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Predatory and trophobiont-tending ants respond differently to fig and fig wasp volatiles

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The interaction between figs and their pollinating or parasitic fig wasps is mediated largely by chemical communication. These fig wasps are often preyed upon by predatory ants. In this study, we found that predatory ants (*Oecophylla smaragdina*) patrolling *Ficus racemosa* trees were attracted to the odour from fig syconia at different developmental phases, as well as to the odours of fig wasps, whereas other predatory ants (*Technomyrmex albipes*) responded only to odours of syconia from which fig wasps were dispersing and to fig wasp odour. However, trophobiont-tending ants (*Myrmicaria brunnea*) patrolling the same trees and exposed to the same volatiles were unresponsive to fig or fig wasp odours. The predatory ants demonstrated a concentration-dependent response towards volatiles from figs receptive to pollinators and those from which wasps were dispersing while the trophobiont-tending ants were unresponsive to such odours at all concentrations. Naïve predatory ants failed to respond to the volatiles to which the experienced predatory ants responded, indicating that the response to fig-related odours is learned. We suggest that predatory ants could use fig-associated volatiles to enhance their probability of wasp encounter and can eavesdrop on signals meant for pollinators.

Figs (*Ficus* spp., Moraceae) have an obligate mutualism with pollinating fig wasps (Hymenoptera, Agaonidae). This 90-million-year-old nursery pollination mutualism (Machado et al. 2001), in which pollinators breed within fig syconia, is largely mediated by chemical communication via volatiles produced by the fig syconia (Hossaert-McKey et al. 1994; Gibernau et al. 1998; Grison-Pigé et al. 2002). The mutualistic interaction between figs and their pollinating wasps is susceptible to exploitation by parasites. The parasitic (nonpollinating) fig wasps use chemical cues emanating from fig syconia (Proffit et al. 2007) and oviposit from the exterior into the fig syconium through the syconial wall, thereby deriving benefits from having their offspring develop at the expense of a potential fig seed or pollinator. Fig trees are also occupied by many nonagaonid insects including sap-sucking trophobionts and ants (Compton & Robertson 1988). Predatory ants patrolling fig trees could function as indirect mutualists of the fig–wasp interaction by capturing parasitic wasps (Schatz et al. 2006), while the sap-sucking insects are often brought into fig trees by trophobiont-tending ants which cultivate them for the honeydew that they produce (Compton & Robertson 1991).

Perception of odours emanating from host plants, host animals and prey is of fundamental importance to many insects (Mustaparta 1984). Use of volatile infochemicals has been proven to be important in locating food and shelter by many insect species (Vet & Dicke 1992; Vos et al. 2006). Ants involved in an obligate mutualistic interaction with plants have been shown to respond to the volatiles emitted from their host plants (Fiala & Maschwitz 1990; Brouat et al. 2000; Edwards et al. 2006, 2007; Jurgens et al. 2006). In the case of seed dispersal mutualisms involving ants (myrmecochory), the mutualistic ant partner responds to seed odours (Sheridana et al. 1996; Youngsteadt et al. 2008) or to compounds present in seed-associated structures (elaiosomes; Gammans et al. 2006; Fischer et al. 2008). Even in facultative interactions between ants and plants, ants respond to herbivore damage-induced volatiles (Agrawal 1998; Agrawal & Rutter 1998; Agrawal & Dubin-Thaler 1999; Bruna & Vasconcelos 2003), indicating that the ability to respond to particular compounds could equip certain ants to form symbiotic interactions with plants.

Since fig syconia produce different volatile signatures at different stages in their developmental cycle (Borges et al. 2008; Proffit et al. 2008), it is possible that predators of fig wasps such as ants may use these phase-specific odours to locate syconia that are attracting fig wasps or from which wasps are dispersing (Bronstein 1988). For example, predatory ants have been shown to be present

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in greater numbers on male trees in dioecious fig species (Schatz et al. 2008), since in dioecious species it is only the male trees in which wasps (pollinators and parasites) breed and from which they exit. Furthermore, *Crematogaster scutellaris* ants were better at capturing pollinating wasps rather than parasitic wasps in the dioecious *Ficus carica* (Schatz & Hossaert-McKey 2003). Behavioural choice experiments on *C. scutellaris* using fig and fig wasp odours demonstrated that these predatory ants patrolling fig trees were significantly attracted only to odours emitted by pollinating fig wasps either alone or in association with odours of male figs releasing wasps (Schatz et al. 2003). Therefore, whether predatory ants can be attracted by odours from figs alone is not known. Furthermore, ants may vary in their exposure to fig volatiles, depending on whether or not they forage or nest in fig trees. Naïve *C. scutellaris*, that is, those that did not patrol fig trees, were not attracted to fig wasp odours (Schatz et al. 2003). Also, fig trees may be occupied by trophobiont-tending nonpredatory ant species that may be exposed to fig and/or wasp odours, but may be unresponsive to them since fig wasps are not a food source for these ants. Learning of relevant cues by ants may be crucial in governing the potential for net positive or negative interactions between ants and fig trees.

In this study, we addressed the following questions. (1) Do predatory and trophobiont-tending ants on fig trees respond to fig and fig wasp volatiles? (2) Does this response vary with the fig syconium cycle? (3) Is there a difference between experienced and naïve predatory ants in their response to fig and/or fig wasp odours?

METHODS

Study System and Study Site

Ficus racemosa Linn., a monoecious fig species, is pollinated by the wasp *Ceratosolen fusciceps* Mayr. *Ficus racemosa* produces cauliflorous syconia (i.e. borne on the trunk) throughout the year, and one fruiting crop cycle takes approximately 60 days to complete. Each cycle can be partitioned into distinct phases depending on the developmental phase of the syconia, namely (1) prereceptive: production of new flush of syconia; (2) receptive: female flowers inside the syconia are ready to receive pollen brought by the pollinating fig wasps that enter the syconia through the open ostiole, with simultaneous oviposition in some flowers; (3) interfloral: fig seeds and fig wasp larvae develop; (4) wasp dispersal: fully developed female pollinators collect pollen and exit the syconia through the exit hole made by the male pollinators; and (5) fruit dispersal: syconia become attractive to frugivores which aid in seed dispersal (Galil & Eisikowitch 1968). Externally ovipositing (nonpollinating) fig wasps arrive at different times from the prereceptive to the mid-interfloral phase of the syconium developmental cycle, while the internally ovipositing pollinating wasps are present in large numbers only at the receptive and wasp dispersal stages. The trunk, main branches and syconia of *F. racemosa* are patrolled by many species of ants, but each *F. racemosa* tree is occupied by a single dominant ant species (Y. Ranganathan, M. Ghara & R. M. Borges, unpublished data). The location of the cauliflorous syconia facilitates interactions with ants. Syconia, fig wasps and associated ant species from *F. racemosa* trees were collected from within the campus of the Indian Institute of Science, Bangalore, India (12°58′N, 77°35′E), where the behavioural experiments were also conducted.

Odour Sources

As odour sources, we used, from several trees, fig wasps and *Ficus racemosa* syconia in distinctly different developmental phases: prereceptive (at least 5 days before pollen receptivity), receptive, interfloral, wasp dispersal (after the fig wasps have exited). Thirty freshly harvested syconia of each phase were used for the initial choice experiments ($N = 48$ trials for each odour and for each ant species). If the odour of 30 figs did not elicit a significant response, we increased the number of figs for that odour source to 50, 70 and 90 ($N = 16$ trials for each odour and for each ant species) to determine a concentration-dependent response, if any. To determine the lower limit of the sensitivity of the ants towards odours of figs in the wasp dispersal phase, we reduced the odour concentration by decreasing the number of figs in this phase from 30 to 10 and four ($N = 16$ for each group). For choice experiments with fig wasps as the odour source, we used live wasps that emerged from 10 wasp dispersal phase syconia. The numbers of these wasps varied but on average 70–100 wasps emerged from each wasp dispersal phase syconium. The dispersal phase syconia, from which wasps were collected for use in the wasp odour experiments, were not used as dispersal phase odour sources. For the dispersal phase trials, separate syconia were collected.

Ants of the Fig Community

Ficus racemosa trees at this site were patrolled by three dominant species of ants: *Oecophylla smaragdina* (Fabricius) (Formicinae), *Technomyrmex albipes* (Smith) (Dolichoderinae) and *Myrmicaria brunnea* Saunders (Myrmicinae). Several other species of ants were also observed to patrol the trunks, but these were limited in their presence either temporally (seen only for a few days) or spatially (localized to a small region of a trunk). These ‘minor’ ant species were: *Solenopsis* sp., *Tapinoma melanocephalum*, *Camponotus pariah*, *Paratrechina* sp. and *Crematogaster subnuda*. *Oecophylla smaragdina* is an aggressive ant with huge arboreal colonies often spanning several branches, and with multiple nests within fig trees. It is largely predatory, but also scavenges. *Technomyrmex albipes*, although much smaller than *O. smaragdina*, is also a predatory ant species. Although this ant has smaller arboreal colonies than *O. smaragdina*, quick recruitment facilitates efficient monopoly of ephemeral food resources. *Myrmicaria brunnea* is predominantly a honeydew-feeder and scavenger, and builds underground nests.

Behavioural Experiments

We used a Y-tube glass olfactometer (arms 13 cm long and stem regions of 4.5 cm in diameter) to conduct volatile choice experiments. Odourless polyethylene terephthalate bags (Nalophan) contained the odour source (fig syconia or fig wasps). Air was allowed to flow (20 ml/min flow rate) through a bag containing the odour source to one arm of the olfactometer (odour arm), while the other arm (no-odour arm) received air at the same flow rate from an empty bag. Air was drawn from the basal arm with the same flow rate to avoid backdraught of volatiles. The odour sources were kept 0.8 m below the plane of the olfactometer, which ensured that no visual cues were available to the test ants. Furthermore, the ends of the Y-tube were closed with fine cloth mesh that ensured laminar air flow but prevented wasps from entering the olfactometer. Each trial consisted of an ant forager being singly introduced into the basal arm of the olfactometer, and lasted 5 min. To control for directional bias, the odour arm was reversed between left and right sides of the Y-tube, in successive trials. Additionally, control trials with blank air were conducted, to examine directional bias if any ($N = 16$ trials for each odour and for each ant species). The olfactometer was rinsed with water, followed by 70% ethanol, and air dried between trials. All the choice experiments were done from 0900 to 1300 hours which corresponded with the peak activity of