

Chemical mediation and niche partitioning in non-pollinating fig-wasp communities

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Summary

1. The parasitic chalcidoid wasps associated with the species-specific and obligatory pollination mutualisms between *Ficus* spp. and their agaonid wasp pollinators provide a good model to study the functional organization of communities.
2. However, communities of non-pollinating fig wasps (NPFWs) remain little characterized, and their functioning and evolutionary dynamics are still poorly understood.
3. We studied the communities of NPFWs associated with the monoecious *F. racemosa* and the dioecious *F. hispida*. Associated with these two fig species are a total of seven wasp species belonging to three genera. These species present contrasts in life history traits and in timing of oviposition. The species studied are thus broadly representative of the communities of NPFWs associated specifically with fig–pollinator mutualisms.
4. In our study systems, there is temporal segregation of oviposition time among members of NPFW communities.
5. We tested the role of volatile chemicals in the attraction of NPFWs associated with these two fig species, and tried to determine if chemical mediation can explain the organization of the communities.
6. We conducted odour choice tests using a Y-tube olfactometer. All the NPFWs studied were shown to use volatile chemicals produced by the fig to locate their host. Furthermore, the signals used by each species depended on the phenological stage of the fig they exploit.
7. Results demonstrated that the pattern of oviposition results from the utilization of volatile signals produced by figs that vary in their composition at different stages of fig development. Thus, chemical mediation allows resource partitioning in the NPFW communities associated with fig–pollinator mutualisms, and suggests hypotheses to explain coexistence in other parasite communities.

Key-words: community ecology, fig–fig wasp mutualism, host localization, olfactory perception of volatile compounds.

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Introduction

Understanding the ecological determinants of community structure and functioning is one of the major challenges in ecology today. Various groups of insects have long inspired the development of theory in community ecology and have long been recognized as convenient

models for testing hypotheses in this domain (Heil & McKey 2003). Owing to their species richness and trophic diversity, insects parasitic on plants or on other insects constitute complex webs of interaction (Hawkins & Compton 1992; Hawkins & Mill 1996). In such systems one host is often parasitized by several species, as in some parasitoids associated with leaf-mining insect communities (Memmott, Godfray & Gauld 1994), in oak gall cynipid wasps (Stone *et al.* 2002), in the parasitoids of grass-feeding chalcidoid wasps (Dawah, Hawkins & Claridge 1995) or in fig-associated chalcidoid wasps (Kerdelhué, Rossi & Rasplus 2000).

Understanding which parameters influence the organization of the community requires information about parasitic strategies of each of the community's members. One key component is the capacity to localize appropriate hosts (Godfray 1994). Interspecific variation in this capacity may be a determinant factor of community structure.

In insect–plant interactions, transmission of information between two partners is often based on chemical cues. In a proximate sense, interactions are mediated by chemical signalling (chemical mediation). Plant-produced chemicals may also give rise to tritrophic interactions, attracting natural enemies of herbivores (reviewed extensively by Vet & Dicke 1992; Godfray 1994). However, when host plants are distributed patchily, the searching insect must be able to distinguish the particular signals emitted by its host plant (or the host plant of its prey) from the myriad of other naturally occurring volatile compounds concurrently present. Many plants are suitable for insects only at a particular stage of their development (Jaeger, Till-Bottraud & Desprès 2000; Schiestl & Ayasse 2001). Insects must thus be able to recognize not only their host plant but also the appropriate phenological stage.

Like other mutualisms, the specific and obligatory interaction between figs and their pollinators is exploited by a specific community of parasite species, which provide valuable model systems for studying community ecology (Hawkins & Compton 1992; West & Herre 1994; Kerdelhué *et al.* 2000). All the ~750 *Ficus* species are associated with at least one specific species of pollinating agaonid wasp, which also depends strictly on its host *Ficus* for reproduction (Wiebes 1979). Recent phylogenies of *Ficus* and pollinating fig wasps support the monophyly of both, implying that the fig–pollinator mutualism arose just once (Machado *et al.* 2001; Jousset, Rasplus & Kjellberg 2003; Datwyler & Weiblen 2004). The defining feature of *Ficus* is the enclosed urn-like inflorescence of figs with its entrance, the ostiole, closed by bracts. Female pollinator wasps penetrate into the fig cavity through the ostiolar bracts, oviposit in the ovaries of some of the female flowers and pollinate female flowers. After several weeks, their offspring emerge in the fig cavity and female wasps leave the fig, all at the same time (for details, see 'Natural history of fig-pollinating wasps' in Materials and methods).

Several species of chalcidoid wasps that do not carry pollen also mature within ovaries in fig inflorescences (Hawkins & Compton 1992; West & Herre 1994; Kerdelhué & Rasplus 1996). Most of these non-pollinating fig wasps (NPFWs) are assumed to be associated specifically with a single *Ficus* species (Cook & Rasplus 2003). However, the poorly developed taxonomy of NPFW means that we have poor knowledge of their biodiversity and ecology. Unlike pollinators, most NPFWs oviposit into ovaries of female flowers by inserting their ovipositor through the fig wall (without entering the figs). Kerdelhué & Rasplus (1996) distinguished three functional categories of parasites: gall-makers, which

transform ovaries of female flower into galls (their larvae feeding on these galled ovaries); inquiline, which feed on the gall tissue induced by pollinator larvae or by non-pollinating gall-makers; and parasitoids, whose larvae feed on the larvae of other fig wasps. Several independent colonizations of figs by different chalcidoid lineages are indicated by molecular studies (Rasplus *et al.* 1998). All the species of NPFWs maturing in a fig are strongly constrained to synchronize their development with emergence of the pollinator.

Compared to other parasitic systems (Godfray 1994), NPFWs are unique in their strict dependence on the developmental cycles of their host system. All the specific fig wasps emerging from one fig begin the process of host localization at the same time. A single fig species can host up to 30 NPFW species from diverse lineages (Cook & Rasplus 2003). Within the fig, niche space could be partitioned among the different parasite species via the existence of different subsets of flowers or by differences in timing of oviposition. Each NPFW species can lay its eggs only during a precisely defined interval, depending on the developmental cycle of the host fig and on the timing of pollination (Kerdelhué & Rasplus 1996; Kerdelhué *et al.* 2000). Such communities of NPFWs constitute a very good opportunity for investigating the stability of species coexistence. They also pose the question of how 'finely tuned' NPFWs can be in their capacity to locate figs at the proper developmental stage.

Pollinators are attracted specifically by volatile chemicals emitted by receptive figs (van Noort, Ware & Compton 1989; Hossaert-McKey, Gibernau & Frey 1994; Ware & Compton 1994; Grison-Pigé, Bessière & Hossaert-McKey 2002). However, while chemical mediation has been studied in pollinating fig wasps, the involvement of volatile chemicals in host localization has never been investigated in NPFWs.

Our aims were (1) to test whether NPFWs associated with two different fig species, *F. racemosa* (monoecious) and *F. hispida* (dioecious) are attracted by volatile chemicals emitted by their host and (2) to determine whether each wasp species is attracted only by the volatile compounds produced by the fig at the developmental phase it exploits. We discuss the implications of discrimination ability in resource localization as a factor structuring the complex communities of fig wasps.

Materials and methods

NATURAL HISTORY OF FIG-POLLINATING WASPS

Galil & Eisikowitch (1968) first recognized five distinct phases of fig development. In the prefemale phase, female flowers in the young fig have not yet become receptive. This is followed by the female phase, during which the female flowers mature and the stigmas become receptive. Female pollinator wasps, attracted by volatile signals specific to the host fig (Grison-Pigé

Table 1. Taxonomy, biology and timing of oviposition of fig wasps associated with the two species of *Ficus* studied. All wasps were identified by Jean-Yves Rasplus. Each *Ficus* species was associated with the same wasp species in China and India. The Agaonidae is not monophyletic (Rasplus *et al.* 1998). The placement of Sycoryctinae and Sycophaginae is not yet established. Gall-makers transform ovaries of female flowers into galls (their larvae feeding on these galled ovaries). Inquilines feed on the gall tissue induced by pollinator larvae or by non-pollinating gall-makers. Larvae of parasitoids feed on the larvae of other fig wasps. Species biologies are assigned to the genus, but they still need to be confirmed at the species level

FAMILY	Subfamily	Genus	<i>F. hispida</i>	<i>F. racemosa</i>	Biology	Stage of oviposition (figs)
AGAONIDAE (monophyletic)						
Agaoninae						
	<i>Ceratosolen</i>		<i>solmsi marchali</i> Mayr		Pollinators	Receptive
				<i>fusciceps</i> Mayr	Pollinators	Receptive
AGAONIDAE (non-monophyletic)						
Sycoryctinae						
	<i>Apocrypta</i>		<i>bakeri</i> Joseph		Parasitoids	Late interfloral
				<i>westwoodi</i> Grandi sp. 2	Parasitoids	Interfloral
	<i>Philotrypesis</i>		<i>pilosa</i> Mayr sp. 2		Parasitoids	Interfloral
					Inquilines	Receptive
					Inquilines	Interfloral
Sycophaginae						
	<i>Apocryptophagus</i>			<i>testacea</i> Mayr	Gall-makers	Prefemale receptive
				<i>fusca</i> Girault	Gall-makers	Receptive
				<i>agraensis</i> Joseph	Gall-makers	Around 10 days after pollination

et al. 2002), penetrate into the fig cavity through the ostiolar bracts. Once inside the fig, the female pollinator wasps pollinate the female flowers and lay eggs in the ovaries of some of them. Figs then enter the interfloral phase, during which both seeds and wasp larvae (in all gall-transformed ovaries) develop. During the male phase, male flowers mature and wingless male wasps emerge inside the fig, mating with the female wasps still enclosed in their natal galls. After emerging from their galls, female wasps acquire (actively or passively) a load of pollen. They then leave their natal fig through a hole chewed through the fig wall by males, in search of an attractive fig in female phase. Finally the fig ripens (post-floral phase) and attracts various avian and mammalian frugivores which disperse the seeds. In monoecious fig species, all figs are similar and produce both wasps and seeds. Half of all fig species are functionally dioecious (anatomically gynodioecious) (Berg 1989), with separate male and female trees. Male figs contain both male and female flowers but produce many wasps and no seeds, whereas female figs contain only female flowers and produce only seeds. Fig production is often synchronous within a tree, but asynchronous among trees, allowing the permanent presence of receptive and male-phase figs required for the persistence of the wasp population (Anstett, Hossaert-McKey & McKey 1997).

STUDY SYSTEM

We investigated the behaviour of NPFWs (Hymenoptera, Chalcidoidea) associated with two *Ficus* species (Moraceae) of subgenus *Sycomorus* (Jousselin *et al.* 2003): *F. hispida* (dioecious) and *F. racemosa* (monoecious). Table 1 presents all taxa of figs and wasps. *Ficus hispida* is pollinated by *Ceratosolen solmsi marchali* and

F. racemosa by *C. fusciceps*. Most of the wasp species associated with members of the subgenus *Sycomorus* are species-specific. We also recorded the biology and timing of oviposition of each parasite species. Two species of *Philotrypesis* and one species of *Apocrypta* (family not yet established, subfamily Sycoryctinae) are associated with *F. hispida* in the two sites (see below). Three species of *Apocryptophagus* (family not yet established, subfamily Sycophaginae) and two species of *Apocrypta* are associated with *F. racemosa*. All these species of NPFWs oviposit from outside the fig into ovaries of female flowers. In both systems, the different species of NPFWs exploit the same figs but oviposit at different times during fig development (Table 1; Proffit *et al.* unpublished data). Because NPFWs live longer (2 or 3 weeks) than the pollinator (from a few hours to 2 days), they have more time to locate their host. Very few studies of the larval ecology of NPFWs exist (Cook & Rasplus 2003). Biologies of the studied NPFWs (Table 1) are based on the assumption that these are similar to biologies of congeneric species whose larval ecology has been studied.

STUDY SITES

The study of *F. racemosa* was carried out on the campus of the Indian Institute of Science (12°58' N, 77°35' E), Bangalore, Karnataka State, India. For the two *Philotrypesis* species associated with *F. hispida*, the site was located in Agumbe (13°30' N, 75°05' E), Karnataka State, India. The tests on the *Apocrypta* species associated with *F. hispida* were performed in Xishuangbanna Tropical Botanical Garden (21°55' N, 101°15' E), Yunnan Province, China. Each NPFW species was studied in only one of these two sites, depending on the availabilities of figs and wasps. In any case, the NPFW

Table 2. Behavioural bioassays conducted. For each non-pollinating fig wasp (NPFW) species, the two different phases of fig maturation chosen for comparison depended on the timing of the oviposition observed for that wasp species *in situ* (Proffitt *et al.* unpublished data). We indicate the number of figs used for each pair of tested odours. We considered as 'interfloral' those figs that had been pollinated and were between female and male phases. Within this developmental phase of the fig, we distinguished three groups: 'early interfloral' (those figs that had been pollinated less than 10 days before the bioassays), '10 days after pollination' (those figs that had been observed to be pollinated around 10 days before bioassays), and 'late interfloral' (those figs pollinated more than 20 days before the bioassays)

Parasite species	Host species	Tested odours (figs)	No. of figs
<i>Apocryptophagus testacea</i>	<i>F. racemosa</i>	Prefemale and receptive	6
<i>Apocryptophagus fusca</i>		Receptive and interfloral	8
<i>Apocryptophagus agraensis</i>		Receptive and 10 days after pollination	6
<i>Apocrypta westwoodi</i>	<i>F. hispida</i>	Receptive and interfloral	8
<i>Philotrypesis pilosa</i>		Receptive and interfloral (male figs)	6
<i>Philotrypesis</i> sp. 2		Receptive and interfloral (male figs)	6
<i>Apocrypta bakeri</i>		Early and late interfloral (male figs)	6

species associated with *F. hispida* were the same in the two sites.

INSECT BEHAVIOURAL TESTS

We tested whether NPFWs responded to volatile compounds from figs at different phases of maturation (Table 2), using a glass Y-tube olfactometer under laboratory conditions in which only chemical attraction was possible (Dufay, Hossaert-McKey & Anstett 2003; Schatz *et al.* 2003). The olfactometer was positioned on a table with the arms directed toward facing windows. The Y-tube was 4 cm in diameter and each arm was 14 cm long; the basal stem was 8 cm long. Air (humidified and purified with activated charcoal) was blown into the tube (75 mL/min in each arm). Air was extracted from the basal stem (95 mL/min). Air was blown through an odour-free plastic bag [polyethylene terephthalate (Nalophan®)] containing figs in one arm of the Y-tube, while air only was blown into the bag of the other arm. In order to eliminate visual cues, a piece of opaque nylon fabric was arranged on the top of each branch. The Y-tube and the nylon were changed and cleaned with acetone before each trial. To avoid a directional bias, the position of control and odour items was inverted between the two arms for each successive trial. In addition, control runs were performed with only air in both arms of the Y-tube. In order to compare wasp choice frequencies between the treatments, we alternated the different bioassays and controls. For 10 min, the behavioural choice made by each individually tested wasp was recorded in three categories: choice for odour, choice for no odour or no choice. We considered that wasps made 'no choice' when they stayed motionless for 3 min in the basal stem before the bifurcation. When wasps always made final choices (indicated by remaining motionless in one arm towards the end closest to the air source) in a time shorter than 10 min, we reduced the duration of the test.

The different species of NPFWs were collected from figs in the male phase taken haphazardly from different individual trees. Wasps were kept and fed with honey

and water for between 1 and 3 days before the tests. All tested individuals were 'naive' to fig odours. For each NPFW species, we tested the behavioural response of individuals when confronted with volatile compounds emitted by figs at two different stages (for stage definition see Table 2). For each NPFW species, the two compared phases of fig development were chosen according to the timing of oviposition in the field (Table 1). The figs were taken haphazardly from different individual plants. Figs continue to emit volatile compounds for four hours after being cut from the tree (L. Grison-Pigé, personal communication). Each plant sample was replaced every 3 h.

STATISTICAL ANALYSIS

For all the comparisons, the response of wasps was compared using two-tailed Fisher's exact tests. First, we tested if a preference of wasps for one of the arms (left vs. right) occurred in the controls. Then, to determine if wasps were attracted by fig odours, for each series of tests data were arranged in a 2 × 2 contingency table. We compared the proportion of wasps that chose the right olfactometer arm or the left, when the tested fig odour was on the left vs. on the right (excluding the 'no choice' response). We compared the proportion of wasps that made 'choice' vs. 'no choice' between the control tests and tests in which fig odours were added in the olfactometer. For each species, we first determined which of the tested odours was the more attractive. We then examined, for all tests (all species pooled), whether the proportion of specific wasps that made 'no choice' was different between the two tested odours (attractive vs. non-attractive odour).

Results

Control tests showed no directional trends in the olfactometer for any of the tested species (Fisher's exact tests): *Apocryptophagus testacea* ($n = 17$, $P = 1$); *Aph. fusca* ($n = 26$, $P = 0.15$); *Aph. agraensis* ($n = 14$, $P = 0.71$); *Apocrypta westwoodi* ($n = 15$, $P = 0.26$); *P. pilosa*

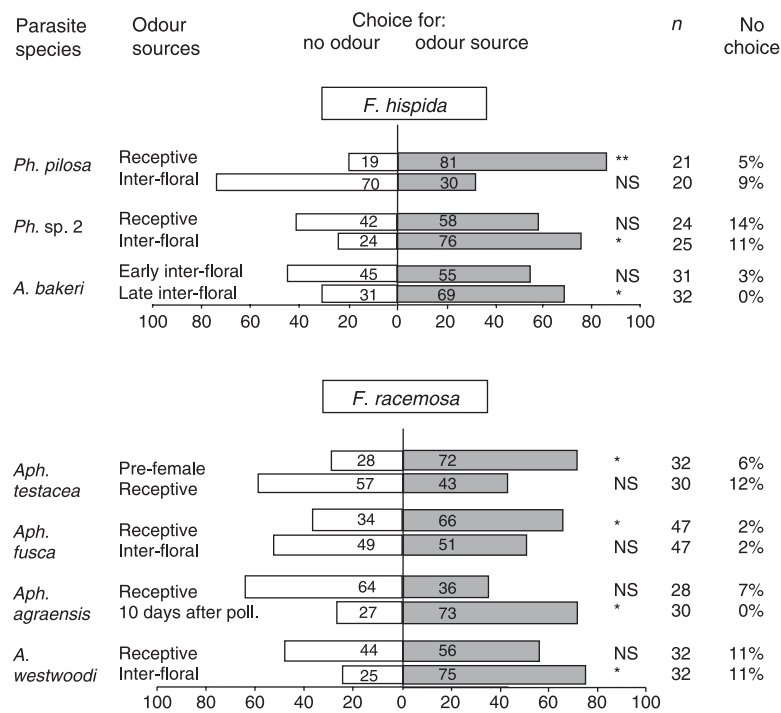


Fig. 1. Responses of the different species of non-pollinating fig wasps (NPFW) in Y-tube experiments when presented with odours from fig at different stages (see Table 2). Wasps and figs were collected haphazardly from different trees. To determine if wasps were attracted by fig odours, for each series of tests data were arranged in a 2×2 contingency table. Using a Fisher's exact test, we compared the proportion of wasps that choose the right olfactometer arm or the left, when the tested fig odour was on the left vs. on the right (excluding the 'no choice' response). [NS, non-significant difference ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *Aph.*, *Apocryptophagus*; *A.*, *Apocrypta*; *P.*, *Philotrypesis*].

($n = 10$, $P = 1$), *P. sp. 2* ($n = 11$, $P = 1$) and *A. bakeri* ($n = 16$, $P = 1$). For all wasp species, no differences were recorded in the proportion of wasps that made 'choice' vs. 'no choice', when each fig odour tested is compared with the controls (Fisher's exact tests, $P > 0.33$ for all species). When a wasp species was attracted significantly by the odour, there was a trend towards a smaller proportion of wasps making 'no choice' compared to the case of non-attractive odour (Wilcoxon's signed-rank test, two-tailed, $n = 7$, $S = 7.50$, $P = 0.06$). Moreover, the NPFW displayed significantly more frequently a choice for no odour, when the odour placed in the other arm was not the one associated with the stage when they oviposit, compared to tests with odour corresponding to the appropriate stage for oviposition (Mann-Whitney test, two-tailed, $n_1 = n_2 = 7$, $Z = 3.13$, $P < 0.001$).

All NPFW species associated with *F. racemosa* were attracted by volatile compounds associated with the phase during which they were observed to oviposit *in situ* (Table 1), and not by volatiles emitted during the other tested phase (Fig. 1). In all cases we used two-tailed Fisher's exact tests. Neither *Aph. testacea*, *Aph. agraisensis* nor *A. westwoodi* were attracted by receptive figs ($P = 0.71$, $P = 0.15$ and $P = 0.72$, respectively). However, these species were, respectively, attracted by prefemale figs ($P = 0.03$), by figs about 10 days after pollination ($P = 0.02$) and by interfloral figs ($P = 0.01$). For *Aph. fusca*, receptive figs were attractive ($P = 0.04$) while interfloral figs were not ($P = 1$).

Similar results were obtained in the case of NPFWs associated with *F. hispida* (Fig. 1). Interfloral figs were not attractive to *P. pilosa* ($P = 0.09$), which was attracted by receptive figs ($P = 0.009$). *Philotrypesis* sp. 2 was attracted significantly by the odours of pollinated figs ($P = 0.02$) but not by odours of receptive figs ($P = 0.44$). Early interfloral figs were not attractive to *A. bakeri* ($P = 0.70$), which was attracted by late interfloral figs ($P = 0.04$).

Discussion

To our knowledge, this study is the first to demonstrate that (1) NPFWs use chemical mediation to locate their host and (2) NPFWs are able to discriminate among volatile chemicals emitted by figs to recognize the appropriate stage of development for oviposition. These results were obtained for seven species of wasps belonging to three genera, presenting contrasts in their biology and timing of oviposition, and associated with two different host fig species, one monoecious and one dioecious. Moreover, the species studied are broadly representative of the communities of NPFWs associated specifically with fig-pollinator mutualisms. Species that specifically exploit different fig developmental stages respond to different bouquets of volatile chemicals. The capacity to detect by olfaction particular developmental stages of figs has evolved independently in different wasp lineages, suggesting that in this case adaptation is not strongly constrained by phylogeny.

Three broad groups of NPFWs can be distinguished in the community depending on the chemical cues to which they respond: (1) those using volatile chemicals emitted by the fig before pollinator arrival; (2) those using the message emitted by the fig to attract the pollinator; or (3) those using volatile chemicals emitted by the fig after pollination. Members of the first group depend on volatile chemicals produced by plant tissues, perhaps as incidental by-products of metabolic activity. Members of the second group can rely on fig volatile compounds whose function is to attract the specific pollinator. We may therefore expect that the message is strong (quantitatively) and specific (Grison-Pigé *et al.* 2002). The third group depends on volatile chemicals released by the fig after pollinator visitation. The specificity of post-pollination cues could result, in a proximate sense, from the presence of developing wasp larvae, developing seeds or both. Specific experiments are necessary to test factors influencing these cues.

As a function of particular life history, figs are suitable only at a particular developmental stage for each NPFW species. As for the pollinator, we can expect that only certain compounds are active in stimulating the wasps. Our study has shown that each wasp can recognize its host at the appropriate stage using the volatile chemicals emitted by the fig at that stage. Thus, the composition of volatile chemicals produced by the plant must change over time. Currently, no information exists about such stage-specific changes. Changes in floral odour following pollination have seldom been examined, but a common trend seems to be a strong quantitative decline of the whole bouquet after pollination, as shown a few days after pollination in *F. hispida* (M. Proffit, unpublished data) or more rapidly in *Silene latifolia* (Dotterl, Wolfe & Jurgens 2005). Despite this post-pollination decrease in scent production, the quantity of chemical volatiles produced by figs is sufficient to be perceptible by NPFWs, from the prefemale phase to the end of the interfloral phase.

The main function of floral fragrances is obviously to attract pollinators (Pellmyr & Thien 1986; Dobson 1994), as stated previously for several fig species (van Noort *et al.* 1989; Hossaert-McKey *et al.* 1994; Ware & Compton 1994; Grison-Pigé *et al.* 2002). The pollinators of *F. hispida* and of *F. racemosa* are also attracted by chemical signals produced by receptive figs of their respective specific hosts (C. Chen and M. Proffit, unpublished data). NPFWs that arrive when host figs are receptive probably use the chemical signal that mediates the encounter of the mutualistic partners. We can expect that there is strong selection to conserve this signal and to produce it in sufficient amounts to facilitate partner encounter. Thus, cueing on to this signal should be a stable strategy for a parasite of the mutualism. For other fig developmental stages, the function of the production of volatile chemicals is unknown, except in the attraction of seed dispersers by mature figs (Kalko, Herre & Handley 1996). The constraints that may act on evolutionary modification of scents at all fig

developmental stages other than receptive figs are thus also unclear.

It is supposed that floral scents have evolved from herbivore deterrents and have been a factor in the diversification of angiosperms and pollinating insects (Pellmyr & Thien 1986). Chemical compounds released by plants attacked by herbivores may attract the natural enemies of herbivores (Turlings, Tumlinson & Lewis 1990; Vet & Dicke 1992; Dicke, van Poecke & de Boer 2003). We have shown in this study that figs – i.e. inflorescences – that have not been attacked by parasites (at least up to receptivity) release volatile chemicals that allow precise detection of the developmental stage of the inflorescence by NPFWs. Several studies have shown that NPFWs have a negative effect on the reproductive success of the pollinators and of their host figs (West & Herre 1994; Kerdelhué & Rasplus 1996; Weiblen, Yu & West 2001). Thus, the production of any scent that parasites can use as a cue to locate hosts at the appropriate stage of development should be counter-selected. Such counter-selection might depend on a delicate balance of costs and benefits (Dicke & Sabelis 1992). We may propose four hypotheses about changes in production of volatile chemicals of figs in phases other than receptive stage: (1) NPFWs may be tolerated because their presence inflicts no (or negligible) fitness costs on the fig, as proposed for some fig species by Bronstein (1991). (2) Post-pollination change in volatile chemicals is an effect of the presence of the pollinator and cannot be controlled by the plant. (3) In the post-floral phase, figs have to produce volatile chemicals to attract seed dispersers, so that defences against NPFWs might have to be traded off against attraction of seed-dispersing frugivores (Patel & McKey 1998). (4) Change in the quantity or composition of scent after pollination can function to repel the pollinator, as demonstrated in other plant–pollinator interactions [e.g. the deceptive orchid *Ophrys sphegodes* Mill. (Schiestl & Ayasse 2001)]. Similarly, in monoecious fig species there is a conflict of interest between the plant and the pollinator in the allocation of flowers to produce seeds or pollinator offspring (Anstett, Bronstein & Hossaert-McKey 1996). Figs stay receptive up to the point where the fig is entered by the number of foundresses that maximizes reproductive success of the fig species (Khadari *et al.* 1995). Post-pollination modification in floral scent could function to limit the number of foundresses entering the fig. Post-pollination modification of the odour bouquet may have been used secondarily by NPFWs as cues to locate hosts at appropriate stages, and could thus be an important proximal factor in structuring NPFW communities.

In tritrophic interactions, chemical cues have been shown to be crucial in host specificity (De Moraes *et al.* 1998) or in the timing of parasite attack (Takabayashi *et al.* 1995). Our work goes further in showing the importance of variation in host localization mechanisms in the structure of parasitic insect communities. In our study systems, there is a temporal segregation of

oviposition time among members of NPFW communities. This segregation depends on the detection by NPFWs of variations in the composition of the volatile compounds emitted by figs. The entire community is organized around events before, during and after pollinator arrival. Similar patterns have been found in other communities of NPFWs (Kerdelhué & Rasplus 1996), but prior to our study the proximate mechanisms leading to such patterns were unexplored. Here we show that this segregation results from the utilization of volatile chemicals produced by figs as cues that vary in their composition at different stages of fig development. Thus, chemical mediation allows resource partitioning in the NPFW communities associated with fig–pollinator mutualisms, and suggests hypotheses to explain coexistence in other parasite communities.

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References

- Anstett, M.C., Bronstein, J.L. & Hossaert-McKey, M. (1996) Resource allocation: a conflict in the fig/fig wasp mutualism? *Journal of Evolutionary Biology*, **9**, 417–428.
- Anstett, M.C., Hossaert-McKey, M. & McKey, D. (1997) Modeling the persistence of small populations of strongly interdependent species: figs and fig wasps. *Conservation Biology*, **11**, 204–213.
- Berg, C.C. (1989) Classification & Distribution of *Ficus*. *Experientia*, **45**, 605–611.
- Bronstein, J.L. (1991) The non-pollinating wasp fauna of *Ficus pertusa* – exploitation of a mutualism. *Oikos*, **61**, 175–186.
- Cook, J.M. & Rasplus, J.Y. (2003) Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology and Evolution*, **18**, 241–248.
- Datwyler, S.L. & Weiblen, G.D. (2004) On the origin of the fig: phylogenetic relationships of Moraceae from *ndhF* sequences. *American Journal of Botany*, **91**, 767–777.
- Dawah, H.A., Hawkins, B.A. & Claridge, M.F. (1995) Structure of the parasitoid communities of grass-feeding chalcid wasps. *Journal of Animal Ecology*, **64**, 708–720.
- De Moraes, C.M., Lewis, W.J., Paré, P.W., Alborn, H.T. & Tumlinson, J.H. (1998) Herbivore-infested plants selectively attract parasitoids. *Nature*, **393**, 570–573.
- Dicke, M. & Sabelis, M.W. (1992) Costs and benefits of chemical information conveyance. *Insect Chemical Ecology, an Evolutionary Approach* (eds B.D. Roitberg & M.B. Isman), pp. 122–155. Chapman & Hall, New York.
- Dicke, M., van Poecke, R.M.P. & de Boer, J.G. (2003) Inducible indirect defence of plants: from mechanisms to ecological functions. *Basic and Applied Ecology*, **4**, 27–42.
- Dobson, H.E.M. (1994) Floral volatiles in insect biology. *Insect–Plant Interactions* (ed. E.A. Bernays), pp. 47–81. CRC Press, Boca Raton.
- Dotterl, S., Wolfe, L.M. & Jurgens, A. (2005) Qualitative and quantitative analyses of flower scent in *Silene latifolia*. *Phytochemistry*, **66**, 203–213.
- Dufaÿ, M., Hossaert-McKey, M. & Anstett, M.C. (2003) When leaves act like flowers: how dwarf palms attract their pollinators. *Ecology Letters*, **6**, 28–34.
- Galil, J. & Eisikowitch, D. (1968) Flowering cycles and fruit types of *Ficus sycomor* in Israel. *New Phytologist*, **67**, 745–758.
- Godfray, H.C.J. (1994) *Parasitoids: Behavioural and Evolutionary Ecology*. Princeton University Press, Princeton.
- Grison-Pigé, L., Bessière, J.M. & Hossaert-McKey, M. (2002) Specific attraction of fig-pollinating wasps: role of volatile compounds released by tropical figs. *Journal of Chemical Ecology*, **28**, 283–295.
- Hawkins, B.A. & Compton, S.G. (1992) African fig wasp communities – undersaturation and latitudinal gradients in species richness. *Journal of Animal Ecology*, **61**, 361–372.
- Hawkins, B.A. & Mills, N.J. (1996) Variability in parasitoid community structure. *Journal of Animal Ecology*, **65**, 501–516.
- Heil, M. & McKey, D. (2003) Protective ant–plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution and Systematics*, **34**, 425–453.
- Hossaert-McKey, M., Gibernau, M. & Frey, J.E. (1994) Chemosensory attraction of fig wasps to substances produced by receptive figs. *Entomologia Experimentalis et Applicata*, **70**, 185–191.
- Jaeger, N., Till-Bottraud, I. & Desprès, L. (2000) Evolutionary conflict between *Trollius europaeus* and its seed-parasite pollinators *Chiastocheta* flies. *Evolutionary Ecology Research*, **2**, 885–896.
- Jousselin, E., Rasplus, J.Y. & Kjellberg, F. (2003) Convergence and coevolution in a mutualism: evidence from a molecular phylogeny of *Ficus*. *Evolution*, **57**, 1255–1269.
- Kalko, E.K.V., Herre, E.A. & Handley, C.O.J. (1996) Relation of fig fruit characteristics to fruit-eating bats in the new and old world tropics. *Journal of Biogeography*, **23**, 565–576.
- Kerdelhué, C. & Rasplus, J.Y. (1996) Non-pollinating Afro-tropical fig wasps affect the fig–pollinator mutualism in *Ficus* within the subgenus *Sycomor*. *Oikos*, **75**, 3–14.
- Kerdelhué, C., Rossi, J.P. & Rasplus, J.Y. (2000) Comparative community ecology studies on old world figs and fig wasps. *Ecology*, **81**, 2832–2849.
- Khadari, B., Gibernau, M., Anstett, M.C., Kjellberg, F. & Hossaert-McKey, M. (1995) When figs wait for pollinators – the length of fig receptivity. *American Journal of Botany*, **82**, 992–999.
- Machado, C., Jousselin, E., Kjellberg, F., Compton, S.G. & Herre, E.A. (2001) Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proceedings of the Royal Society of London, Series B*, **268**, 685–694.
- Memmott, J., Godfray, H.C.J. & Gauld, I.D. (1994) The structure of a tropical host parasitoid community. *Journal of Animal Ecology*, **63**, 521–540.
- van Noort, S., Ware, A.B. & Compton, S.G. (1989) Pollinator-specific volatile attractants released from the figs of *Ficus burtt-davyi*. *South African Journal of Science*, **85**, 323–324.
- Patel, A. & McKey, D. (1998) Sexual specialization in two tropical dioecious figs. *Oecologia*, **115**, 391–400.
- Pellmyr, O. & Thien, L.B. (1986) Insect reproduction and floral fragrances – keys to the evolution of the angiosperms. *Taxon*, **35**, 76–85.
- Rasplus, J.Y., Kerdelhué, C., Le Clainche, I. & Mondor, G. (1998) Molecular phylogeny of fig wasps. Agaonidae are

- not monophyletic. *Comptes Rendus de l'Académie Des Sciences de Paris*, **321**, 517–527.
- Schatz, B., Anstett, M.C., Out, W. & Hossaert-McKey, M. (2003) Olfactive detection of fig wasps as prey by the ant *Crematogaster scutellaris* (Formicidae; Myrmicinae). *Naturwissenschaften*, **90**, 456–459.
- Schiestl, F.P. & Ayasse, M. (2001) Post-pollination emission of a repellent compound in a sexually deceptive orchid: a new mechanism for maximising reproductive success? *Oecologia*, **126**, 531–534.
- Stone, G.N., Schonrogge, K., Atkinson, R.J., Bellido, D. & Pujade-Villar, J. (2002) The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual Review of Entomology*, **47**, 633–668.
- Takabayashi, J., Takahashi, S., Dicke, M. & Posthumus, M.A. (1995) Developmental stage of herbivore *Pseudaletia separata* affects production of herbivore-induced synomone by corn plants. *Journal of Chemical Ecology*, **21**, 273–287.
- Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odours by host-seeking parasitic wasps. *Science*, **250**, 1251–1253.
- Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141–172.
- Ware, A.B. & Compton, S.G. (1994) Responses of fig wasps to host plant volatile cues. *Journal of Chemical Ecology*, **23**, 785–802.
- Weiblen, G.D., Yu, D.W. & West, S.A. (2001) Pollination and parasitism in functionally dioecious figs. *Proceedings of the Royal Society of London, Series B*, **268**, 651–659.
- West, S.A. & Herre, E.A. (1994) The ecology of the New World fig-parasitizing wasps *Idarnes* and implications for the evolution of the fig–pollinator mutualism. *Proceedings of the Royal Society of London, Series B*, **258**, 67–72.
- Wiebes, J.T. (1979) Co-evolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics*, **10**, 1–12.

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