators (Tsuji *et al.* 2004, Junker *et al.* 2007). As a response to detrimental flower-visits by ants, many plant species defend their flowers via mechanical barriers (Galen and Cuba 2001, Herrera 2001), unpalatable nectar(Feinsinger and Swarm 1978, Guerrant and Fiedler 1981, Junker and Blüthgen 2008), and by repellent floral scents (Junker and Blüthgen 2008, Willmer *et al.* 2009, Junker and Blüthgen 2010b). Endemic Hawaiian plants might lack such defense mechanisms due to the absence of ants during their evolution.

Flower visitation by ants can also positively affect the plant's reproduction by means of ant-pollination (Gomez and Zamora 1992, Beattie 2006) or an increase in cross-pollination through disturbance of pollinators (Altshuler 1999). Furthermore, several studies have shown that ants effectively protect flowers from damage by herbivores (Rico-Gray and Oliveira 2007). In these studies, ants were attracted to EFNs on pedicels, bracts, or flower buds and increased fruit set by reducing herbivore damage to flowers and buds. Flower protection by ants that consume floral nectar has also been documented (Lach 2008b) but seems to be less common.

In this study, we quantified the consumption of nectar by invasive ants on flowers of the endemic shrub *Vaccinium reticulatum* (Ericaceae) and investigated its associated effects on the plants' reproduction and on flower parasitism by caterpillars of the introduced plume moth *Stenoptilodes littoralis* which occurred in high abundances and parasitized flower buds and flowers of this plant species leading to their abortion.

Materials and methods

Study sites and organisms

The study was conducted at two woodland sites dominated by *Metrosideros polymorpha* (Myrtaceae) within Hawai'i Volcanoes National Park (HAVO) between March and May 2009. One site, Kīpuka Kahali'i (897 m a.s.l.; N 19° 20.70', W 155° 12.65'), had a cinder substrate, very sparse ground vegetation, and was inhabited by the ant species *Pheidole megacephala*, *Paratrechina bourbonica* and *Plagiolepis alluaudi*, all of which were observed visiting flowers of *Vaccinium reticulatum*. The other site, Broomsedge Burn Area (1230 m a.s.l.; N 19° 26.23', W 155° 17.97'), had a weathered pāhoehoe lava substrate. The dense ground vegetation consisted mainly of invasive grasses such as *Andropogon virginicus* and *Schizachyrium condensatum* (both Poaceae), and was inhabited by a single ant species, *Linepithema humile*, which also visited flowers of *V. reticulatum*.
V. reticulatum (Ericaceae, Hawaiian: 'ōhelo) is a small shrub (10-130 cm in height) usually occurring as a member of the pioneer flora on lava flows, ash dunes and cinder beds or as a member of communities at exposed sites such as alpine and subalpine shrubland. It is common on the islands of Maui and Hawai'I (Wagner *et al.* 1990). *V. reticulatum* is an important plant for Hawaiian tradition being the inherbation of goddess Pele's sister Ka'ōhelo. Branches of the plant are traditionally used as offerings to the goddess and berries are eaten raw or in jams, jellies, or pies. The berries are also an important food source for native animals like the endangered Hawaiian Goose Nēnē (*Branta sandvicensis*) (Black *et al.* 1994) which in turn is an important disperser of *V. reticulatum* seeds (Wagner *et al.* 1990). Yellow-faced bees (*Hylaeus* spp.) have been recorded to be by far the most abundant flower visitors (Heather Sahli, pers. comm.) and probably pollinate the flowers. Imagines of the caterpillars which parasitized in flower buds and flowers were identified as *Stenoptilodes littoralis* (Pterophoridae) by Bernard Landry (Muséum d' histoire naturelle, Geneva). The species has been reported to occur on *Vaccinium* spp. and is considered to be introduced (Henneman and Memmott 2001).

Treatment of plants

Four haphazardly selected branches of ten *V. reticulatum* plants at Kīpuka Kahali'i and of 15 plants at Broomsedge Burn Area were treated as follows: (1) *Ants excluded*: the basal section of the branch was covered with a sticky barrier (Raupenleim, Schacht, Germany) to prevent ants from accessing inflorescences. (2) *All visitors allowed*: no modifications were done. (3) *Flying visitors excluded*: a plastic cup was put over the branch's inflorescence with the bottom facing to the basis of the branch. The bottom had a small opening for the stem and a crescent-shaped one of about a third of the bottom's diameter through which ants were able to access the flowers. The top of the cup reaching beyond the tip of the inflorescence was covered with pollen mesh (Pollen Protection Sheet, GCM, Germany) to keep out flying visitors and pollen. (4) *All visitors excluded*: the inflorescence of each branch was covered entirely with pollen mesh to keep out all visitors and pollen. Additionally, eleven plants at Broomsedge Burn Area were treated with categories one and two only, for nectar measurements. We checked all flowers and flower buds for evidence of presence of the caterpillars.

Availability of nectar

On two days from 07.45 to 09.00 four flowers from ant-free and four flowers from ant-visited branches of a total of eleven plants at Broomsedge Burn Area were haphazardly chosen and nectar volume [µl] was measured using microcapillary tubes (5 µl). Means of the four measurements per plant were compared in a paired design with a Wilcoxon signed rank (n = 11 pairs).

Flower parasitism

V. reticulatum readily self pollinates (Wagner *et al.* 1990) and self pollination in this species leads to 100 % fruit set (Vander Kloet 1993). Thus, we considered fruit set (developed fruits per initial number of flower buds) to be independent from pollination but dependent on the rate of flower abortion through parasitism. Therefore, fruit set was used as a measure for flower parasitism. Self pollination in *V. reticulatum* leads to very low proportions of viable seeds (Vander Kloet 1993). Thus, we considered seed set (proportion of viable seeds per fruit) to be dependent on pollination but independent from flower parasitism and used it as a measure for pollination. The ant density on flowers of each plant was estimated by selecting haphazardly up to 30 flowers of control- and other untreated branches and counting ants inside the corolla once a week. For analysis of fruit set we

counted flower buds and, if present, open flowers on the four treated branches prior to the preparation of the branches. Then, on a weekly basis, all branches were checked for newly developed buds. Finally, when the last flower of each branch had senesced, we counted the developing fruits. Fruits of treatment 4 (*all excluded*) were not included in the statistical analysis because most of the flowers were fed upon by caterpillars that were trapped inside the pollen meshes. Groups were compared with a Friedman test, and the ants' density effect on fruit set was investigated with a Spearman rank correlation.

Seed Set

After all flowers had senesced, treatments (3) and (4) were removed in order to provide equal microclimatic conditions to all branches in terms of fruit development. The berries were collected about two months after bloom. We washed out the seeds from the flesh and counted viable seeds containing an embryo and unviable seeds without an embryo. Both were easily distinguishable under a dissecting microscope (Vander Kloet 1993). For each experimental plant, the proportions of viable seeds in the berries of each treatment were averaged. Seed set was compared with a Kruskal-Wallis test.

Results

We observed numerous pierced flower buds that were prone to die off. Some pierced flower buds, however, still developed into flowers that lacked reproductive structures. We observed a caterpillar of the plume moth *Stenoptilodes littoralis*, apparently coming from a consumed bud, moving to a new bud and entering it through a hole, pierced into the petals. During those interchanges of host buds, caterpillars are particularly exposed to predation by ants. Additionally, we observed an argentine ant (*L. humile*) entering a flower bud through a caterpillar hole and reappearing with the head of a caterpillar. We offered caterpillars of *S. littoralis* to both *P. megacephala* and *P. bourbonica* ants outside flowers. They were readily taken and transported to the nest. On flowering branches of *V. reticulatum*, new buds appear regularly, so nectar producing flowers and buds are most often in direct proximity to each other (own observation). This ensures contact of flower-visiting ants with bud-feeding caterpillars. Furthermore, the ants may drive off adult females and thus prevent them from ovipositing.

Availability of nectar

Ants excessively exploited the floral nectar of *V. reticulatum.* No nectar could be extracted from flowers on ant-accessible branches in any of the samples. Consequently, the volume of available nectar in ant-excluded flowers (median, quartiles: 0.13, 0.00 – 0.56 µl flower^{-1}) was significantly higher than in flowers that were accessible for ants (Wilcoxon signed rank test z = -2.13, p = 0.022, n = 11).

Flower parasitism

At both sites, ant free branches produced fewer fruits (% of initial flower bud set) than ant-visited branches, i.e. more flowers and flower buds were lost to parasitism on branches without ants. On average, the presence of ants increased fruit set more than five-fold compared to the fruit set on ant-excluded branches irrespective of other allowed visitors (Friedman Test, chi^2 = 17.69, p < 0.01, n = 19; Fig. 1). Fruit set was generally higher at Broomsedge Burn area, where ant density was higher (median, quartiles: 0.72, 0.45 – 0.93) ants flower^{-1}, compared to 0.06 (0.03 – 0.35) ants flower^{-1} at Kīpuka Kahali'i). Fruit set was significantly correlated with ant density in flowers of individual plants. Plants with high ant densities in their flowers set more fruit than individuals with lower ant densities (Spearman rank correlation, r = 0.56, p < 0.01, n = 23; Fig. 2).

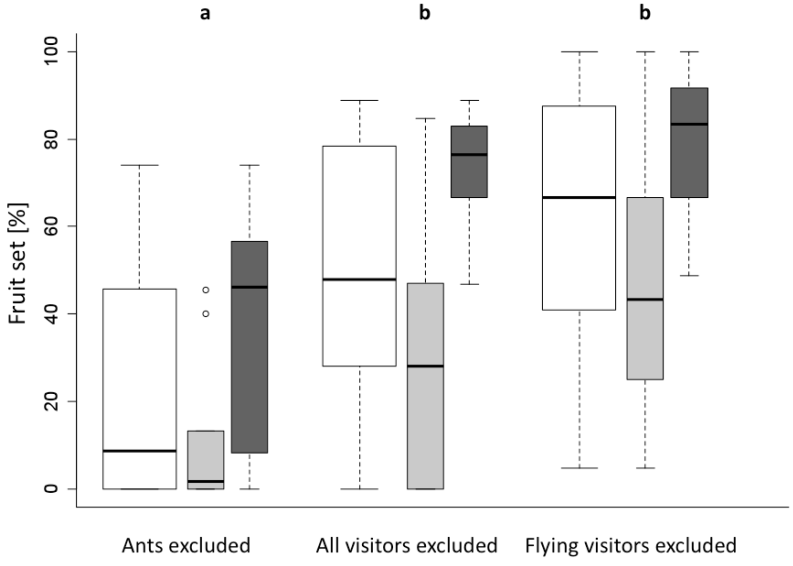

Fig. 1 Fruit set of inflorescences with experimentally altered visitor spectrums averaged across both field sites (white boxes), at Kīpuka Kahali'I (light gray boxes) and at Broomsedge Burn Area (dark gray boxes). Boxplots show medians (lines), quartiles (boxes), range (whiskers) and outliers (cycles). Letters indicate statistically significant differences (p < 0.05) among treatments for pooled as well as for non-pooled data according to pairwise Wilcoxon rank sum tests.

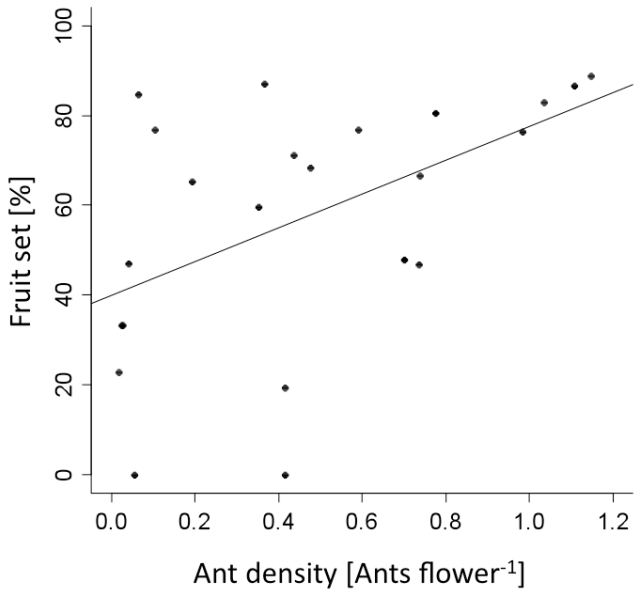

Fig. 2 Relationship between ant density in flowers [ants flower^{-1}] and fruit set of the control (*all allowed*) branches.

Seed set

No flower visitors other than ants and caterpillars of *S. littoralis* were observed during the whole study period. *Hylaeus* spp. bees, the most common visitors of *V. reticulatum* elsewhere (Heather Sahli, pers. comm.) and probably the main pollinator of *V. reticulatum*, were very rare (Broomsedge Burn Area) or absent (Kīpuka Kahali'i) from the study sites (own observations on other flowers in the same habitat). The proportion of viable seeds was low (median: < 10 %) for all treatments (Fig. 3). Consequently, the different treatments showed no effects on seed set of *V. reticulatum* neither at both sites separately nor combined (Kruskal-Wallis test, all $chi^2 \leq 4.09$, $p \geq 0.25$, n (for Kīpuka Kahali'i and Broomsedge Burn Area) = 3 and 11 (ants excluded), 9 and 14 (all allowed), 6 and 8 (ants only), 3 and 2 (all excluded)). The proportion of viable seeds was weakly positively correlated with berry diameter (Spearman rank correlation, $r = 0.27$, $p < 0.01$, $n = 100$ berries). However, neither treatment had an effect on berry diameter (Kruskal-Wallis test, $chi^2 = 0.91$, $p = 0.63$, $n = 52$ berries (all allowed), 30 (ants excluded), 18 (ants only), nor ant density (Pearson's product-moment correlation, $r^2 = 0.03$, $p = 0.57$, $n = 13$ plants).

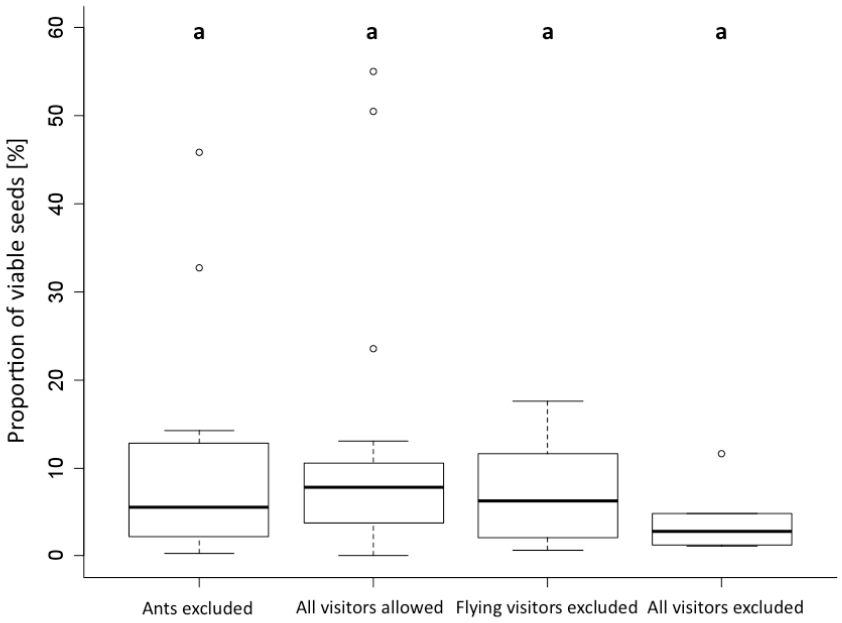

Fig. 3 Proportions of viable seeds in berries from inflorescences with experimentally altered visitor spectrums averaged across both field sites. Boxplots show medians (lines), quartiles (boxes) and range (whiskers), range (whiskers) and outliers (cycles). Letters indicate statistically significant differences ($p < 0.05$) among treatments according to pairwise Wilcoxon rank sum tests.

Discussion

Ants were frequent flower-visitors and depleted floral nectar of *Vaccinium reticulatum.* The presence of ants in flowers did not affect the proportion of viable seeds per fruit but had a positive effect on the plant's reproduction by means of decreasing flower parasitism leading to an increased fruit set. Thus, invasive ants efficiently protected flower buds and flowers of an endemic Hawaiian plant species that is neither adapted to the introduced moth nor to ants. These results constitute a contrasting example to other studies that demonstrated devastating effects of invasive ants on native organisms in Hawai'I (Krushelnycky and Gillespie 2008). The magnitude of floral ant-protection for *V. reticulatum* (fruit set increased more than fivefold) is even greater than shown in studies of coevolved ant-plant mutualisms, where ants increased fruit set usually less than fourfold (Rico-Gray and Thien 1989). However, coevolved systems of ant-flower protection fundamentally differ from the system studied here. In coevolved systems, ants are often attracted to extrafloral nectaries on pedicels, bracts or flower buds (Rico-Gray and Thien 1989) but are mostly repelled from flowers of the same plant during anthesis (Willmer and Stone 1997). Thus, protection of the reproductive structures is enhanced while an interference of ants with pollination is avoided. *V. reticulatum,* that did not coevolve with ants, apparently lacks floral defense mechanisms. The plant profits from ant-protection of its reproductive structures

but cannot avoid interference with pollination. Both, *P. megacephala* (Lach 2008b) and *L. humile* (Blancafort and Gomez 2005, Lach 2007, 2008a) are well known to negatively interfere with other flower visitors. However, we were not able to observe this proposed interference with pollinators as we never observed any potential pollinators visiting the flowers. This apparent lack of pollinators suggests that there was virtually no cross-pollination in any treatment. Our finding that the treatments which allowed visitation of flying insects did not produce more viable seeds than the *all visitors excluded* treatment, supports this assumption. Pollination by ants is unlikely because ant-visited treatments did not produce more viable seeds. We propose three potential reasons for the apparent lack of flower visitors. (1) Reduced activity of pollinators caused by uncommonly cold temperatures from March to May 2009 (mean monthly maximum temperature March-May: 17.3 °C instead of 19.8°C, which is usual during that time of the year, Karin Schlappa, pers. comm.). (2) Decline of populations of potential pollinators caused by competition with ants and honeybees for floral resources. (3) Decline of populations of potential pollinators caused by ant-predation. Numerous authors demonstrated that ants are responsible for an intense decline of native arthropods in Hawai'i (Krushelnycky and Gillespie 2008) and particularly of *Hylaeus* spp. bees (Daly and Magnacca 2003). In a study on the seed set of *V. reticulatum* in HAVO and Haleakalā National Park, Maui, Vander Kloet (1993) found 32.6 % of the seeds from field collections to be viable – four times more than what we found. A studies by Medeiros *et al.* (1986) indicate that *L. humile* ants were generally present at Vander Kloet's collection sites, but one third of these sites hosted only two locally restricted populations of *L. humile* and were mostly uninvaded at that time (Krushelnycky *et al.* 2005). Lower predatory pressure by ants in this area may have supported a denser population of *Hylaeus* spp. bees. This may be one explanation for the higher ratio of viable seeds. *Hylaeus* spp. bees were very rare (Broomsedge Burn Area) or absent (Kīpuka Kahali'i) from our study sites. However, it remains unclear whether ants influenced their populations in this case.

Despite the fact that ants effectively protected the flowers of *V. reticulatum* in our study, their strong resource exploitation and their negative effect on native pollinator populations (Daly and Magnacca 2003) may outweigh this positive effect suggesting overall negative consequences for the reproductive fitness of *V. reticulatum* and other native Hawaiian plant species. Therefore, potential positive effects (e.g. reduction of flower parasitism) by ants and potential negative effects on pollination need to be considered in concert to assess net effects of ants on the reproduction of endemic Hawaiian plants.

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XVI. Lebenslauf

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Würzburg, den 07.07.2010

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XVII. Authors' contributions

(V.) Floral scents deter facultative florivores

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R.R. Junker and N. Blüthgen designed the study. R.R. Junker and I.M.M. Heidinger performed the experiments. R.R. Junker made the statistical analysis and drafted the first version of the manuscript, all authors contributed to writing the manuscript.

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(VI.) Contrasting responses to floral scents by flower and leaf dwelling spiders

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R.R. Junker, S. Bretscher and N. Blüthgen designed the study. R.R. Junker and S. Bretscher performed the experiments. S. Dötterl analysed flower and leave extracts. R.R. Junker made the statistical analysis and drafted the first version of the manuscript, all authors contributed to writing the manuscript.

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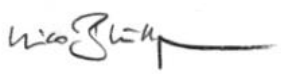

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Robert R. Junker

(VII.) Floral scents contribute to the establishment of flower- and leaf-specific bacterial communities

This chapter is in preparation for publication as:

Junker RR, Loewel C, Gross R, Dötterl S, Keller A and Blüthgen N (in preparation) Composition of epiphytic bacterial communities differs on flowers and leaves

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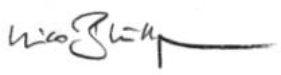

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(VIII.) Historical absence of antagonistic ants lead to an absence of specific floral defences in Hawaii including repellent scents

This chapter has been submitted for publication as:

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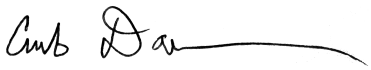

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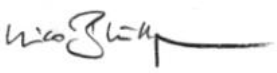

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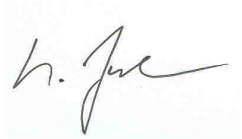

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(X.) How to attract and defend? Dependency on floral resources determines the animals' responses to floral scents

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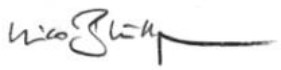

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(XI.) Adding empirical evidence: Responses of obligate and facultative flower visitors to floral scent compounds

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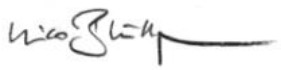

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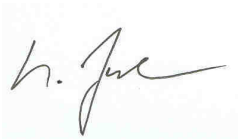

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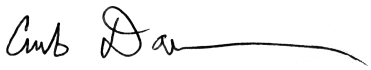

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(XIII.) Beneficial effect of flower-visiting invasive ants on an endemic Hawaiian plant?

This chapter has been submitted for publication as:

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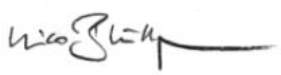

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XVIII. List of publications

Junker RR, Höcherl N & Blüthgen N (2010) Responses to olfactory signals reflect network structure of flower-visitor interactions. ***Journal of Animal Ecology*** 79: 818-823

Junker RR & Blüthgen N (2010) Floral scents repel facultative flower visitors, but attract obligate ones. ***Annals of Botany*** 105: 777-782

Junker RR, Blüthgen (2010) Dependency on floral resources determines the animals' responses to floral scents. ***Plant Signaling and Behavior*** 5

Junker RR, Heidinger IMM, Blüthgen N (2010) Floral scent terpenoids deter the facultative florivore *Metrioptera bicolor* (Ensifera, Tettigoniidae, Decticinae). ***Journal of Orthoptera Research*** 19: 99-104

Junker RR, Blüthgen (2009) Blütenduüfte in doppelter Mission. ***Deutsches Bienen Journal*** 2: 34

Junker RR, Itioka T, Bragg PE, Blüthgen N (2008) Feeding preferences of phasmids (Insecta: Phasmida) in a Bornean Dipterocarp forest. ***Raffles Bulletin of Zoology*** 56: 235-242

Junker RR, Blüthgen N (2008) Floral scents repel potentially nectar-thieving ants. ***Evolutionary Ecology Research*** 10: 295-308

Junker RR, Chung AY, Blüthgen N (2007) Interaction between flowers, ants and pollinators: additional evidence for floral repellence against ants. ***Ecological Research*** 22: 665-670

Congress Contributions

Junker RR, Bleil R, Daehler C, Blüthgen N (2010) Invasive ants as flower visitors in Hawai'i: patterns, explanations, and unexpected consequences. Talk at the ***Multitrophic Interactions Workshop*** in Göttingen, Germany

Junker RR, Daehler C, Blüthgen N (2009) Alien ants on alien plants? What determines the distribution of nectar-feeding ants on flowers in Hawaii? Talk at the ***SCAPE meeting*** in Seili, Finland

Junker RR, Blüthgen N (2009) Floral volatile defences against unbidden guests. Invited talk and poster at the ***Gordon Research Conference "Floral and Vegetative Volatiles"*** in Oxford, United Kingdom

Junker RR, Blüthgen N (2008) Scents as floral filters: the challenge to attract *and* defend. Talk at the ***SCAPE meeting*** in Kaupanger, Norway

Junker RR, Blüthgen N (2008) Floral traits as defensive traits against antagonists. Talk at the ***93rd Annual Meeting of the Ecological Society of America (ESA)*** in Milwaukee, USA

Junker RR, Blüthgen N (2008) Attraction and defence: A different view on floral scents. Poster at the **"*The Ecology and Evolution of Plant-Pollinator Interactions*"** Conference in Milwaukee, USA

Junker RR, Blüthgen N (2008) Floral scents are not only attractive signals. Talk at the ***Multitrophic Interactions Workshop*** in Göttingen, Germany

Junker RR, Blüthgen N (2005) Do flowers repel ants? A comparative study in a Bornean rainforest. Poster at the ***4th International Canopy Conference*** in Leipzig, Germany

XIX. Appendices

Appendices to Chapter VII:

- APPENDIX A: Methods
- APPENDIX B: Results

Appendices to Chapter VIII:

- APPENDIX C: Plant Species list
- APPENDIX D: Commands for R to calculate significance levels of residuals
- APPENDIX E: Mobile olfactometer - technical details and pictures
- APPENDIX F: Floral scent sampling and results
- APPENDIX G: GenBank accession numbers
- APPENDIX H: Ant-flower networks of all 10 study sites
- APPENDIX I: Sources for flower-visitor networks from other islands and continents
- APPENDIX J: Potential links encountered, results

Appendices to Chapter IX:

- APPENDIX K: Network information
- APPENDIX L: Mobile olfactometer
- APPENDIX M: Olfactometer trials
- APPENDIX N: Floral basic colours and results
- APPENDIX O: Flower types after Kugler and results
- APPENDIX P: Correlation between response to scents and link temperature

Appendices to Chapter X:

- APPENDIX Q: Data set used for the meta-analysis
- APPENDIX R: Results of extended dataset
- APPENDIX S: Extended dataset

APPENDIX A

Plant material and isolation of bacterial strains

The bacterial communities colonizing flowers and leaves of *Lotus corniculatus* (Fabaceae) and *Saponaria officinalis* (Caryophyllaceae) were sampled from July to September 2008 in Würzburg and Reichenberg, Germany. Individual samples of plant species were taken from patches that were spatially separated (10 - 100 m). In order to reduce the effect of different colonization time periods of bacteria on leaves and flowers only very young flowers and leaves (1 to 2 days) in an apical position were used. For each sample, several petals and leaves were pooled: *L. corniculatus* leaves (mean ± SE 284.7 ± 21.3, n = 6), flowers (65.3 ± 10.9, n = 6), *S. officinalis* leaves (145.3 ± 18.7, n = 3) and flowers (73.3 ± 2.3, n = 3). In order to estimate the area and density of bacteria on petals and leaves [colony forming units cm^{-2}], 20 leaves and petals from each species were scanned and number of pixels were compared to a defined area and average area of one leaf or flower was multiplied by two (top- and bottom side) and number of leaves flowers used. Forceps and examination gloves were sterilized with 75% ethanol prior to handling of plant material. Plant material was transported from the field to the laboratory in unused plastic bags and in less than 30 min. Plant material was placed in 50 ml polypropylene tubes filled with 30 ml phosphate buffered saline (PBS). Bacteria were washed off the plant surface in an ultrasonic bath for seven minutes at room temperature. PBS including epiphytic bacteria was diluted (10^{0}, 10^{-1}, 10^{-2} and 10^{-3}) and an aliquot of the dilutions (100 µl) were plated on agar that contained cycloheximide (30 mg l^{-1}) to prevent growth of fungi. After incubation of agar plates at 30°C for 48 h, plates with discrete and countable numbers of colonies (typically 100 – 300 colony forming units) were chosen. Plates resulting from other dilutions were discarded. Of each phenologically distinct type of colonies (appearance, colony size and colour, see Krimm (2005) three colonies were haphazardly picked and separately cultivated on an individual LB agar plate without cycloheximide. These LB agar plates were again cultivated for 48 h and afterwards stored in a refrigerator (4°C) until further use.

Identification of bacteria by 16S rDNA sequences

From isolated bacteria strains one colony was dissolved in 120 µl sterile aqua dest. and incubated at 105°C for 10 min. The lysates were centrifuged (14000 g for 3 min) and the supernatant containing bacterial DNA was used as template for Polymerase chain reaction

(PCR). The primer pair 27f and 1492r was used for PCR (Tab. 1). The following PCR conditions were used: 2 µl genomic DNA (different concentrations), 5 µl of 10-fold reaction buffer (Moltaq PCR Kit, Molzym GmbH & Co.KG, Bremen, Germany), 10 mM of dNTP Mix (Thermo Scientific, Germany), 100 pM of each Primer (Metabion, Martinsried, Germany) and 5 U/ µl Taq polymerase (Molzym GmbH & Co.KG, Bremen, Germany) dissolved in a total volume of 50 µl. In case the PCR did not work properly under these conditions, PCR was replicated with addition of 1 µl enhancer (Molzym GmbH & Co. KG, Bremen, Germany). Templates, which showed no product in either PCR, were used in another PCR reaction using the following PCR conditions: 2 µl genomic DNA (different concentrations), 10 µl of 5-fold buffer B (KAPA2G Robust Kit, Peqlab, Erlangen, Germany), 10 µl of 5-fold enhancer 1 (Peqlab, Erlangen, Germany) 10 mM of dNTP Mix (Thermo Scientific, Germany), 100 pM of each Primer (Metabion, Martinsried, Germany) and 5 U/ µl KAPA2G Robust DNA polymerase (Peqlab, Erlangen, Germany) dissolved in a total volume of 50 µl. Positive and negative controls with and without genomic DNA were made for each set of samples during the PCR. A thermocycler (T3 Thermocycler, Biometra, Göttingen, Germany) with the following programme was used: initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 100 s and a final extension step at 72°C for 5 min. PCR products were purified with the SeqLab PCR purification kit (SeqLab, Göttingen, Germany) according to the instructions of the producer. All cleaned PCR products were sent to SeqLab (Sequence Laboratories, Göttingen, Germany) for sequencing.

Tab. 1 Oligonucleotides used during PCR reactions.

Oligonucleotide	**Sequence (5´-3´)**	**Target gene**
27 f	GAG TTT GAT CCT GGC TCA	16S rRNA
1492 r	TAC GGY TAC CTT GTT ACG ACT T	16S rRNA

Wobble base: Y= C/T

Bioinformatics

As a method for validation of sequencing results we used netBLAST (Benson *et al.* 2009) to compare all retained sequences with the GenBank nucleotide database (accessed 23. March 2010) (Benson *et al.* 2009). Sequences without significant BLAST hits (E-value < 10-10) were removed from the dataset (n = 19). The remaining sequences were complimented by three outgroup sequences of the genus Deinococcus (GI:219846824, GI:222083990 and GI:110277976). In addition to sequence information, secondary structures were used in the following analyses: secondary structures were predicted by

homology modeling at the ITS2 database (Koetschan *et al.* 2010). For this, the 16S structure obtained from the RNA STRAND database (http://www.ncbi.nlm.nih.gov/pubmed/18700982?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum) of *Deinococcus radiodurans* (CRW_00105) was used as a template. Pseudoknots were removed prior to prediction, as the tool for homology modeling is not able to handle such. Homology modeling was performed with the identity matrix and a minimum helix transfer of 50%. All sequences were cut to a 402 bp region of the template for which secondary structures were reliably obtainable. Sequences and secondary structures were synchronously aligned using 4SALE (Seibel *et al.* 2008), making use of a sequence-structure scoring matrix. Based on primary and secondary structure information, phylogenetic relationships were reconstructed by Profile Neighbor-Joining (Wolf *et al.* 2008) with a general time reversible model and 100 bootstrap replicates.

Volatile collection

Picked flowers and leaves of both species were placed in vases and enchased in a polyethylene oven bag (Toppits, Germany). Stress induced scents were pumped out the bag for five minutes prior to a 30 minutes period were the bag was closed were scents accumulated. Afterwards, scents were sucked through a microvial (Varian, Darmstadt, Germany) from which the bottom was removed and which was filled with a mixture (1:1) of Tenax-TA (mesh 60-80) and Carbotrap (mesh 20-40) for five minutes. Microvial with trapped scents was stored in a glass vial in a refrigerator until further use. Samples were analysed in a Varian Saturn 2000 system that was equipped with a ChromatoProbe kit. For further details see Dötterl *et al.* (2005). The individual substances were identified by comparison of their retention indices and mass spectra with those of standard substances. In the case no standard compounds were available substances were matched between the samples by comparing retention indices and mass spectra as well, in combination with available databases.

Agar diffusion assay

The following bacteria strains were used for the agar diffusion assay: *Serratia* sp. (SR1-2-f), *Agromyces* sp. (SR2-13-f), *Curtobacterium* sp. (SR1-31-l), *Chryseobacterium* sp. (SR3-14-l), *Microbacterium* sp. (SR3-19-l). Plastic tubes (PP-tubes, 14 ml, Greiner Bio One GmbH, Essen, Germany) were filled with 5 ml double concentrated LB medium and inoculated with 100 µl of a bacterial suspension with OD 0.4 (OD = optical density;

absorption measurement at 600 nm). 5 ml top agar (50°C) were mixed with the inoculated LB medium. The mixture was poured upon dried LB agar plates.

Three substances that were almost exclusively emitted by flowers of *S. officinalis* were used: benzyl nitrile (Purity: 98%, Sigma-Aldrich GmbH, Germany), methyl benzoate (Purity: 98%, Sigma-Aldrich GmbH, Germany) and 2-phenylethyl alcohol (Purity: 99%, Sigma-Aldrich GmbH, Germany). Additionally, two substances that were predominantly emitted by the leaves of this plant species were used: cis-3-hexen1-ol (Purity: 98%, Roth, Germany) and (Z)-3-Hexen-1-ol acetate (Purity: 98%, SAFC, Japan). Firstly, a concentration of 0.06 mMol of each substance was tested for its growth-inhibitory effect on each bacteria strain used. In positive cases, a lower concentration (0.04 mMol) was also tested. Substances were dissolved and diluted with acetone p.A. and 15 µl of the solutions were applied onto sterile cellulose discs (Ø 6 mm, Oxoid, Hampshire, United Kingdom). After the top agar was hardened, prepared cellulose discs were put onto the agar plates. In order to control for the inhibitory effect of acetone, additionally to growth-inhibition tests with floral scent compounds, pure acetone was used for the same tests. These control-treatments never inhibited the growth of any bacteria and were therefore excluded from statistical analysis. Agar plates were sealed with parafilm and incubated at 4°C for one hour to guarantee a consistent diffusion of scents before bacteria started growing. Incubation for 48h at room temperature followed and the diameter of the inhibition zone was measured. All preparative steps were done under a clean bench.

APPENDIX B

Tab. 1 Mean relative amounts (%) of floral scent comounds in bold and the number of samples that the compounds were detected in (n) for S. officinalis and L. corniculatus. Compounds are listed according to their biosynthetic origin, and subsequently in order of retention time. Abbreviations: Lf: flower scents of L. corniculatus; Ll: leaf scents of L. corniculatus; Sf: flower scents of S. officinalis; Sl: leaf scents of S. officinalis.

	Species and plant parts							
	Lf		**Ll**		**Sf**		**Sl**	
No. of flowers and leaves (samples)	11-15 (6)		>50 (5)		5-8 (4)		23-50 (6)	
Mean total amount of flower or leaf scent emitted (ng hr^{-1} dry weight $[g]^{-1}$)	817.22		1114.04		6859.63		941.34	
Total no. of compounds	8		8		24		14	
Compounds	%	n	%	n	%	n	%	n
Fatty acid derivatives								
(Z)-3-Hexen-1-ol	**1.02**	6	**4.15**	5	**0.15**	4	**2.48**	6
(Z)-3-Hexen-1-ol acetate	**41.30**	6	**54.74**	5	**6.13**	4	**88.27**	6
Benzenoid compounds								
Benzaldehyde					**1.72**	4	**2.50**	6
Benzyl alcohol					**7.40**	4	**0.52**	6
Phenylacetaldehyde					**0.004**	1		
Methyl benzoate					**59.87**	4	**3.87**	6
2-Phenylethyl alcohol					**4.16**	4	**0.20**	2
Benzyl nitrile					**10.48**	4		
Unknown 1					**2.93**	4		
Unknown 2					**2.40**	4		
Methyl anthranilate					**0.55**	4		
β-Bourbonene					**0.27**	4		
Benzyl benzoate					**0.08**	4	**0.08**	5
Monoterpenes								
Camphene	**32.05**	6	**6.77**	5	**0.64**	3	**1.52**	6
3-Carene	**1.28**	6	**0.22**	5	**0.06**	3	**0.02**	3
(E)-β-Ocimene	**1.25**	3	**31.61**	5	**0.50**	4	**0.15**	6
(E)-β-Caryophyllene	**14.92**	6	**0.01**	5	**0.61**	3	**0.24**	5
α-Terpineol	**3.26**	5	**2.07**	3				
Sesquiterpenes								
Sesquiterpene 1					**0.07**	3		
β-Cubebene	**4.92**	5	**0.44**	3				
Aromadendrene					**0.15**	3	**0.05**	2
Germacrene D					**0.63**	3	**0.10**	5
(E,E)-α-Farnesene					**0.30**	4		
δ-Cadinene					**0.07**	4		
Nitrogen containing compounds								
Indole					**0.05**	4	**0.01**	1
Unknown 3					**0.79**	4		

Tab. 2 Classification of the scent emitted by flowers and leaves of *Saponaria officinalis* and *Lotus corniculatus* using random forest. Confusion matrix shows number of correctly assigned scent compositions and proportional class error. Scents that were important in the classification (i.e. variable importance E > 0) are listed in decreasing order. Additionally, number of samples in which each scent was identified is given and in parenthesis the proportion of ng/h of this compound within the whole composition. Samples were compared with ANOVA, asterisks indicate significance level with *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Confusion matrix

	LF	LL	SF	SL	Class error
LF	6	0	0	0	0
LL	0	5	0	0	0
SF	0	0	3	1	0.25
SL	0	0	0	6	0

Variable importance

VOC	*E*	*F*	
Methyl benzoate	83.63	55.62	***
Benzyl alcohol	70.25	5.49	**
3-Hexen-1-ol acetate	62.28	12.73	***
Phenylethyl alcohol	60.34	60.92	***
Unknown 3	59.80	120.72	***
Benzyl nitrile	59.76	8.47	**
Benzaldehyde	58.43	2.02	
Unknown 2	56.15	34.4	***
Methyl anthranilate	55.97	5.79	**
Unknown 1	55.16	31.34	***
δ-Cadinene	54.83	4.44	*
E-α-Farnesene	54.21	9.11	***
Camphene	54.13	10.79	***
Z-3-Hexen-1-ol	53.79	2.89	
Indole	39.73	25.23	***
E-β-Caryophyllene	29.16	5.4	**
E-β-Ocimene	28.74	17.7	***
Benzyl benzoate	27.10	1.73	
Carene	24.21	9.49	***
β-Cubebene	22.93	11.4	***
Sesquiterpene 1	21.37	9.7	***
α-Terpineol	18.76	4.68	*
Aromadendrene	9.75	4.35	*
β-Bourbonene	7.26	4.38	*
Germacrene D	2.79	2.06	

APPENDIX C

Complete list of plant species used in this study including information on their origin (endemic, indigenous or introduced) and their typical pollinators.

Family	Plant species	Origin	Typicall pollinators	Reference
Acanthaceae	*Odontonema* sp.	introduced	bird	NA
	Asystasia gangetica	introduced	insect	http://www.plantzafrica.com/plantab/asystasiagan.htm
	Pachystachys lutea	introduced	insect	Taura *et al.* 2007. Acta Biol. Par., Curitiba, 36 (3-4): 175-192
	Ruellia chartacea	introduced	bird	Tripp *et al.* 2009. Evolution 62:1712-1737.
Campanulaceae	*Hippobroma longiflora*	introduced	insect	Haber and Frankie. 1989. Biotropica 21:155-172.
Combretaceae	*Combretum* sp.	introduced	bird	http://www.butterflyworld.com/ECOMMERCE/products.php?cat=3
Convolvulaceae	*Ipomoea indica*	indigenous	insect	Ann Bot. 2004 Aug;94:269-80
	Ipomoea pes-caprae	indigenous	insect	Devall and Thien. 1992. American Midland Naturalist, 28:22-29
	Jacquemontia ovalifolia	indigenous	insect	Sakai *et al.* 1995. Ecology 76:2517-2529.
Ericaceae	*Vaccinium reticulatum*	endemic	insect	Sakai *et al.* 1995. Ecology 76:2517-2529.
Fabaceae	*Caesalpinia kavaiensis*	endemic	bird	Sakai *et al.* 1995. Ecology 76:2517-2529.
	Canavalia hawaiiensis	endemic	insect	http://en.wikipedia.org/wiki/Canavalia
	Crotalaria retusa	introduced	insect	Jacobi *et al.* 2005. Biotropica 37:357-363.
	Mucuna nova-guineensis	introduced	bird	Ferguson and Skvarla. 1982. Botanical Journal of the Linnean Society 84:183-193.
	Saraca asoca	introduced	bird	http://besgroup.talfrynature.com/2008/01/30/saraca-and-sunbirds/
	Sesbania tomentosa	endemic	insect	Hopper 2002. Ph.D. dissertation thesis, University of Hawai'i at Manoa, Honolulu, HI.
	Sophora chrysophylla	endemic	bird	Van Riper 1980. Biotropica 12:282-291.
Goodeniaceae	*Scaevola taccada*	indigenous	insect	http://escholarship.org/uc/item/8z07027k
Hydrophyllaceae	*Nama sandwicensis*	endemic	insect	Sakai *et al.* 1995. Ecology 76:2517-2529.
Lamiaceae	*Rosmarinus officinalis*	introduced	insect	Herrera 1988. Journal of Ecology 76:274-287.
Malvaceae	*Abutilon eremitopetalum*	endemic	insect	Hiyard 2001. Marshall Cavendish Corporation , Tarrytown, NY
	Abutilon menziesii	endemic	bird	Price and Wagner 2009. Evolution 58:2185-2200.
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	bird	http://www.sfds.net/Academics/Student_Projects/2002-2003/7th_Grade_Flowers/pua_alo.htm; http://www.botany.hawaii.edu/faculty/duffy/DPW/2003_MIP/Sec_1/HibBra.pdf
	Kokia drynarioides	endemic	bird	http://www.calhortsociety.org/recaps/recaps-04/3-04-hawaii.htm
	Sida fallax	indigenous	insect	http://www.fs.fed.us/global/iitf/pdf/shrubs/Sida%20fallax.pdf
Musaceae	*Musa velutina*	introduced	bird	Nur 1976. Annals of Botany (London) 40:167-177.

Myoporaceae	*Myoporum sandwicense*	indigenous	insect	http://www.hear.org/naturalareas/kanahabeach/index.html
Myrtaceae	*Metrosideros polymorpha*	endemic	bird	Carpenter 1976. Ecology 57:1125-1444.
Nyctaginaceae	*Boerhavia repens*	indigenous	insect	NA
Plumbaginaceae	*Plumbago zeylanica*	indigenous	insect	http://www.helium.com/items/
Rosaceae	*Osteomeles anthyllidifolia*	indigenous	insect	http://www.pfaf.org/database/plants.php?Osteomeles+subrotunda
Rubiaceae	*Gardenia brighamii*	endemic	insect	Sakai *et al.* 1995. Ecology 76:2517-2529.
	Gardenia sp.	introduced	insect	http://anthony.darrouzet-nardi.net/works/mothpaper.pdf
	Ixora sp.	introduced	insect	Rajaseger *et al.* 1999. Annals of Botany 84:253-257.
	Morinda citrifolia	indigenous	insect	Kato and Kawakita. 2004. American Journal of Botany 91:1814-1827.
	Portlandia platantha	introduced	insect	NA
Scrophulariaceae	*Russelia equisetiformis*	introduced	bird	http://titanarum.uconn.edu/pol_bird.html
Turneraceae	*Turnera ulmifolia*	introduced	insect	Schlindwein and Medeiros. 2006. Flora (Jena) 201:178-188.
Verbenaceae	*Lantana camara*	introduced	insect	Weiss 1991. Nature 354:227-229.

APPENDIX D

Commands for R (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) for Monte-Carlo statistics for the calculation of significance levels of the deviation of the Residuals R_{ij} from zero.

```
### expected matrix

matexp=read.table(file.choose())

### observed matrix

matob=read.table(file.choose())


### total number of ants observed in the matrix

size<-sum(matob)

### number of simulations

simnum=1000000

### Randomisation

rc=dim(matexp)[1]*dim(matexp)[2]
x=seq(1:rc)
p=as.vector(as.matrix(matexp))
o=as.vector(as.matrix(matob))
r=dim(matexp)[1]
c=dim(matexp)[2]

matsim=matrix(NA,simnum,rc)
for (n in 1:simnum) {
    ziehen=sample(x, size, replace = T, prob = p)
    vektor=rep(NA,rc)
    for (n2 in 1:rc) vektor[n2]=length(ziehen[ziehen==n2])
    matsim[n,]=vektor }
```

```
p.values<-rep(NA,rc)
for(n in 1:rc) {
     dif<-matsim[,n]-o[n]
     sm<-length(dif[dif>0])
     la<-length(dif[dif<0])
     eq<-length(dif[dif==0])
     psm<-(sm+eq)/simnum
     pla<-(la+eq)/simnum
     pval<-ifelse(psm<pla, psm, pla)
     p.values[n]=pval }

pm<-matrix(p.values, nrow=r)
pm
write.table(pm, "la p.values.txt")


### calculation of variances

variances<-rep(NA,simnum)
for(n in 1:simnum){
     propsim<-matsim[n,]/size
     ressim<-propsim-p
     varsim<-var(ressim)
     variances[n]<-varsim }

varreal<-var((o/size)-p)
varreal
mean(variances)
sd(variances)
sd(variances)/length(variances)
confint(aov(variances~1), level=0.95, trace=F)
```

APPENDIX E

Mobile olfactometer - technical details and pictures.

Setup

For the biotests, we used a newly established mobile olfactometer (Fig. 1). The system allowed us to conduct the tests in the field with scents from naturally growing, unpicked flowers and insects that did not live in captivity and just recently foraged for resources. The airstream was produced by a battery driven electronic pump (Thomas Gardner Denver, G 24/08 30W; Fig. 3 p). Air was sucked in through a particle filter (Fig. 3 fi). Teflon tubes lead the airstream via a valve (Fig. 3 v) into a flask filled with charcoal (Fig. 3) to clean the air which was than directed into a flask filled with distilled water (Fig. 3) to moisten the air. The valve needs to be opened before switching off the pump in order to reduce the pressure in the system. The cleaned and moistened air was than directed to four flowmeters (Analyt-MTC, 112-08SA; Fig. 3 f) that allowed adjusting a constant flow [ml min^{-1}]. Scent application was accomplished in the following way (Fig. 2): flower or inflorescence stems were wrapped with Teflon tape and a hose of oven bag (Toppits, PET, Fig. 1 A o; Fig. 3 o) was tightly affixed at the Teflon tape using masking tape without damaging the plant tissue. The top end of the hose was thrust through the top part of washing flask which was clearly cut at the top (Fig. 2). Into the overlapping hose a Teflon washing flask topping (Fig. 3 w/t) was tightly pressed into the top part of the cut washing flask. The Teflon topping had two connections for spiral Teflon tubes: One supplied the air from the flowmeters the other one transported the scented air to the arenas (Fig. 3 arena). The whole assemblage was carried by a post and a laboratory clamp (Fig. 2).

We used a smaller version of the four field arena as described in Junker and Blüthgen (2008) (Fig. 4). A glass plate covered the arena. All holes for aerial in and out flow in the arena were obstructed with metal sieves to prevent ants from escaping. Air flew off a central hole. The whole apparatus was fitted into an aluminium box so that the whole setup can be transported to the field site.

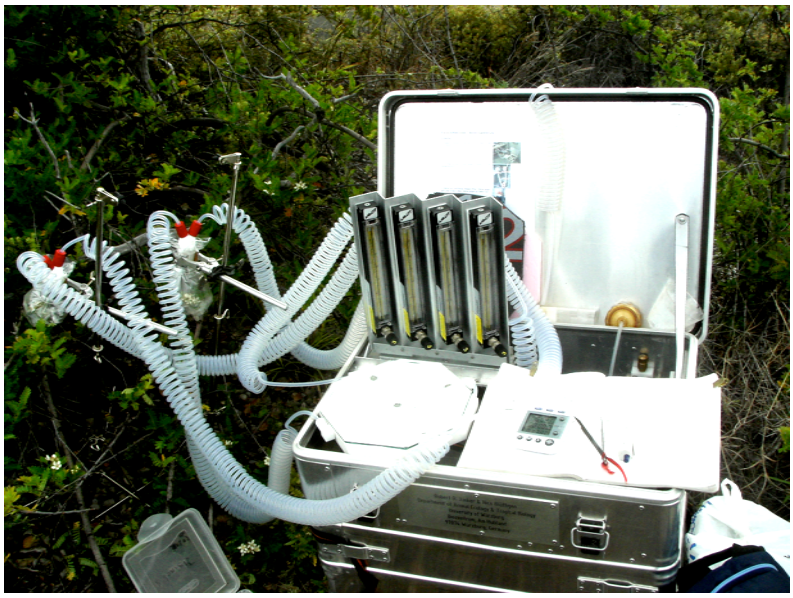

Fig. 1 Mobile Olfactometer.

Fig. 2 Odour source that supplies the arena of the olfactometer with floral scent.

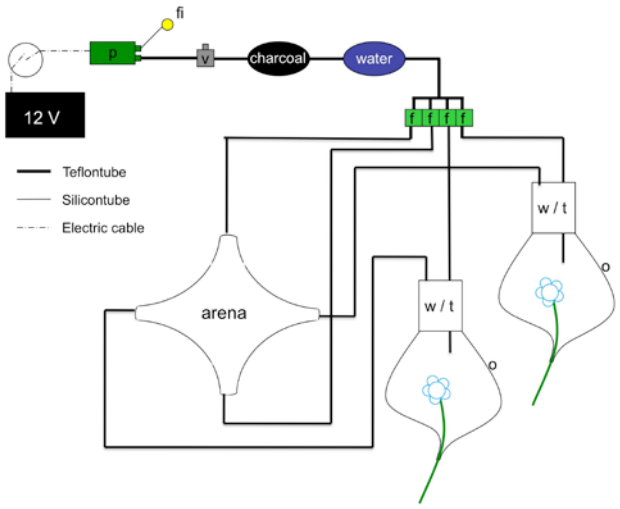

Fig. 3 Schematic drawing of the mobile olfactometer, showing the battery (12 V), power button, electric pump (p), filter (fi), valve (v), washing flasks filled with charcoal and pure water, flowmeters (f), assemblage to apply the scent (w/t , o) and the Y-shaped arena. The two remaining flowmeters are used only when the four field arena is operated.

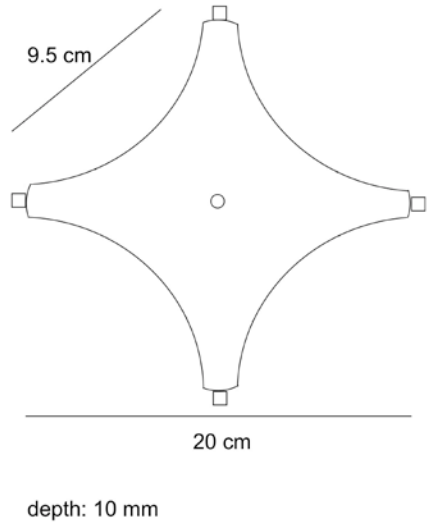

Fig. 4 Dimensions of the arena used for the biotests.

APPENDIX F

Floral scent sampling and results. Time the flowers were in enclosed in bags, time scent was sucke through the volatile trap, total time of scent collection, dryweight of flowers, the origin of plants, total hourly emission [ng] and amount [ng h^{-1}] of Benzenoids (B), fatty acid derivates (FAD), others, monoterpenes (MT), oxidized monoterpenes (MTO), sesquiterpenes (ST) and oxidized sesquiterpenes (STO) are given.

#	Plant species	Family	bag [min]	trapping [min]	total [min]	dryweight [g]	Origin	Total emission	B		FAD		others		MT		MTO		ST		STO	
1	*Asystasia gangetica*	Acanthaceae	0	64	64	0.022	introduced	145	0	7	0	82	0	56	0
2	*Asystasia gangetica*	Acanthaceae	33	27	60	0.118	introduced	711	0	0	0	699	0	12	0
3	*Odontonema* sp.	Acanthaceae	0	92	92	0.360	introduced	121	4	71	14	32	0	0	0
4	*Ochrosia haleakalae*	Apocynaceae	27	5	32	0.590	endemic	3865	2921	100	287	98	452	7	0
5	*Bidens hawaiiensis*	Asteraceae	39	5	44	0.883	endemic	3157	622	39	83	1220	80	1055	59
6	*Combretum* sp.	Combretaceae	89	19	99	1.190	introduced	127	0	0	5	43	76	3	0
7	*Combretum* sp.	Combretaceae	101	10	111	1.477	introduced	47	0	6	2	33	5	0	0
8	*Ipomoea indica*	Convolvulaceae	54	5	59	0.100	indigenous	54	0	0	7	18	0	29	0
9	*Ipomoea indica*	Convolvulaceae	45	5	50	0.093	indigenous	77	0	0	0	53	0	24	0
10	*Ipomoea pes-caprae*	Convolvulaceae	29	16	45	0.096	indigenous	501	406	0	0	60	0	35	0
11	*Vaccinium reticulatum*	Ericaceae	38	5	43	0.758	endemic	835	51	0	126	528	129	0	0
12	*Vaccinium reticulatum*	Ericaceae	45	5	50	0.767	endemic	890	37	0	28	757	67	0	0
13	*Acacia koa*	Fabaceae	38	5.5	43.5	8.030	endemic	311	0	20	7	24	0	259	0
14	*Crotalaria retusa*	Fabaceae	47	5	52	1.174	introduced	70	0	36	3	29	0	2	0
15	*Crotalaria retusa*	Fabaceae	46	5	56	0.610	introduced	115	0	63	4	29	0	19	0
16	*Leucaena leucocephala*	Fabaceae	20	5	25	0.756	introduced	635	216	66	236	105	0	12	0
17	*Saraca asoca*	Fabaceae	0	101	101	0.138	introduced	25	0	0	9	12	0	4	0
18	*Sophora chrysophylla*	Fabaceae	40	5	45	1.583	endemic	209	0	8	4	127	0	68	2
19	*Sophora chrysophylla*	Fabaceae	0	91	91	0.415	endemic	36	0	20	4	6	0	5	0
20	*Scaevola taccada*	Goodeniaceae	4	21	25	0.051	indigenous	1627	1508	49	5	64	0	0	0
21	*Nama sandwicensis*	Hydrophyllaceae	0	74	74	0.060	endemic	845	647	144	18	12	17	8	0
22	*Nama sandwicensis*	Hydrophyllaceae	6	22	28	0.461	endemic	1527	728	452	147	123	75	1	0
23	*Hyptis pectinata*	Lamiaceae	36	5	41	0.591	introduced	3658	0	2	77	1984	10	1583	1
24	*Abutilon eremitopetalum*	Malvaceae	0	65	65	0.181	endemic	152	11	0	6	120	0	15	0
25	*Abutilon menziesii*	Malvaceae	0	79	79	1.108	endemic	547	111	88	162	112	12	62	0
26	*Hibisucus brackenridgei* subsp. *brackenridgei*	Malvaceae	71	5	76	3.776	endemic	1173	882	120	0	102	8	62	0
27	*Kokia drynarioides*	Malvaceae	30	5	35	2.162	endemic	2953	33	0	2	2321	21	566	8
28	*Sida fallax*	Malvaceae	0	68	68	0.356	indigenous	696	40	541	82	17	0	16	0
29	*Sida fallax*	Malvaceae	0	15	15	1.975	indigenous	1443	53	370	460	328	25	207	0
30	*Metrosideros polymorpha*	Myrtaceae	34	5	39	1.769	endemic	211	63	0	45	72	0	30	1
31	*Metrosideros polymorpha*	Myrtaceae	62	20	82	1.124	endemic	2369	1198	4	490	180	405	92	0
32	*Metrosideros polymorpha*	Myrtaceae	76	28	104	1.234	endemic	2162	753	11	149	498	320	412	19
33	*Metrosideros polymorpha*	Myrtaceae	111	9	120	1.099	endemic	795	238	3	280	101	84	78	11
34	*Metrosideros polymorpha*	Myrtaceae	60	15	75	0.850	endemic	417	103	0	58	219	6	30	0
35	*Plumbago zeylanica*	Plumbaginaceae	90	5	95	0.298	indigenous	68	3	24	3	35	4	0	0
36	*Osteomeles anthyllidifolia*	Rosaceae	39	5	44	0.711	indigenous	5314	320	0	0	4989	0	5	0
37	*Osteomeles anthyllidifolia*	Rosaceae	0	120	130	0.621	indigenous	1616	1420	0	0	180	14	1	0
38	*Gardenia brighamii*	Rubiaceae	43	5	48	2.006	endemic	1177	842	158	66	100	0	12	0
39	*Morinda citrifolia*	Rubiaceae	0	49	49	0.372	indigenous	2749	2334	118	40	137	4	117	0
40	*Morinda citrifolia*	Rubiaceae	50	11	61	1.276	indigenous	1583	1139	98	49	179	69	49	0
41	*Myoporum sandwicense*	Scrophulariaceae	42	5	47	1.785	indigenous	319	26	4	34	118	128	8	0
42	*Myoporum sandwicense*	Scrophulariaceae	0	45	45	2.273	indigenous	1391	84	92	60	54	1090	11	0
43	*Russelia equisetiformis*	Scrophulariaceae	97	15	112	0.111	introduced	95	0	0	15	76	0	4	0
44	*Lantana camara*	Verbenaceae	30	5	35	0.133	introduced	2006	139	34	147	567	78	1005	37

APPENDIX G

GenBank accession numbers and representative taxa for phylogenetic analysis.

Plant species	Representative	Accession number
Abutilon eremitopetalum	-	EF219363
		EF219364
Abutilon menziesii	-	EF219365
Asystasia gangetica	-	GQ465765
Boerhavia repens	-	EF079477
		EF079480
Caesalpinia kavaiensis	*Caesalpinia sappan*	EU243573
Canavalia hawaiiensis	*Canavalia grandiflora*	AY293840
	Canavalia ensiformis	EU288913
Combretum sp.	*Combretum alfredii*	AF160471
	Combretum wallichii	AF208731
Crotalaria retusa	-	AJ313501
Gardenia brighamii	*Gardenia hansemannii*	FM204691
Gardenia sp.	*Gardenia hunbergia*	AJ224833
Hibisucus brackenridgei subsp. *brackenridgei*	-	AY962409
Hippobroma longiflora	*Codonopsis_tangshen*	AF134861
	Campanula glomerata	AF090723
	Adenophora potaninii	AF090705
Ipomoea indica	-	AY538295
		AY538296
		AY538297
Ipomoea pes-caprae	*Ipomoea wrightii*	AF110916
	Ipomoea pes-tigridis	AF110912
	Ipomoea plebeia	AF110911
Ixora sp.	*Ixora parviflora*	AJ224840
	Ixora coccinea	AJ224826
Jacquemontia ovalifolia	-	DQ394075
Kokia drynarioides	-	U56784
Lantana camara	-	GQ478094
Metrosideros polymorpha	*Metrosideros collina*	AF328070
Morinda citrifolia	-	FJ907060
		FJ907061
		FJ907062
Mucuna nova-guineensis	*Phaseolus vulgaris*	L36635
Musa velutina	-	FJ428092
Myoporum sandwicense	*Myoporum parvifolium*	DQ444241
Nama sandwicensis	*Nama undulatum*	AF091182
	Nama stenocarpum	AF091181
Osteomeles anthyllidifolia	-	AY864895
Pachystachys lutea	-	AF169844
Plumbago zeylanica	*Armeria villosa*	AJ240015
	Armeria hispalensis	AY179786

Portlandia platantha	-	AY763922
Rosmarinus officinalis	-	DQ667241
		EU796893
Ruellia chartacea	-	EF214461
		EF214462
Russelia equisetiformis	-	AY492118
		AF375152
Saraca asoca	*Bikinia letestu*	AF513679
	Aphanocalyx pectinatus	AF513667
Scaevola taccada	-	AY102780
		AY102781
		AY102782
Sesbania tomentosa	-	AF536357
		AF536358
		AF536359
Sida fallax	-	GQ478107
	Sida poeppigiana	AJ251610
Sophora chrysophylla	-	AY056070
Turnera ulmifolia	-	AY973366
		DQ521284
Vaccinium reticulatum	-	AF382737
		GU011989
		AY274578

APPENDIX H

Ant-flower networks. Data show number of ants and residuals R_{ij} of each link, row R_i and column R_j totals. Plant and ant species are arranged in descending order of their proportional sugar amount offered and proportional abundance on sugar baits, respectively. R_{ij} that significantly deviate from the expectation are framed.

 # 1 Amy B.H. Greenwell Ethnobotanical Garden

		Abutilon eremitopetalum		*Kokia drynarioides*		*Plumbago zeylanica*		*Caesalpinia kavaiensis*		*Abutilon menziesii*		*Metrosideros polymorpha*		*Osteomeles anthyllidifolia*		*Canavalia hawaiensis*		*Gardenia brighamii*		*Hibiscus brackenridgei*		sum
	prop	n.25		n.19		n.18		n.17		n.14		n.05		n.01		n.01		n.01		n.00		0.75
Plagiolepis alluaudi	0.528	0	-0.129	0	-0.100	0	-0.094	0	-0.090	0	-0.075	0	-0.025	2	0.000	0	-0.004	0	-0.006	0	-0.001	-0.523
Technomyrmex albipes	0.256	61	0.085	76	0.136	0	-0.045	0	-0.044	204	0.459	32	0.066	0	-0.003	9	0.020	0	-0.003	0	-0.001	0.671
Solenopsis geminata	0.177	0	-0.043	0	-0.033	0	-0.031	0	-0.030	0	-0.025	0	-0.008	0	-0.002	0	-0.001	0	-0.002	0	0.000	-0.177
Ochetellus glaber	0.040	0	-0.010	0	-0.007	0	-0.007	0	-0.007	0	-0.006	0	-0.002	12	0.029	0	0.000	0	0.000	3	0.007	-0.003
Cardiocondyla obscurior	0.000	0	0.000	0	0.000	0	0.000	7	0.017	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000	0.017
Tetramorium insolens	0.000	0	0.000	0	0.000	0	0.000	0	0.000	4	0.010	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000	0.010
Brachymyrmex obscurior	0.000	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000	2	0.005	0	0.000	0	0.000	0	0.000	0.005
sum	1.000	61	-0.097	76	-0.004	0	-0.177	7	-0.153	208	0.363	32	0.031	16	0.029	9	0.015	0	-0.012	3	0.005	0.000

 # 2 Hawaii Volcanoes National Park

		Lantana camara		*Ipomoea indica*		*Crotalaria retusa*		sum
	prop	n.555		n.371		n.074		1.000
Anoplolepis gracilipes	0.655	0	-0.363	0	-0.243	0	-0.049	-0.655
Pheidole megacephala	0.253	0	-0.140	0	-0.094	0	-0.019	-0.253
Tapinoma melanocephalum	0.080	0	-0.045	20	0.803	0	-0.006	0.753
Plagiolepis alluaudi	0.011	0	-0.006	4	0.162	0	-0.001	0.155
sum	1.000	0	-0.555	24	0.629	0	-0.074	0.000

 # 3 Hawaii Volcanoes National Park

		Metrosideros polymorpha		*Vaccinium reticulatum*		sum
	prop	0.875		0.125		1.000
Linepithema humile	1.000	641	0.009	84	-0.009	0.000
sum	1.000	641	0.009	84	-0.009	0.000

 # 4 Hawaii Volcanoes National Park

		Metrosideros polymorpha		*Vaccinium reticulatum*		sum
	prop	n.876		n.124		1.000
Plagiolepis alluaudi	0.424	8	-0.302	36	0.490	0.187
Pheidole megacephala	0.315	17	-0.071	5	0.062	-0.010
Paratrechina bourbonica	0.261	2	-0.227	4	0.049	-0.178
sum	1.000	27	-0.601	45	0.601	0.000

 # 6 Hawaii Volcanoes National Park

		Metrosideros polymorpha		*Sophora chrysophylla*		*Osteomeles anthyllidifolia*		sum
	prop	0.933		0.061		0.006		1.000
Linepithema humile	1.000	11	-0.017	1	0.022	0	-0.006	0.000
sum	1.000	11	-0.017	1	0.022	0	-0.006	0.000

 # 8 Hawaii Volcanoes National Park

		Lantana camara		*Myoporum sandwicense*		*Osteomeles anthyllidifolia*		*Scaevola taccada*		*Sida fallax*		sum
	prop	n.822		n.107		n.036		n.035		n.000		1.000
Pheidole megacephala	0.849	0	-0.697	3	-0.043	0	-0.031	4	0.034	0	0.000	-0.738
Plagiolepis alluaudi	0.151	0	-0.124	0	-0.016	0	-0.005	56	0.884	0	0.000	0.738
sum	1.000	0	-0.822	3	-0.059	0	-0.036	60	0.917	0	0.000	0.000

 # 7 McBryde Garden

		Mucuna nova-guineensis		*Plumbago zeylanica*		*Sida fallax*		*Abutilon menziesii*		*Gardenia sp.*		*Sesbania tomentosa*		*Ixora sp.*		*Portlandia platantha*		*Myoporum sandwicense*		sum
	prop	n.488		n.296		n.105		n.037		n.028		n.016		n.010		n.009		n.000		1.000
Tetramorium tonganum	0.407	0	-0.203	0	-0.120	0	-0.043	0	-0.015	0	-0.011	0	-0.006	0	-0.004	8	0.125	0	0.000	-0.278
Brachymyrmex obscurior	0.296	0	-0.148	0	-0.088	29	0.437	0	-0.011	0	-0.008	0	-0.005	0	-0.003	0	-0.003	0	0.000	0.171
Tetramorium simillum	0.204	0	-0.102	0	-0.060	0	-0.021	0	-0.008	0	-0.006	0	-0.003	0	-0.002	0	-0.002	0	0.000	-0.204
Technomyrmex albipes	0.093	0	-0.046	0	-0.027	0	-0.010	17	0.271	0	-0.003	8	0.128	0	-0.001	0	-0.001	0	0.000	0.311
sum	1.000	0	-0.499	0	-0.296	29	0.363	17	0.237	0	-0.028	8	0.113	0	-0.010	8	0.120	0	0.000	0.000

 # 8 Sandy Beach

		Scaevola taccada		*Ipomoea pes-caprae*		*Sida fallax*		*Jaquemontia ovalifolia*		*Boerhavia repens*		*Nama sandwicensis*		sum
	prop	n.487		n.399		n.092		n.013		n.005		n.002		1.000
Pheidole megacephala	0.943	8	-0.278	2	-0.331	30	0.595	0	-0.013	0	-0.005	0	-0.002	-0.034
Ochetellus glaber	0.029	0	-0.014	1	0.011	2	0.043	0	0.000	0	0.000	0	0.000	0.040
Paratrechina longicornis	0.014	0	-0.007	0	-0.006	0	-0.001	0	0.000	0	0.000	0	0.000	-0.014
Cardiocondyla kagutsuchi	0.007	0	-0.003	1	0.020	0	-0.001	0	0.000	0	0.000	0	0.000	0.016
Cardiocondyla wroughtonii	0.007	0	-0.003	0	-0.003	0	-0.001	0	0.000	0	0.000	0	0.000	-0.007
sum	1.000	8	-0.305	4	-0.308	32	0.635	0	-0.013	0	-0.005	0	-0.002	0.000

 # 9 University of Hawaii at Manoa

		Combretum sp.		*Russelia equisetiformis*		*Hippobroma longiflora*		*Myoporum sandwicense*		*Morinda citrifolia*		*Asystasia gangetica*		*Scaevola taccada*		*Rosmarinus officinalis*		*Turnera ulmifolia*		sum
	prop	0.789		0.119		0.030		0.016		0.014		0.010		0.006		0.001		0.001		1.000
Pheidole megacephala	1.000	87	-0.348	0	-0.119	0	-0.030	5	0.009	46	0.219	0	-0.010	42	0.207	5	0.025	12	0.060	0.000
sum	1.000	87	-0.348	0	-0.119	0	-0.030	5	0.009	46	0.219	0	-0.010	42	0.207	5	0.025	12	0.060	0.000

 # 10 Lyon Arboretum

		Pachystachys lutea		*Musa velutina*		*Odontonema sp.*		*Saraca asoca*		*Ruellia chartacea*		sum
	prop	n.364		n.330		n.146		n.134		n.026		1.000
Solenopsis papuana	0.397	0	-0.145	0	-0.131	0	-0.058	0	-0.055	0	-0.008	-0.397
Technomyrmex albipes	0.315	20	0.719	0	-0.104	0	-0.046	0	-0.044	4	0.160	0.685
Plagiolepis alluaudi	0.288	0	-0.105	0	-0.095	0	-0.042	0	-0.040	0	-0.006	-0.288
sum	1.000	20	0.469	0	-0.330	0	-0.146	0	-0.139	4	0.146	0.000

APPENDIX I

Flower-visitor webs used for the analysis of the proportion of flowering plant species visited by ants within different habitats. Citation, location, number of ant species, number of plant species and number of ant-visited flowering plant species are given.

#	Source	Location	# ants	# plants	#flowers with ants
1	Aizen (2008) Plos Biology 6(2): e31	Argentinien	1	12	4
2	Aizen (2008) Plos Biology 6(2): e32	Argentinien	1	17	6
3	Aizen (2008) Plos Biology 6(2): e33	Argentinien	1	11	2
4	Aizen (2008) Plos Biology 6(2): e34	Argentinien	1	14	4
5	Chacoff and Vazquez pers. com.	Argentinien	5	52	13
6	Herrera, J. (1988) Journal of Ecology 76: 274-287.	Spain	7	26	9
7	Junker *et al.* (2010) Journal of Animal Ecology doi: 10.1111/j.1365-2656.2010.01698.x	Germany	3	35	15
8	Junker *et al.* (2010) Journal of Animal Ecology doi: 10.1111/j.1365-2656.2010.01698.x	Germany	2	23	3
9	Kaiser-Bunbury (2009) and (2006) pers. com.	Mauritius	3	74	49
10	Kaiser-Bunbury (2009) and (2006) pers. com.	Mauritius	3	64	27
11	Kaiser-Bunbury (submitted) pers. com.	Seychellen	4	18	11
12	Kaiser-Bunbury (submitted) pers. com.	Seychellen	4	20	11
13	Kaiser-Bunbury (submitted) pers. com.	Seychellen	5	23	12
14	Kaiser-Bunbury (submitted) pers. com.	Seychellen	4	16	11
15	Kaiser-Bunbury (submitted) pers. com.	Seychellen	3	20	9
16	Kaiser-Bunbury (submitted) pers. com.	Seychellen	4	21	13
17	Kato *et al.* (1990). Contrib. Biol. Lab., Kyoto, Univ., 27, 309-375.	Japan	6	91	10
18	McMullen 1993 extracted from Rezende *et al.* (2007) Nature 448: 925-929	Galapagos	8	105	11
19	Memmott J. (1999) Ecology Letters 2:276-280.	Great Britan	1	25	1
20	Olesen unpubl. extracted from Rezende *et al.* (2007) Nature 448: 925-929	Flores	2	10	4
21	Percival, M. (1974). Biotropica, 6, 104-129.	Jamaica	2	61	34
22	Primack, R.B. (1983). J. Bot. 21, 317-333. Cass	New Zealand	1	41	2
23	Primack, R.B. (1983). J. Bot. 21, 317-333. Craigieb.	New Zealand	1	49	1
24	Robertson (1928) Flowers andinsects:Lists of visitors of four hundredandfifty-three flowers. Carlinville,IL.	Ilioins	18	552	13
25	Vázquez 2003 Ecol. Lett 6: 1077	Argentina	4	15	3

APPENDIX J

Potential links encountered in the ten habitats. Accessibility: 1 = Nectar holder tube width > head capsule width; 0 = Nectar holder tube width < head capsule width. Mechanical barrier: Other structures that prevent ants from consuming nectar additional to accessibility. Palatability: 1 = nectar offered outside flowers accepted by ants; 0 = nectar not accepted. In cases where a ant and plant species occurred in two or more habitats, the mean value of R_{ij} und Q_{ij} are given.

Family	Plant species	Origin	Nectar holder tube width [mm]	Ant species	Family	Head capsule width [mm]	Residual R_{ij}	Accessibility	Mechanical barrier	Response index Q_{ij}	Palatability
Acanthaceae	*Asystasia gangetica*	introduced	0.00	*Pheidole megacephala*	Myrmicinae	0.49	-0.010	0	0	-0.160	1
	Odontonema sp.	introduced	1.67	*Plagiolepis alluaudi*	Formicinae	0.28	-0.042	1	1	NA	NA
	Odontonema sp.	introduced	1.67	*Solenopsis papuana*	Myrmicinae	0.33	-0.058	1	1	-0.201	1
	Odontonema sp.	introduced	1.67	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.046	1	1	-0.104	1
	Pachystachys lutea	introduced	1.80	*Plagiolepis alluaudi*	Formicinae	0.28	-0.105	1	1	NA	NA
	Pachystachys lutea	introduced	1.80	*Solenopsis papuana*	Myrmicinae	0.33	-0.145	1	1	NA	1
	Pachystachys lutea	introduced	1.80	*Technomyrmex albipes*	Dolichoderinae	0.55	0.719	1	1	NA	1
	Ruellia chartacea	introduced	3.80	*Plagiolepis alluaudi*	Formicinae	0.28	-0.006	1	1	NA	NA
	Ruellia chartacea	introduced	3.80	*Solenopsis papuana*	Myrmicinae	0.33	-0.008	1	1	NA	NA
	Ruellia chartacea	introduced	3.80	*Technomyrmex albipes*	Dolichoderinae	0.55	0.160	1	1	NA	1
Campanulaceae	*Hippobroma longiflora*	introduced	0.00	*Pheidole megacephala*	Myrmicinae	0.49	-0.030	0	0	NA	1
Combretaceae	*Combretum* sp.	introduced	0.77	*Pheidole megacephala*	Myrmicinae	0.49	-0.348	1	1	-0.451	1
Convolvulaceae	*Ipomoea indica*	indigenous	0.47	*Anoplolepis gracilipes*	Formicinae	0.70	-0.243	0	0	0.021	1
	Ipomoea indica	indigenous	0.47	*Pheidole megacephala*	Myrmicinae	0.49	-0.094	0	0	-0.035	1
	Ipomoea indica	indigenous	0.47	*Plagiolepis alluaudi*	Formicinae	0.28	0.162	1	1	NA	NA
	Ipomoea indica	indigenous	0.47	*Tapinoma melanocephalum*	Dolichoderinae	0.38	0.803	1	1	-0.125	1
	Ipomoea pes-caprae	indigenous	0.00	*Cardiocondyla kagutsuchi*	Myrmicinae	0.43	0.020	0	0	NA	NA
	Ipomoea pes-caprae	indigenous	0.00	*Cardiocondyla wroughtonii*	Myrmicinae	0.34	-0.003	0	0	NA	NA
	Ipomoea pes-caprae	indigenous	0.00	*Ochetellus glaber*	Dolichoderinae	0.46	0.011	0	0	NA	NA
	Ipomoea pes-caprae	indigenous	0.00	*Paratrechina longicornis*	Formicinae	0.53	-0.006	0	0	NA	NA
	Ipomoea pes-caprae	indigenous	0.00	*Pheidole megacephala*	Myrmicinae	0.49	-0.331	0	0	-0.215	1
	Jacquemontia ovalifolia	indigenous	0.50	*Cardiocondyla kagutsuchi*	Myrmicinae	0.43	0.000	1	1	NA	NA
	Jacquemontia ovalifolia	indigenous	0.50	*Cardiocondyla wroughtonii*	Myrmicinae	0.34	0.000	1	1	NA	NA
	Jacquemontia ovalifolia	indigenous	0.50	*Ochetellus glaber*	Dolichoderinae	0.46	0.000	1	1	NA	NA
	Jacquemontia ovalifolia	indigenous	0.50	*Paratrechina longicornis*	Formicinae	0.53	0.000	0	0	NA	NA
	Jacquemontia ovalifolia	indigenous	0.50	*Pheidole megacephala*	Myrmicinae	0.49	-0.013	1	1	NA	NA
Ericaceae	*Vaccinium reticulatum*	endemic	3.50	*Linepithema humile*	Dolichoderinae	0.61	-0.009	1	1	-0.007	1
	Vaccinium reticulatum	endemic	3.50	*Paratrechina bourbonica*	Formicinae	0.65	0.049	1	1	NA	NA
	Vaccinium reticulatum	endemic	3.50	*Pheidole megacephala*	Myrmicinae	0.49	0.062	1	1	-0.007	1
	Vaccinium reticulatum	endemic	3.50	*Plagiolepis alluaudi*	Formicinae	0.28	0.490	1	1	-0.042	1
Fabaceae	*Caesalpinia kavaiensis*	endemic	0.40	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	0	0	NA	NA
	Caesalpinia kavaiensis	endemic	0.40	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.017	0	0	NA	NA
	Caesalpinia kavaiensis	endemic	0.40	*Ochetellus glaber*	Dolichoderinae	0.46	-0.007	0	0	NA	NA
	Caesalpinia kavaiensis	endemic	0.40	*Plagiolepis alluaudi*	Formicinae	0.28	-0.090	1	1	NA	NA
	Caesalpinia kavaiensis	endemic	0.40	*Solenopsis geminata*	Myrmicinae	0.76	-0.030	0	0	NA	NA
	Caesalpinia kavaiensis	endemic	0.40	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.044	0	0	NA	NA
	Caesalpinia kavaiensis	endemic	0.40	*Tetramorium insolens*	Myrmicinae	0.76	0.000	0	0	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Ochetellus glaber*	Dolichoderinae	0.46	0.000	1	1	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Plagiolepis alluaudi*	Formicinae	0.28	-0.004	1	1	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Solenopsis geminata*	Myrmicinae	0.76	-0.001	1	1	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Technomyrmex albipes*	Dolichoderinae	0.55	0.020	1	1	NA	NA
	Canavalia hawaiiensis	endemic	3.00	*Tetramorium insolens*	Myrmicinae	0.76	0.000	1	1	NA	NA
	Crotalaria retusa	introduced	0.00	*Anoplolepis gracilipes*	Formicinae	0.70	-0.049	0	0	0.014	1
	Crotalaria retusa	introduced	0.00	*Pheidole megacephala*	Myrmicinae	0.49	-0.019	0	0	-0.056	1
	Crotalaria retusa	introduced	0.00	*Plagiolepis alluaudi*	Formicinae	0.28	-0.001	0	0	NA	NA
	Crotalaria retusa	introduced	0.00	*Tapinoma melanocephalum*	Dolichoderinae	0.38	-0.006	0	0	-0.010	1
	Mucuna nova-guineensis	introduced	0.00	*Brachymyrmes obscurior*	Formicinae	0.38	-0.148	0	0	NA	NA
	Mucuna nova-guineensis	introduced	0.00	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.046	0	0	NA	NA
	Mucuna nova-guineensis	introduced	0.00	*Tetramorium simillium*	Myrmicinae	0.48	-0.102	0	0	NA	NA
	Mucuna nova-guineensis	introduced	0.00	*Tetramorium tonganum*	Myrmicinae	0.52	-0.203	0	0	NA	NA
	Saraca asoca	introduced	0.50	*Plagiolepis alluaudi*	Formicinae	0.28	-0.040	1	1	NA	NA
	Saraca asoca	introduced	0.50	*Solenopsis papuana*	Myrmicinae	0.33	-0.055	1	1	-0.326	0
	Saraca asoca	introduced	0.50	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.044	0	0	-0.021	0
	Sesbania tomentosa	endemic	2.00	*Brachymyrmes obscurior*	Formicinae	0.38	-0.005	1	1	NA	NA
	Sesbania tomentosa	endemic	2.00	*Technomyrmex albipes*	Dolichoderinae	0.55	0.128	1	1	NA	NA
	Sesbania tomentosa	endemic	2.00	*Tetramorium simillium*	Myrmicinae	0.48	-0.003	1	1	NA	NA
	Sesbania tomentosa	endemic	2.00	*Tetramorium tonganum*	Myrmicinae	0.52	-0.006	1	1	NA	NA
	Sophora chrysophylla	endemic	1.00	*Linepithema humile*	Dolichoderinae	0.61	0.022	1	1	0.014	1
Goodeniaceae	*Scaevola taccada*	indigenous	0.83	*Cardiocondyla kagutsuchi*	Myrmicinae	0.43	-0.003	1	1	NA	NA
	Scaevola taccada	indigenous	0.83	*Cardiocondyla wroughtonii*	Myrmicinae	0.34	-0.003	1	1	NA	NA
	Scaevola taccada	indigenous	0.83	*Ochetellus glaber*	Dolichoderinae	0.46	-0.014	1	1	NA	NA
	Scaevola taccada	indigenous	0.83	*Paratrechina longicornis*	Formicinae	0.53	-0.007	1	1	NA	NA
	Scaevola taccada	indigenous	0.83	*Pheidole megacephala*	Myrmicinae	0.49	-0.012	1	1	-0.146	1
	Scaevola taccada	indigenous	0.83	*Plagiolepis alluaudi*	Formicinae	0.28	0.884	1	1	NA	1
Hydrophyllaceae	*Nama sandwicensis*	endemic	0.25	*Cardiocondyla kagutsuchi*	Myrmicinae	0.43	0.000	0	0	NA	NA
	Nama sandwicensis	endemic	0.25	*Cardiocondyla wroughtonii*	Myrmicinae	0.34	0.000	0	0	NA	NA
	Nama sandwicensis	endemic	0.25	*Ochetellus glaber*	Dolichoderinae	0.46	0.000	0	0	NA	NA
	Nama sandwicensis	endemic	0.25	*Paratrechina longicornis*	Formicinae	0.53	0.000	0	0	NA	NA
	Nama sandwicensis	endemic	0.25	*Pheidole megacephala*	Myrmicinae	0.49	-0.002	0	0	-0.125	NA

Lamiaceae	*Rosmarinus officinalis*	introduced	0.65	*Pheidole megacephala*	Myrmicinae	0.49	0.025	1	1	NA	NA
Malvaceae	*Abutilon eremitopetalum*	endemic	3.00	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Abutilon eremitopetalum	endemic	3.00	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Abutilon eremitopetalum	endemic	3.00	*Ochetellus glaber*	Dolichoderinae	0.46	-0.010	1	1	NA	NA
	Abutilon eremitopetalum	endemic	3.00	*Plagiolepis alluaudi*	Formicinae	0.28	-0.129	1	1	NA	NA
	Abutilon eremitopetalum	endemic	3.00	*Solenopsis geminata*	Myrmicinae	0.76	-0.043	1	1	NA	NA
	Abutilon eremitopetalum	endemic	3.00	*Technomyrmex albipes*	Dolichoderinae	0.55	0.085	1	1	-0.069	NA
	Abutilon eremitopetalum	endemic	3.00	*Tetramorium insolens*	Myrmicinae	0.76	0.000	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Brachymyrmes obscurior*	Formicinae	0.38	-0.011	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Ochetellus glaber*	Dolichoderinae	0.46	-0.006	1	1	NA	1
	Abutilon menziesii	endemic	1.45	*Plagiolepis alluaudi*	Formicinae	0.28	-0.075	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Solenopsis geminata*	Myrmicinae	0.76	-0.025	1	1	-0.111	NA
	Abutilon menziesii	endemic	1.45	*Technomyrmex albipes*	Dolichoderinae	0.55	0.365	1	1	0.035	1
	Abutilon menziesii	endemic	1.45	*Tetramorium insolens*	Myrmicinae	0.76	0.010	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Tetramorium simillium*	Myrmicinae	0.48	-0.008	1	1	NA	NA
	Abutilon menziesii	endemic	1.45	*Tetramorium tonganum*	Myrmicinae	0.52	-0.015	1	1	NA	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Ochetellus glaber*	Dolichoderinae	0.46	0.007	1	1	NA	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Plagiolepis alluaudi*	Formicinae	0.28	-0.001	1	1	-0.083	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Solenopsis geminata*	Myrmicinae	0.76	0.000	0	0	-0.479	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.001	1	1	NA	NA
	Hibisucus brackenridgei subsp. *brackenridgei*	endemic	0.70	*Tetramorium insolens*	Myrmicinae	0.76	0.000	0	0	NA	NA
	Kokia drynarioides	endemic	8.00	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Kokia drynarioides	endemic	8.00	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Kokia drynarioides	endemic	8.00	*Ochetellus glaber*	Dolichoderinae	0.46	-0.007	1	1	NA	NA
	Kokia drynarioides	endemic	8.00	*Plagiolepis alluaudi*	Formicinae	0.28	-0.100	1	1	NA	NA
	Kokia drynarioides	endemic	8.00	*Solenopsis geminata*	Myrmicinae	0.76	-0.033	1	1	-0.275	1
	Kokia drynarioides	endemic	8.00	*Technomyrmex albipes*	Dolichoderinae	0.55	0.136	1	1	0.007	1
	Kokia drynarioides	endemic	8.00	*Tetramorium insolens*	Myrmicinae	0.76	0.000	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Brachymyrmes obscurior*	Formicinae	0.38	0.437	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Cardiocondyla kagutsuchi*	Myrmicinae	0.43	-0.001	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Cardiocondyla wroughtonii*	Myrmicinae	0.34	-0.001	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Ochetellus glaber*	Dolichoderinae	0.46	0.043	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Paratrechina longicornis*	Formicinae	0.53	-0.001	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Pheidole megacephala*	Myrmicinae	0.49	0.297	1	1	-0.067	NA
	Sida fallax	indigenous	0.80	*Plagiolepis alluaudi*	Formicinae	0.28	0.000	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.010	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Tetramorium simillium*	Myrmicinae	0.48	-0.021	1	1	NA	NA
	Sida fallax	indigenous	0.80	*Tetramorium tonganum*	Myrmicinae	0.52	-0.043	1	1	NA	NA
Musaceae	*Musa velutina*	introduced	0.00	*Plagiolepis alluaudi*	Formicinae	0.28	-0.095	0	0	NA	NA
	Musa velutina	introduced	0.00	*Solenopsis papuana*	Myrmicinae	0.33	-0.131	0	0	NA	NA
	Musa velutina	introduced	0.00	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.104	0	0	NA	1
Myoporaceae	*Myoporum sandwicense*	indigenous	0.80	*Brachymyrmes obscurior*	Formicinae	0.38	0.000	1	1	NA	NA
	Myoporum sandwicense	indigenous	0.80	*Pheidole megacephala*	Myrmicinae	0.49	-0.017	1	1	-0.132	1
	Myoporum sandwicense	indigenous	0.80	*Plagiolepis alluaudi*	Formicinae	0.28	-0.016	1	1	0.056	0
	Myoporum sandwicense	indigenous	0.80	*Technomyrmex albipes*	Dolichoderinae	0.55	0.000	1	1	NA	NA
	Myoporum sandwicense	indigenous	0.80	*Tetramorium simillium*	Myrmicinae	0.48	0.000	1	1	NA	NA
	Myoporum sandwicense	indigenous	0.80	*Tetramorium tonganum*	Myrmicinae	0.52	0.000	1	1	NA	NA
Myrtaceae	*Metrosideros polymorpha*	endemic	5.00	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Metrosideros polymorpha	endemic	5.00	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Metrosideros polymorpha	endemic	5.00	*Linepithema humile*	Dolichoderinae	0.61	-0.004	1	1	0.090	1
	Metrosideros polymorpha	endemic	5.00	*Ochetellus glaber*	Dolichoderinae	0.46	-0.002	1	1	NA	NA
	Metrosideros polymorpha	endemic	5.00	*Paratrechina bourbonica*	Formicinae	0.65	-0.227	1	1	NA	NA
	Metrosideros polymorpha	endemic	5.00	*Pheidole megacephala*	Myrmicinae	0.49	-0.071	1	1	-0.167	1
	Metrosideros polymorpha	endemic	5.00	*Plagiolepis alluaudi*	Formicinae	0.28	-0.163	1	1	-0.167	1
	Metrosideros polymorpha	endemic	5.00	*Solenopsis geminata*	Myrmicinae	0.76	-0.008	1	1	-0.326	1
	Metrosideros polymorpha	endemic	5.00	*Technomyrmex albipes*	Dolichoderinae	0.55	0.066	1	1	0.007	1
	Metrosideros polymorpha	endemic	5.00	*Tetramorium insolens*	Myrmicinae	0.76	0.000	1	1	NA	NA
Nyctaginaceae	*Boerhavia repens*	indigenous	0.60	*Cardiocondyla kagutsuchi*	Myrmicinae	0.43	0.000	1	1	NA	NA
	Boerhavia repens	indigenous	0.60	*Cardiocondyla wroughtonii*	Myrmicinae	0.34	0.000	1	1	NA	NA
	Boerhavia repens	indigenous	0.60	*Ochetellus glaber*	Dolichoderinae	0.46	0.000	1	1	NA	NA
	Boerhavia repens	indigenous	0.60	*Paratrechina longicornis*	Formicinae	0.53	0.000	1	1	NA	NA
	Boerhavia repens	indigenous	0.60	*Pheidole megacephala*	Myrmicinae	0.49	-0.005	1	1	NA	NA
Plumbaginaceae	*Plumbago zeylanica*	indigenous	0.40	*Brachymyrmes obscurior*	Formicinae	0.41	0.000	0	0	NA	NA
	Plumbago zeylanica	indigenous	0.40	*Brachymyrmes obscurior*	Formicinae	0.38	-0.088	1	0	NA	NA
	Plumbago zeylanica	indigenous	0.40	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	0	0	NA	NA
	Plumbago zeylanica	indigenous	0.40	*Ochetellus glaber*	Dolichoderinae	0.46	-0.007	0	0	-0.031	1
	Plumbago zeylanica	indigenous	0.40	*Pheidole megacephala*	Myrmicinae	0.49	-0.013	0	0	0.042	1
	Plumbago zeylanica	indigenous	0.40	*Plagiolepis alluaudi*	Formicinae	0.28	-0.094	1	0	NA	NA

	Plumbago zeylanica	indigenous	0.40	*Solenopsis geminata*	Myrmicinae	0.76	-0.031	0	0	NA	NA
	Plumbago zeylanica	indigenous	0.40	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.036	0	0	0.021	1
	Plumbago zeylanica	indigenous	0.40	*Tetramorium insolens*	Myrmicinae	0.76	0.000	0	0	NA	NA
	Plumbago zeylanica	indigenous	0.40	*Tetramorium simillium*	Myrmicinae	0.48	-0.060	0	0	NA	NA
	Plumbago zeylanica	indigenous	0.40	*Tetramorium tonganum*	Myrmicinae	0.52	-0.120	0	0	NA	NA
Rosaceae	*Osteomeles anthyllidifolia*	indigenous	1.50	*Brachymyrmex obscurior*	Formicinae	0.41	0.005	1	1	NA	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Linepithema humile*	Dolichoderinae	0.61	-0.006	1	1	-0.129	0
	Osteomeles anthyllidifolia	indigenous	1.50	*Ochetellus glaber*	Dolichoderinae	0.46	0.029	1	1	NA	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Pheidole megacephala*	Myrmicinae	0.49	-0.031	1	1	0.069	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Plagiolepis altuaudi*	Formicinae	0.28	-0.003	1	1	NA	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Solenopsis geminata*	Myrmicinae	0.76	-0.002	1	1	NA	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.003	1	1	NA	NA
	Osteomeles anthyllidifolia	indigenous	1.50	*Tetramorium insolens*	Myrmicinae	0.76	0.000	1	1	NA	NA
Rubiaceae	*Gardenia brighamii*	endemic	0.50	*Brachymyrmex obscurior*	Formicinae	0.41	0.000	1	1	NA	NA
	Gardenia brighamii	endemic	0.50	*Cardiocondyla obscurior*	Myrmicinae	0.41	0.000	1	1	NA	NA
	Gardenia brighamii	endemic	0.50	*Ochetellus glaber*	Dolichoderinae	0.46	0.000	1	1	NA	NA
	Gardenia brighamii	endemic	0.50	*Plagiolepis altuaudi*	Formicinae	0.28	-0.006	1	1	-0.056	0
	Gardenia brighamii	endemic	0.50	*Solenopsis geminata*	Myrmicinae	0.76	-0.002	0	0	NA	NA
	Gardenia brighamii	endemic	0.50	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.003	0	0	NA	NA
	Gardenia brighamii	endemic	0.50	*Tetramorium insolens*	Myrmicinae	0.76	0.000	0	0	NA	NA
	Gardenia sp.	introduced	0.10	*Brachymyrmex obscurior*	Formicinae	0.38	-0.008	0	0	NA	NA
	Gardenia sp.	introduced	0.10	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.003	0	0	NA	NA
	Gardenia sp.	introduced	0.10	*Tetramorium simillium*	Myrmicinae	0.48	-0.006	0	0	NA	NA
	Gardenia sp.	introduced	0.10	*Tetramorium tonganum*	Myrmicinae	0.52	-0.011	0	0	NA	NA
	Ixora sp.	introduced	0.02	*Brachymyrmex obscurior*	Formicinae	0.38	-0.003	0	0	NA	NA
	Ixora sp.	introduced	0.02	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.001	0	0	NA	NA
	Ixora sp.	introduced	0.02	*Tetramorium simillium*	Myrmicinae	0.48	-0.002	0	0	NA	NA
	Ixora sp.	introduced	0.02	*Tetramorium tonganum*	Myrmicinae	0.52	-0.004	0	0	NA	NA
	Morinda citrifolia	indigenous	1.98	*Pheidole megacephala*	Myrmicinae	0.49	0.219	1	1	0.052	NA
	Portlandia platantha	introduced	0.10	*Brachymyrmex obscurior*	Formicinae	0.38	-0.003	0	0	NA	NA
	Portlandia platantha	introduced	0.10	*Technomyrmex albipes*	Dolichoderinae	0.55	-0.001	0	0	NA	NA
	Portlandia platantha	introduced	0.10	*Tetramorium simillium*	Myrmicinae	0.48	-0.002	0	0	NA	NA
	Portlandia platantha	introduced	0.10	*Tetramorium tonganum*	Myrmicinae	0.52	0.125	0	0	NA	NA
Scrophulariaceae	*Russelia equisetiformis*	introduced	1.50	*Pheidole megacephala*	Myrmicinae	0.49	-0.119	1	0	-0.208	1
Turneraceae	*Turnera ulmifolia*	introduced	0.57	*Pheidole megacephala*	Myrmicinae	0.49	0.060	1	1	NA	NA
Verbenaceae	*Lantana camara*	introduced	0.75	*Anoplolepis gracilipes*	Formicinae	0.70	-0.363	1	1	-0.375	1
	Lantana camara	introduced	0.75	*Pheidole megacephala*	Myrmicinae	0.49	-0.419	1	1	-0.222	1
	Lantana camara	introduced	0.75	*Plagiolepis altuaudi*	Formicinae	0.28	-0.065	1	1	-0.208	NA
	Lantana camara	introduced	0.75	*Tapinoma melanocephalum*	Dolichoderinae	0.38	-0.045	1	1	-0.188	NA

APPENDIX K

In the first network we observed 2251 interactions between 35 plant species and 164 animal species (Tab. 1 a, b). The second one comprised 1080 interaction between 23 plant species and 64 animal species (Tab. 2 a, b). The degree of network-level specialisation (Blüthgen *et al.* 2006) (H_2') was 0.47 in the first and 0.52 in the second network, respectively. This level is typical for flower-visitor networks in general (Blüthgen *et al.* 2007).

Tab. 1 a *Link temperatures* T_{ij} of network #1. Animals and plant species are sorted by number of observed interactions. For a better clarity, only those species with five recorded interactions are shown. Significant hot T_{ij}s are marked in red, cold T_{ij}s in blue. Those interactions used for the olfactometer tests are framed in bold. 'Total' provides the total number of interactions observed for each species including those that are not shown.

		Tilia cordata	Rubus fruticosus	Galium mollugo	Achillea millefolium	Reseda lutea	Erigeron annuus	Verbascum pulverulentum	Geranium molle	Rosa canina	Potentilla reptans	Convolvulus arvensis	Papaver rhoeas	Matricaria recutita	Echium vulgare	Medicago x varia	Geum urbanum	Hypericum perforatum	Sonchus arvensis	Knautia arvensis	Medicago lupulina	Trifolium repens	Cornus sanguinea	Total
	#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Meligethes aeneus	1	0.49	-0.18	-0.12	-0.04	-0.04	-0.04	-0.02	0.02	-0.02	0.00	-0.01	-0.01	-0.01	0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	588
Apis mellifera	2	0.25	0.03	-0.13	-0.05	-0.04	-0.03	0.03	-0.02	0.00	-0.01	-0.01	-0.01	0.00	-0.01	-0.01	0.00	0.00	0.00	0.02	0.00	0.01	0.00	293
Corymbia rubra	3	-0.35	0.19	0.01	0.19	-0.04	0.13	-0.03	-0.02	0.01	-0.02	-0.02	-0.01	0.00	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	264
Lasius niger	4	-0.37	0.29	0.11	-0.05	0.04	-0.03	-0.01	-0.02	-0.01	0.00	0.08	-0.01	0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	239
Bombus terrestris	5	0.33	-0.08	-0.15	-0.05	-0.04	-0.04	0.06	-0.02	0.00	-0.02	-0.02	0.05	-0.01	-0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	122
Myrmica rubra	6	-0.28	0.41	0.17	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	117
Oedemera virescens	7	-0.37	0.02	0.10	0.01	-0.03	0.07	0.01	0.04	0.06	0.02	0.04	0.06	0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	106
Hylaeus signatus	8	-0.37	-0.17	-0.15	-0.05	0.94	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	75
Bombus lapidarius	9	0.63	-0.18	-0.15	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40
Oedemera femorata	10	-0.37	-0.05	0.37	0.05	-0.04	-0.04	-0.03	-0.02	0.08	0.11	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.00	31
Formica rufibarbis	11	-0.37	-0.18	0.85	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20
Byturus tomentosus	12	-0.37	0.35	-0.15	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	0.25	-0.02	-0.01	-0.01	-0.01	-0.01	0.20	0.00	0.00	0.00	0.00	0.00	0.00	15
Lasioglossum sp. 6	13	-0.37	-0.12	0.12	-0.05	-0.04	-0.04	-0.03	0.18	-0.02	0.05	-0.02	0.32	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.06	0.00	0.00	15
Coleoptera sp. 4	14	-0.37	0.20	-0.15	0.10	-0.04	0.12	-0.03	-0.02	0.21	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.07	13
Mordellidae sp. 1	15	-0.29	-0.18	0.39	-0.05	-0.04	-0.04	-0.03	0.06	-0.02	0.29	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13
Colletes sp. 1	16	-0.37	-0.08	-0.15	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	0.19	-0.01	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10
Agrilus angustulus	17	-0.37	-0.18	-0.15	-0.05	-0.04	-0.04	-0.03	-0.02	0.98	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8
Lasioglossum sp. 4	18	-0.37	-0.18	-0.15	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	0.99	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8
Miarus sp. 1	19	-0.37	-0.18	-0.15	-0.05	-0.04	-0.04	0.10	0.35	0.11	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.37	0.00	0.00	0.00	0.00	0.00	8
Stenopterus rufus	20	-0.37	-0.18	-0.15	0.95	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8
Curculionidae sp. 4	21	-0.37	-0.18	-0.15	-0.05	-0.04	-0.04	0.97	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7
Hylaeus sp. 1	22	-0.37	-0.18	-0.15	0.38	-0.04	-0.04	-0.03	0.41	-0.02	-0.02	-0.02	-0.01	-0.01	0.14	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7
Lasioglossum sp. 3	23	-0.37	-0.18	0.85	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7
Mordellidae sp. 2	24	-0.37	-0.18	0.85	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7
Amblytylus nasutus	25	-0.37	-0.18	0.52	-0.05	-0.04	-0.04	-0.03	0.14	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.16	0.00	0.00	0.00	0.00	6
Clytra quadripunctata	26	-0.37	-0.18	0.68	-0.05	0.12	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6
Syrphus ribesii	27	-0.37	-0.18	0.68	-0.05	-0.04	-0.04	-0.03	-0.02	-0.02	0.15	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6
Anthrenus verbasci	28	-0.37	-0.18	-0.15	0.95	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5
Chrysanthia nigricornis	29	-0.37	0.22	-0.15	-0.05	0.16	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	0.19	-0.01	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5
Hylaeus sp. 2	30	-0.37	-0.18	-0.15	0.15	-0.04	-0.04	-0.03	0.18	-0.02	0.38	-0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	0.00	0.20	0.00	5
Total		833	410	336	114	99	82	59	49	45	43	37	33	17	12	12	9	8	8	7	6	6	5	2251

Tab. 1 b Observed interactions in network #1. Animals and plant species are sorted by total number of observed interactions. For clarity, only those species with five recorded interactions are shown. 'Total' is the total number of interactions observed for each species including those that are not shown.

			Tilia cordata	*Rubus fruticosus*	*Galium mollugo*	*Achillea millefolium*	*Reseda lutea*	*Erigeron annuus*	*Verbascum pulverulentum*	*Geranium molle*	*Rosa canina*	*Potentilla reptans*	*Convolvulus arvensis*	*Papaver rhoeas*	*Matricaria recutita*	*Echium vulgare*	*Medicago x varia*	*Geum urbanum*	*Hypericum perforatum*	*Sonchus arvensis*	*Knautia arvensis*	*Medicago lupulina*	*Trifolium repens*	*Cornus sanguinea*	Total
	#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Meligethes aeneus	1		503	4	18	5	0	0	2	22	0	9	4	0	0	7	0	5	0	2	0	1	0	0	588
Apis mellifera	2		183	62	5	1	0	3	16	0	7	2	1	1	1	0	0	0	0	0	6	0	3	0	293
Corymbia rubra	3		4	99	42	63	1	44	0	0	7	0	0	0	1	0	0	0	0	0	0	2	0	1	264
Lasius niger	4		0	114	61	0	20	1	5	0	2	4	22	2	4	0	0	0	0	0	0	0	0	2	239
Bombus terrestris	5		85	13	0	0	0	0	10	0	3	0	0	8	0	0	2	0	0	0	0	0	0	0	122
Myrmica rubra	6		10	69	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	117
Oedemera virescens	7		0	21	26	6	2	11	4	7	8	4	6	8	2	0	0	0	0	0	0	1	0	0	106
Hylaeus signatus	8		0	1	0	0	74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75
Bombus lapidarius	9		40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40
Oedemera femorata	10		0	4	16	3	0	0	0	0	3	4	0	0	0	0	0	0	1	0	0	0	0	0	31
Formica rufibarbis	11		0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
Byturus tomentosus	12		0	8	0	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0	0	0	0	15
Lasioglossum sp. 6	13		0	1	4	0	0	0	0	3	0	1	0	5	0	0	0	0	0	0	0	1	0	0	15
Coleoptera sp. 4	14		0	5	0	2	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	13
Mordellidae sp. 1	15		1	0	7	0	0	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	13
Colletes sp. 1	16		0	1	0	0	0	0	0	0	0	0	0	0	2	0	7	0	0	0	0	0	0	0	10
Agrilus angustulus	17		0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	8
Lasioglossum sp. 4	18		0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	8
Miarus sp. 1	19		0	0	0	0	0	0	1	3	1	0	0	0	0	0	0	0	3	0	0	0	0	0	8
Stenopterus rufus	20		0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
Curculionidae sp. 4	21		0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Hylaeus sp. 1	22		0	0	0	3	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	7
Lasioglossum sp. 3	23		0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Mordellidae sp. 2	24		0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Amblytylus nasutus	25		0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6
Clytra quadripunctata	26		0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Syrphus ribesii	27		0	0	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6
Anthrenus verbasci	28		0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Chrysanthia nigricornis	29		0	2	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	5
Hylaeus sp. 2	30		0	0	0	1	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	1	0	5
Total			833	410	336	114	99	82	59	49	45	43	37	33	17	12	12	9	8	8	7	6	6	5	2251

Tab. 2 a *Link temperatures* T_{ij} of network #2. Animals and plant species are sorted by number of observed interactions. For a better clarity, only those species with five recorded interactions are shown. Significant hot T_{ij}s are marked in red, cold T_{ij}s in blue. Those interactions used for the olfactometer tests are framed. 'Total' is the total number of interactions observed for each species.

			Crepis vesicaria	*Cichorium intybus*	*Origanum vulgare*	*Erigeron annuus*	*Medicago lupulina*	*Ballota nigra*	*Echium vulgare*	*Cirsium arvense*	*Reseda lutea*	*Senecio jacobaea*	*Cirsium vulgare*	*Geranium molle*	*Hypericum perforatum*	*Centaurea scabiosa*	*Convolvulus arvensis*	*Plantago lanceolata*	*Trifolium repens*	*Galium verum*	Total
	#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Apis mellifera	1		-0.23	-0.02	0.09	-0.07	-0.03	0.15	0.03	0.02	0.01	0.00	0.02	-0.02	0.02	0.01	-0.01	0.01	0.05	-0.01	163
Episyrphus balteatus	2		0.25	0.27	-0.07	-0.07	-0.07	-0.07	-0.06	-0.03	-0.04	-0.02	-0.03	-0.02	-0.02	-0.01	0.00	0.00	-0.01	-0.01	148
Meligethes aeneus	3		0.71	-0.11	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	-0.04	-0.04	-0.03	-0.01	-0.02	-0.02	-0.02	0.02	-0.01	-0.01	117
Aphantopus hyperanthus	4		-0.25	-0.11	0.36	0.26	-0.06	-0.07	-0.06	0.08	-0.04	-0.03	-0.02	-0.02	-0.02	0.02	-0.02	-0.01	-0.01	-0.01	100
Sphaerophoria scipta	5		0.19	0.35	-0.10	-0.06	-0.06	-0.07	-0.05	-0.04	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	0.03	-0.01	-0.01	-0.01	65
Thymelicus sylvestris	6		-0.12	-0.11	-0.05	-0.08	0.50	0.01	0.04	-0.04	-0.04	-0.04	0.00	-0.02	-0.02	-0.02	0.02	-0.01	-0.01	-0.01	61
Bombus pascuorum	7		-0.25	-0.11	-0.10	-0.08	-0.01	0.37	0.40	-0.04	-0.04	-0.04	-0.03	-0.02	0.00	0.00	-0.02	-0.01	-0.01	-0.01	52
Oedemera virescens	8		0.19	-0.07	-0.08	0.08	-0.05	-0.07	-0.06	-0.03	-0.04	-0.02	-0.03	0.17	-0.02	0.00	0.00	-0.01	-0.01	0.05	52
Melanargia galathea	9		-0.25	-0.11	0.20	-0.05	-0.07	-0.07	-0.06	-0.02	-0.04	-0.01	0.40	-0.02	-0.02	0.17	-0.02	-0.01	-0.01	-0.01	37
Hylaeus signatus	10		-0.25	-0.11	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	0.96	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	30
Corymbia rubra	11		-0.25	-0.11	-0.06	0.38	-0.07	-0.07	-0.06	0.29	-0.04	-0.04	-0.03	-0.02	0.02	-0.02	-0.02	0.07	-0.01	-0.01	24
Bombus terrestris	12		-0.25	-0.11	0.14	-0.08	-0.07	0.12	0.03	-0.04	-0.04	0.16	0.16	-0.02	0.08	-0.02	-0.02	-0.01	-0.01	-0.01	21
Lygocoris sp. 1	13		-0.21	-0.06	-0.10	-0.03	0.31	-0.07	-0.06	-0.04	-0.04	0.25	0.11	-0.02	-0.02	0.03	-0.02	-0.01	-0.01	-0.01	21
Osmia adunca	14		-0.25	-0.11	-0.10	-0.08	-0.07	-0.07	0.94	-0.04	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	18
Agrilus angustulus	15		-0.05	-0.11	-0.10	0.26	-0.07	-0.07	-0.06	-0.04	-0.04	0.16	-0.03	-0.02	0.05	-0.02	-0.02	-0.01	-0.01	-0.01	15
Oedemera femorata	16		-0.11	-0.11	-0.10	0.42	-0.07	-0.07	-0.06	0.10	-0.04	0.18	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	14
Halictinae sp. 1	17		0.29	0.16	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	-0.04	-0.04	-0.03	0.16	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	11
Lasius niger	18		-0.25	-0.11	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	-0.04	0.42	-0.03	-0.02	-0.02	-0.02	0.53	-0.01	-0.01	-0.01	11
Pieris rapae	19		-0.25	-0.11	0.35	-0.08	0.29	0.02	-0.06	-0.04	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	0.08	-0.01	-0.01	-0.01	11
Adelphocoris seticornis	20		-0.25	-0.11	-0.10	0.21	0.50	-0.07	-0.06	-0.04	-0.04	0.11	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	7
Bombus hotorum	21		-0.25	-0.11	-0.10	-0.08	-0.07	0.93	-0.06	-0.04	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	7
Celastrina agriolus	22		-0.25	-0.11	-0.10	0.07	0.78	-0.07	-0.06	-0.04	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	7
Hylaeus sp.1	23		-0.25	-0.11	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	-0.04	0.39	-0.03	0.55	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	7
Myrmica rubra	24		-0.25	-0.11	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	-0.04	-0.04	-0.03	-0.02	0.98	-0.02	-0.02	-0.01	-0.01	-0.01	7
Rhagonycha fulva	25		-0.25	-0.11	-0.10	-0.08	0.21	-0.07	0.08	0.24	-0.04	0.25	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	7
Halictinae sp. 2	26		-0.25	0.89	-0.10	-0.08	-0.07	-0.07	-0.06	-0.04	-0.04	-0.04	-0.03	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	5
Total			273	117	110	84	78	75	68	48	39	38	35	21	21	20	17	10	10	9	1080

Tab. 2 b Observed interactions in network #2. Animals and plant species are sorted by total number of observed interactions. For clarity, only those species with five recorded interactions are shown. 'Total' is the total number of interactions observed for each species including those that are not shown.

			Crepis vesicaria	*Cichorium intybus*	*Origanum vulgare*	*Erigeron annuus*	*Medicago lupulina*	*Ballota nigra*	*Echium vulgare*	*Cirsium arvense*	*Reseda lutea*	*Senecio jacobaea*	*Cirsium vulgare*	*Geranium molle*	*Hypericum perforatum*	*Centaurea scabiosa*	*Convolvulus arvensis*	*Plantago lanceolata*	*Trifolium repens*	*Galium verum*	Total
	#		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Apis mellifera	1		3	15	31	1	7	35	15	10	7	5	9	0	7	4	1	3	10	0	163
Episyrphus balteatus	2		75	56	5	1	1	0	0	2	0	2	0	0	0	2	3	1	0	0	148
Meligethes aeneus	3		113	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	117
Aphantopus hyperanthus	4		0	0	46	34	1	0	0	12	0	1	1	0	0	4	0	0	0	0	100
Sphaerophoria scipta	5		29	30	0	1	1	0	1	0	0	0	0	0	0	0	3	0	0	0	65
Thymelicus sylvestris	6		8	0	3	0	35	5	6	0	0	0	2	0	0	0	2	0	0	0	61
Bombus pascuorum	7		0	0	0	0	3	23	24	0	0	0	0	0	1	1	0	0	0	0	52
Oedemera virescens	8		23	2	1	8	1	0	0	1	0	1	0	10	0	1	1	0	0	3	52
Melanargia galathea	9		0	0	11	1	0	0	0	1	0	1	16	0	0	7	0	0	0	0	37
Hylaeus signatus	10		0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	30
Corymbia rubra	11		0	0	1	11	0	0	0	8	0	0	0	0	1	0	0	2	0	0	24
Bombus terrestris	12		0	0	5	0	0	4	2	0	0	4	4	0	2	0	0	0	0	0	21
Lygocoris sp. 1	13		1	1	0	1	8	0	0	0	0	6	3	0	0	1	0	0	0	0	21
Osmia adunca	14		0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	18
Agrilus angustulus	15		3	0	0	5	0	0	0	0	0	3	0	0	1	0	0	0	0	0	15
Oedemera femorata	16		2	0	0	7	0	0	0	2	0	3	0	0	0	0	0	0	0	0	14
Halictinae sp. 1	17		6	3	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	11
Lasius niger	18		0	0	0	0	0	0	0	0	0	5	0	0	0	0	6	0	0	0	11
Pieris rapae	19		0	0	5	0	4	1	0	0	0	0	0	0	0	0	1	0	0	0	11
Adelphocoris seticornis	20		0	0	0	2	4	0	0	0	0	1	0	0	0	0	0	0	0	0	7
Bombus hotorum	21		0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	7
Celastrina agriolus	22		0	0	0	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Hylaeus sp.1	23		0	0	0	0	0	0	0	0	0	3	0	4	0	0	0	0	0	0	7
Myrmica rubra	24		0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	7
Rhagonycha fulva	25		0	0	0	0	2	0	1	2	0	2	0	0	0	0	0	0	0	0	7
Halictinae sp. 2	26		0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Total			273	117	110	84	78	75	68	48	39	38	35	21	21	20	17	10	10	9	1080

APPENDIX L

Setup

For the biotests, we used a newly established mobile olfactometer (Fig. 1 A). The system allowed us to conduct the tests in the field with scents from naturally growing, unpicked flowers and insects that did not live in captivity and just recently foraged for resources. The airstream was produced by a battery driven electronic pump (Thomas Gardner Denver, G 24/08 30W; Fig. 1 p). Air was sucked in through a particle filter (Fig. 2 fi). Teflon tubes lead the airstream via a valve (Fig. 2 v) into a flask filled with charcoal (Fig. 2) to clean the air which was than directed into a flask filled with distilled water (Fig. 2) to moisten the air. The valve needs to be opened before switching off the pump in order to reduce the pressure in the system. The cleaned and moistened air was than directed to four flowmeters (Analyt-MTC, 112-08SA, Fig. 1 f; Fig. 2 f) that allowed adjusting a constant flow [ml min^{-1}]. Scent application was accomplished in the following way (Fig. 1 B): flower or inflorescence stems were wrapped with Teflon tape and a hose of oven bag (Toppits, PET, Fig. 1 A o; Fig. 2 o) was tightly affixed at the Teflon tape using masking tape without damaging the plant tissue. The top end of the hose was thrust through the top part of washing flask which was clearly cut at the top (Fig. 1 w). Into the overlapping hose a Teflon washing flask topping (Fig. 1 t) was tightly pressed into the top part of the cut washing flask. The Teflon topping had two connections for spiral Teflon tubes (Fig. 1 s): One supplied the air from the flowmeters the other one transported the scented air to the arenas (Fig. 1 a, Fig. 2 a). The whole assemblage was carried by a post and a laboratory clamp (Fig. 1 c).

Two different arenas were used, each suited for different animals (Fig. 1). A smaller version of a four field arena was used as described in Junker and Blüthgen (2008) (Junker and Blüthgen 2008). Secondly, a new type of y-shaped arena was used (for dimensions of both arenas see Fig. 3). Glass plates covered the arenas. All holes for aerial in and out flow in the arenas were obstructed with metal sieves to prevent insects from escaping. Air flew off a central hole in the four field arena and in a hole in the back wall in the Y-shaped olfactometer. In the Y-shaped olfactometer, insects were introduced via a closable hole in the back wall. The hole was closed with a Teflon plug.

The whole apparatus was fitted into an aluminium box so that the whole setup can be transported to the field site.

Method

After tests with one plant species, the arena and the Teflon tubes that came in contact with floral scents were thoroughly cleaned with hexane and acetone. Oven bags were discarded after each treatment.

For the biotests, protocols needed to be modified for different insects depending on their ability to fly and other behavioural characteristics.

Four field olfactometer:

In each corner of the arena, scented (two opposite corners) or neutral air was blown with a constant flow of 160 ml min^{-1}. After each trial, scented and neutral fields were changed to avoid effects of potential site-preferences of the animals tested. Animals were released into the centre of the arena before the glass plate was placed onto the arena.

Ants:

Six ants were simultaneously placed into the arena were they could choose between the scented and the neutral fields. After two minutes of acclimatisation, for three minutes every 30 seconds the number of ants in the odour fields was counted. In each test with a given plant species, ants from three different colonies were used. Results of each group of six ants were condensed to one mean value. Number of ants in scented fields of the arena was tested against the null-hypothesis assuming equal number of ants in control and scented fields (i.e. three individuals in control and treatment each) with a paired *t*-test.

Beetles:

One beetle was placed in the arena. After two minutes of acclimatisation, for three minutes the time spent in the scented fields was taken. Each beetle was used only once. Time spend in the scented fields of the arena was tested against the null-hypothesis assuming that beetles spend the same time in control and scented fields (i.e. 90 s each) with a paired *t*-test.

Y-shaped olfactometer:

Bees, bumblebees and hoverflies were tested in this kind of arena. In each arm of the arena, scented or neutral air was blown with a constant flow of 200 ml min^{-1}. After each trial, scented and neutral arms were changed to avoid effects of potential site-preferences of the animals tested.

Insects were released in the arena through the hole in the back wall which was than closed by the Teflon plug. Animals were free to choose the scented or neutral arm of the Y-shaped arena. The first decision was recorded for the statistical analysis. Animals that did not fly or crawl in either of the arms were not considered. Numbers of animals that chose the scented or control arm were tested against the null-hypothesis that animals choose both treatment and control equally often with a *Chi*^{2}-test.

Fig. 1

Fig. 1 Mobile Olfactometer (A) and scent application (B), including the four field arena (a1), Y-shaped arena (a2), flowmeters (f), spiral Teflon tubes for air transport (s), oven bag (o), laboratory clamp (c), cut washing flask top (w) and the Teflon washing flask topping (t).

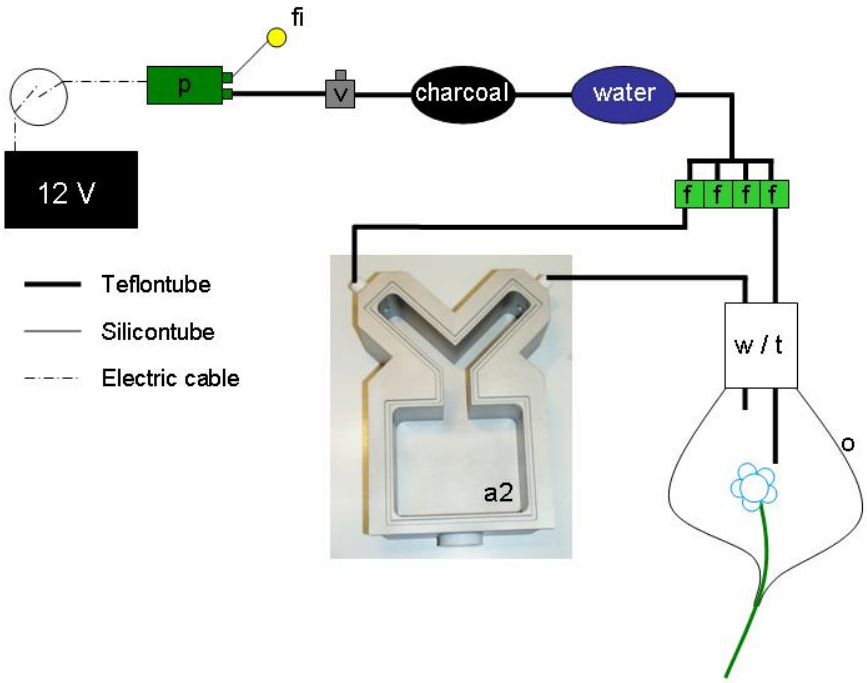

Fig. 2 Schematic drawing of the mobile olfactometer, showing the battery (12 V), power button, electric pump (p), filter (fi), valve (v), washing flasks filled with charcoal and pure water, flowmeters (f), assemblage to apply the scent (w/t , o) and the Y-shaped arena. The two remaining flowmeters are used only when the four field arena is operated.

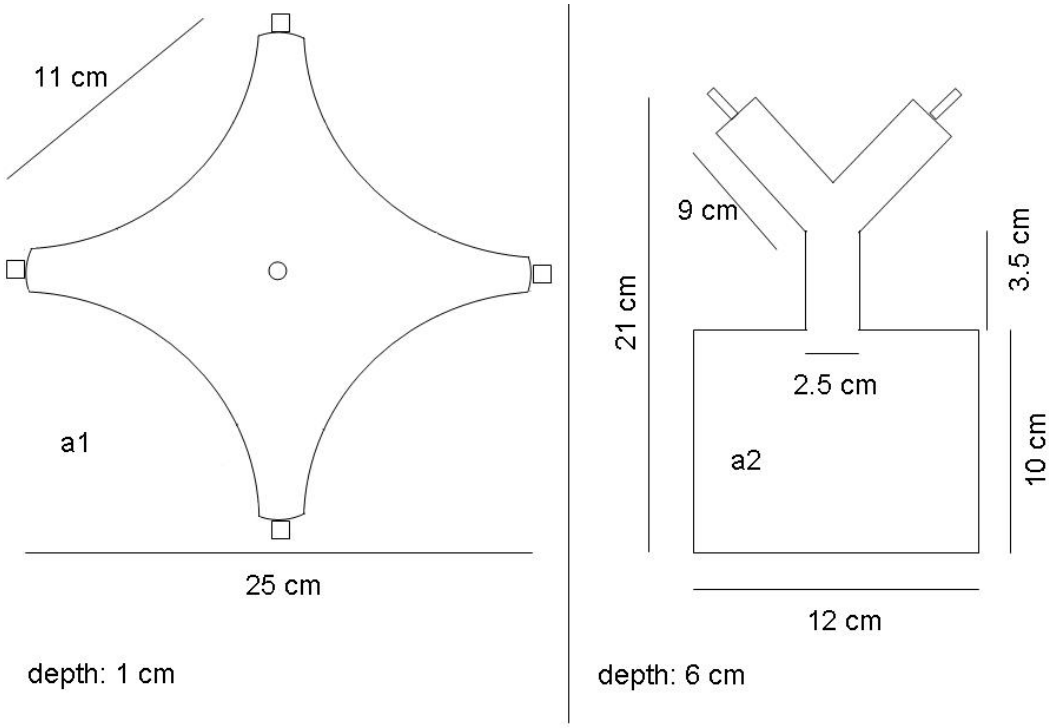

Fig. 3 Dimensions of the arenas used for the biotests. The four field arena (a1) was used for crawling insects like ants and beetles, the Y-shaped arena for flying insects like bees and bumblebees.

APPENDIX M

Olfactometer trials and results

Interactions that were tested in the olfactometer trials. Here we show the *link temperature* T_{ij} of each interaction and the *p*-value received from Monte-Carlo statistics expressing the deviation from the neutral model, the *response index* R_{ij}, the response indices of animals that were caught from another species than the focal species $R_{ij}^{\$}$ and of animals that were caught from the focal species $R_{ij}^{\S}$, the sample size *n* of the biotests and the *p*-value showing whether the response R_{ij} significantly deviates from 0. Additionally, the plant species on which the animals used for the biotests foraged when they where caught is given including the link temperature $T_{ij}^{\$}$ of this specific interaction. Plant species marked with an asterisk (*) had to be picked for the bioassays and were placed in vases. Both networks are included (nw = network number).

#	Plant family	Plant species	Animal species	Animal order	Animal family	T_{ij}	p-value of T_{ij}	R_{ij}	$R_{ij}^{\$}$	$R_{ij}^{\S}$	n	p-value of R_{ij}	Animals caught from	$T_{ij}^{\$}$	nw
1	Asteraceae	*Achillea millefolium*	*Apis mellifera*	Hymenoptera	Apidae	-0.047	0.000	-0.545	-0.545	NA	22	0.011	*Tilia cordata*	hot	1
2		*Achillea millefolium*	*Bombus terrestris*	Hymenoptera	Apidae	-0.051	0.004	-0.727	-0.727	NA	22	0.001	*Tilia cordata*	hot	1
3		*Achillea millefolium*	*Corymbia rubra*	Coleoptera	Cerambycidae	0.188	0.000	-0.175	-0.294	-0.063	16	0.174	*Rubus fruticosus*	hot	1
4		*Achillea millefolium*	*Lasius niger*	Hymenoptera	Formicoidea	-0.051	0.000	-0.368	-0.368	NA	15	0.001	NA	NA	1
5		*Erigeron annuus*	*Apis mellifera*	Hymenoptera	Apidae	-0.026	0.001	-0.143	-0.143	NA	14	0.593	*Tilia cordata*	hot	1
6		*Erigeron annuus*	*Corymbia rubra*	Coleoptera	Cerambycidae	0.130	0.000	0.028	NA	0.028	16	0.841	*Erigeron annuus*	hot	1
7		*Erigeron annuus*	*Hylaeus signatus*	Hymenoptera	Apidae	-0.036	0.040	-0.067	-0.067	NA	15	0.796	*Reseda lutea*	hot	1
8		*Erigeron annuus*	*Meligethes aeneus*	Coleoptera	Nitidulidae	-0.036	0.000	-0.334	-0.334	NA	14	0.032	*Sonchus arvensis*	neutral	1
9	Dipsacaceae	*Knautia arvensis*	*Apis mellifera*	Hymenoptera	Apidae	0.017	0.000	0.128	-0.053	0.3	39	0.423	*Origanum vulgare*	hot	1
10	Fabaceae	Trifolium repens*	*Apis mellifera*	Hymenoptera	Apidae	0.052	0.035	0.055	0.029	0.1	55	0.686	*Origanum vulgare*	hot	1
11	Geraniaceae	*Geranium molle**	*Hylaeus signatus*	Hymenoptera	Apidae	-0.022	0.199	0.000	0.000	NA	10	1.000	*Reseda lutea*	hot	1
12		*Geranium molle**	*Lasius niger*	Hymenoptera	Formicoidea	-0.022	0.005	-0.200	-0.200	NA	15	0.000	NA	NA	1
13	Resedaceae	*Reseda lutea*	*Hylaeus signatus*	Hymenoptera	Apidae	0.943	0.000	0.600	NA	0.600	20	0.007	*Reseda lutea*	hot	1
14		*Reseda lutea*	*Lasius niger*	Hymenoptera	Formicoidea	0.040	0.001	-0.054	-0.054	NA	15	0.378	NA	NA	1
15		*Reseda lutea*	*Meligethes aeneus*	Coleoptera	Nitidulidae	-0.044	0.000	-0.236	-0.236	NA	15	0.062	*Tilia cordata*	hot	1
16	Rosaceae	*Rubus fruticosus**	*Apis mellifera*	Hymenoptera	Apidae	0.029	0.045	-0.067	0.000	-0.200	15	0.796	*Tilia cordata*	hot	1
17		*Rubus fruticosus**	*Bombus terrestris*	Hymenoptera	Apidae	-0.076	0.041	-0.143	0.000	-0.500	14	0.593	Verbascum pulverulantum	hot	1
18		*Rubus fruticosus**	*Corymbia rubra*	Coleoptera	Cerambycidae	0.193	0.000	-0.371	-0.489	-0.253	18	0.002	*Erigeron annuus*	hot	1
19		*Rubus fruticosus**	*Lasius niger*	Hymenoptera	Formicoidea	0.295	0.000	-0.124	-0.124	NA	15	0.114	NA	NA	1
20	Rubiaceae	*Galium mollugo*	*Corymbia rubra*	Coleoptera	Cerambycidae	0.010	0.388	-0.038	-0.192	0.193	20	0.750	*Rubus fruticosus*	hot	1
21		*Galium mollugo*	*Lasius niger*	Hymenoptera	Formicoidea	0.106	0.000	0.149	0.149	NA	15	0.124	NA	NA	1
22	Scrophulariaceae	*Verbascum pulverulantum*	*Apis mellifera*	Hymenoptera	Apidae	0.028	0.002	0.600	0.600	NA	15	0.020	*Tilia cordata*	hot	1
23		*Verbascum pulverulantum*	*Bombus terrestris*	Hymenoptera	Apidae	0.056	0.000	-0.167	-0.167	NA	12	0.564	*Tilia cordata*	hot	1
24		*Verbascum pulverulantum*	*Hylaeus signatus*	Hymenoptera	Apidae	-0.026	0.145	-0.083	-0.083	NA	24	0.683	*Reseda lutea*	hot	1
25		*Verbascum pulverulantum*	*Meligethes aeneus*	Coleoptera	Nitidulidae	-0.023	0.000	-0.056	-0.056	NA	14	0.687	*Sonchus arvensis*	neutral	1
26	Tiliaceae	*Tilia cordata**	*Apis mellifera*	Hymenoptera	Apidae	0.255	0.000	-0.250	-0.500	0.000	24	0.221	*Tilia cordata*	hot	1
27		*Tilia cordata**	*Bombus lapidarius*	Hymenoptera	Apidae	0.630	0.000	0.000	NA	0.000	12	1.000	*Tilia cordata*	hot	1
28		*Tilia cordata**	*Bombus terrestris*	Hymenoptera	Apidae	0.327	0.000	-0.200	NA	-0.200	15	0.439	*Tilia cordata*	hot	1
29		*Tilia cordata**	*Corymbia rubra*	Coleoptera	Cerambycidae	-0.355	0.000	-0.192	-0.221	-0.023	20	0.053	*Rubus fruticosus*	hot	1
30		*Tilia cordata**	*Lasius niger*	Hymenoptera	Formicoidea	-0.370	0.000	-0.200	-0.200	NA	15	0.055	NA	NA	1
31		*Tilia cordata**	*Meligethes aeneus*	Coleoptera	Nitidulidae	0.485	0.000	0.195	NA	0.195	15	0.007	*Tilia cordata*	hot	1
32	Asteraceae	*Cichorium intybus*	*Apis mellifera*	Hymenoptera	Apidae	-0.016	0.446	0.154	0.143	0.167	26	0.433	*Origanum vulgare*	hot	2
33		*Cichorium intybus*	*Bombus pascuorum*	Hymenoptera	Apidae	-0.108	0.008	-0.200	-0.200	NA	20	0.371	*Ballota nigra*	hot	2
34		*Cichorium intybus*	*Episyrphus balteatus*	Diptera	Syrphidae	0.270	0.000	0.081	0.111	0.053	37	0.622	*Crepis vesicaria*	hot	2
35		*Cichorium intybus*	*Sphaerophoria scripta*	Diptera	Syrphidae	0.353	0.000	-0.125	-0.125	NA	16	0.617	*Crepis vesicaria*	hot	2
36		*Cirsium vulgare*	*Bombus pascuorum*	Hymenoptera	Apidae	-0.032	0.248	0.333	0.333	NA	18	0.157	*Ballota nigra*	hot	2
37		*Crepis vesicaria*	*Apis mellifera*	Hymenoptera	Apidae	-0.234	0.000	0.273	0.273	NA	22	0.201	*Origanum vulgare*	hot	2
38		*Crepis vesicaria*	*Bombus pascuorum*	Hymenoptera	Apidae	-0.253	0.000	-0.556	-0.556	NA	18	0.018	*Ballota nigra*	hot	2
39		*Crepis vesicaria*	*Bombus terrestris*	Hymenoptera	Apidae	-0.253	0.000	-0.111	-0.111	NA	18	0.637	*Ballota nigra*	hot	2
40		*Crepis vesicaria*	*Episyrphus balteatus*	Diptera	Syrphidae	0.254	0.004	0.153	-0.100	0.282	59	0.241	*Senecio jacobaea*	hot	2
41		*Crepis vesicaria*	*Meligethes aeneus*	Coleoptera	Nitidulidae	0.713	0.000	0.236	NA	0.236	15	0.144	*Crepis vesicaria*	hot	2
42		*Senecio jacobaea*	*Apis mellifera*	Hymenoptera	Apidae	-0.005	0.489	-0.455	-0.455	NA	22	0.033	*Origanum vulgare*	hot	2
43		*Senecio jacobaea*	*Bombus pascuorum*	Hymenoptera	Apidae	-0.035	0.214	-0.222	-0.222	NA	18	0.346	*Ballota nigra*	hot	2
44		*Senecio jacobaea*	*Episyrphus balteatus*	Diptera	Syrphidae	-0.022	0.187	-0.091	-0.189	0.111	55	0.500	*Crepis vesicaria*	hot	2
45		*Senecio jacobaea*	*Meligethes aeneus*	Coleoptera	Nitidulidae	-0.035	0.028	-0.256	-0.256	NA	15	0.049	*Crepis vesicaria*	hot	2
46	Boraginaceae	*Echium vulgare*	*Bombus pascuorum*	Hymenoptera	Apidae	0.399	0.000	-0.010	-0.127	0.130	101	0.921	*Ballota nigra*	hot	2
47	Fabaceae	*Medicago lupulina*	*Apis mellifera*	Hymenoptera	Apidae	-0.029	0.248	0.200	0.200	NA	20	0.371	*Arctium* sp.	hot	2
48		*Medicago lupulina*	*Bombus pascuorum*	Hymenoptera	Apidae	-0.015	0.583	0.045	-0.071	0.250	88	0.670	*Origanum vulgare*	cold	2
49		*Medicago lupulina*	*Meligethes aeneus*	Coleoptera	Nitidulidae	-0.072	0.001	-0.166	-0.166	NA	15	0.094	*Crepis vesicaria*	hot	2
50	Lamiaceae	*Ballota nigra*	*Apis mellifera*	Hymenoptera	Apidae	0.145	0.000	-0.016	-0.150	0.217	63	0.900	*Origanum vulgare*	hot	2
51		*Ballota nigra*	*Bombus pascuorum*	Hymenoptera	Apidae	0.373	0.000	0.212	0.136	0.273	99	0.035	*Echium vulgare*	hot	2
52		*Ballota nigra*	*Bombus terrestris*	Hymenoptera	Apidae	0.121	0.026	0.333	NA	0.333	18	0.157	*Ballota nigra*	hot	2
53		*Ballota nigra*	*Episyrphus balteatus*	Diptera	Syrphidae	-0.069	0.000	-0.158	-0.158	NA	19	0.491	*Crepis vesicaria*	hot	2
54		*Ballota nigra*	*Sphaerophoria scripta*	Diptera	Syrphidae	-0.069	0.021	-0.368	-0.368	NA	19	0.108	*Crepis vesicaria*	hot	2
55		*Origanum vulgare*	*Apis mellifera*	Hymenoptera	Apidae	0.088	0.000	0.200	0.050	0.350	80	0.074	*Arctium* sp.	hot	2
56		*Origanum vulgare*	*Bombus pascuorum*	Hymenoptera	Apidae	-0.102	0.009	0.200	-0.100	0.500	40	0.414	*Ballota nigra*	hot	2
57		*Origanum vulgare*	*Bombus terrestris*	Hymenoptera	Apidae	0.136	0.027	0.100	-0.200	0.400	40	0.683	*Cirsium vulgare*	hot	2
58		*Origanum vulgare*	*Lasius niger*	Hymenoptera	Formicoidea	-0.102	0.378	-0.241	-0.241	NA	15	0.001	NA	NA	2
59		*Origanum vulgare*	*Meligethes aeneus*	Coleoptera	Nitidulidae	-0.102	0.000	-0.398	-0.398	NA	15	0.004	*Crepis vesicaria*	hot	2

APPENDIX N.

Network #1:

Plant	Basic colour
Tilia cordata	yellow
Rubus fruticosus	white
Galium mollugo	white
Achillea millefolium	white
Reseda lutea	yellow
Erigeron annuus	white
Verbascum pulverulentum	yellow
Geranium molle	pink
Rosa canina	pink
Potentilla reptans	yellow
Convolvulus arvensis	white
Papaver rhoeas	red
Matricaria recutita	white
Echium vulgare	pink
Medicago x varia	various
Geum urbanum	yellow
Hypericum perforatum	yellow
Sonchus arvensis	yellow
Knautia arvensis	blue
Medicago lupulina	yellow
Trifolium repens	white
Cornus sanguinea	white

Network #2:

Plant	Basic colour
Crepis vesicaria	yellow
Cichorium intybus	blue
Origanum vulgare	purple
Erigeron annuus	white
Medicago lupulina	yellow
Ballota nigra	purple
Echium vulgare	pink
Cirsium arvense	pink
Reseda lutea	yellow
Senecio jacobaea	yellow
Cirsium vulgare	purple
Geranium molle	pink
Hypericum perforatum	yellow
Centaurea scabiosa	purple
Convolvulus arvensis	white
Plantago lanceolata	brown
Trifolium repens	white
Galium verum	yellow

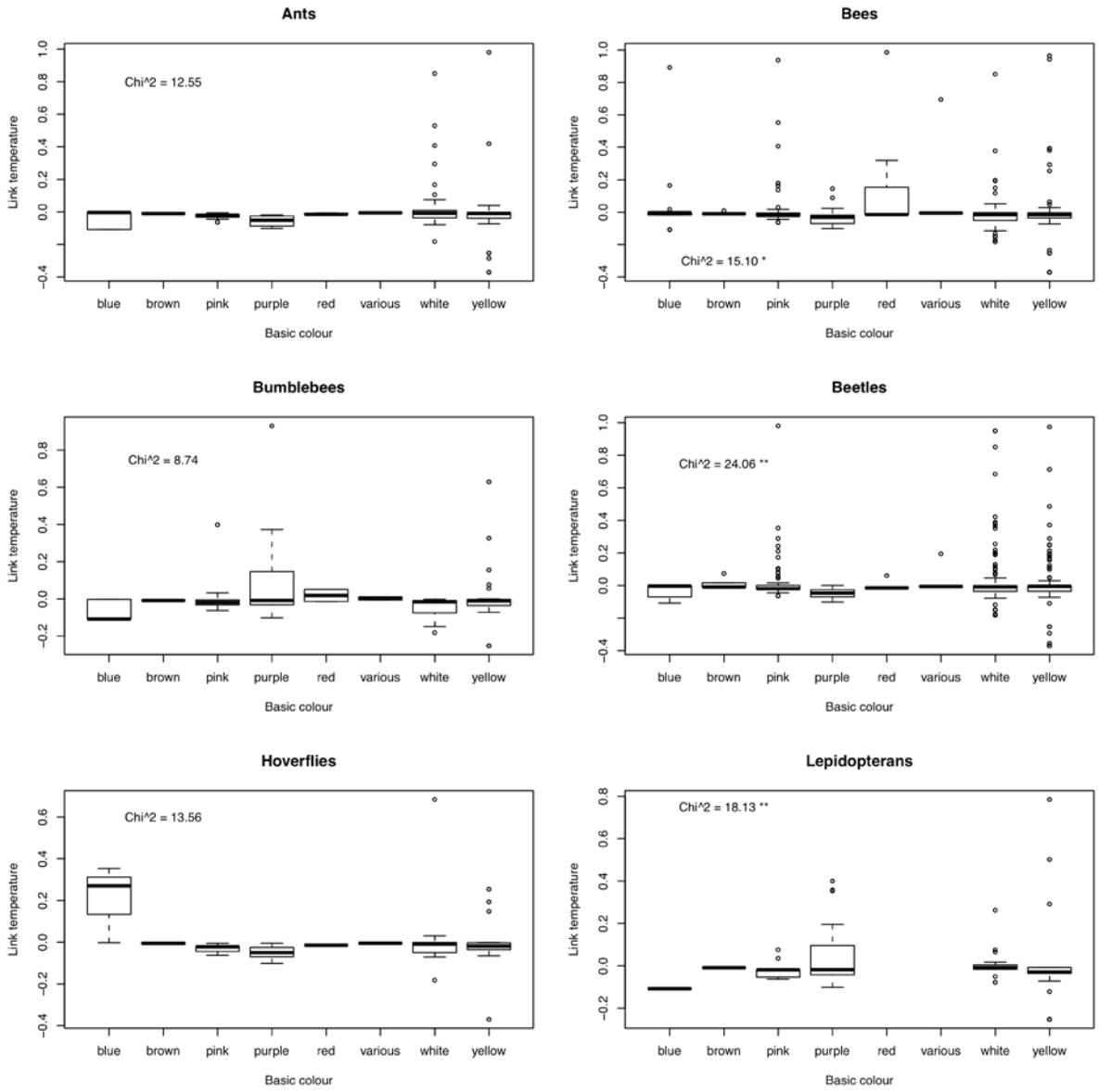

Fig. 1 Link temperatures T_{ij} of different insect groups in dependency of floral basic colours. Results of Kruskal-Wallis rank sum test are given in each figure.

APPENDIX O

Network #1:

Plant	Flower type after Kugler
Tilia cordata	K1
Rubus fruticosus	K1
Galium mollugo	K1
Achillea millefolium	K7
Reseda lutea	K1
Erigeron annuus	K7
Verbascum pulverulentum	K5
Geranium molle	K1
Rosa canina	K1
Potentilla reptans	K1
Convolvulus arvensis	K2
Papaver rhoeas	K1
Matricaria recutita	K7
Echium vulgare	K5
Medicago x varia	K6
Geum urbanum	K1
Hypericum perforatum	K1
Sonchus arvensis	K7
Knautia arvensis	K7
Medicago lupulina	K6
Trifolium repens	K6
Cornus sanguinea	K1

Network #2:

Plant	Flower type after Kugler
Crepis vesicaria	K7
Cichorium intybus	K7
Origanum vulgare	K5
Erigeron annuus	K7
Medicago lupulina	K6
Ballota nigra	K5
Echium vulgare	K5
Cirsium arvense	K7
Reseda lutea	K1
Senecio jacobaea	K7
Cirsium vulgare	K7
Geranium molle	K1
Hypericum perforatum	K1
Centaurea scabiosa	K7
Convolvulus arvensis	K2
Plantago lanceolata	K0
Trifolium repens	K6
Galium verum	K1

Flower types after Kugler:

K0	not applicable
K1	disk- and bowlshaped flowers
K2	funnel flowers
K5	lip flowers
K6	flag blossom
K7	flower heads

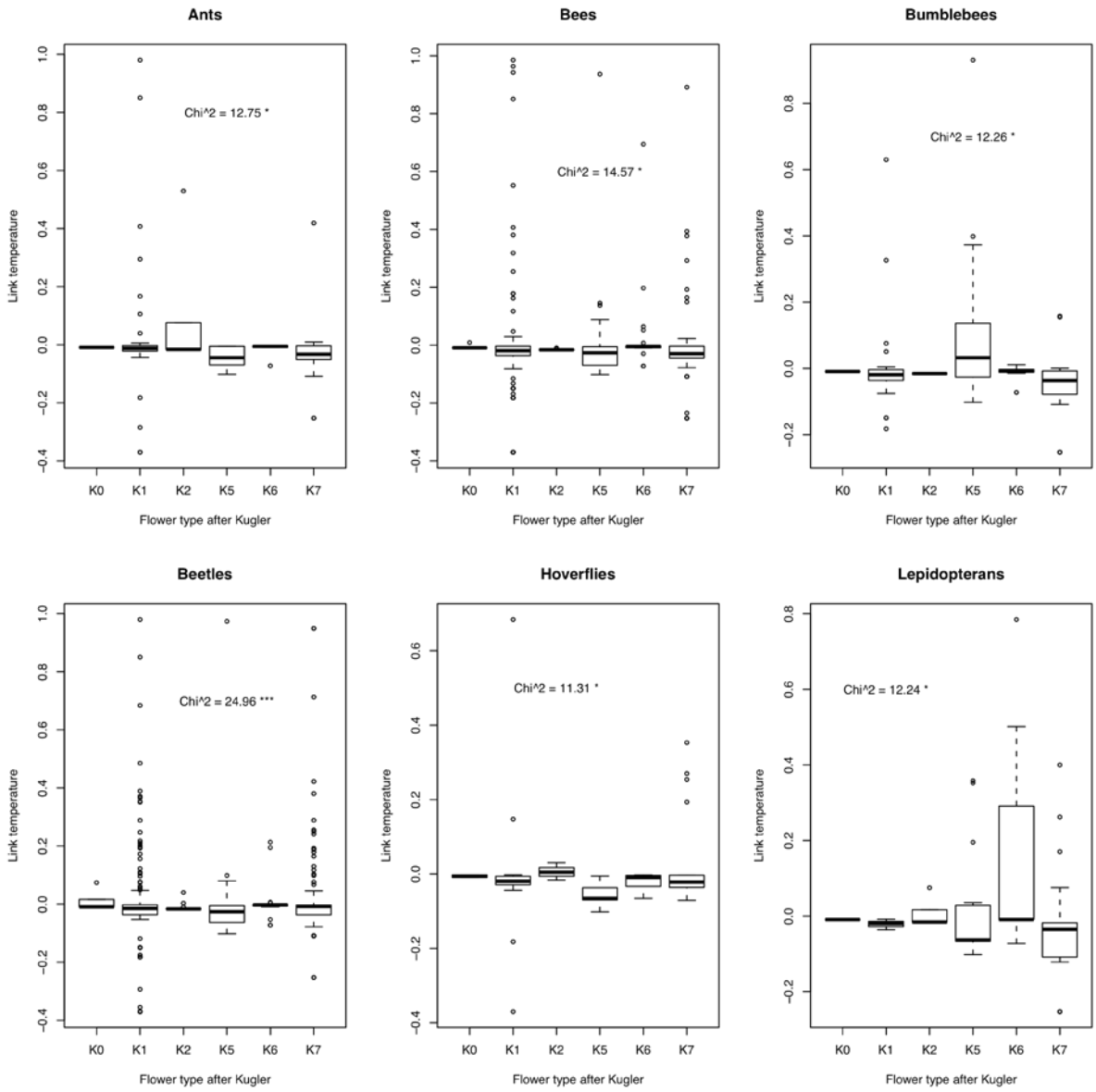

Fig. 1 Link temperatures T_{ij} of different insect groups in dependency of Flower types. Results of Kruskal-Wallis rank sum test are given in each figure.

APPENDIX P

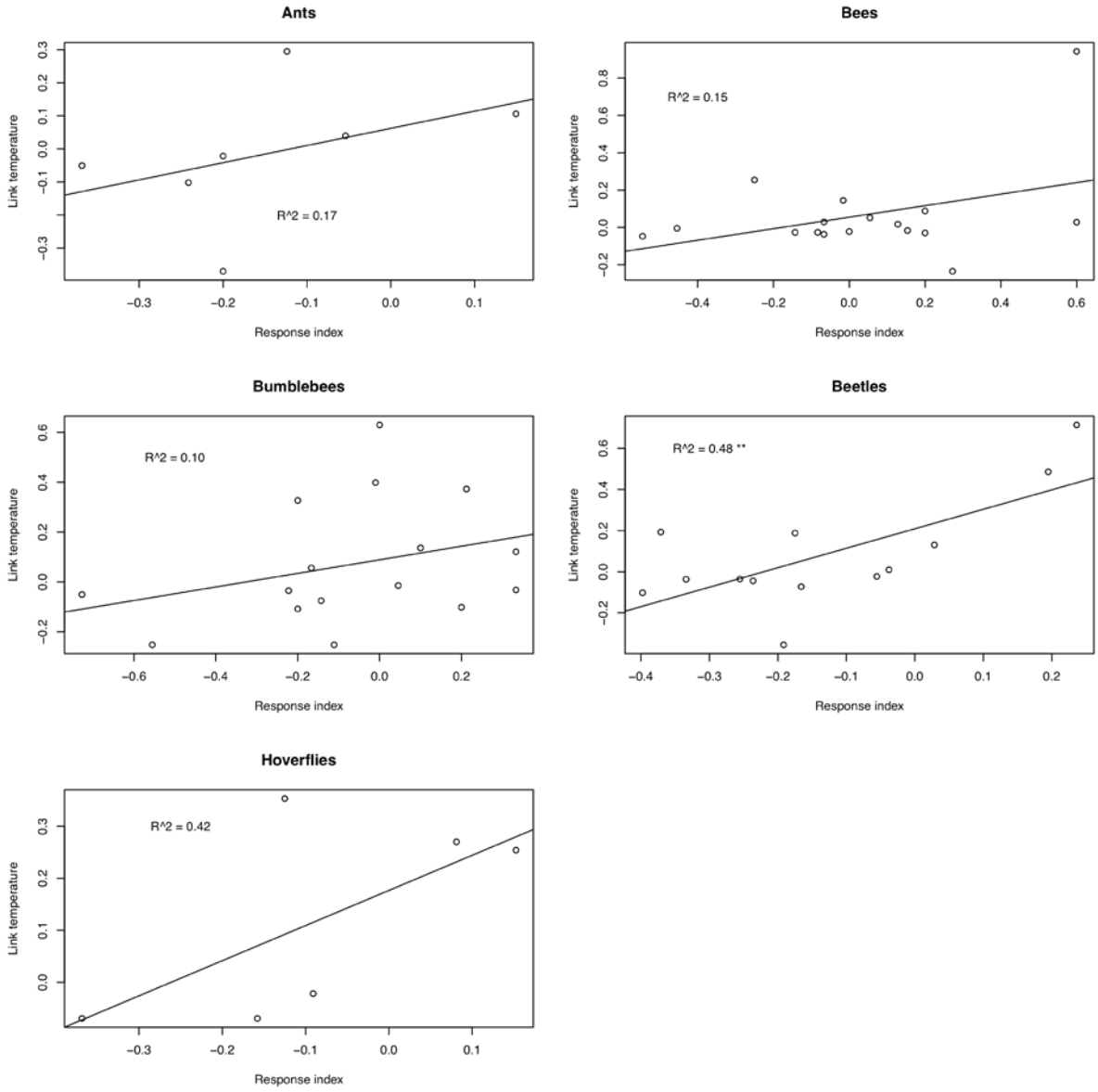

Fig. 1 Correlation between response index R_{ij} and link temperature T_{ij} for different groups of insects. Pearson R^2 and significance are given in each figure.

APPENDIX Q

Interaction: m = mutualist; a = antagonist; u = unknown; mixed = depends on plant species. Dependency: o = obligate flower visitor; f = facultative flower visitor.
*) **) Animal species or at least families were asigned to facultative or obligate flower visitors by John Hilty (*) or Konrad Fiedler (**)

source	Order	Family	animal species	Interaction	Dependency	chemical_class	Substance / bouquet	functgroup	Volume / Mass	L	VarL	confirmation of interaction and status
Andrews *et al.* (2007) J Chem Ecol 33:1682-1691	Coleoptera	Chrysomelidae	*Acalymma vittatum*	mixed	f	Benzenoid	1,2,4-trimethoxybenzene	Ether	20µl	0.1398	0.1523	Andrews et al. (2007) J Chem Ecol 33:1682-1691, *)
	Coleoptera	Chrysomelidae	*Acalymma vittatum*	mixed	f	Benzenoid	(*E*) - cinnamaldehyde	Aldehyde	20µl	-0.3567	0.3287	
	Coleoptera	Chrysomelidae	*Acalymma vittatum*	mixed	f	N-containing_compound	indole	Indole	20µl	0.7419	0.4810	
	Hymenoptera	Apidae	*Peponapis pruinosa*	m	o	Benzenoid	1,2,4-trimethoxybenzene	Ether	20µl	4.1997	0.1000	Andrews et al. (2007) J Chem Ecol 33:1682-1691, *)
	Hymenoptera	Apidae	*Peponapis pruinosa*	m	o	Benzenoid	(*E*) - cinnamaldehyde	Aldehyde	20µl	4.7593	0.7061	
	Hymenoptera	Apidae	*Peponapis pruinosa*	m	o	N-containing_compound	indole	Indole	20µl	3.9120	0.4667	
Hammack (1996) J Chem Ecol 22: 1237-1253	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	Aliphates	(E,E)-2,4-Decadienal	Aldehyde	100mg	-0.2442	0.2368	*)
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	irregular_terpenes	geranylacetone	Ketone	100mg	1.9781	0.1121	
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	irregular_terpenes	β-cyclocitral	Aldehyde	100mg	-0.4502	0.1800	
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	irregular_terpenes	β-ionone	Ketone	100mg	-0.0870	0.1565	
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	Monoterpenoid	citral	Aldehyde	100mg	-0.2336	0.2760	
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	Monoterpenoid	α-terpineol	Alcohol	100mg	1.0643	0.2106	
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	Monoterpenoid	limonene	Hydrocarbon	100mg	-0.2535	0.4385	
	Coleoptera	Chrysomelidae	*Diabrotica barberi*	mixed	f	Monoterpenoid	1,8-cineole	Ether	100mg	-0.0503	0.1903	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	Aliphates	(E,E)-2,4-Decadienal	Aldehyde	100mg	0.0892	0.4339	*)
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	irregular_terpenes	geranylacetone	Ketone	100mg	0.9908	0.3683	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	irregular_terpenes	β-cyclocitral	Aldehyde	100mg	0.2364	0.3624	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	irregular_terpenes	β-ionone	Ketone	100mg	1.8352	0.5179	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	Monoterpenoid	citral	Aldehyde	100mg	-0.3662	0.7285	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	Monoterpenoid	α-terpineol	Alcohol	100mg	2.4071	0.5581	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	Monoterpenoid	limonene	Hydrocarbon	100mg	0.0818	0.5038	
	Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	mixed	f	Monoterpenoid	1,8-cineole	Ether	100mg	0.0765	0.3684	
Imai et al. (2001) Appl Entomol Zool 36: 475-478	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl anthranilate	N-compounds	1g	0.0000	2.0000	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	ethyl anthranilate	N-compounds	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl m-aminobenzoate	N-compounds	1g	5.8091	1.4000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl p-aminobenzoate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	o-toluate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	o-aminophenol	Alcohol	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	o-anisidine	N-compounds	1g	6.5023	1.5500	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl benzoate	Ester	1g	6.5023	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl toluate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl salicylate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	o-aminoacetophenone	Ketone	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Chirothrips manicatus*	u	o	Benzenoid	anisaldehyde	Aldehyde	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl anthranilate	N-compounds	1g	9.7794	1.1687	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	ethyl anthranilate	N-compounds	1g	5.8091	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl m-aminobenzoate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl p-aminobenzoate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	o-toluate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	o-aminophenol	Alcohol	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	o-anisidine	N-compounds	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl benzoate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl toluate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl salicylate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	5.8091	1.4000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	o-aminoacetophenone	Ketone	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Megalurothrips distalis*	u	o	Benzenoid	anisaldehyde	Aldehyde	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl anthranilate	N-compounds	1g	5.8091	1.4000	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	ethyl anthranilate	N-compounds	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl m-aminobenzoate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl p-aminobenzoate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	o-toluate	N-compounds	1g	5.8091	1.4000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	o-aminophenol	Alcohol	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	o-anisidine	N-compounds	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl benzoate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl toluate	Ester	1g	5.1160	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl salicylate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	5.8091	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	5.8091	2.0000	
	Thysanoptera	Thripidae	*Ponteculothrips* sp.	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	0.0000	2.0000	

	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	methyl benzoate	Ester	1g	0.4055	1.2000	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	methyl toluate	Ester	1g	3.8918	1.0431	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	methyl salicylate	Ester	1g	0.0000	1.4000	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	1.8718	1.1905	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	1.3863	1.2500	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	2.1972	1.1259	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	o-aminoacetophenone	Ketone	1g	0.6931	1.2500	
	Thysanoptera	Thripidae	*Stenchaetothrips biformis*	u	o	Benzenoid	anisaldehyde	Aldehyde	1g	-0.6931	2.0000	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl anthranilate	N-compounds	1g	11.0692	1.1684	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	ethyl anthranilate	N-compounds	1g	6.7254	1.3280	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl m-aminobenzoate	N-compounds	1g	10.1659	1.1261	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl p-aminobenzoate	N-compounds	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	o-toluate	N-compounds	1g	6.7254	1.1360	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	o-aminophenol	Alcohol	1g	6.2146	1.4667	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	o-anisidine	N-compounds	1g	11.7598	1.1963	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl benzoate	Ester	1g	9.8434	1.0837	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl toluate	Ester	1g	10.6843	1.1060	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl salicylate	Ester	1g	6.9078	1.2667	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	9.5348	1.2618	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	7.8240	1.4773	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	7.4186	1.2080	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	o-aminoacetophenone	Ketone	1g	8.1117	1.1180	
	Thysanoptera	Thripidae	*Thrips coloratus*	u	o	Benzenoid	anisaldehyde	Aldehyde	1g	6.9078	1.2000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl anthranilate	N-compounds	1g	5.6240	1.1491	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	ethyl anthranilate	N-compounds	1g	2.4849	1.5500	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl m-aminobenzoate	N-compounds	1g	0.6931	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl p-aminobenzoate	N-compounds	1g	-5.1160	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	o-toluate	N-compounds	1g	-5.1160	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	o-aminophenol	Alcohol	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	o-anisidine	N-compounds	1g	0.6931	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl benzoate	Ester	1g	2.6391	1.4000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl toluate	Ester	1g	1.0986	1.4667	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl salicylate	Ester	1g	-5.1160	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	0.0000	2.0000	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	2.9957	1.2860	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	3.1355	1.3195	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	o-aminoacetophenone	Ketone	1g	2.9957	1.1180	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	anisaldehyde	Aldehyde	1g	2.1972	1.3185	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl anthranilate	N-compounds	1g	6.3578	0.1534	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	ethyl anthranilate	N-compounds	1g	3.7554	0.1969	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl m-aminobenzoate	N-compounds	1g	1.3863	0.4250	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl p-aminobenzoate	N-compounds	1g	0.6931	0.1625	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	o-toluate	N-compounds	1g	1.3218	0.2147	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	o-aminophenol	Alcohol	1g	0.0000	0.2000	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	o-anisidine	N-compounds	1g	5.1930	0.1700	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl benzoate	Ester	1g	3.0796	0.2030	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl toluate	Ester	1g	3.5835	0.4157	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl salicylate	Ester	1g	2.1401	0.2114	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl o-methoxybenzoate	Ester	1g	4.6658	0.1966	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl N-methylanthranilate	Ester	1g	4.0860	0.1556	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	methyl N,N-dimethylanthranilate	N-compounds	1g	3.1884	0.2391	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	o-aminoacetophenone	Ketone	1g	5.1314	0.1816	
	Thysanoptera	Thripidae	*Thrips hawaiiensis*	u	o	Benzenoid	anisaldehyde	Aldehyde	1g	4.4248	0.1957	
James (2005) J Chem Ecol 31:481-495	Diptera	Syrphidae	miscellaneous	m	o	Aliphates	cis-3-hexenol	Alcohol	1ml	0.3365	0.0604	*)
	Diptera	Syrphidae	miscellaneous	m	o	Aliphates	trans-2-hexen-1-al	Aldehyde	1ml	-0.1278	0.0917	
	Diptera	Syrphidae	miscellaneous	m	o	Aliphates	(Z)-3-hexenyl actetate	Ester	1ml	0.0000	0.0800	
	Diptera	Syrphidae	miscellaneous	m	o	Benzenoid	benzaldehyde	Aldehyde	1ml	0.1823	0.0678	
	Diptera	Syrphidae	miscellaneous	m	o	Benzenoid	methyl salicylate	Ester	1ml	0.4187	0.0573	
	Diptera	Syrphidae	miscellaneous	m	o	Benzenoid	methyl anthranilate	N-compounds	1ml	-0.0408	0.0834	
	Diptera	Syrphidae	miscellaneous	m	o	Monoterpenoid	linalool	Alcohol	1ml	-0.4463	0.0556	
	Diptera	Syrphidae	miscellaneous	m	o	Monoterpenoid	geraniol	Alcohol	1ml	-0.2231	0.1025	
	Diptera	Syrphidae	miscellaneous	m	o	NA	cis-jasmone	Ketone	1ml	0.0770	0.0743	
	Diptera	Syrphidae	miscellaneous	m	o	NA	methyl jasmonate	Ester	1ml	-0.5798	0.0604	
	Diptera	Syrphidae	miscellaneous	m	o	N-containing_compound	indole	Indole	1ml	0.1133	0.0719	
Junker and Blüthgen (2008) Evol Ecol Res 10:295-308	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	Benzenoid	benzaldehyde	Aldehyde	600 ng h-1	-0.1503	0.0311	*)
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	Monoterpenoid	geraniol	Alcohol	15 ng h-1	-0.7167	0.0436	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	Monoterpenoid	β-pinene	Hydrocarbon	3000 ng h-1	-0.2513	0.0447	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	Monoterpenoid	limonene	Hydrocarbon	600 ng h-1	-0.0125	0.0306	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	Monoterpenoid	1,8-cineole	Ether	5000 ng h-1	-0.3151	0.0610	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	Monoterpenoid	linalool	Alcohol	742 ng h-1	-0.8772	0.0160	

	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Brassica napus* (Brassicaceae)	NA		-0.6129	0.0097	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Brillantaisia nyanzarum* (Acanthaceae)	NA		-0.6328	0.0336	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Catharanthus roseus* (Apocynaceae)	NA		-0.7766	0.0217	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Convolvulus arvensis* (Convolvuaceae)	NA		-0.8924	0.0302	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Cryptophragmium ceylanicum* (Acanthaceae)	NA		-0.3847	0.0635	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Daucus carota* (Aplaceae)	NA		-0.7309	0.0183	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Echinacea pallida* (Asteraceae)	NA		-0.7452	0.0318	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Euphorbia cyparissias* (Euphorbiaceae)	NA		-0.5430	0.0509	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Euphorbia milii* (Euphorbiaceae)	NA		-0.3536	0.0499	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Juanulloa aurantiaca* (Solanaceae)	NA		-0.5376	0.0275	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Kolkwitzia amabilis* (Caprifoliaceae)	NA		-0.7768	0.0138	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Lotus corniculatus* (Fabaceae)	NA		-0.6633	0.0329	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Matricaria reticulata* (Asteraceae)	NA		-0.7768	0.0609	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Murraya paniculata* (Rutaceae)	NA		-1.3863	0.0474	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Nerium oleander* (Apocynaceae)	NA		-0.7423	0.0489	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Nicotiana rustica* (Solanaceae)	NA		-0.2007	0.0727	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Paeonia officinalis* (Paeoniaceae)	NA		-0.4790	0.0079	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Paulownia tomentosa* (Scrophulariaceae)	NA		-0.5645	0.0121	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Phlox* spec. (Polemoniaceae)	NA		-0.4055	0.0478	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Salvia officinalis* (Lamiaceae)	NA		-0.5430	0.0367	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Silene alba* (Caryophyllaceae)	NA		-1.0330	0.0259	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Spartium junceum* (Fabaceae)	NA		-0.6300	0.0510	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Spiraea trilobata* (Rosaceae)	NA		-0.4055	0.0077	
	Hymenoptera	Formicidae	*Camponotus floridanus*	a	f	NA	bouquet of *Trachelospermum jasminoides* (Apocynaceae)	NA		-0.5761	0.0325	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Benzenoid	benzaldehyde	Aldehyde	600 ng h-1	0.0125	0.0028	*)
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Monoterpenoid	geraniol	Alcohol	15 ng h-1	-0.1503	0.0198	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Monoterpenoid	β-pinene	Hydrocarbon	3000 ng h-1	-0.2386	0.0084	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Monoterpenoid	limonene	Hydrocarbon	600 ng h-1	-0.2386	0.0050	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Monoterpenoid	1,8-cineole	Ether	5000 ng h-1	-0.0375	0.0086	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Monoterpenoid	linalool	Alcohol	742 ng h-1	-0.5242	0.0116	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Monoterpenoid	α-pinene	Hydrocarbon	1200 ng h-1	-0.0250	0.0051	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	Sesquiterpenoid	β-caryophyllene	Hydrocarbon	1200 ng h-1	-0.2133	0.0263	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Agapanthus africanus* (Alliaceae)	NA		-0.0125	0.0563	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Agave ferox* (Agavaceae)	NA		-0.1704	0.0095	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Aloe plicatilis* Aloaceae	NA		-0.0300	0.0168	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Anemone hupehensis* var. *japonica* (Ranunculaceae)	NA		-0.0500	0.0073	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Aristolochia gigantea* (Aristolochiaceae)	NA		0.0200	0.0394	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Begonia grandis* ssp. *evansiana* (Begoniaceae)	NA		-0.1906	0.0195	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Brillantaisia nyanzarum* (Acanthaceae)	NA		-0.2615	0.0066	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Catharanthus roseus* (Apocynaceae)	NA		0.3665	0.0323	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Convolvulus arvensis* (Convolvuaceae)	NA		0.1001	0.0173	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Cryptophragmium ceylanicum* (Acanthaceae)	NA		-0.3023	0.0023	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Daucus carota* (Aplaceae)	NA		0.1629	0.0324	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Echinacea pallida* (Asteraceae)	NA		-0.0100	0.0152	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Euphorbia cyparissias* (Euphorbiaceae)	NA		-0.0750	0.0107	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Euphorbia milii* (Euphorbiaceae)	NA		-0.2768	0.0219	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Juanulloa aurantiaca* (Solanaceae)	NA		0.3925	0.0219	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Kolkwitzia amabilis* (Caprifoliaceae)	NA		-0.1704	0.0140	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Lotus corniculatus* (Fabaceae)	NA		-0.3125	0.0100	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Matricaria reticulata* (Asteraceae)	NA		-0.0300	0.0083	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Murraya paniculata* (Rutaceae)	NA		-0.7538	0.0142	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Nerium oleander* (Apocynaceae)	NA		-0.3640	0.0118	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Nicotiana rustica* (Solanaceae)	NA		0.0500	0.0033	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Phlox* spec. (Polemoniaceae)	NA		-0.1001	0.0228	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Salvia officinalis* (Lamiaceae)	NA		-0.2209	0.0106	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Silene alba* (Caryophyllaceae)	NA		-0.2921	0.0029	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Spartium junceum* (Fabaceae)	NA		-0.2865	0.0045	
	Hymenoptera	Formicidae	*Lasius fuliginosus*	a	f	NA	bouquet of *Trachelospermum jasminoides* (Apocynaceae)	NA		-0.3951	0.0092	
	Hymenoptera	Formicidae	*Monomorium floricola*	a	f	Monoterpenoid	linalool	Alcohol	1 mMol kg-1	-0.8490	0.0521	*)
Junker *et al.* (submitted)	Ensifera	Tettigoniidae	*Metrioptera bicolor*	a	f	Aliphates	1-hexanol	Alcohol	1 mMol kg-1	-0.3106	0.0989	Ingrisch and Köhler (1998) Westarp Wissenschaften- Verlagsgesellschaft mbH, Magdeburg
	Ensifera	Tettigoniidae	*Metrioptera bicolor*	a	f	Monoterpenoid	α-pinene	Hydrocarbon	1 mMol kg-1	-0.0468	0.2503	
	Ensifera	Tettigoniidae	*Metrioptera bicolor*	a	f	Monoterpenoid	1,8-cineole	Ether	1 mMol kg-1	0.2227	0.0720	
	Ensifera	Tettigoniidae	*Metrioptera bicolor*	a	f	Monoterpenoid	linalool	Alcohol	1 mMol kg-1	-1.5227	0.3120	
	Ensifera	Tettigoniidae	*Metrioptera bicolor*	a	f	Sesquiterpenoid	β-caryophyllene	Hydrocarbon	1 mMol kg-1	-0.8136	0.1069	
Kessler and Baldwin (2006) Plant J 49: 840-854	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Aliphates	1-hexanol	Alcohol	0.1 mM	-0.7105	0.0888	Kessler and Baldwin (2006) Plant J 49: 840-854, *) **)
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Aliphates	cis-3-hexenol	Alcohol	0.1 mM	-1.3491	0.1278	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Benzenoid	benzaldehyde	Aldehyde	0.1 mM	0.1844	0.0934	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Benzenoid	benzylacetone	Ketone	0.1 mM	0.9513	0.1467	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Benzenoid	methyl benzoate	Ester	0.1 mM	0.2103	0.0494	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Benzenoid	methyl salicylate	Ester	0.1 mM	0.5376	0.0528	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Benzenoid	cis-3-hexenyl benzoate	Ester	0.1 mM	0.5236	0.0539	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Diterpenoid	phytol	Alcohol	0.1 mM	-0.3222	0.0379	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Monoterpenoid	limonene	Hydrocarbon	0.1 mM	0.1840	0.0518	Kessler and Baldwin (2006) Plant J 49: 840-854
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Monoterpenoid	ocimene	Hydrocarbon	0.1 mM	-0.5664	0.0543	
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Monoterpenoid	linalool	Alcohol	0.1 mM	-0.1845	0.0809	

	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Sesquiterpenoid	cis-α-bergamotene	Hydrocarbon	0.1 mM	1.2759	0.1506		
	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	Sesquiterpenoid	trans-caryophyllene	Hydrocarbon	0.1 mM	-0.4707	0.0694		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Aliphates	1-hexanol	Alcohol	0.1 mM	-0.4814	0.0361	Kessler and Baldwin (2006) Plant J 49: 840-854, *)	
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Aliphates	cis-3-hexenol	Alcohol	0.1 mM	-0.2183	0.0285		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Benzenoid	benzaldehyde	Aldehyde	0.1 mM	0.5643	0.0546		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Benzenoid	benzylacetone	Keton	0.1 mM	0.7544	0.0249		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Benzenoid	methyl benzoate	Ester	0.1 mM	-0.7319	0.0442		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Benzenoid	methyl salicylate	Ester	0.1 mM	-0.4776	0.0574		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Benzenoid	cis-3-hexenyl benzoate	Ester	0.1 mM	0.0729	0.0228		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Diterpenoid	phytol	Alcohol	0.1 mM	-0.0705	0.0272		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Monoterpenoid	limonene	Hydrocarbon	0.1 mM	-0.5482	0.0611		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Monoterpenoid	ocimene	Hydrocarbon	0.1 mM	0.0952	0.0558		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Monoterpenoid	linalool	Alcohol	0.1 mM	-0.3757	0.0812		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Monoterpenoid	geraniol	Alcohol	0.1 mM	-0.2546	0.0634		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	NA	cis-3-hexenyl butyrate	Ester	0.1 mM	-0.5490	0.0180		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	N-containing_compound	nicotine	Pyridine	0.1 mM	-0.4230	0.0276		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Sesquiterpenoid	cis-α-bergamotene	Hydrocarbon	0.1 mM	0.5453	0.0265		
	Trochiliformes	Trochilidae	*Archilochus alexandri / Selasphorus rufus*	m	o	Sesquiterpenoid	trans-caryophyllene	Hydrocarbon	0.1 mM	0.4625	0.0409		
Kirk (1985) J Chem Ecol 11: 35-43	Thysanoptera	Thripidae	*Frankliniella intonsa*	u	o	Benzenoid	eugenol	Ether	NA	-0.7538	0.7012	Kirk (1985) J Chem Ecol 11: 35-43, *)	
	Thysanoptera	Thripidae	*Frankliniella intonsa*	u	o	Benzenoid	anisaldehyde	Aldehyde	NA	1.0380	0.4234		
	Thysanoptera	Thripidae	*Frankliniella intonsa*	u	o	Monoterpenoid	myrcene	Hydrocarbon	NA	-0.5306	0.7745		
	Thysanoptera	Thripidae	*Frankliniella intonsa*	u	o	Monoterpenoid	geraniol	Alcohol	NA	0.0572	0.5428		
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	eugenol	Ether	NA	0.8183	0.3624	Imai et al. (2001) Appl Entomol Zool 36: 475-478, *)	
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Benzenoid	anisaldehyde	Aldehyde	NA	1.0761	0.3257		
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Monoterpenoid	myrcene	Hydrocarbon	NA	-5.2338	1.1467		
	Thysanoptera	Thripidae	*Thrips flavus*	u	o	Monoterpenoid	geraniol	Alcohol	NA	1.1206	0.4813		
	Thysanoptera	Thripidae	*Thrips major*	u	o	Benzenoid	eugenol	Ether	NA	0.0000	0.5734	Kirk (1985) J Chem Ecol 11: 35-43, *)	
	Thysanoptera	Thripidae	*Thrips major*	u	o	Benzenoid	anisaldehyde	Aldehyde	NA	2.1972	0.4867		
	Thysanoptera	Thripidae	*Thrips major*	u	o	Monoterpenoid	myrcene	Hydrocarbon	NA	-1.2528	0.5053		
	Thysanoptera	Thripidae	*Thrips major*	u	o	Monoterpenoid	geraniol	Alcohol	NA	-0.8473	0.5794		
	Thysanoptera	Thripidae	*Thrips pillichi*	u	o	Benzenoid	eugenol	Ether	NA	-0.6061	0.5573	Kirk (1985) J Chem Ecol 11: 35-43, *)	
	Thysanoptera	Thripidae	*Thrips pillichi*	u	o	Benzenoid	anisaldehyde	Aldehyde	NA	1.4733	0.3073		
	Thysanoptera	Thripidae	*Thrips pillichi*	u	o	Monoterpenoid	myrcene	Hydrocarbon	NA	0.0870	0.2611		
	Thysanoptera	Thripidae	*Thrips pillichi*	u	o	Monoterpenoid	geraniol	Alcohol	NA	0.0870	0.3351		
Koschier *et al.* (2000) J Chem Ecol 26: 2643-2655	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	benzaldehyde	Aldehyde	0.01 - 0.1 µl	0.4055	0.0144	Rhainds (2005) Ecol Entomol 30: 96-104, *)	
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	o-anisaldehyde	Aldehyde	0.01 - 0.1 µl	0.4055	0.0048		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	m-anisaldehyde	Aldehyde	0.01 - 0.1 µl	0.4599	0.0017		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	p-anisaldehyde	Aldehyde	0.01 - 0.1 µl	0.5194	0.0017		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	salicylaldehyde	Aldehyde	0.01 - 0.1 µl	-0.0800	0.0300		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	eugenol	Ether	0.01 - 0.1 µl	0.1886	0.0452		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	(E) - cinnamic aldehyde	Aldehyde	0.01 - 0.1 µl	0.4055	0.0048		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Benzenoid	3-phenylpropionaldehyde	Aldehyde	0.01 - 0.1 µl	-0.0280	0.0270		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	geraniol	Alcohol	0.01 - 0.1 µl	-0.0800	0.0043		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	nerol	Alcohol	0.01 - 0.1 µl	-0.0280	0.0526		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	linalool	Alcohol	0.01 - 0.1 µl	0.2412	0.0134		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	1,8-cineole	Ether	0.01 - 0.1 µl	-0.3516	0.0765		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	trans-β-ocimene	Hydrocarbon	0.01 - 0.1 µl	-0.3640	0.0392		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	citronellal	Aldehyde	0.01 - 0.1 µl	0.1886	0.0190		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	citronellol	Alcohol	0.01 - 0.1 µl	0.0280	0.0227		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	myrcene	Hydrocarbon	0.01 - 0.1 µl	0.2412	0.0178		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	limonene	Hydrocarbon	0.01 - 0.1 µl	0.0280	0.0100		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Monoterpenoid	sabinene	Hydrocarbon	0.01 - 0.1 µl	0.4055	0.0048		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	N-containing_compound	ethyl nicotinate	Pyridine	0.01 - 0.1 µl	0.2412	0.0580		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Sesquiterpenoid	(-)-(E)-Caryophyllene	Hydrocarbon	0.01 - 0.1 µl	0.2412	0.0045		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Sesquiterpenoid	β-Farnesene	Hydrocarbon	0.01 - 0.1 µl	0.4599	0.0000		
	Thysanoptera	Thripidae	*Frankliniella occidentalis*	u	o	Sesquiterpenoid	α-bisabolol	Alcohol	0.01 - 0.1 µl	-0.4055	0.0144		
Landolt et al. (2004) Florida Etomol 87: 294-299	Lepidoptera	Noctuidae	*Trichoplusia ni*	m	o	Monoterpenoid	linalool	Alcohol	2 ml	9.1590	1.0869	Haynes et al. (1991) J Chem Ecol 17: 637-646, **)	
Lee *et al.* (2003) J Strored Prod Res 39: 77-85	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	carvacrol	Alcohol	50 µg ml-1 of air	-9.2103	0.0100	*)	
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	carveol	Alcohol	50 µg ml-1 of air	-2.1972	0.4544		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	citronellol	Alcohol	50 µg ml-1 of air	0.8473	0.1315		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	linalool	Alcohol	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	menthol	Alcohol	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	terpineol	Alcohol	50 µg ml-1 of air	1.3863	0.3574		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	verbenol	Alcohol	50 µg ml-1 of air	-2.1972	0.3406		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	carvone	Ketone	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	fenchone	Ketone	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	menthone	Ketone	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	pulegone	Ketone	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	thujone	Ketone	50 µg ml-1 of air	-9.2103	0.0100		
	Diptera	Muscidae	*Musca domestica*	m	f	Monoterpenoid	verbenone	Ketone	50 µg ml-1 of air	-9.2103	0.0100		

	Lepidoptera	Noctuidae	*Mocis latipes*	m	o	Aliphates	3-methyl-1-butanol	Alcohol	NA	-0.6931	1.5625	**)
	Lepidoptera	Noctuidae	*Mocis* ssp.	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	NA	7.2654	1.1941	**)
	Lepidoptera	Noctuidae	*Mocis disseverans*	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	NA	3.4012	1.4444	**)
	Lepidoptera	Noctuidae	*Mocis latipes*	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	NA	3.4012	1.4444	**)
Omura and Honda (2005) Oecologia 142: 588-596	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	Aliphates	hexenol	Alcohol	5 mg	-0.1250	0.1152	Omura and Honda (2005) Oecologia 142: 588-596, *)
	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	Benzenoid	benzaldehyde	Aldehyde	5 mg	1.3740	0.0555	
	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	Benzenoid	methyl salicylate	Ester	5 mg	1.6648	0.0651	
	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	Monoterpenoid	linalool	Alcohol	5 mg	1.5719	0.2770	
	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	Sesquiterpenoid	nerolidol	Alcohol	5 mg	1.6134	0.0493	
Raguso and Willis (2002) Animal Behaviour 64: 685-695	Lepidoptera	Sphingidae	*Manduca sexta*	m	o	NA	bouquet of *Oenothera neomexicana* (Onagraceae)	NA	NA	7.9374	1.0459	Kessler and Baldwin (2006) Plant J 49: 840-854, *) **)
Roy and Raguso (1997) Oecologia 109: 414-426	Hymenoptera	Halictidae	Halictids	m	o	Benzenoid	benzaldehyde	Aldehyde	NA	-0.5700	1.0293	Roy and Raguso (1997) Oecologia 109: 414-426, *)
	Hymenoptera	Halictidae	Halictids	m	o	Benzenoid	methyl benzoate	Ester	NA	1.0072	1.0679	
	Hymenoptera	Halictidae	Halictids	m	o	Benzenoid	2-phenylethanol	Alcohol	NA	-0.2594	1.1925	
	Hymenoptera	Halictidae	Halictids	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	NA	0.8163	0.8588	
	Hymenoptera	Halictidae	Halictids	m	o	N-containing_compound	indole	Indole	NA	0.0440	1.3995	
Shorey *et al.* (1996) Environ Entomol 25: 114-119	Hymenoptera	*Formicidae*	*Linepithema humile*	a	f	Benzenoid	methyl eugenol	Ether	NA	-0.1860	0.6000	*)
	Hymenoptera	*Formicidae*	*Linepithema humile*	a	f	Sesquiterpenoid	farnesol	Alcohol	NA	-2.4560	0.5136	
	Hymenoptera	*Formicidae*	*Linepithema humile*	a	f	Sesquiterpenoid	nerolidol	Alcohol	NA	-1.2677	0.1999	
Theis (2006) J Chem Ecol 32: 917-1000	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	-1.3863	0.0391	Theis (2006) J Chem Ecol 32: 917-927
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	0.0000	2.0000	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	4.6052	1.0625	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	0.0000	2.0000	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	-0.9808	0.0437	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	-0.9808	0.0252	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	-1.3863	0.0807	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	0.0000	2.0000	
	Colecptera	Cerambycidae	*Tetraopes tetrophthalamus*	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	0.4855	0.0275	
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	0.4055	0.0278	Theis (2006) J Chem Ecol 32: 917-927
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	1.9459	0.0281	
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	1.5041	0.0298	
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	1.8718	0.0370	
	Colecptera	Mordellidae	Mordellidae	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	1.0986	0.0278	
	Colecptera	Mordellidae	Mordellidae	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	0.0000	0.0417	
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	0.4055	0.0278	
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	0.0000	0.0833	
	Colecptera	Mordellidae	Mordellidae	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	-0.6931	0.0833	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	0.5108	0.0161	Theis (2006) J Chem Ecol 32: 917-927
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	0.2877	0.0359	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	-1.0986	0.0880	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	-1.0986	0.0880	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	-1.0986	0.0694	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	0.0000	0.0324	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	0.0000	0.0324	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	0.2877	0.0463	
	Colecptera	Scarabaeidae	*Popillia japonica*	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	-0.4055	0.0278	
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	5.5511	1.0363	Theis (2006) J Chem Ecol 32: 917-927
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	0.0000	0.0556	
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	0.0000	0.0556	
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	0.0000	0.1389	
	Diptera	Syrphidae	Syrphid	m	o	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	4.6052	1.1111	
	Diptera	Syrphidae	Syrphid	m	o	Monoterpenoid	linalool	Alcohol	430 µg h-1	5.7038	1.0370	
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	methyl salicylate	Ester	769 µg h-1	4.6052	1.1111	
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	1.9459	0.0658	
	Diptera	Syrphidae	Syrphid	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	4.6052	1.1111	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	-1.6094	0.0717	Theis (2006) J Chem Ecol 32: 917-927
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	0.0000	0.2293	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	-4.6052	1.0001	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	0.0000	0.2501	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	-6.2146	2500.0092	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	-0.9163	0.0665	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	-0.5108	0.0346	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	0.6931	0.0157	
	Heteroptera	Lygaeidae	*Lygus kalmii*	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	-6.2146	1666.6758	
	Hymenoptera	Apidae	*Apis mellifera*	m	o	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	0.5108	0.1213	Theis (2006) J Chem Ecol 32: 917-927
	Hymenoptera	Apidae	*Apis mellifera*	m	o	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	1.0296	0.0913	
	Hymenoptera	Apidae	*Apis mellifera*	m	o	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	0.3365	0.0919	
	Hymenoptera	Apidae	*Apis mellifera*	m	o	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	-0.2231	0.1200	
	Hymenoptera	Apidae	*Apis mellifera*	m	o	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	-0.1823	0.1100	
	Hymenoptera	Apidae	*Apis mellifera*	m	o	Monoterpenoid	linalool	Alcohol	430 µg h-1	1.2040	0.1212	

Hymenoptera	Apidae	Augochlorella	m	o	Benzenoid	methyl salicylate	Ester	769 µg h-1	0.0000	2.0000	
Hymenoptera	Apidae	Augochlorella	m	o	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	1.7047	0.1002	
Hymenoptera	Apidae	Augochlorella	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	6.8024	1.0534	
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	0.1054	0.0275	Theis (2006) J Chem Ecol 32: 917-927
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	0.4249	0.0468	
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	0.0572	0.0307	
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	-1.4469	0.0383	
Hymenoptera	Apidae	Lasioglossum	m	o	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	0.2007	0.0332	
Hymenoptera	Apidae	Lasioglossum	m	o	Monoterpenoid	linalool	Alcohol	430 µg h-1	-0.1178	0.0284	
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	methyl salicylate	Ester	769 µg h-1	-0.7309	0.0256	
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	2.1155	0.0512	
Hymenoptera	Apidae	Lasioglossum	m	o	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	0.9946	0.0286	
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	0.0000	0.0417	Theis (2006) J Chem Ecol 32: 917-927
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	2.3979	0.0692	
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	2.5649	0.0646	
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	1.3863	0.0625	
Hymenoptera	Formicidae	Formicidae	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	0.9163	0.0567	
Hymenoptera	Formicidae	Formicidae	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	0.4055	0.0463	
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	1.0986	0.0417	
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	0.0000	0.1250	
Hymenoptera	Formicidae	Formicidae	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	1.8718	0.0261	
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	0.0000	0.1250	Theis (2006) J Chem Ecol 32: 917-927
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	0.2231	0.0198	
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	0.1178	0.0287	
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	-1.3863	0.0365	
Orthoptera	Acrididae	Acrididae	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	0.0000	0.1250	
Orthoptera	Acrididae	Acrididae	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	0.0000	0.1250	
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	-4.6052	1.0625	
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	-0.2877	0.0226	
Orthoptera	Acrididae	Acrididae	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	0.6931	0.0633	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	2-phenylethanol	Alcohol	363 µg h-1	-5.9915	1.0104	Theis (2006) J Chem Ecol 32: 917-927
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	benzaldehyde	Aldehyde	703 µg h-1	1.3863	0.0127	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	benzyl alcohol	Alcohol	415 µg h-1	0.1823	0.0294	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	dimethyl salicylate	Ester	631 µg h-1	0.4700	0.0518	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Monoterpenoid	furanoid linalool oxide	Ether	533 µg h-1	-0.6931	0.0312	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Monoterpenoid	linalool	Alcohol	430 µg h-1	-5.9915	1.0104	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	methyl salicylate	Ester	769 µg h-1	0.0000	0.0417	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	p-anisaldehyde	Aldehyde	343 µg h-1	0.5878	0.0123	
Orthoptera	Tettigoniidae	Tettigoniidae	a	f	Benzenoid	phenylacetaldehyde	Aldehyde	453 µg h-1	0.0000	0.0208	

APPENDIX R

For an extended dataset comprising data from the analysis in the main text and data from additional fifteen studies we performed an ANOVA. From the fifteen additional studies no standard-deviation could be extracted (see **APPENDIX S**).

The results of both analyses (Fig. 1 in main paper and Fig. S1 below) reveal similar results (Tab. S1).

Tab. 1. Effect of the animal's dependency on floral scents. Results of the ANOVA for effects of floral scents on animals with different dependencies in floral resources (extended data set).

a) Meta-analysis - Parameter	*df*	*F*	*p*
Dependency	1	114.1	**< 0.001**
Residuals	502		

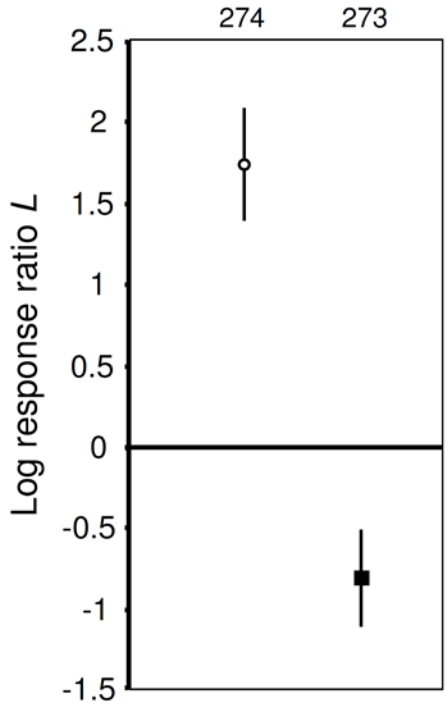

Fig. 1. Effects of floral scents on obligate (open circles) and facultative flower visitors (black squares). Shown are mean and 95% confidence intervals of the log response ratio L which describes the proportional difference between the mean effect of the scent treatment and the control. Sample sizes are given at the top of the figure.

APPENDIX S

Interaction: m = mutualist; a = antagonist; u = unknown; mixed = depends on plant species. Dependency: o = obligate flower visitor; f = facultative flower visitor.
*) **) Animal species or at least families were assigned to facultative or obligate flower visitors by John Hilty (*) or Konrad Fiedler (**)

source	Order	Family	animal species	interaction	Dependency	Substance / bouquet	L	confirmation of interaction and status
Bartlet *et al.* (2004) J Chem Ecol 30: 913-925	Coleoptera	Nitidulidae	*Meligethes aeneus*	a	o	bouquet of *Brassica napus* (Brassicaceae)	1.8386	Bartlet et al. (2004) J Chem Ecol 30: 913-925
	Coleoptera	Nitidulidae	*Meligethes aeneus*	a	o	bouquet of *Iberis amara* (Brassicaceae)	1.2897	
	Coleoptera	Nitidulidae	*Meligethes aeneus*	a	o	2-Phenylethyl isothiocyanate	2.2031	
Carroll and Berenbaum (2002) J Chem Ecol 25: 2191-2201	Lepidoptera	Oecophoridae	*Depressaria pastinacella*	a	o	octyl acetate	7.6309	Carroll and Berenbaum (2002) J Chem Ecol 28: 2191-2201. **)
	Lepidoptera	Oecophoridae	*Depressaria pastinacella*	a	o	bouquet of *Pastinaca sativa* (Apiaceae)	7.5670	
	Lepidoptera	Oecophoridae	*Depressaria pastinacella*	a	o	octyl butyrate	2.8904	
Cook *et al.* (2002) Entomol Exp Appl 104: 43-50	Coleoptera	Nitidulidae	*Meligethes aeneus*	a	o	bouquet of *Brassica nacus* (Brassicaceae)	1.0986	Bartlet et al. (2004) J Chem Ecol 30: 913-925
Dötterl and Schäffler (2007) J Chem Ecol 33: 441-446	Hymenoptera	Apidae	*Macropis fulvipes*	m	o	bouquet of *Lysimachia punctata* (Myrsinoceae)	6.9078	Dötterl and Schäffler (2007) J Chem Ecol 33: 441-445, *)
Dötterl *et al.* (2005) J Chem Ecol 31: 2993-2996	Hymenoptera	Andrenidae	*Andrena nasa*	m	o	1,4-dimethoxybenzene	5.5390	Müller, A. et al. (1987) Beiwe. Naturbeob.-Verlag, Augsburg, *)
Gäbel *et al.* (1992) J Chem Ecol 18: 693-701	Lepidoptera	Tortricidae	*Lobesia botrana*	m	o	bouquet of *Tanacetum vulgare* (Asteraceae)	1.5081	**)
Grison-Pigé (2002) J Chem Ecol 28: 283-295	Hymenoptera	Chalcidoidea	*Blastophaga compacta*	m	o	bouquet of *Ficus tiliia* (Moraceae)	1.3063	Grison-Pigé (2002) J Chem Ecol 28: 283-295, *)
	Hymenoptera	Agaonidae	*Ceratosolen constrictus*	m	o	bouquet of *Ficus condorisa* (Moraceae)	1.9217	Grison-Pigé (2002) J Chem Ecol 28: 283-295, *)
	Hymenoptera	Agaonidae	*Eupristina verticillata*	m	o	bouquet of *Ficus microcarpa* (Moaceae)	1.4110	Grison-Pigé (2002) J Chem Ecol 28: 283-295, *)
	Hymenoptera	Agaonidae	*Wiedeosia* sp.	m	o	bouquet of *Ficus dotoidea* (Moraceae)	0.0000	Grison-Pigé (2002) J Chem Ecol 28: 283-295, *)
Harborne (1997) Annals of Botany 68: 39-57	Hymenoptera	Formicidae	*Atta cephalotes*	a	f	β-ocimene	-0.3944	*)
Hick *et al.* (2005) US Patent No.: US 6,890,525 B2	Coleoptera		*pollen beetle*	a	o	cis-jasmone	-0.8115	*)
Honda *et al.* (1998) J Chem Ecol 24: 2167-2180	Lepidoptera	Pieridae	*Pieris rapae*	m	o	6-methylhept-5-en-2-one	1.8458	Omura et al. (1999) J Chem Ecol 25: 1895-1906, *)
	Lepidoptera	Pieridae	*Pieris rapae*	m	o	hexenol	0.4055	
Hwang et al. (1985) J Chem Ecol 11: 1297-1306	Diptera	Culicidae	*Aedes aegypti*	a	f	3-nonanone	-0.1609	Müller, G. and Schlein, Y. (2006) Med. Vet. Entom 19: 413-422, *)
	Diptera	Culicidae	*Aedes aegypti*	a	f	(-)-borneol	-1.2179	
	Diptera	Culicidae	*Aedes aegypti*	a	f	(-)-camphor	-0.7024	
	Diptera	Culicidae	*Aedes aegypti*	a	f	(+)-camphor	-0.4655	
	Diptera	Culicidae	*Aedes aegypti*	a	f	(±)-camphor	-0.4863	
	Diptera	Culicidae	*Aedes aegypti*	a	f	bornyl acetate	-0.1870	
	Diptera	Culicidae	*Aedes aegypti*	a	f	cineole	0.0896	
	Diptera	Culicidae	*Aedes aegypti*	a	f	isoborneol	-1.5543	
	Diptera	Culicidae	*Aedes aegypti*	a	f	isobornyl acetate	-0.6040	
	Diptera	Culicidae	*Aedes aegypti*	a	f	limonene	-0.2127	
	Diptera	Culicidae	*Aedes aegypti*	a	f	linalool	-1.1778	
	Diptera	Culicidae	*Aedes aegypti*	a	f	myrcene	-0.2830	
	Diptera	Culicidae	*Aedes aegypti*	a	f	terpinen-4-ol	-1.8516	
	Diptera	Culicidae	*Aedes aegypti*	a	f	thujone	-0.3064	
	Diptera	Culicidae	*Aedes aegypti*	a	f	α-terpinene	-0.1753	
	Diptera	Culicidae	*Aedes aegypti*	a	f	β-pinene	-0.0993	
Kessler and Baldwin (2006) Plant J 49: 840-854	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	1-hexanol	0.5596	Kessler and Baldwin (2006) Plant J 49: 840-854, *)
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	cis-3-hexenol	0.1310	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	benzaldehyde	0.1398	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	benzylacetone	0.4055	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	cis-3-hexenyl benzoate	0.0563	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	methyl benzoate	-0.5276	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	methyl salicylate	-1.1394	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	phytol	-0.3147	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	geraniol	-0.0513	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	limonene	0.2211	Kessler and Baldwin (2006) Plant J 49: 840-854
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	linalool	-0.1355	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	ocimene	0.2251	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	cis-3-hexenyl butyrate	-0.3857	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	nicotine	-0.2231	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	cis-α-bergamotene	-0.1165	
	Hymenoptera	Formicidae	*Solenopsis xyloni*	a	f	trans-caryophyllene	0.0488	
Omura and Honda (2003) Oecologia 142: 588-596	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	bouquet of *Cirsium lanceolatum* (Asteraceae)	1.7918	Omura and Honda (2003) Oecologia 142: 588-596, *)
	Lepidoptera	Nymphalidae	*Vanessa indica*	m	o	bouquet of *Taraxacum officinale* (Asteraceae)	3.4500	
Omura *et al.* (1999) J Chem Ecol 25: 1895-1906	Lepidoptera	Pieridae	*Pieris rapae*	m	o	benzaldehyde	1.7405	Omura et al. (1999) J Chem Ecol 25: 1895-1906, *)
	Lepidoptera	Pieridae	*Pieris rapae*	m	o	Benzyl Alcohol	0.6931	
	Lepidoptera	Pieridae	*Pieris rapae*	m	o	Indole	0.3365	
	Lepidoptera	Pieridae	*Pieris rapae*	m	o	2-phenylethanol	1.5081	
	Lepidoptera	Pieridae	*Pieris rapae*	m	o	phenylacetaldehyde	1.4663	
	Lepidoptera	Pieridae	*Pieris rapae*	m	o	Phenylacetonitrile	1.2763	
Pair *et al.* (1997) US Patent No.: 5,665,344	Lepidoptera	Noctuidae	*Helicoverpa zea*	m	o	bouquet of *Lonicera japonica* (Caprifoliaceae)	1.6284	Culin (1995) Ento Experi et Appl 74: 165-176, *)
	Lepidoptera	Sphingidae	*Manduca sso.*	m	o	bouquet of *Lonicera japonica* (Caprifoliaceae)	2.4849	*) **)
	Lepidoptera	Noctuidae	*Pseudoplusia includens*	m	o	bouquet of *Lonicera japonica* (Caprifoliaceae)	3.5041	Groman and Pellmyr (1999) Oikos 87: 373-380, **)
	Lepidoptera	Noctuidae	*Trichoplusia ni*	m	o	bouquet of *Lonicera japonica* (Caprifoliaceae)	3.1094	Hawes et al. (1991) J Chem Ecol 17: 637-645, **)
Russell *et al.* (1994) Phytochemistry 35: 1455-1456	Hymenoptera	Formicidae	*Crematogaster opuntiae*	a	f	(2S,3R)-2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	-0.6931	*)
Shorey *et al.* (1997) US Patent No.: 5,662,914	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	benzaldehyde	0.1402	Stone and Anderson (1988) Proc. Verteb. Pest Conf. *)
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	eugenol	-1.7819	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	methyl anthranilate	-0.0120	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	methyl salicylate	-0.7650	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	3-carene	0.4601	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	bornyl acetate	-2.4179	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	citral	-0.7865	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	limonene	-0.5721	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	limonene oxide	0.0000	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	linalool	-0.5042	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	myrcene	0.1298	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	α-pinene	0.2132	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	α-terpineol	0.1298	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	β-citronellol	-2.6692	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	β-pinene	0.2302	
	Hymenoptera	Formicidae	*Iridomyrmex bumitis*	a	f	methyl eugenol	-2.8274	