The mechanism of pollinator specificity between two sympatric fig varieties: a combination of olfactory signals and contact cues

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INTRODUCTION

Adaptations that favour pollination by a limited range of animal vectors have been a key factor in the diversification of flowering plants, with some of the most species-rich plant groups characterized by extreme pollinator specificity (Darwin, 1876; Johnson, 2006; Raguso, 2008). Pollinator specificity is a pre-zygotic isolating mechanism that often determines the extent of gene flow among taxa and consequently determines their species boundaries (Grant, 1994; Sargent, 2004; Waser and Ollerton, 2006). Plants with plant-specific pollinators often also display weak post-zygotic isolating mechanisms, as in some Orchidaceae species (Schiestl and Schluter, 2009) and those genera associated with obligate nursery pollination mutualisms, such as Ficus (Ramirez, 1970; Hassaert-McKey et al., 2010), Yucca (Pellmyr, 2003) and Glochidion (Kato et al., 2003). Consequently, traits responsible for maintaining pollinator specificity in these plant groups with highly specific pollinators influence both reproductive isolation and the generation of novel diversity.

Specific floral scents are known to be a key element in pollinator attraction for many plants that have just one or a small number of species of pollinators, and thus contribute to the plants’ reproductive isolation. In the fig–fig wasp mutualism, the volatile blends released from the figs of >40 Ficus species have been investigated (Ware et al., 1993; Grison et al., 1999; Song et al., 2001; Grison-Pige et al., 2002; Chen et al., 2009; Proffit et al., 2009; Soler et al., 2011), and their role in attracting their specific pollinators has been demonstrated in field trapping (Bronshtein, 1987; van Noort et al., 1989; Ware and Compton, 1994b) and laboratory tests (Grison-Pige et al., 2002; Chen and Song, 2008; Chen et al., 2009; Proffit et al., 2009). It is believed that species specificity in the fig–fig wasp system is generally mediated by species-specific volatile signals (van Noort et al., 1989; Chen et al., 2009; Hassaert-McKey et al., 2010). Pollinator specificity that is driven primarily by plant volatiles is also found in some other systems. Euglossine bee-pollinated neotropical orchids, and sexually deceptive orchids in Australia and Europe are examples (Ayasse et al., 2003; Schiestl et al., 2003; Eltz et al., 2005). Similarly, the host-specific Epicephala moths that pollinate Breynia vitis-idaea (Euphorbiaceae) have been shown to be attracted by floral scent using both behavioural and electrophysiological methods (Svensson et al., 2010).
The one pollinator—one host Ficus relationship is not universal, and there are several reports of Ficus species with multiple pollinator species (Rasplus, 1996; Kerdelhue et al., 1999; Molbo et al., 2003; Compton et al., 2009), or one pollinator species may be associated with more than one host taxon (Cornille et al., 2012). Those reports suggest that a relatively loose form of coevolution has been occurring, at least in some lineages. Entry of pollinators into the figs of atypical Ficus hosts often results in fertile seeds, even among sympatric Ficus species that are clearly distinct in terms of their ecology and morphology. This shows that post-zygotic barriers to hybridization are often weak in this genus (Ramirez, 1970; Janzen, 1979; Parrish et al., 2003; Kasumi et al., 2012). Therefore, the strength of pollinator specificity of each fig wasp largely determines species boundaries of figs (Machado et al., 2005). Factors among Ficus species that are likely to increase the likelihood of figs being visited by the ‘wrong’ fig wasps include living in sympatry, co-flowering phenologies, similarities in fig size and shared compounds in the floral scents released from their receptive figs (Whitehead and Peakall, 2009; Hossaert-McKey et al., 2010). The extent to which phylogeny determines the components of floral scents among Ficus species is unclear (Hossaert-McKey et al., 2010), but if closely related taxa produce more similar volatile blends, then pollinator ‘mistakes’ that result in genetic exchange may be more likely to occur among sister taxa.

Sympatric or sister taxa can provide powerful insights into floral scent-mediated reproductive isolation (Whitehead and Peakall, 2009). Ideal systems for studying the consequences of floral fragrance variation should control for other confounding abiotic and biotic variables, such as geographical and temporal isolation, mechanical and ethological isolation and phylogenetic constraints (Knudsen, 1999; Schiestl and Ayasse, 2002; Okamoto et al., 2007). Whitehead and Peakall (2009) have summarized examples of studies that more or less meet the requirements for sympatric, co-flowering and morphologically similar taxa, and emphasize their rarity. In the fig–fig wasp system, although co-flowering sympatric taxa are common (Berg and Corner, 2005), studies of the maintenance of pollinator specificity among sympatric closely related fig species rarely combine both behavioural and chemical investigations.

Contact cues from the surfaces of plants or insects have a role in host attraction and selection among both parasitoids and phytophagous insects (Vinson, 1976; Visser, 1988; Espelie et al., 1991; Giudice et al., 2010). Chemical and physical features on the surface of figs are thought to contribute to host recognition in fig wasps (Ware and Compton, 1994a; Gibernau et al., 1998), but their role in the maintenance of host specificity is largely unknown. Furthermore, mechanical isolation for specificity in the fig–fig wasp system is also common. Fig wasp females need to have an appropriate shape and size to allow passage through the ostiole into figs, as well as an ovipositor of appropriate length for laying eggs into the ovaries via the styles (Nefft and Compton, 1996; van Noort and Compton, 1996). Combinations of long-range floral scents, short-range contact cues and physical matching may act together to help ensure host specificity among fig wasps, especially among closely related fig taxa.

Pollinator responses to volatiles have generally been monitored using insects walking in Y-tube olfactometers, though the responses of flying insects in the field have also been tested (Bronstein, 1987; van Noort et al., 1989; Ware and Compton, 1994b; Chen and Song, 2008; Chen et al., 2009; Profiti et al., 2009). Those studies have consistently detected pollinator attraction to species-specific and developmental stage-specific floral scents emanating from figs. However, attraction of fig wasps to their normal host does not necessarily equate with the maintenance of absolute pollinator specificity, because infrequent arrivals at non-host figs have been recorded under field conditions, as has the entry of pollinators into figs of atypical host species (Bronstein, 1987; Ware and Compton, 1994b; Moe et al., 2011). In addition to these ‘mistakes’ where long-range cues have been by-passed, some fig wasps may also be attracted to figs of species other than their typical hosts, as with the pollinator of F. microcarpa, which is attracted to figs of F. fulva as well as to those of its normal pollinator (Grison-Pige et al., 2002). Furthermore, for those closely related fig species that are living in sympathy, behavioural tests of fig wasp responses to floral scent cues could supply more powerful evidence to understand the mechanism of pollinator specificity.

Here, we use two sympatric varieties of an Asian fig tree, Ficus semicordata, that are host to two different pollinators, to investigate mechanisms of pollinator specificity among sympatric closely related fig taxa. Both volatile and short-range contact cues were investigated and the following specific questions were addressed. (1) Are the floral scents emitted by receptive-phase figs of the two varieties distinct? (2) Do floral scents alone maintain pollinator specificity or are contact cues on the surface of the figs also utilized for host recognition by the fig wasps? (3) Do morphological barriers prevent entry into figs by the fig wasps associated with the other variety? (4) What are the reproductive consequence for the plants of being pollinated by fig wasps carrying alien pollen?

**Materials and Methods**

**Study species and sites**

Ficus semicordata is a functionally dioecious small freestanding tree distributed in the Sino-Himalayan region, southwards to the Malay Peninsula (Berg and Corner, 2005). It has large leaves with asymmetrical heart-shaped leaf bases and fagelliflorous or geocarpic figs. Molecular data suggest that its two varieties [Ficus semicordata Buch.-Ham. ex Sm. var. semicordata (FSS) and Ficus semicordata var. montana Amatya (FSM)] are sister taxa within subgenus Sycomorus, Section Hemicardia [G. Wang, Xishuangbanna Tropical Botanical Garden (XTBG), China, unpubl. res.; Fig. 1]. The two have often been recorded under the same name, so any differences in their distributions are poorly understood, but the two taxa are known to be sympatric in Nepal (Amatya, 1996), central and northern Laos (G. Wang, XTBG, China, pers. observ.) and south-western China [herbarium records of the Chinese Virtual Herbarium (www.cvh.org.cn); G. Wang, XTBG, China, pers. obs.]. The two varieties have similar growth forms, heights and leaf shape, but their figs and
habitats are different (Amatya, 1996). Receptive figs of FSS are green or light brown in colour and about 16 mm in diameter with 1611 ± 18 (mean ± s.e.; n = 20) female flowers per female fig, whereas receptive figs of FSM are bright red, about half the diameter of receptive FSS figs and the number of female flower per female fig is 730 ± 26 (n = 20). The figs of FSM at XTBG are also typically almost free of surface hairs, whereas FSS figs are more pubescent. FSS favours well-lit open areas, such as secondary forests, whereas FSM prefers shadier habitats in or adjacent to rain forests, but it is also common to find the two varieties as both have receptive figs used was 57 for FSS and 355 for FSM – this reflected the difference in fig size and quantity of volatiles released by the two varieties. The volatiles they were releasing were collected in situ using the adsorption–desorption (dynamic headspace) technique described in Chen et al. (2009). To detect any environmental contamination during the volatiles collections, controls were conducted with ambient air collected at the same place using the same dynamic headspace technique. Any volatile compounds shared with this control treatment were removed before analyses. Two internal standards, octane and decyl acetate, were added into each sample, using the same method as Chen et al. (2009).

The extracts were analysed in a coupled gas chromatography–mass spectrometry (GC-MS) system (Agilent HP6890GC/5973MS) equipped with a HP-5MS column (length 30 m; inner diameter 0.25 mm; film thickness 0.25 μm) with helium as the carrier gas. Ionization was by electron impact (70 eV; source temperature 230 °C). For each sample, 2 μL was injected (split with a 10:1 ratio), with the injector temperature at 250 °C. The column temperature began at 60 °C, was increased at 3 °C min⁻¹ up to 80 °C, then up to 260 °C at 5 °C min⁻¹, and this temperature was then maintained for 15 min.

Compound identification was based on automated matching of the mass spectra with Wiley7n.1 libraries. Some components were confirmed by comparison of the retention data with published data. The absolute amounts of all compounds were estimated using the average peak area of the two internal standards as a reference scale. The relative proportions of each compound were also calculated.

**Behavioural bioassays**

Adult female fig wasps were obtained by placing mature male figs into mesh bags during the afternoon before the experiments. These emerged from the figs the following morning. Two potential components of chemosensory recognition were examined: (1) an olfactory phase, where wind-borne volatiles could be used for long-range host localization; and (2) a contact stimuli phase operating once a female wasp had landed on the surface of a suitable fig (Ware and Compton, 1994b; Smadja and Butlin, 2009).

Male and female dioecious figs have broadly similar volatile profiles, reflecting intersexual chemical mimicry (Chen et al., 2009; Hossaert-McKey et al., 2010), but receptive female figs were used in most of the olfactory tests and all the contact stimuli tests.

**Olfactory tests**

These were carried out using Y-tube olfactometry. The equipment and procedures were as described in Chen et al. (2009). The responses of C. gravelyi and Ceratosolen sp. to three scent combinations were examined: receptive host figs vs. air, atypical host figs vs. air, and host figs vs. atypical
host figs. The first two combinations simulated conditions when receptive figs of only one variety were available locally, which is likely to be the more common situation in nature, and the third where volatiles from receptive figs of both varieties were being perceived simultaneously, as might occur at ecotones when the two varieties are flowering in close proximity. Because of their difference in size and the numbers of flowers they contain, volatiles from one receptive FSS fig and six FSM figs were used in the Y-tube olfactometry. Experiments were performed between 0900 and 1100 h within 3 h of the figs being removed from the trees, and the figs were replaced with new ones every hour. Each experiment (comprising one scent combination for each wasp species) was replicated 23–41 times, with no more than five females from any one male fig utilized.

Contact stimuli on the surface of figs

The fig surface experiments were conducted on receptive figs in situ between 0900 and 1200 h. Individual newly emerged female C. gravelyi and Ceratosolen sp. were introduced separately onto the surface of receptive figs of their own or the atypical host. Each wasp was used only once. Their behaviour was observed; the number of wasps which entered figs and wasps which left after walking around on the fig surface for >10 s or after trying to enter but failing to do so were recorded as either ‘Entered’ and ‘Failed to enter’, respectively. The time from the wasp arriving in the ostiole for the first time to the point when its entire body had disappeared inside the ostiolar bracts was also recorded. Wasps that spent <10 s on the fig surface before leaving or flying away were considered to have displayed ‘no choice’ and were excluded from the analyses.

Artificial pollination and seed germination

Cross-pollination and subsequent germination experiments were performed to assess the consequences for the trees of being pollinated by atypical wasp species carrying pollen from a different variety of F. semicordata. Pre-receptive figs on female trees were enclosed in mesh bags to prevent natural pollinator entry. Once the figs were receptive, two recently emerged adult females of either C. gravelyi or Ceratosolen sp. were introduced into figs of FSS, and single individuals of each species into the smaller figs of FSM. The figs were located on two trees of each variety. The pollinators were placed separately on the surface of the figs and allowed to enter unaided. The figs were then bagged again to prevent entry by further pollinators. The figs matured 50–65 d later, and their contents were then recorded. Seeds belonging to each of four types from two female trees were mixed together separately; groups of 300 normal-looking seeds were chosen at random for germination trials. Germination rates of the seeds were compared in Petri dishes lined with moistened filter paper in a continuously illuminated incubator maintained at 30 °C. The trial was repeated three times for each of the four seed sources (two varieties, each with their own or heterovarietal pollen), with each replicate consisting of 100 seeds in a single Petri dish. Germination was recorded daily until no further seeds had germinated for 5 d.

Data analysis

Analyses were mainly performed using R version 2.12.0 (R Development Core Team, 2011). Non-metric multidimensional scaling (NMDS) ordination using the Vegan package (Oksanen et al., 2011) was used to compare scent composition between the two fig varieties. A multiple response permutation procedure (MRPP; Vegan package) was used in a matrix of mean dissimilarities with 999 permutations to test the null hypothesis that there was no difference in the scent profiles. The relative proportions of all the compounds recorded from the seven FSM and six FSS samples were included in the multivariate analyses.

χ² and binominal tests were used to determine whether fig wasps were attracted to one of two odour sources and to determine fig wasp entry preferences after being placed on the surface of figs. Wilcoxon rank-sum tests were used to compare the total absolute quantities of scent emitted by the two fig varieties and the time spent by fig wasps on the fig surfaces. Seed production was compared using t-tests.

RESULTS

Variation in floral scents

The absolute quantity of scent emitted over the course of 1 h by a single FSS fig (mean ± s.d. = 37.72 ± 22.87 ng (n = 6) [56.04 ± 35.23 ng (n = 2), 28.56 ± 11.06 ng (n = 4) for male and female figs, respectively]) was >100 times higher than that from the smaller FSM figs [0.34 ± 0.13 ng (n = 7) [0.38 ± 0.21 ng (n = 3) and 0.32 ± 0.03 ng (n = 4) for male and female, respectively]. A total of 35 different compounds, including fatty acid derivatives, terpenoids and shikimic compounds, were found in the scents emitted by the receptive figs of the two varieties (see Supplementary Data Table S1). As has been recorded previously, the shikimic compound, 4-methylanisole, contributed almost 98 % (94.48–99.55 %) of the total quantity of volatiles released from receptive FSS figs. A further ten compounds were present in small quantities (male and female figs combined). This dominance of a single component contrasts with the more diverse floral scent of FSM, which included 31 compounds (male and female figs combined), of which nine, representing 8.29 % of the total scent emitted, remain unidentified. Of the 30 compounds, 18 were recorded from both sexes (76.47 % of the total quantity of volatiles), with five compounds only recorded from male figs and eight only from female figs. Five compounds (β-caryophyllene, α-caryophyllene, 4-methylanisole, 1,8-cineole and d-limonene), each representing >5 % of the total quantity, together constituted about 67.4 % of the total scent emitted from FSM figs. 4-Methylanisole was present in all three samples from male figs, but with very large variation in its relative percentage (5.47, 17.46, and 66.48 %), and it was not detected at all in the four scent profiles obtained from female figs. Seven of the 11 compounds from FSS figs were shared with FSM figs, but six of these were in trace amounts and together represented <1 % of the total quantity of volatiles.

An ordination (NMDS with Bray–Curtis distances) of the 13 volatile samples illustrated the contrast between the volatile blends of the two varieties (Fig. 2). The overall scent profiles...
of the receptive figs differed significantly between varieties (MRPP, $A = 0.6314$, $P = 0.002$).

**Y-tube experiments**

Female *Ceratosolen* sp. were attracted to receptive female figs of both FSM (its regular host) and FSS when given a choice between them and clean air, but displayed no preference between the scents of receptive figs of the two varieties (Fig. 3). *Ceratosolen gravelyi* females were also attracted to receptive figs of both varieties when clean air was the alternative, but in contrast to *Ceratosolen* sp. they showed a strong preference for their normal host figs (FSS) when given a choice between these and receptive figs of FSM (Fig. 3).

**Contact stimuli tests**

The searching behaviour of female *Ceratosolen* sp. on receptive figs mainly involved walking across the surface while keeping the head lower than the body. The antennae were swept across the fig surface, while keeping their tips in contact with the fig surface. *Ceratosolen gravelyi* females tended to walk more quickly and often kept their heads further from the substrate, and there was noticeably less antennal contact with the fig surface. These differences in behaviour on the general surface of the figs were consistent, irrespective of the fig variety. Once the ostiole was encountered, both species pressed about half the length of their antennae against the outer ostiolar bracts, before deciding whether to enter or not. *Ceratosolen* sp. females were more likely to resume walking and then return to the ostiole later than were *C. gravelyi* females, which tended to enter the ostiole on the first occasion they came into contact.

When *C. gravelyi* and *Ceratosolen* sp. females were placed on the surface of receptive figs, they were significantly more likely to enter their normal host figs than those of the alternative variety (Fig. 4A). Some individuals of each species nonetheless entered atypical host figs (34% of *C. gravelyi* on FSM figs and 17% of *Ceratosolen* sp. on FSS figs, compared with 92 and 100% entry when the wasps were placed on their normal hosts). There was a non-significant tendency for a higher proportion of *C. gravelyi* than *Ceratosolen* sp. females to be willing to enter an atypical host once placed on the fig surface ($\chi^2$ with Yates’ correction $= 3.2$, d.f. = 1, $P < 0.001$).

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Fig. 2. Non-metric multidimensional scaling (NMDS) ordination of scent profiles of receptive phase figs of *F. semicordata* var. *montana* ($M, n = 7$), and *F. semicordata* var. *semicordata* ($S, n = 6$). Female and male figs are indicated by f and m. Stress $= 0.057$.

Tab. 1. Proportion of wasps choosing either odour.

<table>
<thead>
<tr>
<th>Wasp tested</th>
<th>Odour 1</th>
<th>Choice for:</th>
<th>Odour 2</th>
<th>$P$</th>
<th>NC</th>
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<tbody>
<tr>
<td><em>Ceratosolen</em> sp.</td>
<td>FSM♀</td>
<td>16</td>
<td>18</td>
<td>ns</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>FSM♂</td>
<td>26</td>
<td>2</td>
<td>***</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>3</td>
<td>20</td>
<td>***</td>
<td>6</td>
</tr>
<tr>
<td><em>C. gravelyi</em></td>
<td>FSM♀</td>
<td>1</td>
<td>40</td>
<td>***</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>FSM♂</td>
<td>2</td>
<td>22</td>
<td>***</td>
<td>7</td>
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<td></td>
<td>FSM♀</td>
<td>32</td>
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**Fig. 3.** Behavioural responses of female *Ceratosolen* wasps to olfactory stimuli in Y-tube tests. Numbers in or beside bars indicate the absolute numbers of wasps that chose each odour. Wasps that did not respond within 5 min (NC) were excluded from the statistical analysis. ($\chi^2$ tests: ns, $P > 0.05$, ***,$P < 0.001$). FSS = receptive figs of *Ficus semicordata* var. *semicordata*; FSM = receptive figs of *F. semicordata* var. *montana*; Air = purified air. Male and female figs are indicated as ‘♀’ and ‘♂’. **Fig. 4.** Behavioural responses of male *Ceratosolen* sp. wasps to olfactory stimuli in Y-tube tests. Numbers in or beside bars indicate the absolute numbers of wasps that chose each odour. Wasps that did not respond within 5 min (NC) were excluded from the statistical analysis. ($\chi^2$ tests: ns, $P > 0.05$, ***,$P < 0.001$). FSS = receptive figs of *Ficus semicordata* var. *semicordata*; FSM = receptive figs of *F. semicordata* var. *montana*; Air = purified air. Male and female figs are indicated as ‘♀’ and ‘♂’.
FIG. 4. Behavioural responses of female Ceratosolen placed on the surface of receptive figs. (A) The willingness of female fig wasps to enter receptive figs of normal or atypical hosts after they had been placed on the surface of the figs. Shaded bars indicate the wasps that entered the figs; open bars the wasps that did not. Sample sizes (n wasps) are also indicated. (B) The time spent by female fig wasps on the surface of receptive figs of the two varieties of F. semicordata, measured from when the wasps first arrived at the ostiole to when their entire body had entered the figs (mean ± s.e.). Note that after coming into contact with the ostiole for the first time, some females wandered away before then returning and attempting entry. Shaded bars indicate atypical host figs, open bars the normal host figs. Sample sizes (n wasps) are also indicated. (A) χ² tests (*P < 0.05; ***P < 0.001) and (B) Wilcoxon rank-sum tests, with different letters indicating significant differences. FSS = Ficus semicordata var. semicordata, FSM = F. semicordata var. montana.

DISCUSSION

Ficus semicordata var. semicordata and F. semicordata var. montana are two very closely related and frequently sympatric taxa that are pollinated by two different species of Ceratosolen fig wasps. Their receptive figs release floral scents that contain different combinations of volatiles, but both scents are attractive to both pollinators, and one species showed no preference for volatiles released from figs of its normal host over those released by the other variety. These results contrast with the generally accepted basis for pollinator specificity in the fig–fig wasp mutualism, that volatiles released from receptive figs are long-range olfactory cues that attract only the pollinators associated with that particular species of fig tree. Short-range cues have largely been ignored in previous studies of pollinator specificity, but the two pollinators of the F. semicordata varieties displayed clear preferences for their usual hosts when they were placed on the surfaces of receptive figs, and these contact cues, rather than long-range volatile attractants, may be more critical in determining which pollinators enter the figs. Some pollinators were nonetheless willing to enter the ‘wrong’ figs and, once inside, their pollination resulted in the production of viable hybrid seeds, suggesting that gene flow between the two varieties of F. semicordata could take place where plants are growing together. Hybrid viability was not assessed beyond the seedling stage, but there is clearly the potential for introgression between the two varieties of F. semicordata.

Molecular evidence suggests that the two varieties of F. semicordata are sister taxa, yet their volatile profiles are very different. The diverse scent of FFS is typical of figs in general (Hossaert-McKey et al., 2010), whereas the very simple composition of the FSS bouquet is rare and possibly unique among Ficus species (Chen et al., 2009). However, only a small proportion of the volatile compounds that make up the scent of FSS may be attractive to its Ceratosolen sp. pollinator. 4-Methylisohene, which contributes the vast majority of the volatiles released from FSS figs, is clearly not essential for attraction of Ceratosolen sp. to FSS figs, because not all FSS figs release it. Furthermore, the scent of FSS figs was attractive to C. gravaeleyi females, despite the rarity or absence of 4-methylisohene, so this pollinator will clearly respond to other volatiles. Similarly, the simple FSS blend also attracted Ceratosolen sp. females. Some compounds are components of both floral scents (β-caryophyllene, α-caryophyllene, 4-methylisohene and α-copaene) and these may be responsible for the figs of the two varieties being attractive to both species of pollinators.

Y-tube olfactometry is widely used to investigate pollinator attraction to floral scents (Okamoto et al., 2007; Chen and Song, 2008; Chen et al., 2009; Proffit et al., 2009; Svensson et al., 2010, 2011), but it is clearly a poor model for host choice under natural situations – the insects are usually
Walking rather than flying, and concentrations of volatiles may be unnaturally high. Trapping of fig wasps under more natural field conditions has nonetheless given similar results to Y-tube experiments, and supported the conclusion that pollinator-specific responses to host-specific volatiles are largely responsible for maintaining fig wasp host specificity (Bronstein, 1987; van Noort et al., 1989; Ware and Compton, 1994b). However, small numbers of unexpected fig wasp species reported in the above studies can nonetheless be trapped around receptive trees, and this may not necessarily reflect only chance encounters. In F. semicordata, the marked differences in floral scents between the two varieties do not appear to be sufficient to prevent both Ceratosolen species being attracted to both varieties. This result conflicts with previous assumptions that specific floral scent alone can maintain Ficus pollinator specificity (van Noort et al., 1989; Grison-Pige et al., 2002; Chen et al., 2009; Hossaert-McKey et al., 2010), a difference that may reflect the paucity of comparisons between very closely related fig trees.

In the absence of clear-cut differences in responses to host fig volatiles, contact cues from receptive figs appear to help the two pollinator species distinguish between the figs of FSS and FSM. Both physical (the fig surfaces differ in hairiness) and tactile chemical cues may be involved (Vinson, 1976). As with other pollinating fig wasps (Ware and Compton, 1994a; Gibernau et al., 1998), females of both Ceratosolen species repeatedly tapped the surface of the figs with their antennae as they walked around, but their responses to cues emanating from the ostioles appeared to be more decisive than from the fig surface, with both species much more likely to avoid entry if the ostiole belonged to an atypical host variety. Although the relative significance of visual, chemical and physical cues was not examined, contact chemical cues may be particularly important, based on studies of both parasitoids and phytophagous insects in general (Vinson, 1976; Visser, 1988; Espelie et al., 1991; Giudice et al., 2010). Additionally, Gibernau et al. (1998) showed that extracts of whole receptive figs of F. carica are attractive to its pollinator (Blastophaga psenes), but these extracts may have contained compounds from the fig surface that elicit behavioural responses, as well as those responsible for long-distance attraction to the figs.

There is increasing evidence of hybridization and introgression among fig trees (Parrish et al., 2003; Machado et al., 2005; Renoult et al., 2009; Kusumi et al., 2012). Sympatry, similar flowering phenologies, a lack of pollinator specificity in response to long-range olfactory cues, a high misdirection rate once pollinators are on the fig surface and weak post-zygotic isolation (at least up to the seedling stage) will facilitate gene flow between the two varieties of F. semicordata. Undirectional hybrids resulting from Ceratosolen sp. (with FSM as its normal host) visiting FSS figs are particularly likely. Ongoing genetic studies will test this assumption. An understanding of how the two varieties maintain their identities will also help assess the likely role of hybridization and introgression in Ficus speciation and diversification (Machado et al., 2005; Whitehead and Peakall, 2009).

In conclusion, our study shows that both long-range volatile attractants and surface cues contribute to host specificity in two closely related and frequently sympatric species of fig trees. Species-specific floral scents, the major mechanism for pollinator isolation in Ficus (Whitehead and Peakall, 2009; Hossaert-McKey et al., 2010), were unable on their own to maintain the species-specific relationship between the fig wasps and their hosts. Contact stimuli from the surface of the figs played a complementary role in host recognition, but failed to ensure that pollinators only entered their typical host figs. Putative weak post-zygotic isolation between these fig species suggests that hybrid offspring production is likely. The extent to which fig surface characters are important for host recognition among other species of fig wasps remains to be determined.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: relative abundances and occurrence of the volatile compounds found in floral scents emitted by figs of two varieties of F. semicordata at their receptive phase.

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LITERATURE CITED


