Original article

A coat of many scents: Cuticular hydrocarbons in multitrophic interactions of fig wasps with ants

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ABSTRACT

The fig–fig wasp system of *Ficus racemosa* constitutes an assemblage of galler and parasitoid wasps in which tritrophic interactions occur. Since predatory ants (*Oecophylla smaragdina* and *Technomyrmex albipes*) or mostly trophobiont-tending ants (*Myrmicaria brunnea*) were previously shown to differentially use volatile organic compounds (VOCs) from figs as proximal cues for predation on fig wasps, we examined the response of these ants to the cuticular hydrocarbons (CHCs) of the wasps. CHC signatures of gallers were distinguished from those of parasitoids by the methyl-branched alkanes 5-methylpentacosane and 13-methylnonacosane which characterised trophic group membership. CHC profiles of wasp predator and wasp prey were congruent suggesting that parasitoids acquire CHCs from their prey; the CHC composition of the parasitoid *Apocrypta* sp 2 clustered with that of its galler host *Apocryptophagus fusca*, while the CHC profile of the parasitoid *Apocryptophagus agraensis* clustered with its galler prey, the fig pollinator *Ceratosolen fusciceps*. In behavioural assays with ants, parasitoid CHC extracts evoked greater response in all ant species compared to galler extracts, suggesting that parasitoid CHC extracts contain more elicitors of ant behaviour than those of plant feeders. CHCs of some wasp species did not elicit significant responses even in predatory ants, suggesting chemical camouflage. Contrary to earlier studies which demonstrated that predatory ants learned to associate wasp prey with specific fig VOCs, prior exposure to fig wasp CHCs did not affect the reaction of any ant species to these CHCs.

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1. Introduction

Ants have varied foraging strategies and food sources (Carroll and Janzen, 1973; Traniello, 1989) depending on which they employ a variety of food recognition modalities that are primarily chemical but may also be assisted by visual cues (Eriksson, 1985). Ants associated with plants as herbivores, seed predators or seed dispersers use plant secondary compounds (Cherrett and Seaforth, 1970; Saverschek et al., 2010), plant nutrients (Marshall et al., 1979; Skidmore and Heithaus, 1988) or other chemical features of their mutualistic partners (Ghazoul, 2001; Pierce et al., 2002; Stadler and Dixon, 2005; Choe and Rust, 2006; Youngsteadt et al., 2008; Willmer et al., 2009; Hojo et al., 2014) as recognition, feedant or anti-feedant cues. Predatory ants that feed on plant-associated insect prey may use volatile, plant-derived compounds to obtain

information about the location and type of insect prey available on plant resources (Ranganathan and Borges, 2009; Schatz and Hossaert-McKey, 2010). However, such predatory ants feeding on insects associated with plants may also use less volatile chemicals such as insect cuticular hydrocarbons (CHCs) as feedant cues since CHCs often play an important role in insect predator–prey relationships (Espelie et al., 1991).

While insect CHCs are involved in several important discriminatory functions in ants such as mate recognition, nestmate recognition, colony regulation, chemical mimicry and camouflage (Howard and Blomquist, 2005; Blomquist and Bagnères, 2010; Tsutsui, 2013; Guillem et al., 2014; Menzel et al., 2014), their role in predator–prey interactions by providing feedant or anti-feedant cues to ants regarding prey has received less attention. Since qualitative or quantitative differences in CHC profiles can elicit aggressive, appeasement, or indifferent behaviour by ants towards conspecific or heterospecific ants (Endo and Itino, 2012, 2013; Menzel et al., 2013; Lenoir et al., 2013), it is possible that CHCs of insect prey may evoke differential responses in ants for varied prey

types and across predatory or non-predatory ants. Indeed, non-predatory, trophobiont-tending ant species were indifferent to plant volatile cues that were used by predatory arboreal ants to locate plant-associated insect prey (Ranganathan and Borges, 2009). Furthermore, the behavioural response of the predatory arboreal ants to plant volatiles was a learned association between the presence of the volatiles and the presence of insect prey (Ranganathan and Borges, 2009); consequently, the response to prey-associated plant volatiles was not innate in these ants.

CHCs of both plant and insect cuticles have been implicated in mediating multitrophic interactions between plants, insect herbivores and their predators and parasitoids (Espelie and Hermann, 1988; Espelie and Brown, 1990; Espelie et al., 1991). Insects may acquire CHCs from their diets (Liang and Silverman, 2000; Richard et al., 2004), via contact (von Beeren et al., 2011), or synthesise them *de novo* (Fan et al., 2003). Since ant feeding behaviour on plant products such as seeds or extrafloral nectar is elicited by resource chemistry (Skidmore and Heithaus, 1988; Shenoy et al., 2012), ants that feed on plant-feeding insects or their parasitoids may exhibit differential responses to CHCs of plant-feeding insect gallers versus carnivorous parasitoids based on differences in their CHC profiles, if any. Also, as in the case of plant volatiles (Ranganathan and Borges, 2009), such differential responses to CHCs by ants may be acquired and may not be innate.

Infochemical use by carnivorous insects in complex tritrophic interactions has scarcely been examined (Steidle and van Loon, 2003). Whether ants with different lifestyles or experience can show similar learning with regard to CHCs has not been examined in these complex multitrophic systems. In order to investigate the differential responses of ants to CHC profiles of a multitrophic prey community, we chose the co-evolved system of figs associated with fig wasps, since galler and parasitoid fig wasps form an important prey resource for arboreal ants (Schatz et al., 2006, 2008; Ranganathan et al., 2010; Zachariades et al., 2010; Bain et al., 2014) and ants are predictably available as dominant predators on fig trees. Stable and predictable plant-based prey sources such as figs can therefore serve as important model systems to understand ant foraging behaviour (Heil and McKey, 2003; Debout et al., 2005; Ranganathan and Borges, 2009), particularly the response of ants to cuticular compounds of their prey.

The fig (*Ficus*: Moraceae) syconium is a specialised globular inflorescence within which fig wasps breed. These wasps could be gallers, kleptoparasites feeding on galled plant tissue, parasitoids or hyperparasitoids and develop within the syconium (Cook and Rasplus, 2003; Herre et al., 2008; Borges, 2015). All wasp species are usually highly specific to their natal fig species (Herre et al., 2008; Jousselin et al., 2008); however, a single parasitoid wasp species may parasitise several wasp species developing within the same syconium or in the same fig species (Ghara and Borges, 2010; Ghara et al., 2014; Borges, 2015). Therefore, in this tritrophic interaction, the predatory parasitoids could be generalists at the prey level but are specialists at the host plant level (*sensu* Vet and Dicke, 1992). Owing to host-plant specificity of fig wasps, and assuming that some CHCs could be acquired from the diet, all gallers could acquire elements of their CHC profiles from the fig species they feed upon, and in turn all parasitoids could acquire components of their CHC signature from the various gallers or parasitoids (in the case of hyperparasitoids) they prey upon within the same fig species (Fig. 1). All else being equal, we expected close correspondence between CHC profiles of predator–prey species pairs.

We selected a reasonably speciose community of fig wasps and ant predators in a common fig species *Ficus racemosa* L. (Moraceae) in India since we had knowledge of the trophic level of the fig wasps (Ghara and Borges, 2010; Ghara et al., 2011, 2014), had
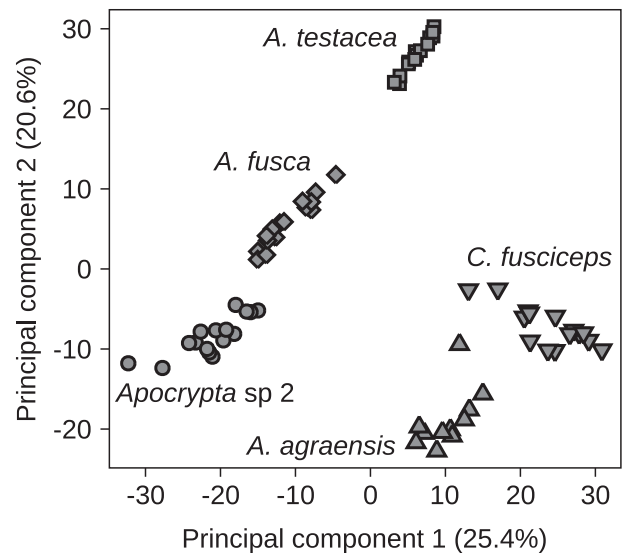

Fig. 1. Principal Component Analysis (PCA) plot based on proportional abundance of fig wasp CHCs.

established that predatory ants but not trophobiont-tending ants in this system learn to associate insect prey with plant volatiles (Ranganathan and Borges, 2009), and we also knew the predation levels of the ants on the different species of fig wasps (Ranganathan et al., 2010). We therefore asked the following questions: 1) What are the CHC profiles of galler and parasitoid fig wasps developing within *F. racemosa* syconia, and how do they differ? 2) Are the CHC profiles of parasitoid fig wasps congruent with those of their prey? 3) What is the response of ants to CHC extracts of the different fig wasps? 4) Is there a difference between predatory and trophobiont-tending ants in this response? 5) Are these responses learned? To the best of our knowledge, this is the first study to address such questions in a highly specific and complex multitrophic interaction such as that of figs and fig wasps interacting with generalist ants.

2. Materials and methods

2.1. Natural history of the fig wasp community

F. racemosa (Moraceae: Subgenus *Sycomorus*) bears globular enclosed inflorescences termed syconia (figs) and produces syconia 5–6 times per year. This fig species is pollinated by an agaonid wasp *Ceratosolen fusciceps* which enters the syconia and breeds by galling some pollinated flowers into which eggs are deposited. The development of syconia goes through distinct phases. In the pre-pollination phase, syconia are small with floral buds; in the pollen receptive phase, syconia contain female flowers that are ready to be pollinated by *C. fusciceps*. Ovipositing pollinators die within a few hours of entering syconia. Pollinator larvae and seeds develop during an inter-floral phase. The wasp-dispersal phase follows when male flowers produce pollen; wingless male fig wasps eclose from galled flowers, and mate with eclosed female pollinating fig wasps while they are still inside their galls. The females collect pollen and exit the syconium via the exit hole chewed out cooperatively by the pollinator males which later die within the syconium.

This obligate brood-site pollination mutualism between *F. racemosa* and its pollinating wasp is subject to parasitism by several species of galling and parasitoid chalcid wasps that do not enter the syconium but oviposit into the fig syconia from the outside using long ovipositors during the various development phases of the