Original article

The occurrence of fig wasps in the fruits of female gynodioecious fig trees

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ABSTRACT

Fig trees are pollinated by wasp mutualists, whose larvae consume some of the plant's ovaries. Many fig species (350+) are gynodioecious, whereby pollinators generally develop in the figs of 'male' trees and seeds generally in the 'females.' Pollinators usually cannot reproduce in 'female' figs at all because their ovipositors cannot penetrate the long flower styles to gall the ovaries. Many non-pollinating fig wasp (NPFW) species also only reproduce in figs. These wasps can be either phytophagous gallers or parasites of other wasps. The lack of pollinators in female figs may thus constrain or benefit different NPFWs through host absence or relaxed competition. To determine the rates of wasp occurrence and abundance we surveyed 11 dioecious fig species on Hainan Island, China, and performed subsequent experiments with Ficus tinctoria subsp. gibbosa to identify the trophic relationships between NPFWs that enable development in female syconia. We found NPFWs naturally occurring in the females of Ficus auriculata, Ficus hainanensis and F. tinctoria subsp. gibbosa. Because pollinators occurred only in male syconia, when NPFWs also occurred in female syconia, overall there were more wasps in male than in female figs. Species occurrence concurred with experimental data, which showed that at least one phytophagous galler NPFW is essential to enable multiple wasp species to coexist within a female fig. Individuals of galler NPFW species present in both male and female figs of the same fig species were more abundant in females than in males, consistent with relaxed competition due to the absence of pollinator. However, these wasps replaced pollinators on a fewer than one-to-one basis, inferring that other unknown mechanisms prevent the widespread exploitation by wasps of female figs. Because some NPFW species may use the holes chewed by pollinator males to escape from their natal fig, we suggest that dispersal factors could be involved.

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1. Introduction

Each of the 750+ species of fig tree (Ficus, Moraceae) has an obligate mutualism with its pollinating wasps (Agaonidae) (Janzen, 1979; Weiblen, 2002; Herre et al., 2008). The trees can be divided roughly equally into species that are monoecious and those that are functionally dioecious (gynodioecy) (Janzen, 1979; Cook and Rasplus, 2003). This is mainly based on the arrangement of the unisexual florets within the enclosed inflorescences (syconia, colloquially 'figs' and often referred to as 'fruits'), and their occurrence on individual trees. In monoecious species the pollinating wasps carry pollen from their natal syconium to pollinate the female flowers in a syconium on another tree. They also lay their eggs individually into the ovaries of some female flowers in the same syconium. Ovaries containing eggs develop into galls on which the larvae feed, whereas those that are pollinated and do not receive eggs become seeds. In dioecious species the male and female reproductive function occurs on different trees. This is because gynodioecy in Ficus has probably evolved, at least in part, to resolve the conflict between tree and pollinator about how many ovaries are allocated to either wasp or seed production (Cook and Rasplus, 2003; Harrison and Yamamura, 2003). Wasps and pollen are thus produced by 'male' trees and seeds produced by 'female' trees.

In additional to their pollinators, all Ficus support a suite of non-pollinating fig wasp (NPFW) species that also only use Ficus syconia

in which to reproduce (Weiblen, 2002; Cook and Rasplus, 2003; Herre et al., 2008). Unlike the pollinators, most NPFWs oviposit from outside the syconium and thus do not transfer pollen (but see Jousselin et al., 2001; Al-Beidh et al., in press for reports on internally ovipositing non-pollinating fig wasps). Externally ovipositing NPFWs can be broadly categorized into four groups: (1) large wasps with phytophagous larvae that develop in galls initiated from syconium wall tissue and (2) their similar sized parasitoids, (3) wasps of a similar small size as the pollinators with phytophagous larvae that gall individual ovaries (or seeds; Pereira et al., 2007) within the syconium, and (4) small parasites (parasitoids or inquilines) of pollinators or other small NPFWs (West et al., 1996; Cook and Rasplus, 2003; Elias et al., 2012). Up to 30 NPFW species can be associated with a single monoecious Ficus species (Compton and Hawkins, 1992). By comparison, fewer NPFW species are associated with dioecious Ficus species, and they have only rarely been recorded as developing successfully in the syconia of 'female' trees (Kerdulhué and Rasplus, 1996a; Weiblen, 2002). Pollinating and non-pollinating wasps are equally attracted to trees of either 'sex' in dioecious species (Weiblen et al., 2001). However, pollinating wasps generally cannot develop in the 'female' syconia because the ovipositors of female wasps are too short to penetrate the long styles to lay their eggs in the ovaries (Nefdt and Compton, 1996; Weiblen, 2002). The absence of pollinators in female syconia thus precludes NPFWs that parasitize the pollinators. A likely prerequisite for successful exploitation by NPFWs of the female syconia of dioecious Ficus is thus to be a phytophagous galler or to be a parasite or inquiline of other phytophagous galler NPFWs, although this has never been confirmed empirically. Moreover, because NPFWs often compete with pollinators within syconia (Kerdulhué and Rasplus, 1996b), if any small galling species is able to colonize both male and female syconia of the same dioecious Ficus species, reduced competition may result in increased abundance of the galler, and possibly its specialist parasites, in female syconia.

We studied 11 species of gynodioecious Ficus in order to answer three questions. 1) How many of the 11 species have wasps successfully developing in their female syconia? 2) Are the wasp species present in female syconia the same species as those that develop in the male trees of the same Ficus species, and of the species that are present in male and female syconia do their numbers differ between syconia of different sexes? 3) What phytophagous galler NPFW species are required for other NPFWs, e.g. parasitoids or inquilines, to successfully develop in the female syconia of dioecious Ficus?

2. Methods

2.1. General fig wasp biology

The spherical Ficus syconium is lined with approximately 100 to several thousand individual flowers. This variation in flower number is due to the considerable interspecific variation in syconium size, which ranges from about the size of a pea to that of a tennis ball (Janzen, 1979; Cook and Rasplus, 2003). Syconium size is usually positively correlated with the number and size of flowers, and the size of the wasps associated with a particular species of fig tree (Herre, 1989).

As they approach maturity syconia become enlarged and soft, which enables the pollinating wasps that have developed within to emerge from their galls. The males emerge before the females by chewing holes in the walls of their own galls. After emerging from their galls into the syconium central cavity (the lumen), the males chew holes in the walls of galls containing females to enable mating. After mating, the males then enlarge the holes to enable

the females to emerge into the lumen to collect pollen from the now mature male flowers. Some male pollinators will chew small tunnels through the syconium wall to enable the pollen-laden females to disperse to another fig tree that has a crop of syconia that are receptive to the pollinators. In most species the female wasps are thus unable to disperse without benevolent male behaviour (Zammit and Schwartz, 2000). Pollinating fig wasps are highly sexually dimorphic (Weiblen, 2002). Females are winged and can disperse long distances to find receptive trees (Nason et al., 1998; Ahmed et al. 2009). Males are blind, wingless, have reduced sized legs and never leave their natal syconium unless they fall out of the exit tunnel they have just chewed.

On emergence from their natal syconium, the female wasps are attracted to a species-specific chemical signal emitted by receptive fig trees (Proffitt et al., 2007, 2008). They will search for a suitable syconium and enter it through a specialized bract-lined tunnel, the ostiole, which is only open during a brief window of receptivity. Depending on the species, from one to >30 'foundress' females will enter a receptive syconium, where they will lay their eggs individually into some of the flowers by inserting their ovipositors down the styles, whilst simultaneously spreading the pollen carried from their natal tree. In most Ficus species foundresses live out the remainder of their short lifespans (<48 h; Dunn et al., 2008b) in the syconium in which they oviposit (but see Moore et al., 2003).

The different species of NPFWs associated with a particular Ficus species will oviposit into syconia that are at different developmental stages relative to the time of pollination, depending on the trophic status of their larvae (West et al., 1996; Proffitt et al., 2007; Elias et al., 2008). For instance, those that can independently induce Ficus ovaries to form galls and have phytophagous larvae often lay their eggs prior to pollination, whereas those that are parasites of the pollinators are constrained to oviposit after pollination. Identifying the developmental stages of syconia relative to pollination that NPFWs oviposit is thus a key determinant in identifying the larval trophic status of different species of NPFWs.

Analogous to the pollinator males, many species of NPFWs are highly sexually dimorphic and have males that have specialized adaptations for a short adult life spent entirely within the lumen of their natal syconium (e.g. they are wingless, have small eyes and legs, and often have enlarged mandibles to facilitate intrasexual combat; Hamilton, 1979; Moore et al., 2009). Although the males of NPFW species possessing such adaptations have the ability to release intra-specific females from their galls, their ability to chew exit tunnels may be negligible or less efficient than pollinator males (Suleman et al., 2011). The females of many NPFW species may thus be partly reliant on the exit tunnels dug by the pollinator males for successful dispersal.

2.2. Screening of NPFWs developing in female syconia

From July 2007 to May 2008, we collected syconia from 11 species of dioecious Ficus (Table 1). These were located in three different areas on Hainan Island, China: 1) Danzhou Campus, Hainan University (19°30′34.92″N, 109°29′33.97″E), 2) Bawang Mountain (19°06′47.44″N, 109°3′58.92″E), and 3) Yingge Mountain (19°01′20.02″N, 109°32′57.08″E). Trees were identified with the aid of Zhou and Gilbert (2003). For each fig species, we haphazardly picked at least 50 male and female syconia from each of two to 30 trees (Table 1). All syconia were mature (D-stage; Galil and Eisikowich, 1968), whereby they were relatively large and soft just prior to wasp dispersal but showed no signs of exit holes dug by male wasps. After removal from the tree, each syconium was placed individually into a plastic vial and returned to the laboratory. All containers were then left for four days at ambient room

Table 1

The 11 gynodioecious fig tree species used for the study. Three species supported NPFWs in their female syconia. Data for syconium size are diameter measurements of mature female (D-stage) syconia taken from Zhou and Gilbert (2003) and Chen and Zhang (1965). Data for trees and syconia reflect the numbers of trees of each sex that syconia were sampled from.

Species	Subgenus	Trees		Syconia		Size (mm)	NPFWs
		Male	Female	Male	Female		
F. hirta	Ficus	5	3	50	30	10–15	No
F. triloba	Ficus	–		–		20–25	No
F. stenophylla	Ficus	–		–		7–8	No
F. formosana	Ficus	–		–		6–9	No
F. auriculata	Sycomorus	6	3	60	30+	30–60	Yes
F. hainanensis	Sycomorus	1	1	10	10	15–20	Yes
F. variegata	Sycomorus	6	3	40	20	25–30	No
F. hispida	Sycomorus	20	10	400	200+	15–25	No
F. fistulosa	Sycomorus	–		–		15–20	No
F. tinctoria subsp. gibbosa	Sycidium	6	3	300	150+	6–10	Yes
F. heterophylla	Sycidium	–		–		10–20	No

Table 2

The wasp species found to have successfully developed in either the male or the female syconia of *F. auriculata* or *F. tinctoria* subsp. *gibbosa*. *Ceratosolen emarginatus* and *Liporrhopalum rutherfordi* are the agaonid pollinators of each tree species, respectively. The numbers presented are the mean number of individuals (±se) per syconium.

Wasps	F. auriculata		F. tinctoria subsp. gibbosa	
	Male	Female	Male	Female
Pollinators	Present[a]	Absent	45.39 (2.76)[e]	Absent
Neosycophila	–	–	1.90 (0.11)[f]	3.36 (0.26)[f]
Sycoscapter	Present[b]	11.50 (15.74)[b]	3.54 (0.31)[g]	Absent
Philotrypesis	Present[c]	17.67 (27.15)[c]	13.57 (0.92)[h]	Absent
Apocryptophagus	Present[d]	21 (37.04)[d]	–	–
Sycophila	–	–	0.98 (0.08)[i]	1.21 (0.11)[i]
Braconid	–	–	0.28 (0.04)[j]	0.44 (0.10)[j]

[a] *Ceratosolen emarginatus*.
[b] *Sycoscapter* sp.
[c] *Philotrypesis* sp.
[d] *Apocryptophagus* sp.
[e] *Liporrhopalum rutherfordi*.
[f] *Neosycophila* sp.
[g] *Sycoscapter nayoshorum*.
[h] *Philotrypesis jacobsoni*.
[i] *Sycophila* sp.
[j] Unidentified braconid species.

temperature and natural light conditions. Each syconium was then removed from its container, and any wasps that had emerged from it were killed with ethanol, and placed individually in small airtight vials filled with 95% ethanol. Each syconium was bisected twice using a scalpel, and each flower was carefully removed using fine forceps. With the aid of a stereomicroscope, flowers containing wasp galls were isolated and the wasps within them removed and also placed individually in small airtight vials containing 95% ethanol for identification. Within syconia, ovules that have become wasp galls can be easily differentiated from those that have become seeds, because the relatively dark wasps within can be seen through the semi-transparent gall walls (Yao et al., 2005).

We found that NPFWs were present in the female syconia of three *Ficus* species: *Ficus auriculata*, *Ficus hainanensis* and *Ficus tinctoria* subsp. *gibbosa* (Table 1). *F. tinctoria* subsp. *gibbosa* is the commonest gynodioecious fig species on Hainan Island (T. Wu, personal observations). We therefore made a more intensive study of the wasps that reproduce in the male and female syconia of *F. tinctoria* subsp. *gibbosa* by collecting a further 513 male and 307 female syconia, and then processing the wasps they contained as described above. We also collected on an *ad hoc* basis a further 12 (six males and six females) *F. auriculata* syconia. Again, the wasps these syconia contained were processed using the same methods as described earlier. For *F. auriculata*, we determined in each male syconium the presence or absence of the different species of wasps that had successfully developed. This was repeated for each female syconium with the addition of counts of the wasps. For *F. tinctoria* subsp. *gibbosa* all wasps for male and female syconia were counted (Table 2). We did not use *F. hainanensis* for this part of the study because the rarity of this species (T. Wu, personal observation) presented us with logistical problems.

2.3. Single or combined species exposure experiments on the female syconia of F. tinctoria subsp. gibbosa

Our general syconium screening showed that at least three species of NPFWs developed in the female syconia of *F. tinctoria* subsp. *gibbosa*. These were a *Neosycophila* sp., a *Sycophila* sp., and an undescribed braconid. We therefore used this tree species to clarify the trophic statuses of these wasp species. First, we haphazardly selected a female tree that had a crop of small syconia that were at a developmental stage prior to receptivity to the pollinators (A-stage; Galil and Eisikowich, 1968). We then monitored daily their development over time whilst simultaneously observing the oviposition behaviour of any NPFWs that were

present. Our observations revealed that *Neosycophila* sp. is attracted to, and oviposits into, small syconia (A-phase; Galil and Eisikowich, 1968) before pollination, whereas *Sycophila* sp. and the unnamed braconid are attracted to, and oviposit into, larger syconia after pollination (the interfloral C-phase; Galil and Eisikowich, 1968).

These findings suggested that *Neosycophila* sp. has phytophagous larvae and may be able to independently induce the formation of galls, whereas *Sycophila* sp. and the unmanned braconid may be parasites of *Neosycophila* sp. We therefore experimentally tested these hypotheses, by introducing freshly emerged wasps of each species to haphazardly selected syconia that had been covered since very early development with fine mesh bags to prevent any natural wasp infestation. When the syconia in a bag reached the appropriate developmental stage(s), we introduced freshly collected wasps of each of the three species using a total number of 125 replicates of six combinations of wasps of one, two or all three species (Table 3). For example, for each of the seven replicates involving only *Neosycophila* sp. an individual wasp was presented using a fine paintbrush to a syconium that was at a developmental stage prior to pollinator receptivity. For the three replicates involving *Neosycophila* sp. and *Sycophila* sp., after *Neosycophila* sp. was introduced as described above, each syconium was re-bagged and left to develop further to reach the size that *Sycophila* sp. was

Table 3

Results of the introduction experiment involving the three wasp species found to be harboured by the female syconia of *F. tinctoria* subsp. *gibbosa*. The numbers in the column for each of the three wasp species, is the number of experimental syconia of each of the introduced combinations that offspring of each species were found to have successfully developed. The data suggest that *Neosycophila* sp. is a phytophagous galler, and that *Sycophila* sp. and the undescribed braconid are its parasites (parasitoids or inquilines).

Species combination introduced	No. figs	Sycophila sp.	Neosycophila sp.	Braconid
Neosycophila sp.	7	0	5	0
Sycophila sp.	85	0	0	0
Braconid	19	0	0	0
Neosycophila sp. + braconid	4	0	4	3
Neosycophila sp. + Sycophila sp.	3	2	2	0
Neosycophila sp. + Sycophila sp. + braconid	7	1	6	2

known to oviposit. A single *Sycophila* sp. was then introduced and allowed to oviposit, then the syconium was re-bagged. All syconia were then allowed to mature until just prior to wasp emergence. Each syconium was then picked and returned to the laboratory for dissection using the methods described above. Each wasp present was removed from its gall to enable identification. If wasps were present, the corresponding syconium was scored as having successfully harboured that NPFW species.

3. Results

3.1. How many of the 11 fig species have wasps successfully developing in their female syconia?

Three out of the 11 (27.3%) fig species, *F. auriculata*, *F. tinctoria* subsp. *gibbosa* and *F. hainanensis*, harboured wasps in the syconia of their female trees (Table 1). Five genera of chalcid NPFWs, and one undescribed braconid species, were present. All wasps in all syconia had reached adulthood, showing that female syconia did not impede normal development in the individual wasps we found.

In the field we found that the female *F. tinctoria* subsp. *gibbosa* growing at all three sites contained all of the three species of wasps we found to be associated with it. However, for *F. auriculata* NPFWs did not occur in female syconia in Dazhou over the three-years of the study.

3.2. Are the wasp species present in female syconia the same species as those that develop in the male trees of the same Ficus species and do their numbers differ?

As expected, only the male trees of *F. auriculata* and *F. tinctoria* subsp. *gibbosa* contained pollinators. However, the compositions of NPFWs in female and male syconia between tree species were more complex. In *F. auriculata*, male trees harboured at least three species of undescribed chalcid NPFW: a *Sycoscapter* sp., a *Philotrypesis* sp., and an *Apocryptophagus* sp. We also found wasps of these species to have successfully developed in the syconia of female trees (Table 2). In *F. tinctoria* subsp. *gibbosa* male trees harboured at least four species of chalcid NPFWs: a *Neosycophila* sp., *Philotrypesis jacobsoni*, *Sycoscapter nayoshorum* and a *Sycophila* sp., and an undescribed braconid. However, *P. jacobsoni* and *Sycos. nayoshorum* were absent from all of the *F. tinctoria* subsp. *gibbosa* female syconia we examined. No NPFW species was present in the female syconia of either *Ficus* species that was not also present in the male syconia (Table 2).

Overall, the total number of wasps developing in *F. tinctoria* subsp. *gibbosa* male syconia (mean $\pm$ se $= 65.66 \pm 4.22$) was greater than in female syconia (mean $\pm$ se $= 5.01 \pm 0.47$; $t_{818} = 11.09, P < 0.001$). This mainly reflected the presence of relatively high numbers of pollinators and their likely parasites *P. jacobsoni* and *Sycos. nayoshorum* in male syconia, and the absence of all three species from female syconia (Table 2). However, of the three other wasp species present in both male and female syconia, male syconia contained significantly fewer *Neosycophila* sp. individuals ($t_{818} = 5.93, P < 0.001$), and marginally significantly fewer *Sycophila* sp. ($t_{818} = 1.72, P = 0.08$) and braconid individuals ($t_{818} = 1.72, P = 0.08$), than did female syconia (Table 2). There was no significant tree effect for any of our measures of wasp abundance (data not shown).

3.3. What phytophagous galler NPFW species are required for other NPFWs to develop in the female syconia of F. tinctoria subsp. gibbosa?

In the single species introductions, only *Neosycophila* sp. could independently develop in female syconia suggesting that its larvae were phytophagous, and the ovipositing adult females of this species have the capability to induce *Ficus* ovaries to form galls. The results of the combined species introductions showed that *Sycophila* sp. and the braconid could produce offspring only when *Neosycophila* sp. was also present, suggesting that these species are parasites (parasitoids or inquilines) of *Neosycophila* sp. (Table 3).

4. Discussion

Our study has three main findings. (1) Contrary to some conclusions (e.g. Harrison and Yamamura, 2003), the female syconia of some dioecious *Ficus* species readily support NPFWs — of the 11 species we studied, three supported NPFWs in their female syconia. (2) Due to the presence of pollinators, more wasps in total and more wasp species were present in male syconia than in females. As expected, female syconia did not support NPFWs that rely on pollinators, i.e. their parasites. Some NPFW species developed in both the male and female syconia of the same fig species, and these wasps were most numerous in female syconia, possibly due to reduced competition due to a lack of pollinators. (3) We clarified experimentally that at least one small NPFW species that oviposits prior to pollination, has phytophagous larvae, and can independently form galls, is necessary to support a wasp community in female syconia.

Two wasp genera generally regarded as being either parasitoids or inquilines, *Sycoscapter* and *Philotrypesis* (Abdurahiman and Joseph, 1978; Weiblen et al., 2001; Dunn et al., 2008a), were present in the male and female syconia of *F. auriculata* and in the male syconia only of *F. tinctoria* subsp. *gibbosa* (Table 2). This suggests that both species associated with *F. auriculata* are parasites of wasps in addition to, or other than, the pollinators, whereas in *F. tinctoria* subsp. *gibbosa* *P. jacobsoni* and *Sycos. nayoshorum* are parasites of the pollinators. Among the NPFWs developing in the female syconia of *F. auriculata*, *Apocryptophagus* sp., a species that is attracted to and oviposits into syconia before pollination, is likely to be a small phytophagous galler (Peng et al., 2005), which may thus provide a host for both *Sycoscapter* and *Philotrypesis*. This confirms, in conjunction with our experimental data for *F. tinctoria* subsp. *gibbosa*, that the presence of at least one species of phytophagous, early-ovipositing NPFW is needed to enable small parasitic NPFWs to exploit female syconia. Moreover, our data also support previous findings of a close association between epichrysomallines (hosts) and eurytomids (parasites) (Compton, 1993). Because we only obtained detailed data for two species, we cannot make general conclusions about the patterns of presence and abundance of the NPFWs supported by male and female trees across dioecious *Ficus*. However, in *F. auriculata* and *F. tinctoria* subsp. *gibbosa* male syconia can harbour all or some of the NPFWs as do the female syconia, and that although female syconia tend to support fewer wasps overall due to the absence of pollinators, they can support more individuals of some NPFW species than male syconia.

Monoecy is the ancestral condition of *Ficus* with gynodioecy, having evolved probably only twice, being derived (Weiblen, 2000; Machado et al., 2001; Harrison and Yamamura, 2003). The evolution of gynodioecy and subsequent radiations of predominantly gynodioecious *Ficus* clades, e.g. the section *Sycomorus*, would have initially resulted in reduced exploitation of their female syconia due to the absence of the pollinators. However, small galling NPFWs with phytophagous larvae may not have suffered any negative effects of pollinator absence. Indeed, niche expansion may have been possible due to reduced competition, enabling radiations within NPFWs specializing in exploiting female syconia and/or providing ecological opportunities to attain large population sizes. Radiations of NPFWs exploiting the female syconia of dioecious *Ficus* either did not happen or they were evolutionary unstable. Moreover, current data, although few, show that overall fewer

wasps develop per syconium in female syconia compared with the male syconia of the same fig species (Weiblen et al., 2001), and with monoecious fig species. Indeed, our data for *F. tinctoria* subsp. *gibbosa* concurred with this trend, and reflected the absence from male syconia of pollinators and NPFW species likely to be their parasites. However, we found more *Neosycophila* sp., *Sycophila* sp., and braconids in female syconia than in males. This suggests that some resources unexploited by pollinators within the female *F. tinctoria* subsp. *gibbosa* syconium are used by *Neosycophila* sp., and the extra *Sycophila* sp. and braconids reflect the increase of *Neosycophila* sp. hosts. The absence of pollinators in female *F. tinctoria* subsp. *gibbosa* syconia does not translate directly into an increase in available resources to *Neosycophila* sp. and its parasites. Overall, this indicates that there may be mechanisms preventing early ovipositing small phytophagous galling NPFWs from fully replacing pollinators, that somehow also preclude or limit the numbers of NPFW species that are able to exploit the female syconia of this *Ficus* species.

We showed that the rate of NPFW presence in the 11 study species was fairly low (27.27%). Approximately half of the 750 species of *Ficus* are gynodioecious (Berg, 1989), meaning that if exploitation rates were crudely extrapolated, about 100 gynodioecious species may support wasps in their female syconia. Kerdulhué and Rasplus (1996a) suggested that dioecy evolved in *Ficus*, at least in part, to reduce costs to the trees imposed by NPFWs disrupting pollinator and seed production. Monoecious *Ficus* support more diverse wasp communities, and more individual wasps, than dioecious species (Kerdulhué and Rasplus, 1996a; Weiblen, 2002). Kerdulhué and Rasplus (1996a) suggested that this may be due to the differences in the arrangements of the style lengths of the flowers between gynodioecious and monoecious species, and invoked the assumption that long-styled flowers, the ovaries of which mature at the outer wall of the syconium and are generally avoided by ovipositing pollinators in monoecious species, are generally 'unbeatable' and are largely immune to wasps (*sensu* West and Herre, 1994). Because the female seed-producing syconia of gynodioecious fig species contain a uniform layer of long-styled flowers to prevent pollinator oviposition (Cook and Rasplus, 2003), this may thus result in fewer reproductive opportunities for small galling NPFWs and their parasites due to an inability to develop in long flowers per se. However, the successful development of some NPFWs in the female syconia of some gynodioecious *Ficus*, and in the long-flowers in the syconia of some monoecious species (e.g. Dunn et al., 2008a), suggests that long-flowers are not always 'unbeatable' to all wasps. Moreover, most NPFWs, including small early ovipositing galling species with phytophagous larvae, oviposit from outside of the syconium, meaning that constraints imposed on the lengths of pollinator ovipositors by long styles are unlikely to affect NPFWs.

Fig trees and their pollinators have coevolved over at least 60 million years (Ronsted et al., 2005), with different groups of NPFWs also having diversified over long time periods. For example, Cruaud et al. (2011) reported that wasps of the sycophaginae have diverged over 15 million years into several clades characterized by diverse larval trophic statuses. The generation times of wasps are also several orders of magnitude faster than that of the trees, a fact that adds to the mystery as to why NPFWs have not evolved mechanisms to exploit the trophic niche left vacant by pollinators in female gynodioecious syconia. More detailed studies are required in more dioecious *Ficus* clades to determine the rates of wasp presence across gynodioecious *Ficus*, and to identify possible mechanisms preventing wasps from exploiting their female syconia.

Non-pollinating fig wasps may fail to fully exploit female syconia due to constraints associated with dispersal, because female NPFWs may totally or partly rely on pollinator males to produce exit tunnels. It is unknown if the males of any of the NPFW species in our study are able to dig exit tunnels, although Suleman et al. (2011) found that male *Sycoscapter* sp. can dig exit holes in their natal syconium, albeit at a reduced efficiency compared with pollinator males. If the males of NPFWs species able to develop in female syconia are unable to dig exit holes, then in the absence of an alternative dispersal mechanism the syconium would clearly become a 'tomb blossom' for any wasps. However, some of the frugivores that eat mature syconia (Shanahan et al., 2001; Sigiura and Yamazaki, 2004; Ma et al., 2009; Heer et al., 2010; Lomáscolo et al., 2010) may facilitate wasp dispersal. Bats and birds are attracted to *Ficus* with large or small syconia respectively (Lomáscolo et al., 2010), because bats eat fruits incrementally (Mello et al., 2005) whereas birds swallow them whole (Lomáscolo et al., 2010). Indeed, within dioecious *Ficus* species female syconia are more attractive to frugivores than male syconia (Lambert, 1992). Bird predation may thus select for wasps to avoid female syconia in *Ficus* with small syconia. Although previous studies have shown that some NPFW species are equally attracted to the male and female syconia of the same *Ficus* species (e.g. Weiblen et al., 2001), differential rates of attraction to male and female syconia may occur between *Ficus* species with different sized syconia. It will thus be informative to compare the occurrence and the mechanism of female NPFW dispersal in different dioecious *Ficus* species with different sized syconia.

In conclusion, our study shows that there are constraints on female syconium use due to the trophic status of NPFW larvae, although the same species of NPFW can be supported by both male and female syconia. More detailed future studies into why the female syconia of dioecious *Ficus* appear to be under-exploited by wasps are needed to fully understand this under-studied branch of mutualism research.

Author contributions

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