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Pollinator sharing and gene flow among closely related sympatric dioecious fig taxa

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Hybridization and insect pollination are widely believed to increase rates of plant diversification. The extreme diversity of figs (*Ficus*) and their obligate pollinators, fig wasps (Agaonidae), provides an opportunity to examine the possible role of pollinator-mediated hybridization in plant diversification. Increasing evidence suggests that pollinator sharing and hybridization occurs among fig taxa, despite relatively strict coevolution with the pollinating wasp. Using five sympatric dioecious fig taxa and their pollinators, we examine the degree of pollinator sharing and inter-taxa gene flow. We experimentally test pollinator preference for floral volatiles, the main host recognition signal, from different figs. All five fig taxa shared pollinators with other taxa, and gene flow occurred between fig taxa within and between sections. Floral volatiles of each taxon attracted more than one pollinator species. Floral volatiles were more similar between closely related figs, which experienced higher levels of pollinator sharing and inter-taxa gene flow. This study demonstrates that pollinator sharing and inter-taxa gene flow occurs among closely related sympatric dioecious fig taxa and that pollinators choose the floral volatiles of multiple fig taxa. The implications of pollinator sharing and inter-taxa gene flow on diversification, occurring even in this highly specialized obligate pollination system, require further study.

1. Introduction

Hybridization has fascinated biologists for many decades [1–3]. It has been considered as evolutionary noise, as a delay in the speciation process [4], or, conversely, as a source of biological novelty, promoting diversification [5]. At least 10% of animal species and 25% of plant species are known to be of hybrid origin [2]. Obligate pollination systems, in which the plant and/or the pollinator are completely dependent on the other member for successful reproduction, are often exceptionally diverse, such as in euglossine bees and orchids [6], *Epicephala* moths and Phyllanthaceae [7], and Agaonidae wasps and figs (*Ficus*) [8]. Host recognition and pollinator choices determine the patterns of plant gene flow in those systems, and pollinator isolation is believed to play a central role in reproductive isolation among sympatric plant species [9–12]. Studies on these obligate pollination systems may also help unravel the role of insect pollinator-mediated hybridization in plant diversification [13], given the association between diversity and apparently close coevolutionary relationships between plants and the obligate insect pollinators.

The mechanism and degree of pollinator specificity is a critical aspect for understanding genetic diversification in flowering plants, particularly among sympatric plants, where this specificity is a primary source for reproductive isolation among plant taxa [9,11,14]. The floral volatile signature, which often consists of a mixture of volatile organic compounds, is believed to be the main

mechanism for maintaining pollinator specificity in many obligate pollination systems [10,15]. Intraspecific variations in floral volatiles may allow multiple pollinator species to coexist on one host [16], while their similarity among different plant species may facilitate pollinator sharing or shifts [17,18]. The extent to which plant taxa in an obligate system maintain the identity of their floral signature, especially among sympatric closely related taxa, may be critical to guarantee the reproductive isolation of plants. Further, the occurrence of multiple pollinators per host, pollinator sharing and pollinator shift events have been reported in many obligate pollination systems [6,19–21]. Temporal isolation [22] or habitat preference [23] have also been mentioned as mechanisms to maintain reproductive isolation. However, the strength of reproductive isolation largely depends on the scale of the pollinator sharing and subsequent inter-taxa gene flow. If a high degree of pollinator sharing exists, the biological identity of those species would be challenged [9,13,24].

Ficus (Moraceae) is one of the most species-rich genera of woody plants, with over 800 species, greater than the total species number in all other 38 genera (approx. 325 species) in the Moraceae family [25]. Figs and pollinating fig wasps form an extreme obligate pollination mutualism [26]. In most cases, this mutualistic system follows the ‘strict matching’ rule, where one plant species is pollinated by one wasp species, with each species completely dependent on the other to achieve reproductive success [8]. The species-specific floral volatiles produced during the receptive phase and the attraction of the obligate fig wasp species have been regarded as the mechanism that ensures pollinator specificity in the fig–fig wasp system, similar to most other obligate pollination systems [9,27]. However, cases of multi-pollinator species per fig species or pollinator sharing among different fig species have also been reported in both monoecious and dioecious figs [19,28–29]. The role of floral volatiles alone in maintaining pollinator specificity is also doubtful as the volatiles of some fig taxa are attractive to pollinators of other figs [30–31]. Furthermore, temporal hybridization and introgression have been detected in several studies due to the pollinator sharing [17,23,32–33]. Thus, the fig–fig wasp system would be an ideal group to evaluate how pollinator sharing and hybridization affect the plant species identity and the co-diversification of plants and their pollinators.

In tropical China and neighbouring areas in southeast Asia, two Asian sections (*Sycomorus* and *Hemicardia*) of *Ficus* subgenus *Sycomorus* provide an excellent study system, where pollinator sharing may lead to inter-taxa gene flow among sympatric dioecious host figs [23,31,34,35]. In southwest China, three cryptic fig wasps have been observed pollinating three morphological fig taxa of the *Ficus auriculata* complex with a certain degree of pollinator sharing [19,23]. In experimental host switching studies, each pollinator could develop well, with some variation of fitness, in an atypical host fig taxon [36], and natural hybrids have been detected in field studies [23]. Pollinators of two varieties of *F. semicordata* also preferred the floral volatile signature of either of two morphologically distinct figs varieties, while viable bidirectional hybrid seeds were produced by artificial host switching without significant reduction in seed fitness and germination [31]. Though the two fig sections may not be sister groups in a phylogenetic context, their pollinators are closely related, as indicated by recent phylogenetic studies [26,37], suggesting

the possibility of pollinator shifts and inter-taxa gene flow between the two sections.

Herein, with the five *Ficus* taxa in these two sections and their pollinating fig wasps as materials, we aim to address the following questions. (1) To what extent does pollinator sharing or pollinator shifts occur among sympatric dioecious fig taxa? (2) How does the degree of pollinator sharing relate to inter-taxa gene flow among sympatric host figs? (3) How does the fig volatile signature influence pollinator behaviour among fig taxa, in terms of host recognition and pollinator sharing? Overall, we aim to evaluate the pattern and causation of pollinator sharing and inter-taxa gene flow among sympatric fig taxa.

2. Material and methods

(a) Study system and sampling

All five fig taxa are functionally dioecious, free-standing trees, and are sympatric across their distribution in the Sino-Himalayan region and southwards to the Malay Peninsula [34]. Three fig taxa, *Ficus auriculata* Lour. (FA), *F. oligodon* Miq. (FO) and *F. hainanensis* Merr. & Chun (FH), are morphologically distinct taxa in the *F. auriculata* complex (section *Sycomorus*; subsection *Neomorpha*) [34]. At least three cryptic wasp taxa of *Ceratosolen emarginatus* pollinate the *F. auriculata* complex [19,23]. The other two taxa are morphologically distinct varieties of *F. semicordata* in section *Hemicardia*: *F. semicordata* var. *semicordata* Buch.-Ham. ex Sm. (FSS) and *F. semicordata* var. *montana* Amatya (FSM) [31,35]. FSS is pollinated by *Ceratosolen gravellyi* [38], and FSM is pollinated by *Ceratosolen* sp. based on our preliminary study [31].

Despite having similar leaf traits and tree forms, identification of the two varieties of *F. semicordata* in the field is rather easy. FSS has white latex and large fruits, while FSM has yellow or pink latex with rather small fruits. Fruit colour during the receptive phase is also different; green or light brown in FSS and dark red in FSM [31,35]. For the taxonomy of the three taxa from *F. auriculata* complex, we mainly followed the criteria described by Wei *et al.* [23]. *Ficus auriculata* has large and broad lamina, large fruits borne on the stout long leafless branchlet (more than 20 cm), and flowers with a white perianth. *Ficus oligodon* (Southern *F. oligodon* type described by Wei *et al.* [23]) has smaller and narrower lamina than that of *F. auriculata*, with larger fruits and whiter perianth, while the fruit branchlet is shorter (less than 10 cm). *Ficus hainanensis* has the smallest and narrowest lamina, fruits are distinctively smaller, with purple perianth, and fruit branchlet longer than 50 cm.

Fig trees and fig wasps were sampled in southwest China (two sites), central Laos (two sites) and Vietnam (three sites) from May 2011 to July 2012 (electronic supplementary material, table S1). Distances among sites range from 70 to 500 km, and all the sites selected were either primary or secondary tropical forests. In total, 278 adult fig trees of all five taxa (30–78 trees each) were sampled for microsatellite analyses. One healthy mature leaf of each tree was collected and dried in silica gel immediately. In total, 87 female pollinating fig wasps (13–22 wasps of each fig taxa) were sampled for pollinator sharing survey. About 54.32% fig samples and 67.82% wasp samples were collected from the Menglun site (21°55' N, 101°15' E, southwest China), including all five taxa (see the electronic supplementary material, table S1 for detailed sampling information). Pollinating fig wasps were sampled from either male syconia of male flower phase, when mature fig wasps were emerging from fig syconia, or from syconia of receptive phase, when fig wasps were entering into receptive fig syconia for pollination and oviposition. Wasps from a single fig syconium were stored in 95% alcohol separately. To avoid sampling sibling wasps, only one wasp individual per male

flower phase syconium or one to three individuals per receptive syconium were used for molecular identification (see below). Dried leaf samples of figs and wasp samples in 95% alcohol were stored at -20°C for DNA extraction.

(b) Pollinator sharing ratio evaluation

(i) Molecular identification of fig wasp

Total genomic DNA of 87 wasp individuals was extracted with DNA Extraction kits (TransGen, Beijing, China). Mitochondrial COI gene fragment was sequenced de novo with primer and protocols of Weiblen *et al.* [39]. Sequences were deposited in GenBank (accession nos. KP325490–KP325576). To identify fig wasps and detect potential pollinator sharing among focal and sympatric non-focal *Ficus* species, a super *Ceratosolen* wasp phylogeny (electronic supplementary material, appendix S1), including almost all *Ceratosolen* species with COI gene deposited in GenBank, was created. In total, of the 795 sequences of the 74 *Ceratosolen* species with COI sequences available in GenBank, 289 sequences of 72 species were used for the analysis. The 87 de novo sequenced sequences in this study were also included. Two *Ceratosolen* species—*C. vissali* (host *F. theophrastoides*) and *C. acutatus* (host *F. vogeliana*), which are taxonomically distantly related with *C. emarginatus* and *C. graveleyi* in a recent super wasp phylogeny [26]—were removed as their aligned sequence are too short (less than 400 bp). One to 10 sequences of each non-focal *Ceratosolen* species were maintained to achieve each species representation. Another two *Tetrapus* sequences were downloaded as outgroups. All 291 COI sequences (electronic supplementary material, appendix S5.1) were aligned in MEGA 5.0 [40]. The Bayesian tree was established using MrBAYES v. 3.1.2 [41] with GTR + G model selected as best-fit model by hierarchical likelihood ratio tests in jMODELTEST1.1 [42]. The Markov chain Monte Carlo analysis was run twice for 20 million generations, sampling a tree every 100 generations and excluding the first 10 000 corresponding to the burn-in period. The stationary distribution converged with a final standard deviation of split frequencies of 0.0105. Posterior probabilities were obtained for each given node of sampled trees. Based on the molecular identification results, wasp individuals that formed a well-supported monophyletic clade were taken as one pollinator species.

The mitochondrial gene (COI), a widely used barcode gene [43], can be effectively used for species identification of *Ceratosolen* fig wasps. Three cryptic species within *C. emarginatus* and two within *C. graveleyi*, which were collected from the same site (XTBG), were detected with either of mitochondrial COI gene and nuclear 28S gene in a recently published study [19]. That suggested the species identification using COI is unlikely to be confounded by issues such as genetic introgression of ancestral polymorphism. All COI sequences in that work were included herein and supported the former result.

(ii) Evaluation of pollinator sharing ratio

Pollinator sharing ratio between two fig taxa was calculated as total number of wasps in alternative host taxon divided by total number of wasps sampled in two fig taxa. Pollinator sharing events were counted not only among five focal fig taxa, but also among focal fig taxa and some non-focal fig taxa whose pollinator sequences were downloaded from GenBank.

(c) Gene flow among fig taxa

(i) Microsatellite genotyping and analyses

DNA extraction of 278 *Ficus* samples was performed using Plant Tissue extraction kit (Qiagen, Beijing, China). For the microsatellite analysis, 11 informative primer pairs, selected from 30 published primer pairs for *Ficus* species, were amplified and genotyped with amplification protocols shown in the electronic

supplementary material, table S2. Microsatellite alleles (electronic supplementary material, appendix S4) were genotyped using the ABI 3730 Sequencer with ROX 500 as the size standard (Applied Biosystems). Allele scoring was conducted using GENEMAPPER v. 3.2 software.

For species identification and determination of possible hybrids among five morphological fig taxa, Bayesian clustering analyses were performed using the STRUCTURE software [44]. Each individual was assigned to one or more ancestral species based on its genotype, and hybrids were identified as those with partial assignment to more than one ancestral species. Without considering prior species identifications, the most appropriate ancestral species number (K) was five, which was consistent with the morphological classification of the five fig taxa. Assuming $K = 5$, with prior information on species identification, the cluster analysis was performed independently for each fig taxon with the inter-taxa migration rate of $v = 0.1, 0.05$ and 0.01 separately to get the genetic structure of five figs.

BayesAss analysis was performed with its default settings to reconfirm the results from STRUCTURE analysis and to calculate the gene flow level among five figs [45]. BayesAss estimates the posterior probability distribution of an individual's ancestry and the recent immigration rates among populations (taxa) with Bayesian method and Markov chain Monte Carlo techniques. Each individual would be assigned as an immigrant from a specific population, a non-migrant, or an offspring of immigrant and non-migrant (hybrids) based on its genotype.

(ii) Fig phylogeny

A fig phylogeny was reconstructed to confirm the traditional classification of *F. auriculata* complex and the two varieties of *F. semicordata*, in which each group forms an independent monophyletic clade without a sister relationship. The ITS and G3pdh gene of several fig individuals of each taxon were de novo sequenced (accession numbers in Genbank: KP325577–KP325602) and a fig phylogeny (electronic supplementary material, figure S2 and appendix S2) was established together with published sequences of six other sympatric fig taxa (electronic supplementary material, appendix S5.2), which are sympatric, closely related fig taxa as suggested by recent *Ficus* phylogenetic studies [26,37] and morphological classification [34]. The procedure herein follows that in Xu *et al.*'s work [37].

(d) Floral volatiles variation of sympatric fig taxa

Floral volatiles produced during the receptive phase were collected *in situ* with the dynamic headspace technique following the method in Chen *et al.*'s work [27] at the Menglun study site between April 2011 and June 2012. Samples were analysed with gas chromatography–mass spectrometry (GC-MS), and compounds were identified and quantified following the procedures of Wang *et al.* [31]. Compounds were mainly identified by matching of mass spectra with the NIST 08 MS libraries, and by comparison of Kovats retention indices with that reported in the NIST chemistry Web Book (<http://webbook.nist.gov>) and published data. The pick area of each compound was used for quantification. The MS data were used for compound identification and GC data for quantification. Floral volatiles from 40 trees of five fig taxa (6–11 trees per fig taxon) were collected (electronic supplementary material, table S3). Volatile profile data of two varieties of *F. semicordata* have been published [31]. The comparison of fig volatile composition among samples was conducted with permutational multivariate analysis of variance (PERMANOVA) using the 'adonis' function of 'vegan' package [46] in R. Relative proportions of all compounds with respect to the total peak area were square root transformed and scaled by the standard deviations. A non-metric multidimensional scaling ordination (NMDS) was conducted with 'metaMDS' function, based on

the Bray–Curtis distance to obtain the best two-dimensional representation of the distance matrix of volatiles.

(e) Behavioural test of fig wasps to sympatric fig taxa

To check whether the wasp pollinators always chose the specific receptive phase floral volatiles released by their host fig taxa, we tested the preference of pollinators for volatiles from different fig taxa with both an experimental Y-tube test and field trapping experiments in the Menglun site. Figs trees for which the volatiles were collected or behaviour responding experiment were performed were genotyped. Two *F. oligodon* trees, for which volatiles were sampled, were evaluated as hybrids with Bayesian clustering analysis (electronic supplementary material, appendix S4 and table S3). Fig syconia of any evaluated hybrid trees were avoided in the behaviour responding experiment.

The Y-tube test was conducted from April 2011 to June 2012. The Y-tube olfactometer (stem 8 cm, arms 9 cm, at 55° angle, i.d. 1.5 cm) was used following the methods given by Chen *et al.* [27]. We trapped healthy adult female pollinators, emerging in the morning from mature male syconia of all five fig taxa, in mesh bags. Fresh receptive syconia of different fig taxa were used to represent the different scent source. To check the responses of pollinator species to receptive syconia of typical host and atypical host independently or simultaneously, three scent combinations were examined: receptive syconia of typical host versus air, receptive syconia of atypical host versus air and receptive syconia of typical host versus receptive syconia of atypical host. The setting is appropriate as many figs often coexist within a limited area without clear phenological divergence. As the quantity of volatile emission and size (diameter: 1.6–6 cm) of receptive syconia vary greatly among five fig taxa based on our preliminary experiment and observation, 1–16 fresh receptive syconia of five taxa were used in different treatments of Y-tube test to supply sufficient scent [27]. All tests were conducted within 3 h after the figs were removed from trees, between 09.00 and 11.00, and syconia were renewed every hour. Each test (one pollinator species to one scent combination) was replicated until 21–67 individuals of pollinators chose either of the scent sources. To exclude position bias, preliminary tests were performed to confirm a suitable position, in which similar numbers of fig wasp were responding evenly to the two chambers of Y-tube olfactometer. During the test, each pollinator individual was tested only one time and no more than five individuals emerging from one syconium were used. The total number of wasps choosing each of two scent sources was recorded. The unresponding fig wasp within 5 min were not included in the total number and have been excluded from the following statistical analysis. Two-sided exact binominal tests, with probability equal to 0.5, were used to determine whether each pollinator species prefers one of the two scent sources.

The field trapping was performed between April and May 2012 mainly following the protocol described by Ware & Compton [47]. Pollinators of three fig taxa (two varieties of *F. semicordata* and *F. hainanensis*) were tested. Two male trees in the receptive phase of each fig taxon were chosen as wasp resource. Sticky traps, consisting of a cylinder (8 cm radius, 18 cm length) covered with odourless yellow sticky insect plate and a mesh bag underside with fresh receptive syconia, were used to investigate the attractiveness of receptive syconia to pollinators. The sticky traps were placed at a height of 1.2 m, 4 m from its nearest fig tree and 3 m interval to nearest neighbour. For each tree, we used two duplications of four types of trap with receptive syconia from different figs: (A) typical host figs, (B) atypical host figs from same section, (C) atypical host figs from other section and (D) empty control. These were set with position order 'ABCDABCD' between 07.00 and 16.00. The trapping experiment for each fig species was conducted two to four times. No trees of the other four fig species in receptive or male flower phase occurred within a 50 m radius.

Similar volume of figs (*F. auriculata*, three figs; FSS or *F. hainanensis*, 15 figs; FSM, 200 figs) were used as odour sources in each trapping trial to avoid the potential influence of inter-taxa variation on fig size. The number of pollinating fig wasp trapped was taken as the focal pollinator, and a limited number of individuals with clear morphological differences were excluded. The possibility of some non-focal pollinators being trapped occasionally should be negligible, because of the relatively limited flying ability of dioecious fig pollinators and the rich population resources of focal wasps near the tested scent traps. Wilcoxon rank-sum test with Bonferroni correction was used to compare whether there is a difference in wasp attraction among receptive syconia of typical and atypical hosts.

3. Results

(a) Pollinator sharing in five fig taxa

Based on sequences acquired from GenBank and de novo sequenced in this study, pollinator sharing events were detected between the five focal fig taxa, and between the focal fig taxa and three non-focal sympatric fig taxa: *F. prostrata*, *F. tikoua* and *F. tsiangii*. When all *Ceratosolen* species were considered, 13.15% of wasp samples (38/289) were detected in alternate hosts. Most pollinator sharing occurred between figs from the same section. Figs of section *Sycomorus* were about five times more likely to share pollinators (pollinator sharing ratio 10.20–34.88%) compared with figs of section *Hemicardia* (two varieties of *F. semicordata*, *F. prostrata* and *F. tikoua*, pollinator sharing ratio: 2.04–4.76%; table 1; electronic supplementary material, figure S1). One case of pollinator sharing between distantly related figs was detected between two figs of *F. auriculata* complex and *F. tsiangii* (subgenus *Sycidium*), with pollinator sharing ratio of 3.03 and 6.25% (table 1; electronic supplementary material, figure S1).

Phylogenetic relationships among pollinators of five focal fig taxa detected from the phylogeny based on exclusively de novo sequences (electronic supplementary material, figure S3) were confirmed with the super-phylogeny of all *Ceratosolen* species recorded in GenBank (electronic supplementary material, figure S1), and the relationship was also congruent with the recent publication of wasp phylogeny [26]. At least six monophyletic clades of focal pollinators were detected with both the de novo sequences and sequences downloaded from GenBank (electronic supplementary material, figure S1; table 1). Pairwise P-distance among the six wasp clades (5.1–11.4%) was markedly greater than within-clade distance (0.1–1.9%; electronic supplementary material, table S4). Three downloaded sequences of wasps from *F. semicordata* var. *semicordata*, *F. oligodon* and *F. hainanensis* were spread out in the wasp phylogeny, suggesting that more pollinator sharing events may exist among distantly related figs (electronic supplementary material, figure S1). *Ficus semicordata* var. *semicordata* and *F. auriculata* complex belong to different sections and form two distant and distinct phylogenetic clades, while their pollinators, *C. graveleyi* and *C. emarginatus*, are two sister species (electronic supplementary material, figures S1–S3), suggesting a pollinator shift event between them. It is unlikely that geographical factors influence the wasp phylogeny, since the pollinators sampled from the same host fig taxa at different sites were often found within the same clade (electronic supplementary material, figure S3).

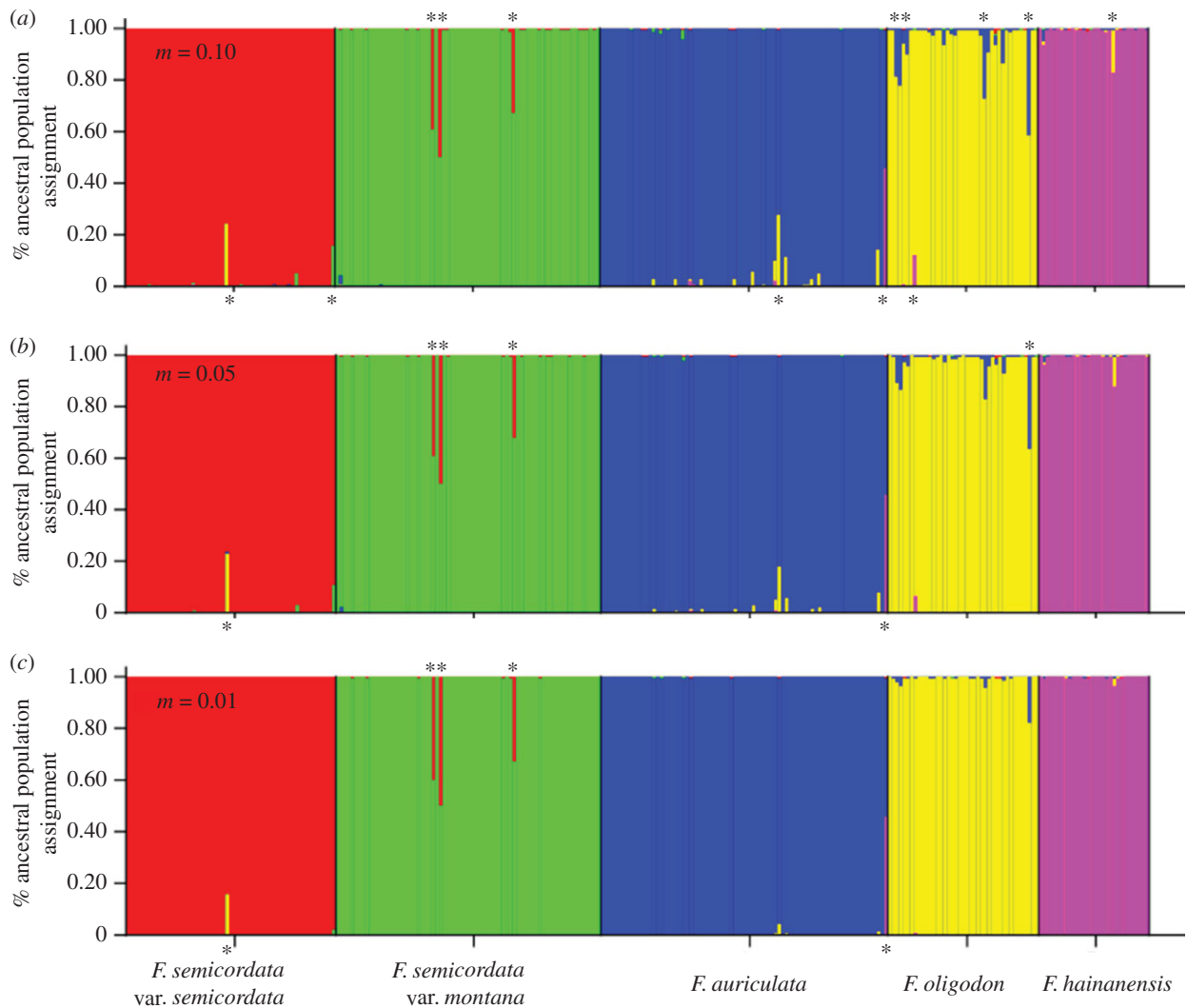


Figure 1. Barplot of ancestral population assignments for 278 individuals of five fig taxa from Bayesian clustering analysis in STRUCTURE software when $K = 5$. Prior population information based on morphological identification was used and assumed migration rates are (a) 0.1, (b) 0.05 and (c) 0.01. Bars with different colours indicate hybrids.

Table 1. Frequency of associations between sympatric *Ficus* species and *Ceratosolen* species based on sequences of all *Ceratosolen* taxa with COI sequences recorded in GenBank. Data include the total number of wasps sampled (N_{ind}), number of wasp species observed on the fig taxon (N_{sp}), number of wasps collected from the common host figs (N_{com}) and number of wasps collected from alternate figs other than common host figs (N_{alter}) of each fig taxon. Number of species (N_{sp}) responding to number of main phylogeny clades with same colour tips in the electronic supplementary material, figure S1. Pollinator sharing ratio between two figs was calculated as total number of wasps in alternative host taxon (N_{alter}) divided by total number of wasps sampled in two fig taxa (N_{ind}). FSM, *Ficus semicordata* var. *montana*; FSS, *F. semicordata* var. *semicordata*; FA, *F. auriculata*; FO, *F. oligodon*; FH, *F. hainanensis*; FP, *F. prostrata*; FT, *F. tikoua*; FTS, *F. tsiangii*. See the electronic supplementary material, figure S1 for more information.

<i>Ficus</i>	dominator pollinator	N_{ind}	N_{sp}	N_{com}	N_{alter} (alternative figs)	pollinator sharing ratio (24.52%: 38/155)
FSS	<i>C. gravey</i> clade 1–2	47	3	45	1 (FP); 1 (FT)	FSS–FP: 2.04% (1/49); FSS–FT: 2.08% (1/48)
FSM	<i>Ceratosolen</i> sp.	21	1	20	1 (FP)	FSM–FP: 4.76% (1/21)
FA	<i>C. emarginatus</i> clade 1	27	3	17	8 (FO); 2 (FH)	FA–FO: 34.88% (15/43); FA–FH: 20% (12/60)
FO	<i>C. emarginatus</i> clade 2	16	4	8	7 (FA); 1 (FTS)	FO–FTS: 6.25% (1/16)
FH	<i>C. emarginatus</i> clade 3	33	3	16	11 (FA); 5 (FO); 1 (FTS)	FH–FO: 10.20% (5/49); FH–FTS: 3.03% (1/33)

(b) Hybridization and gene flow among five fig taxa

Thirteen of the 278 fig individuals (4.68%) were identified as hybrids with high posterior probability in STRUCTURE analysis with an assumed migration rate of 0.10, including six F_1 hybrids and seven F_2 hybrids. Five of these hybrids were detected in all

tested migration rates (0.10, 0.05, 0.01; figure 1). Eight of the identified hybrids were confirmed with the BayesAss analysis results (electronic supplementary material, table S6). Pairwise migration rates among the five fig taxa were asymmetric and vary from 0.40 to 9.61% (figure 2). The highest estimated

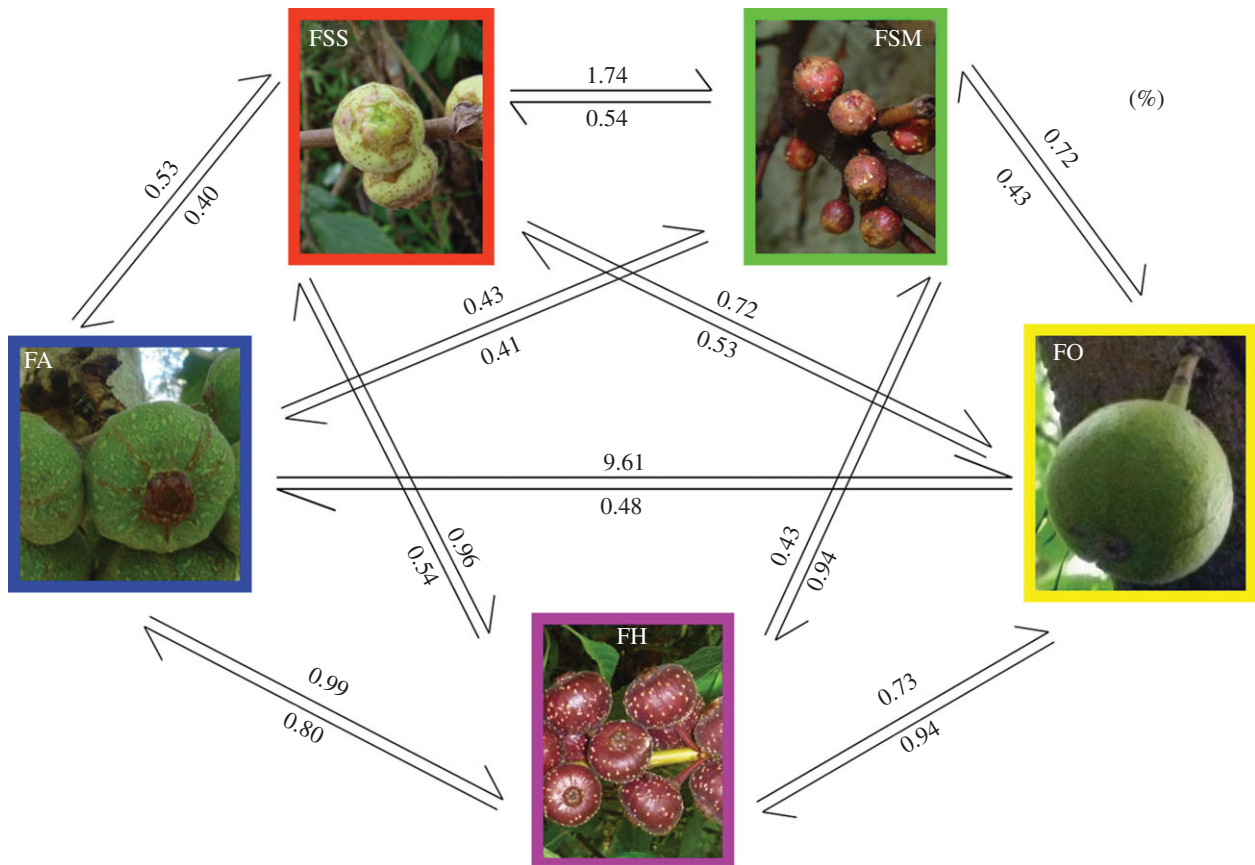


Figure 2. Percentage pairwise migration rates for five sympatric morphologically distinct fig taxa estimated in BayesAss. For species abbreviations, see caption of table 1. For detailed migration rates, see the electronic supplementary material, table S7.

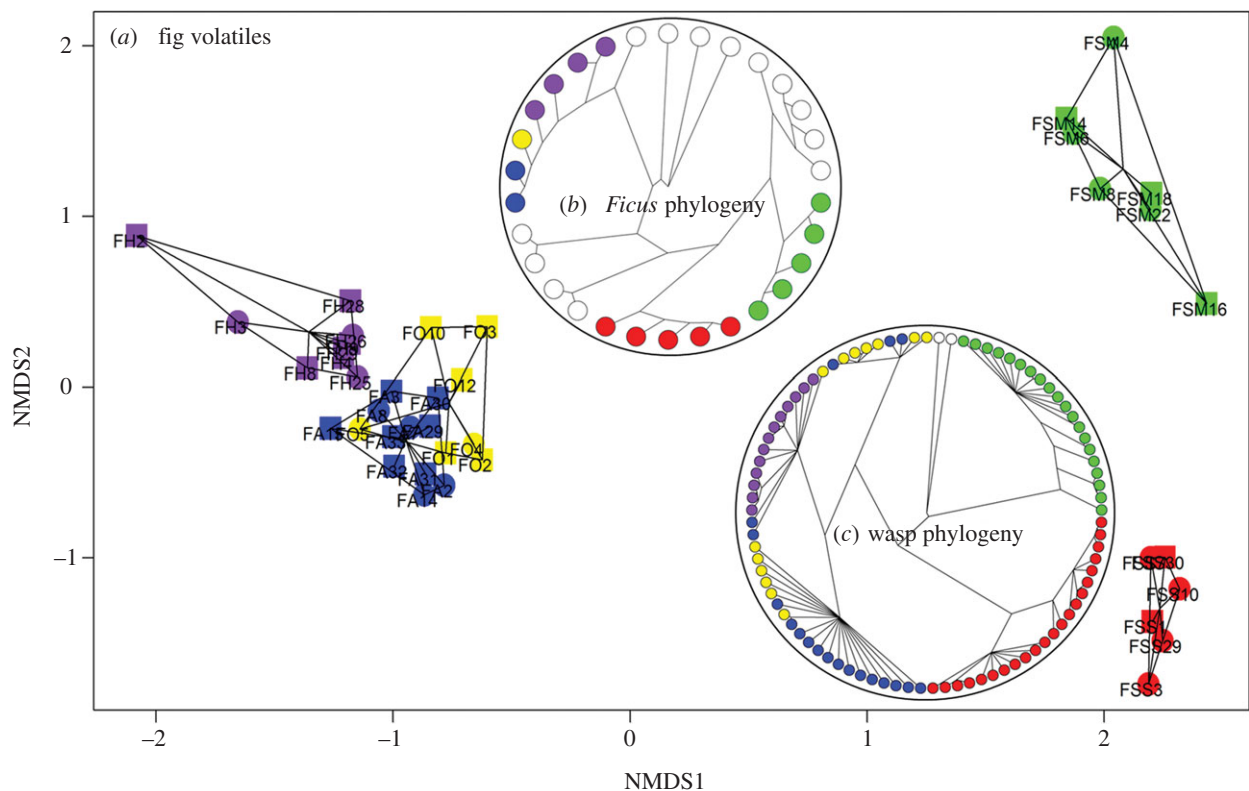


Figure 3. (a) Non-metric multidimensional scaling of receptive floral volatiles of five fig taxa based on Bray–Curtis dissimilarity index (stress = 0.081). (b) *Ficus* phylogeny and (c) fig wasp phylogeny were included. *F. auriculata*, FA, blue; *F. oligodon*, FO, yellow; *F. hainanensis*, FH, purple; *F. semicordata* var. *montana*, FSM, green; *F. semicordata* var. *semicordata*, FSS, red. (a) Symbols of each point correspond to the individual labels of figs in the genetic structure analysis; the circle symbol means male tree and square means female tree. White circle in (b) and (c) means other species or outgroup. For detailed phylogenetic information of (b) and (c), see the electronic supplementary material, appendices S2 and S3.

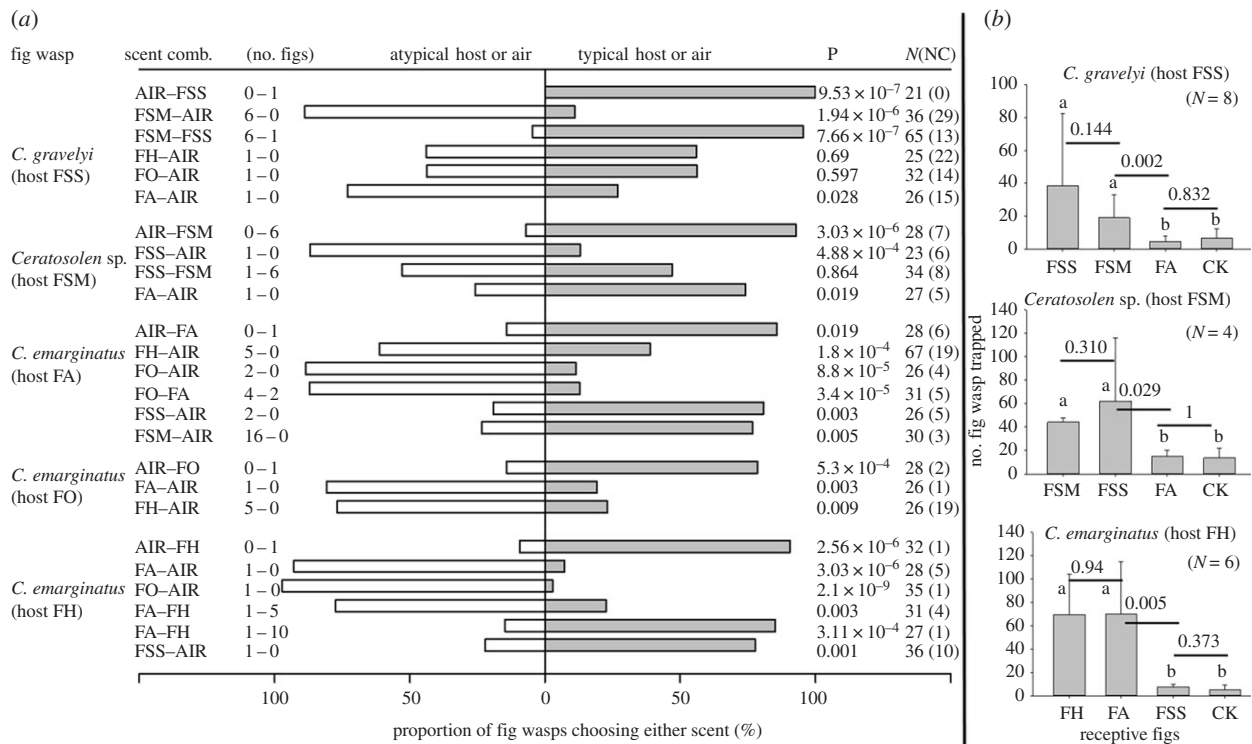


Figure 4. Behavioural responses of female *Ceratosolen* fig wasps to olfactory stimuli in (a) Y-tube test and (b) field trapping experiment. Fresh figs in the receptive phase of each fig taxon were used as odour sources. Behaviour choice to scent combinations in Y-tube test are as follows: typical host versus air, atypical host versus air and typical host versus atypical host (binomial test). Species abbreviations: see caption of table 1. Air: purified air; N: number of fig wasps responding to either odour; NC: number of unresponding fig wasps and not counted. CK = empty bag. One to sixteen fig syconia were used in Y-tube test to supply enough scent. Means are not significantly different if bars share the same letters in (b) (Wilcoxon rank-sum test).

migration rates were found between taxa from the same section, such as from *F. auriculata* to *F. oligodon* at 9.61% (0.48% inversely), and from FSS to FSM at 1.74% (0.54% inversely). Recent migration was also detected among fig taxa from different sections, such as from two varieties of *F. semicordata* to *F. hainanensis* at the rate of 0.94–0.96% (figure 2; electronic supplementary material, table S7).

(c) Floral volatiles signature of five fig taxa

Floral volatile signatures among fig taxa from the same section are more similar and have more common compounds than among fig taxa from different sections (figure 3a; electronic supplementary material, table S8). The highest similarity in floral volatile signatures occurs between *F. auriculata* and *F. oligodon*, with 34 out of 48 compounds shared, and without significant difference in floral volatile profiles (PERMANOVA, $F = 1.891$, $p = 0.062$). *Ficus hainanensis* has relatively independent floral volatile structure within *F. auriculata* complex (PERMANOVA, $F = 1.971$, $p = 0.026$ with *F. auriculata* and $F = 3.81$, $p = 0.001$ with *F. oligodon*). Floral volatile signatures of two varieties of *F. semicordata* are nicely separated (PERMANOVA, $F = 14.59$, $p = 0.002$), while still sharing most of their main compounds, such as 4-methylanisole, β -caryophyllene and α -caryophyllene. Their volatile compositions are distantly related to that of species from *F. auriculata* complex (PERMANOVA, $F = 13.214$, $p = 0.001$; electronic supplementary material, table S8).

(d) Response of fig wasps to floral volatile signatures of different fig taxa

While pollinator species were primarily attracted by floral volatiles of their typical hosts, they were also attracted by at

least one atypical host from either within the same section or in different sections, as demonstrated by both experimental Y-tube preference tests (figure 4a) and field trapping observations (figure 4b). The attractiveness of floral volatiles to pollinators is asymmetric (e.g. the pollinator of *F. hainanensis* was also attracted to *F. auriculata*, but not vice versa; figure 4a). Some pollinator species were unable to distinguish the floral volatiles of typical host figs from those of atypical host figs, such as *Ceratosolen* sp. (host FSM) to two varieties of *F. semicordata* in a Y-tube preference test (figure 4a) and field trapping experiments (figure 4b). In addition, the host recognition ability of *C. emarginatus* (host FH) depends on the quantity of volatile emission when facing selection between a typical host (*F. hainanensis*) and an atypical host (*F. auriculata*) in a Y-tube test (figure 4a). The variation of the proportion of unresponding wasps when confronted with the atypical or typical host is noticeable among five pollinators in a Y-tube test (figure 4a). Behaviour choice between atypical host fig volatiles and empty air shows that most pollinators showed no preference or even repulsion to floral volatiles of atypical hosts from other sections, except *C. graveleyi* (host FSS), which is attracted to *F. auriculata*, but not vice versa (figure 4a).

4. Discussion

The importance of occasional and persistent gene flow during the process of diversification and speciation has been demonstrated in many different groups [2–3,5]. Figs and their pollinating fig wasps perhaps represent an extreme case of an obligate pollination system in plant–insect coevolution [26]. However, the species-specific pollination relationship has been repeatedly challenged [17,19,29,32]. Here, we

combined observational and experimental methods (molecular genetics, chemical composition and pollinator preference tests) for the first time to examine the prevalence of pollinator sharing and interspecific gene flow within a suite of sympatric and closely related fig taxa. We demonstrate that pollinator sharing and inter-taxa gene flow are rather common, and the inter-taxa similarity of fig floral volatile signatures during the receptive phase and the floral volatiles' attractiveness to multiple pollinators might be the main reasons for the patterns.

(a) Inter-taxa pollinator sharing of figs

Previous studies have reported a significant amount of pollinator sharing in monoecious figs [13,17,19,29], while pollinator sharing in dioecious figs was predicted to be rather low [19,28]. However, we found that all five dioecious fig taxa shared a pollinator with at least one other fig taxon with a total pollinator sharing ratio of 13.15% (table 1), suggesting pollinator sharing among dioecious figs does occur frequently. The degree of pollinator sharing was correlated with the relatedness of the fig taxa, with higher frequency among fig taxa within same section, but rarely among figs from different sections. Although pollinator sharing has been reported among figs within numerous sections (*Sycocarpus*, *Pharmacosyceae*, *Galoglychia* and *Americana*) in previous studies [19], only this study experimentally compared the ratio of taxa within same section and that from different sections.

The pollinator sharing ratio may be overestimated with wasps collected from receptive syconia if wasps have lower survival on 'non-typical' hosts, compared with the ratio calculated based on wasps emerging from syconia in the male flower phase [14]. The high pollinator sharing ratio within *F. auriculata* complex may be partially explained by this reason, as all pollinators of FA and half the pollinators of FO were collected from receptive syconia (electronic supplementary material, table S1). However, the pollinator sharing ratio herein should be accurate, as pollinators emerging from each fig taxon of *F. auriculata* complex could develop and mature well in other two atypical host fig taxa without a significant decrease in offspring fitness [36].

(b) Floral volatile signature and its role in pollinator specificity

Our experimental evidence suggests that the relationship between the chemical composition of the floral volatiles and pollinator choice is more complicated than has been generally proposed [10–11]. Floral volatiles produced by each fig taxon did primarily attract its obligate pollinator and rarely attracted pollinators of fig taxa from other sections. However, we observed clear evidence that floral volatiles of each fig taxon could attract multiple pollinators (figure 4). These results indicate that pollinator specificity, even among this most strict case of dioecious obligate mutualists [28], is not completely fixed, and that pollinator sharing among closely related sympatric figs could be rather common. The attraction of specific floral volatiles to other atypical pollinators is understandable, as the specificity of floral volatiles is usually achieved by quantitative differences in relative proportions of many common compounds, and some compounds that actively attract certain pollinators may occur in volatiles of several plants [15]. This flexibility in host preference may also provide an explanation for the atypical pollinators captured in earlier trapping studies

[30,47]. Floral volatiles between two sympatric figs, *F. auriculata* and *F. oligodon*, were notably similar and shared at least three pollinator species, with the highest pollinator sharing ratio being 34.88% (table 1; electronic supplementary material, table S5 and figure S1). Floral volatile similarity and pollinator sharing pattern has also been reported in two African monoecious figs [17]. These patterns indicate that the higher degree of pollinator sharing among closely related plants is largely due to the similarity of floral volatile signatures and plasticity of the pollinator's preference.

Similar patterns have also been observed in other obligate pollination systems. For sexually deceptive orchids, some plants use almost the same volatiles and attract the same pollinators [18]. Many orchid species depend upon a single male euglossine bee species for pollination [48]. Seven *Yucca* plants from section *Chaenocarpa* even share one moth pollinator and at least three of them show similar floral volatiles [49]. Species-specific floral volatiles, though, as the guarantee of pollinator specificity, also seem to facilitate the pollinator sharing among sympatric plants among closely related plants in obligate pollination systems.

Although multiple wasp taxa may have been attracted to one host fig taxon in the behaviour test, the impact of this behaviour on the main conclusion could be negligible. In a field trapping experiment, pollinators of monoecious fig taxa can fly very long distances, in comparison with pollinators of dioecious fig taxa, which typically fly short distances [50]. On rare occasions, potential pollinators of monoecious figs had been detected and excluded from analysis. Furthermore, no sources of fig wasps (fig trees in receptive or male flower phase) of the other four fig taxa were within 50 m of the tree sampled during the trapping experiment, while rich population sources of focal wasps existed only 4 m away from the tested scent traps. So the possibility of other non-focal wasps being attracted in field trapping should be rare, and unlikely to affect interpretations of the behaviour test.

(c) Inter-taxa gene flow and hybrids

Corresponding to the frequent pollinator sharing, we observed a significant number of hybrids (13/278) and a high level of inter-taxa gene flow (migration rate up to 9.61%) among the five dioecious fig taxa in our study. The hybridization and introgression happen not only among fig taxa from the same section but also among taxa from different sections (figures 1 and 2; electronic supplementary material, table S6). Similar to the pattern of floral volatiles and pollinator sharing, gene flow was highest among figs from the same section (migration rate of 1.74% for two varieties of *F. semicordata* and 9.61% for *F. auriculata* to *F. oligodon*). Figs with more similar floral volatiles (*F. auriculata* and *F. oligodon*) had higher frequencies of pollinator sharing and higher levels of inter-taxa gene flow, suggesting a causal relationship among similarity of floral volatiles, pollinator sharing and inter-taxa gene flow of *Ficus* plants. To the best of our knowledge, this is the first study that has shown the connection among the three factors in a fig–fig wasp system. Strong pollinator isolation and weak post-zygotic isolation are a general pattern in many obligate pollination systems; in some cases, no obvious fitness reduction in hybrid seeds was observed in the seed and seedling growth phases [14,24,31]. Recent and early natural hybridization accompanied by pollinator sharing or pollinator

shifts, as shown here and in previous studies [13,14,23,32,33], suggest hybridization and introgression among symmetric fig species should be a natural outcome of pollinator sharing.

The surprisingly high level of pollinator sharing and relatively lower level of genetic introgression among five fig taxa suggests that, beside pollinator isolation, some other barriers possibly maintain the integrity of each fig taxon [22–23]. Based on our field surveys, habitat isolation could be one of the most important isolating barriers. Two varieties of *F. semicordata* clearly have light niche divergence, with FSS preferring an open, light-rich area, while FSM prefers closed primary rainforest or valley sides. Three fig taxa in *F. auriculata* complex show different preferences in soil type and elevation range, with *F. auriculata* preferring rich soils at low elevation, while *F. oligodon* and *F. hainanensis* prefer limestone-rich soil, the former at higher elevation while the latter prefers more moist areas [23]. Habitat specialization may have limited the inter-taxon gene flow mediated by pollinator sharing through outbreeding depression. Asymmetrical gene flow may also be explained partially by the unidirectional hybrid depression [5]. Additionally, some degree of flowering phenology isolation may partially contribute to the establishment of reproductive isolation [23,51].

5. Conclusion

Using experimental, field observation, chemical comparison and molecular DNA evidence, we demonstrate that pollinator sharing and gene flow among sympatric fig taxa are relatively common and potentially mediated by chemical signals in the floral volatiles. These findings may not be limited to the fig–fig wasp systems. The evolutionary relationships between plant species and their obligate pollinators vary among different

obligate pollination systems (e.g. *Epicephala*–Phyllanthaceae [52]; euglossine bees–fragrance orchids [48]; sexually deceptive orchids [11]). However, plants in all such systems mainly depend on specific floral volatiles for establishing relatively strict but not absolute pollinator specificity (thus pre-zygotic isolation) among sympatric plant species [6,7,10,24]. It is likely that a certain degree of pollinator sharing and gene flow among sympatric closely related plants also exists in those obligate pollination systems. How the frequency and extent of hybridization contributes to the diversification of the two partners in these coevolutionary relationships will be the next important question to address.

Data accessibility. Supplementary material can be found in the online version of this article and the Dryad data repository at <http://data.dryad.org>, <http://dx.doi.org/10.5061/dryad.f1d5h>.

Authors' contributions. G.W. and J.C. designed the study. G.W. collected data and conducted the data analyses. G.W., J.C. and C.H.C. wrote the manuscript. All authors gave final approval for publication.

Competing interests. We have no competing interests.

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References

- Darwin CR. 1862 *On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing*. London, UK: John Murray.
- Mallet J. 2005 Hybridization as an invasion of the genome. *Trends Ecol. Evol.* **20**, 229–237. (doi:10.1016/j.tree.2005.02.010)
- Schumer M, Rosenthal GG, Andolfatto P. 2014 How common is homoploid hybrid speciation? *Evolution* **68**, 1553–1560. (doi:10.1111/Evo.12399)
- Wagner Jr W. 1970 Biosystematics and evolutionary noise. *Taxon* **19**, 146–151. (doi:10.2307/1217945)
- Abbott R *et al.* 2013 Hybridization and speciation. *J. Evol. Biol.* **26**, 229–246. (doi:10.1111/j.1420-9101.2012.02599.x)
- Ackerman JD. 1983 Specificity and mutual dependency of the orchid–euglossine bee interaction. *Biol. J. Linn. Soc.* **20**, 301–314. (doi:10.1111/j.1095-8312.1983.tb01878.x)
- Kawakita A. 2010 Evolution of obligate pollination mutualism in the tribe Phyllanthaceae (Phyllanthaceae). *Plant Spec. Biol.* **25**, 3–19. (doi:10.1111/j.1442-1984.2009.00266.x)
- Weiblen GD. 2002 How to be a fig wasp. *Annu. Rev. Entomol.* **47**, 299–330. (doi:10.1146/annurev.ento.47.091201.145213)
- Ayasse M, Gogler J, Stokl J. 2010 Pollinator-driven speciation in sexually deceptive orchids of the genus *Ophrys*. In *Evolution in action: case studies in adaptive radiation, speciation and the origin of biodiversity* (ed. M Glaubrecht), pp. 101–118. New York, NY: Springer.
- Hossaert-McKey M, Soler C, Schatz B, Proffitt M. 2010 Floral scents: their roles in nursery pollination mutualisms. *Chemoecology* **20**, 75–88. (doi:10.1007/s00049-010-0043-5)
- Schiestl FP, Schluter PM. 2009 Floral isolation, specialized pollination, and pollinator behavior in orchids. *Annu. Rev. Entomol.* **54**, 425–446. (doi:10.1146/annurev.ento.54.110807.090603)
- Okamoto T, Kawakita A, Kato M. 2007 Interspecific variation of floral scent composition in *Glochidion* and its association with host-specific pollinating seed parasite (*Epicephala*). *J. Chem. Ecol.* **33**, 1065–1081. (doi:10.1007/s10886-007-9287-0)
- Machado CA, Robbins N, Gilbert MTP, Herre EA. 2005 Critical review of host specificity and its coevolutionary implications in the fig/fig-wasp mutualism. *Proc. Natl Acad. Sci. USA* **102**, 6558–6565. (doi:10.1073/pnas.0501840102)
- Moe AM, Weiblen GD. 2012 Pollinator-mediated reproductive isolation among dioecious fig species (*Ficus*, Moraceae). *Evolution* **66**, 3710–3721. (doi:10.1111/j.1558-5646.2012.01727.x)
- Raguso RA. 2008 Wake up and smell the roses: the ecology and evolution of floral scent. *Annu. Rev. Ecol. Syst.* **39**, 549–569. (doi:10.1146/annurev.ecolsys.38.091206.095601)
- Soler C, Hossaert-McKey M, Buatois B, Bessiere JM, Schatz B, Proffitt M. 2011 Geographic variation of floral scent in a highly specialized pollination mutualism. *Phytochemistry* **72**, 74–81. (doi:10.1016/j.phytochem.2010.10.012)
- Cornille A, Underhill JG, Cruaud A, Hossaert-McKey M, Johnson SD, Tolley KA, Kjellberg F, van Noort S, Proffitt M. 2012 Floral volatiles, pollinator sharing and diversification in the fig-wasp mutualism: insights from *Ficus natalensis*, and its two wasp pollinators (South Africa). *Proc. R. Soc. B* **279**, 1731–1739. (doi:10.1098/rsob.2011.1972)

18. Stokl J, Paulus H, Dafni A, Schulz C, Francke W, Ayasse M. 2005 Pollinator attracting odour signals in sexually deceptive orchids of the *Ophrys fusca* group. *Plant Syst. Evol.* **254**, 105–120. (doi:10.1007/s00606-005-0330-8)
19. Yang LY, Machado CA, Dang XD, Peng YQ, Yang DR, Zhang DY, Liao WJ. 2015 The incidence and pattern of co-pollinator diversification in dioecious and monoecious figs. *Evolution* **69**, 294–304. (doi:10.1111/evo.12584)
20. Starr TN, Gadek KE, Yoder JB, Flatz R, Smith CI. 2013 Asymmetric hybridization and gene flow between Joshua trees (Agavaceae: *Yucca*) reflect differences in pollinator host specificity. *Mol. Ecol.* **22**, 437–449. (doi:10.1111/mec.12124)
21. Zhang J, Wang SX, Li HH, Hu BB, Yang XF, Wang ZB. 2012 Diffuse coevolution between two *Epicephala* species (Gracillariidae) and two *Breynia* species (Phyllanthaceae). *PLoS ONE* **7**, e41657. (doi:10.1371/journal.pone.0041657)
22. Kiestner AR, Lande R, Schemske DW. 1984 Models of coevolution and speciation in plants and their pollinators. *Am. Nat.* **124**, 220–243. (doi:10.1086/284265)
23. Wei ZD, Kobmoo N, Cruaud A, Kjellberg F. 2014 Genetic structure and hybridization in the species group of *Ficus auriculata*: can closely related sympatric *Ficus* species retain their genetic identity while sharing pollinators? *Mol. Ecol.* **23**, 3538–3550. (doi:10.1111/mec.12825)
24. Xu SQ, Schluter PM, Scoope G, Breitkopf H, Gross K, Cozzolino S, Schiestl FP. 2011 Floral isolation is the main reproductive barrier among closely related sexually deceptive orchids. *Evolution* **65**, 2606–2620. (doi:10.1111/j.1558-5646.2011.01323.x)
25. Stevens P. 2001 Angiosperm phylogeny website. Version 12, July 2012. See <http://www.mobot.org/MOBOT/research/APweb/>.
26. Cruaud A *et al.* 2012 An extreme case of plant-insect co-diversification: figs and fig-pollinating wasps. *Syst. Biol.* **61**, 1029–1047. (doi:10.1093/sysbio/sys068)
27. Chen C, Song QS, Proffitt M, Bessiere JM, Li ZB, Hossaert-Mckey M. 2009 Private channel: a single unusual compound assures specific pollinator attraction in *Ficus semicordata*. *Funct. Ecol.* **23**, 941–950. (doi:10.1111/j.1365-2435.2009.01622.x)
28. Moe AM, Rossi DR, Weiblen GD. 2011 Pollinator sharing in dioecious figs (*Ficus*: Moraceae). *Biol. J. Linn. Soc.* **103**, 546–558. (doi:10.1111/j.1095-8312.2011.01669.x)
29. McLeish MJ, van Noort S. 2012 Codivergence and multiple host species use by fig wasp populations of the *Ficus* pollination mutualism. *BMC Evol. Biol.* **12**, 1. (doi:10.1186/1471-2148-12-1)
30. Grison-Pige L, Bessiere JM, Hossaert-Mckey M. 2002 Specific attraction of fig-pollinating wasps: role of volatile compounds released by tropical figs. *J. Chem. Ecol.* **28**, 283–295. (doi:10.1023/A:1017930023741)
31. Wang G, Compton SG, Chen J. 2013 The mechanism of pollinator specificity between two sympatric fig varieties: a combination of olfactory signals and contact cues. *Ann. Bot.* **111**, 173–181. (doi:10.1093/aob/mcs250)
32. Renoult JP, Kjellberg F, Grout C, Santoni S, Khadari B. 2009 Cyto-nuclear discordance in the phylogeny of *Ficus* section *Galoglychia* and host shifts in plant-pollinator associations. *BMC Evol. Biol.* **9**, 248. (doi:10.1186/1471-2148-9-248)
33. Parrish TL, Koelewijn HP, van Dijk PJ, Kruijt M. 2003 Genetic evidence for natural hybridization between species of dioecious *Ficus* on island populations. *Biotropica* **35**, 333–343. (doi:10.1111/j.1744-7429.2003.tb00587.x)
34. Berg CC. 2004 Flora Malesiana precursor for the treatment of Moraceae 6: *Ficus* subgenus *sycomorus*. *Blumea* **49**, 155–200. (doi:10.3767/000651904X486278)
35. Amatya SM. 1996 A new variety of *Ficus semicordata* (Moraceae) from Nepal. *Novon* **6**, 323–324. (doi:10.2307/3392035)
36. Yang P, Li ZB, Peng YQ, Yang DR. 2012 Exchange of hosts: can Agaonid fig wasps reproduce successfully in the figs of non-host *Ficus*? *Naturwissenschaften* **99**, 199–205. (doi:10.1007/s00114-012-0885-5)
37. Xu L, Harrison RD, Yang P, Yang D-R. 2011 New insight into the phylogenetic and biogeographic history of genus *Ficus*: vicariance played a relatively minor role compared with ecological opportunity and dispersal. *J. Syst. Evol.* **49**, 546–557. (doi:10.1111/j.1759-6831.2011.00155.x)
38. Wiebes JT, Vereniging NE. 1963 *Taxonomy and host preferences of Indo-Australian fig wasps of the genus Ceratosolen (Agaonidae)*. Amsterdam, The Netherlands: Nederl. entomol. vereen.
39. Weiblen GD. 2001 Phylogenetic relationships of fig wasps pollinating functionally dioecious *Ficus* based on mitochondrial DNA sequences and morphology. *Syst. Biol.* **50**, 243–267. (doi:10.1080/713849615)
40. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011 MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739. (doi:10.1093/molbev/msr121)
41. Huelsenbeck JP, Ronquist F. 2001 MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* **17**, 754–755. (doi:10.1093/bioinformatics/17.8.754)
42. Posada D. 2008 jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**, 1253–1256. (doi:10.1093/molbev/msn083)
43. Hebert PDN, Cywinka A, Ball SL, DeWaard JR. 2003 Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B* **270**, 313–321. (doi:10.1098/rspb.2002.2218)
44. Pritchard JK, Stephens M, Donnelly P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
45. Wilson GA, Rannala B. 2003 Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**, 1177–1191.
46. Oksanen J *et al.* 2011 vegan: Community ecology package. R package version 2.0-2. See <http://cran.r-project.org/package=vegan>.
47. Ware AB, Compton SG. 1994 Responses of fig wasp to host-plant volatile cues. *J. Chem. Ecol.* **20**, 785–802. (doi:10.1007/BF02059613)
48. Ramirez SR, Eltz T, Fujiwara MK, Gerlach G, Goldman-Huertas B, Tsutsui ND, Pierce NE. 2011 Asynchronous diversification in a specialized plant-pollinator mutualism. *Science* **333**, 1742–1746. (doi:10.1126/science.1209175)
49. Svensson GP, Pellmyr O, Raguso RA. 2011 Pollinator attraction to volatiles from virgin and pollinated host flowers in a yucca/moth obligate mutualism. *Oikos* **120**, 1577–1583. (doi:10.1111/j.1600-0706.2011.19258.x)
50. Harrison RD, Rasplus JY. 2006 Dispersal of fig pollinators in Asian tropical rain forests. *J. Trop. Ecol.* **22**, 631–639. (doi:10.1017/S0266467406003488)
51. Kuaraksa C, Elliott S, Hossaert-Mckey M. 2012 The phenology of dioecious *Ficus* spp. tree species and its importance for forest restoration projects. *Forest. Ecol. Manag.* **265**, 82–93. (doi:10.1016/j.foreco.2011.10.022)
52. Hembry DH, Kawakita A, Gurr NE, Schmaedick MA, Baldwin BG, Gillespie RG. 2013 Non-congruent colonizations and diversification in a coevolving pollination mutualism on oceanic islands. *Proc. R. Soc. B* **280**, 20130361. (doi:10.1098/rspb.2013.0361)