Oviposition sequence of non-pollinating fig wasps associated with *Ficus benjamina* in China

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Abstract: Fig-fig wasp mutualisms are exploited by non-pollinating fig wasps (NPFWs). In most cases, NPFWs oviposit into ovaries of female flowers from outside syconia. In this study, the oviposition sequence of externally ovipositing non-pollinating fig wasps associated with *Ficus benjamina* were studied by direct observation, yellow sticky board and manipulation experiments in Xishuangbanna, southwest of China. The results showed that (ⅰ) Different genera of wasps showed a temporal partition in oviposition sequence among NPFWs. *Acophila, Sycobia* and *Walkerella* wasps colonized syconia in pre-female phase, while *Micronisa, Philotrypesis* and *Sycoscapter* oviposited in the interfloral phase. Further, NPFWs of the same genus also showed difference ovposition period. (ⅱ) Different genera of wasps exhibited various strategies of resource exploitation. *Acophila*, *Sycobia* and *Walkerella* wasps were gallers, while *Philotrypesis* wasps should be inquiline. However, only the galler species *Walkerella* sp.2 had significant negative effective on pollinator and seed.
Keywords: mutualism; non-pollinating fig wasp; ovipositon sequence; effect

1. Introduction

Pollinating fig wasps are obligate mutualists with their *Ficus* hosts [1-4], but they are exploited by a diverse group of non-pollinating fig wasps (NPFWs) that also develop within syconia [5-7]. Most of these NPFWs are also assumed to be associated specifically with a single *Ficus* species [8]. Pollinating fig wasps have been studied extensively, whereas much less is known about the biology of NPFW [9].

The developmental period of the syconium is divided into several phases: pre-female, female, interfloral, male, postfloral [10]. The female phase corresponds to that time when the female flowers are receptive for pollination and oviposition. During this period, the ostiolar scales become loose to facilitate the entrance of the female pollinator [11]. Few NPFWs, like pollinators, have foundresses that enter the figs to oviposit, however, they are unable to transport pollen efficiently for fig trees [7]. Unlike pollinating fig wasp, most NPFWs lay their eggs through the fig wall from the outside and do not transfer pollen [7]. Therefore, oviposition periods of the externally ovipositing fig wasps are not need to be confined to the female phase. Each NPFW species can lay its eggs only during a precisely defined interval, depending on the developmental cycle of the host fig and timing of pollination [7, 8, 12]. Temporal segregation of colonization time between NPFWs is due to the use of different volatile signs produced by syconia at different developmental stages [8, 13].

NPFWs are directly or indirectly dependent on fig tree-fig pollinating fig wasp mutualism for survival [7, 14]. NPFWs include species that gall *Ficus* ovaries, that are inquiline, or that are parasitoids [7]. Gallers oviposit at the same time or before the pollinating females (foundresses); Inquilines lay eggs in the galls made by other fig wasps, but they are not able to induce galls, therefore, they oviposit in the induced galls and compete, even eliminate the galler larvae; Parasitoids feed directly on the larvae of gallers or inqulines [13]. Despite their larvae diet, NPFWs were considered to have a negative effect on fig–pollinator mutualism [7, 15].

In this study, the oviposition sequence of non-pollinating fig wasps associated with *Ficus benjamina* were observed. The effects of non-pollinating fig wasps on fig–pollinator mutualism were also studied.

2. Materials and methods

2.1 Study site and species

The study was carried out at Xishuangbanna Tropical Botanical Garden (XTBG) which is located in south-west China (101°15'E, 21°55'N).

Ficus benjamina L. (Section *Conosycea*) is a large free-standing monoecious fig tree that is native in Xishuangbanna tropical region [16]. In Xishuangbanna, *F. benjamina* produces figs throughout the year in synchronous crops, with different trees fruiting at different times. A crop comprises several thousands of syconia. Mature figs are subglobe, yellow and measure 12–25mm in diameter [16]. Each fig contains around 700 flowers (Mean ± S.E. = 614.71 ± 18.61 female flowers and 59.29 ± 2.16 male flowers, n = 24 syconia).

F. benjamina is actively pollinated by *Eupristina koningsbergeri* Grandi, which also supports 14 species of non-pollinating fig wasps in Xishuangbanna. In this study, we studied nine NPFW species that they are quite common. The fig wasps species studied are shown in Table 1.

Table 1. The species of fig wasps and their Diagnosis

Fig wasp species	Diagnosis	Oviposition
Eupristina koningsbergeri	Female: body black color with a short ovipositor Male: wingless	Internal oviposition
Walkerella sp.1	Female: Short ovipositor, dark blue metallic Male: Wingless, head enlongated	External oviposition
Walkerella sp.2	Female: Short ovipositor, dark blue metallic Male: Wingless, head rounded, with both dark and yellow males	External oviposition
Micranisa sp.	Female: Short ovipositor downward Male: Wingless	External oviposition
Sycoscapter sp.	Female: Green long ovipositor. Male: Wingless with normal tibia III	External oviposition
Philotrypesis tridentata	Female: Yellow, relatively big species Male: Wingless	External oviposition
*Philotrypesi*s sp.1	Female: Yellow long ovipositor with black back Male: Wingless	External oviposition
Philotrypesis sp.2	Female: Black Male: Wingless, blackish	External oviposition
Sycobia sp.	Female: Dark grey, head yellowish Male: Winged	External oviposition
Acophila sp.	Female: Completely black Male: Winged	External oviposition

2.2 Oviposition sequence

We investigated the oviposition sequence of non-pollinating fig wasps by means of yellow sticky board traps and direct observations. From 2008 to 2010, one *F. benjamina* tree was selected as the sampling tree. The oviposition sequence of fig wasps was observed.

When syconia developed into pre-floral phase, thirty syconia from a crop were marked. We observed and recorded the wasp species ovipositing on the marked syconia twice per day. The size of trap was 215×150 mm yellow board with glue. Ten traps were placed on the branches, which are about 1.5 m above the ground. The species and numbers of fig wasps on each trap were daily recorded and moved. The observation lasted from pre-floral to post-floral phases. Here, we only discussed ovipositon sequence of fig wasps on one crop parasitized by nine non-pollinating fig wasp species.

2.3 Natural population of fig wasps

212 near D phase syconia (before wasp emergence) were collected from seven crops of seven *Ficus benjamin*a trees. Each syconium was placed individually in a fine-mesh bag (200×200 mm) and the fig wasps were allowed to emerge from syconia. Each wasp was identified to species and counted. The number of seeds in each syconium was counted as well.

2.4 Experimental manipulation

Pre-female (A) phase syconia were enclosed in fine mesh nylon bags (one syconium into one bag) to prevent any female fig wasps from oviposition. The two treatments were performed: (1) ten *Walkerella* sp.2 and one pollinator per syconium because about ten *Walkerella* sp.2 foundresses were observed ovipositing on one syconia at the same time. When *Walkerella* sp.2 females were found to lay eggs in adjacent syconia at the same developmental stage, *Walkerella* sp.2 were collected from D phase syconia from other trees and 10 *Walkerella* sp.2 females were released into each bag with a syconum. When the syconia developed into female phase, one pollinator foundress was introduced into each syconium which had been oviposited in by the *Walkerella* sp.2.

(2) one pollinator per syconium. one pollinator foundress was introduced into a syconium without oviposited by other fig wasps. At maturity, each manipulation syconium was collected. *Walkerella* sp.2, pollinator, and seeds in the syconia were counted respectively.

2.5 Data analysis

To determine the actual quantitative relationships between species, path analysis was used to study the effect of NPFWs on pollinatiing fig wasp and seed production. In addition, the percentage of wasps coexisting with other species was also used to show the relationships between NPFWs pollinating fig wasps. This amount ranges from zero to one, with a number closer to one meaning a closer relationship [4]. The forum is as followed: Coexistence percentage = a/(a+b+c), where a is the numbers of two species coexisting in a syconium from the total sampled syconia, b is syconium numbers having species 1 but not species 2 among the total sampled syconia, and c is syconium numbers having species 2 but not species 1 among the total sampled syconia. In manipulation syconia, t test was used to analyze the effect of *Walkerella* sp.2 on the fig-pollinator mutualism.

3. Results

3.1 Oviposition sequences of non-pollinating fig wasps associated with *F. benjamina*

Different genus of NPFWs showed a temporal partitioning in oviposition patterns. *Acophila*, *Sycobia* and *Walkerella* colonized syconia at pre-female phase, while *Micronisa*, *Philotrypesis* and *Sycoscapter* oviposited at interfloral phase. At pre-female phase, some NPFWs of the same genus also showed significant difference in oviposition sequence. For example, *Walkerella* sp.1 was observed ovipositing at the early pre-female phase, while *Walkerella* sp.2 oviposit in the middle of pre-female phase. Compared with the genus that oviposit at pre-female phase, wasps oviposit at B and C phase are more overlapped. Take genus *Philotrypesis* as the example, the oviposition periods *Philotrypesis tridentata*, *Philotrypesis* sp.1, and *Philotrypesis* sp.2 were concentrated in a few days just after pollinators entering the syconia. By direct observation and using adhesive traps, the specific oviposition sequence of the fig wasps associated with *F.benjamina* were summarized as follows. *Acophila* sp., *Walkerella* sp.1, *Sycobia* sp., *Walkerella* sp.2, *Micronisa* sp., *Philotrypesis* sp.1, *Philotrypesis* sp.2, *Philotrypesis tridentata*, *Sycoscapter* sp..(Fig.1)

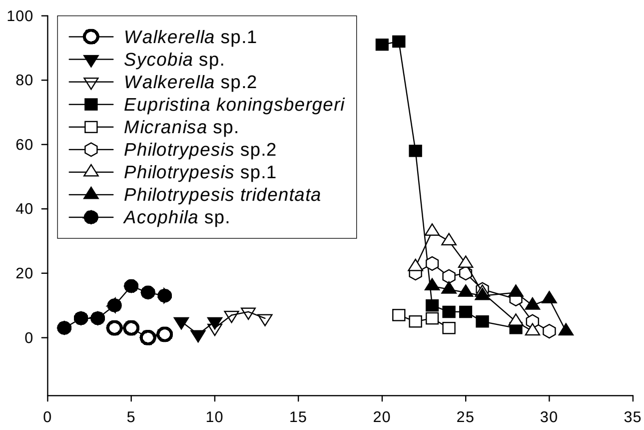

Figure 1. Fig wasp capture sequence on adhesive traps in *F.benjamina*

3.2 Larvae diet of some NPFWs

Acophila sp. was the first NPFW to oviposit on syconia. It indicated that *Acophila* sp. should be a galler species. *Walkerella* sp.1 is the second NPFW oviposited on syconia. The oviposition periods of *Acophila* sp. and *Walkerella* sp.1 are partial overlapped. However, some natural syconia parasitized by *Walkerella* sp.1 did not contain *Acophila* sp., suggesting *Walkerella* sp.1 is also a galler species. *Walkerella* sp.2 was the last NPFW that oviposited in pre-female phase syconia. *Walkerella* sp.2 was able to reproduce in the syconia without other fig wasps, indicating *Walkerella* sp.2 is also a galler species. Moreover, in manipulation experiment, the syconia that *Walkerella* sp.2 was introduced to oviposit, the offsprings normally developed adult, while they could not leave the syconia independently. *Philotrypesis* sp.2 oviposited just after the pollinator entered the syconia and 36 of all natural syconia studied only contain *Philotrypesis* sp.2 and pollinator. These evidences showed that *Philotrypesis* sp.2 should be inquiline.

3.3 The effect of NPFWs on pollinator and seed

The path way analysis revealed that nearly all NPFWs, beside *Walkerella* sp.2 did not have significant negative effect on pollinator and seeds (Table 2). In single natural syconium, offsprings number of *Walkerella* sp.2 ranged from 1 to 33. The Coexisting percentage of *Walkerella* sp.2 and pollinator is the highest, comparing with NPFWs. Pollinator number in manipulation syconia with both *Walkerella* sp.2 and pollinator is significantly less than that in syconia only with pollinator (Table 3). Coexisting percentage of *Philotrypesis* sp.2 and pollinator is the second highest. However, path analyse showed that the correlation between *Philotrypesis* sp.2 and pollinator is not significant, indicating that *Philotrypesis* sp.2 did not have significant impact on pollinator although *Philotrypesis* sp.2 may kill some pollinator larvae by competition. It did not affect the seed number as well (Table 2).

Table 2. Coexisting percentage and path analyses coefficient results.

Species	Coexisting percentage with pollinator	Path coefficient with pollinator	Path coefficient with seed
Acophila sp.	0.02	0.04 NS	-0.01NS
Walkerella sp.1	0.03	-0.03NS	-0.01NS
Sycobia sp.	0.12	0.01 NS	0.05NS
Walkerella sp.2	0.41	-0.36**	-0.65*
Micronisa sp.	0.12	-0.17NS	-0.20NS
Philotrypesis sp.1	0.09	-0.03 NS	-0.02NS
Philotrypesis sp.2	0.32	-0.01 NS	0.04NS
Philotrypesis tridentata	0.09	0.02 NS	-0.02NS
Sycoscapter sp.	0.03	-0.03 NS	-0.01NS

*P<0.01; **P<0.001; NS, not significant.

Table 3. The contents of *F.benjamina* syconia with experimental manipulation of foundresses

Model	Sample size	*Eupristina koningsbergeri* progeny	*Walkerella* sp.2 progeny	seed
1*Eupristina koningsbergeri* +10*Walkerella* sp.2	9	130.11 ± 4.22a	38.00 ± 4.33	67.11 ± 1.05a
1*Eupristina koningsbergeri*	24	150.00 ± 6.61b	_	92.25 ± 7.95b

Different letters indicate significant difference at P=0.05 leve, Mean ± S.E.

4. Discussion

The results showed that the oviposition sequence of NPFW species is similar within genus, while it is, at least to some degree, different between different genera. *Acophila* sp. is the first NPFW oviposited on syconia at prefemale phase. In Xisuangbanna, another *Acophila* species associated with *Ficus altissima* is also the first ovipositing NPFW, indicating that ovipositing at early prefemale phase should be the character of genus *Acophila*. Oviposition period of *Walkerella* sp.1 was a few days later than *Acophila* sp, while *Walkerella* sp.2 is the last non-pollinating fig wasp that oviposit in pre-female phase syconia, which indicated that oviposition sequence of wasps belonging to the same genus exhibit similarity, but there still exist some differences. Genus *Philotrypesis* oviposit on syconia 1 to 3 days later than pollinator. They have to lay eggs in the syconia that containing pollinator. Some *Philotrypesis* can stay on the syconia without pollinator for a while, while they never ovipost on such syconia. In a dioecious fig tree (*Ficus hispida*), two *Philotrypesis* species also exhibited such character [17]. Therefore, we speculate that all *Philotrypesis* species oviposite at early interfloret phase syconia despite in monoecious fig tree or dioecious fig tree. *Micronisa* sp., which have to lay eggs in syconia containing pollinator oviposit at early interfloret phase, is similar to *Philotrypesis*.

To understand fully the effect of NPFWs on fig-pollinating fig wasp mutualism, we have to determine their larval diets [9]. *Acophila* sp. is the first NPFW oviposit on syconia, indicating it must be a galler species. *Walkerella* sp.1 and *Walkerella* sp.2 are both galler species because they can reproduce in syconia without being oviposited by other fig wasps. All *Philotrypesis* species have to lay eggs in syconia containing pollinator offsprings and they oviposit just after the pollinator, suggesting that *Philotrypesis* are inquline of pollinator.

Path analysis showed that there was not significant correlation between the number of *Acophila* sp. and pollinator or seed, suggesting that such competitor did not have significant impact on the mutualism. *Sycobia* sp. and *Walkerella* sp.1 did not significant affect the mutualism as well. The early gallers in this study did not have significant effect on mutualism may be because the low parasitism rate and the few offspring number within single syconium. *Walkerella* sp.2 was the last NPFW that oviposit in pre-female phase syconia, however it have significant negative effect on pollinator number and seed number. Path analyse showed that in nature syconia, *Philotrypesis* sp.2 did not have significant impact on pollinator although *Philotrypesis* sp.2 might kill some pollinator larvae by competition. Here, we concluded that the effect of NPFWs on pollinator number not only depend on oviposition sequence but also depend on their actual reproduction.

Although some of the NPFWs have negative significant effect on fig pollinating fig wasp mutualism, they did not lead to the collapse of mutualism. Most NPFWs have to leave the fig through a hole bitten by pollinating fig wasp males, otherwise, they will die in the syconium cavity. In addition, syconia of *F.benjamina* without being pollinated are easy to drop, suggesting the host sanctions.

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