Reproductive strategies of two *Philotrypesis* species on *Ficus hispida*

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Abstract

Philotrypesis pilosa and *Philotrypesis* sp., the only non-pollinating fig wasps inhabiting the inflorescences of *Ficus hispida*, oviposited from outside the fig wall using their long ovipositors to reach the ovules inside. Both were inquilines of the pollinating wasps. However, each species of *Philotrypesis* has a different reproductive strategy. Foundresses of *P. pilosa* have higher reproductive potential in controlled experiments, but brood sizes were smaller than that of *Philotrypesis* sp. in the field. There was temporal segregation in the oviposition of species, and duration of oviposition was different. Their space distributions were similar; over 50% of individuals for both species were distributed in the ovule layer nearest the fig wall. There was a high degree of variation in the populations of both species in space and time.

Keywords: *Ficus hispida*, non-pollinating fig wasps, coexistence, reproductive differences

1. Introduction

The fig-fig pollinator mutualism is regarded as a classic example of coevolution (Kjellberg et al., 2001; Ronsted et al., 2005). In addition to the fig pollinating wasps, up to over 20 species of non-pollinators inhabit the fig inflorescences, depending on the species. The larvae of these wasps develop within the figs, but the adults do not normally transfer pollen (Jousellin et al., 2001). They include gallers, inquilines, parasitoids of the pollinators, and of other non-pollinating wasp species (Boucek, 1993). Some studies found that non-pollinating fig wasps had negative impacts on the fig-fig pollinator mutualism through predation of pollinator larvae, or through competition with pollinators for ovules (Kerdelhué and Rasplus, 1996). The reproductive behaviour of only a few non-pollinators has been well studied (Abdurahiman, 1986; Murray, 1990). The background natural history of the vast majority of species is unknown and comparative studies are few (Kerdelhué et al., 2000; West et al., 1994).

A key factor in stabilizing host-parasite interactions is parasitoid aggregation in space and time (Pacala et al., 1990). Several studies have found that in monoecious figs ovaries close to the fig wall usually produce seeds, while those next to fig lumen usually contain fig wasps (Anstett, 2001). Some studies, however, found that both pollinators and non-pollinators were distributed in the same ovary layers (Kerdelhué and Rasplus, 1996). In dioecious *Ficus*, seeds and wasps are produced by separate plants. Female trees produce only seeds and non-pollinators rarely oviposit successfully (Harrison and Yamamura, 2001; Weiblen et al., 2001). Competition for ovules is therefore restricted to the male trees and most non-pollinating fig wasps are inquilines and parasitoids (Kerdelhué and Rasplus, 1996), and negative density dependence may stabilize the interaction between pollinating wasps and their parasitoids (Weiblen et al., 2001). In this study, we compare the reproductive strategies of two species of *Philotrypesis* inhabiting on *Ficus hispida* by addressing the following questions: (i) are there differences in oviposition timing and behaviour between species? (ii) which ovary layers does each species occupy? and (iii) what proportion of inflorescences are occupied by each species across space and time?

2. Materials and Methods

Ficus hispida L. (section *Sycocarpus*) is a small- to

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medium-sized free-standing tree. Its inflorescences are pollinated by *Ceratosolen solmsi marchali* Mayr (Wiebes, 1994). In the Xishuangbanna tropical area, Yunnan Province, China, some trees produce figs year-round. The figs harbour two *Philotrypesis* species: *Philotrypesis pilosa* Mayr and an unidentified species referred to as *Philotrypesis* sp. in this paper. Both oviposit in ovules previously galled by the pollinator, by inserting their ovipositors through the fig wall.

Oviposition behaviour

Thirty immature figs were marked on a male tree of *Ficus hispida*, and we observed the oviposition behaviour of both *Philotrypesis* species 1–2 times per day for 30 days. We recorded the species' oviposition behaviour and the temporal sequence of oviposition.

Spacial distribution within the fig

Just before the wasps emerged from their galls, 42 figs were collected and brought to laboratory. Galls were classified into three layers according to pedicel length: wall layer (0.52±0.26 mm, n=271), intermediate (1.89±0.57 mm, n=307), inner layer (3.13±0.55 mm, n=280). We selected and identified the wasps in 20 to 30 galls in each layer in each ovary.

Experimental introduction

Branches bearing prefemale-phase figs were selected and enclosed in fine-meshed nylon bags (200×200 mm) to prevent entry by pollinators. Once the figs reached receptivity (diameter: 18.5±1.6 mm, n=30), we carried out the introduction experiment. *Philotrypesis* are inquilines, and thus depend on the pollinator galls for oviposition sites. To limit oviposition sites, only three pollinator foundresses, from those arriving naturally at the tree, were allowed to enter in a fig. We removed the other non pollinated figs and kept only one fig per branch. Next, the two *Philotrypesis* species were collected from mature figs on other male trees. A single female wasp was placed in a bag and, then, it was tied on the branch which the fig had been previously pollinated. *Philotrypesis pilosa* oviposits on the same or on the day following the entry of *Ceratosolen*, the pollinating wasp. Therefore, *Philotrypesis pilosa* wasps were introduced two hours after the pollinators. *Philotrypesis* sp. is a late colonizer so it was introduced on the 8th day after the entry of pollinators.

When figs were close to maturity, they were collected, individualized in nylon bags and the wasps allowed to emerge. All wasps were identified and carefully counted. We obtained 19 experimental figs for *P. pilosa* and 12 for *Philotrypesis* sp..
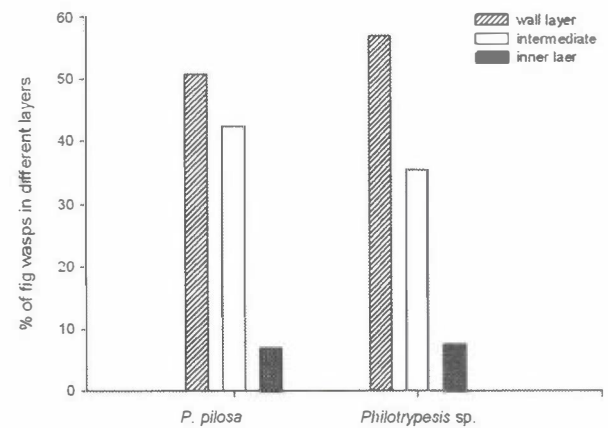

Figure 1. Frequency distribution of occupancy of ovules in different layers for two species of *Philotrypesis* on *Ficus hispida*.
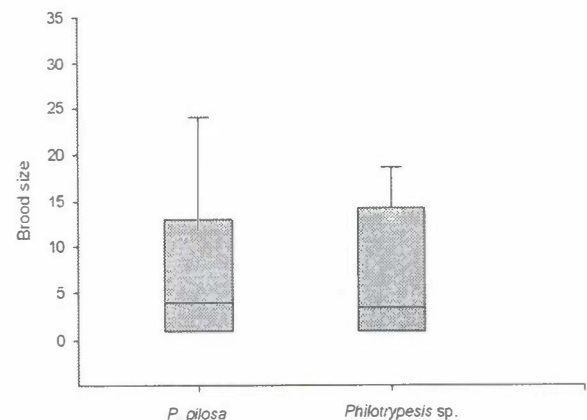

Figure 2. The brood sizes produced by single foundress of two *Philotrypesis* species in controlled introduction experiments on *Ficus hispida*. Boxes delimitate 50% of observations. central traces represent medians and vertical bars indicate the total data amplitude.

Proportions of fig parasitised by each species of Philotrypesis

We collected 117 figs from one single male tree in four fig crops, from January 2003 to April 2005. On September 2004, we collected 83 male figs from four different trees. From all samples, fig wasps were allowed to emerge and then preserved in 70% ethanol. Finally, we identified all the fig wasps and counted them.

Table 1. The percentage of infested figs, and the number of wasps per fig of two *Philotrypesis* species, from the same tree of *Ficus hispida* in different months. Paired samples t-test was used to test the difference of wasp number.

Variables	Samples	P. pilosa	Philotrypesis sp.	t-value*	P
% of parasitised figs	2003.01 (n=40)	52.5%	95%	–	–
	2003.03 (n=25)	48%	84%	–	–
	2004.09 (n=14)	57.1%	100%	–	–
	2005.04 (n=38)	68.4%	92.1%	–	–
Wasp number	2003.01 (n=40)	1.8±0.4	12.7±2.0	5.273	$<10^{-3}$
	2003.03 (n=25)	2.4±0.8	12.6±4.4	2.250	0.034
	2004.09 (n=14)	4.5±1.9	32.7±4.7	7.715	$<10^{-3}$
	2005.04 (n=38)	10.9±2.0	47.9±5.5	7.364	$<10^{-3}$

Table 2. The percentage of infested figs, and the number of wasps per fig of two *Philotrypesis* species collected at the same time (September 2004). from four different trees of *Ficus hispida*. Paired samples t-test was used to test the difference of wasp number.

Variables	Samples	P. pilosa	Philotrypesis sp.	t-value*	P
% of parasitised figs	Tree 1 (n=14)	57.1%	100%	–	–
	Tree 2 (n=16)	75%	75%	–	–
	Tree 3 (n=32)	28.1%	78.1%	–	–
	Tree 4 (n=21)	52.4%	47.6%	–	–
Wasp number	Tree 1 (n=14)	4.5±1.9	32.7±4.7	7.715	$<10^{-3}$
	Tree 2 (n=16)	25.5±9.9	9.6±2.2	1.641	0.122
	Tree 3 (n=32)	5.2±2.8	24.4±5.6	3.472	0.002
	Tree 4 (n=21)	3.4±1.4	5.7±2.4	0.8	0.433

3. Results

Oviposition behaviour of P. pilosa *and* Philotrypesis *sp.*

The ovipositor length of *Philotrypesis pilosa* is 4.6±0.2 mm (n=36). It started ovipositing immediately after pollinators' entry into the fig. Female wasps tapped the fig surface with their antenna, and then remained on the surface of receptive fig for a period. From 1–20 wasps simultaneously oviposited on the same fig. The average oviposition time was 3.6 mins (S.E.=0.5 min, n=32). The oviposition process was occasionally interrupted by ant attacks. Figs remained attractive to *P. pilosa* for two days following pollination. In contrast, the ovipositor of *Philotrypesis* sp. is longer, being 7.1±0.3 mm (n=35) length. It oviposited from the 6 to 13th day after pollination, and spent a longer time ovipositing, 12.5 mins (S.E.=1.4 mins, n=30). Approximately, 1–80 female wasps simultaneously oviposited on the same fig, but were not easily disturbed by ants.

Distribution of ovules utilised by P. pilosa *and* Philotrypesis *sp.*

In both species more than 50% of the individuals were distributed in the galls of wall layer, and few wasps developed in galls of the inner layer (<10%) (Fig. 1). In contrast, the distribution of the pollinating fig wasps was even, with about 32% of the individuals in the wall layer, 38% in the intermediate layer, and 30% in the inner layer. However, the proportion of pollinating wasps (in relation to the total number of wasps) was higher in the inner layers (inner = 96%, intermediate = 84% and wall layer = 76%).

Reproductive potential of P. pilosa *and* Philotrypesis *sp. from controlled introductions*

In the controlled introduction experiment, one single foundress of *P. pilosa* produced on average 7.8±2.1 (S.E.) wasp offspring and 52.6% of the figs contained only daughters. *Philotrypesis* sp. produced on average 6.5±2.1 (S.E.) wasp offspring and 66.7% of the figs produced only female broods. These brood size was not significantly different (Mann-Whitney U test: Z=−0.434, P=0.664) (Fig. 2).

Proportions of figs occupied by P. pilosa *and* Philotrypesis *sp.*

In figs collected from one selected tree on four occasions over two years, the proportion of figs infested by *Philotrypesis* sp. was higher than those infested by *P. pilosa*. Furthermore, the number of wasps per fig was significantly higher in *Philotrypesis* sp. than in *P. pilosa* (Table 1). However, samples taken from different trees at the same time did not always show this pattern. For example, four

trees were sampled in September, 2004, and in two there was no significant difference between average number of wasps with both species showing similar infestation rates. In the other two trees, *Philotrypesis* sp. exhibited both higher infestation rate and brood sizes than *P. pilosa* (Table 2).

4. Discussion

Philotrypesis pilosa and *Philotrypesis* sp. oviposited at different phases of the fig development, but their spatial distributions were similar. This suggested that the wasps depend on the heterogeneity in time as a partitioning factor in this ecological niche. In contrast, the fig-pollinating wasps (*Ceratosolen*) were equally distributed in all the gall layers, and should experience less space competition with *Philotrypesis* species in the inner gall layer. Thus, spatial partitioning is likely a factor that could explain the successful coexistence between pollinating fig wasps and both *Philotrypesis* species.

Observations in nature indicated that *Philotrypesis* sp. wasps have a higher fitness to exploit the fig-wasp mutualism than *P. pilosa*, as generally the former species exhibited both higher infestation rates and brood sizes. However, our experimental data showed that when only one *Philotrypesis* foundress was allowed to oviposit, both species had similar fitness. The higher population density of *Philotrypesis* sp. in nature, may be due to its extended colonization window (~one week vs. two days for *P. pilosa*). Moreover, *Philotrypesis* sp. foundresses were not as disturbed by ants, which could increase their colonization success.

Our data suggest that foundress wasps of both *Philotrypesis* species produce female-biased sex ratios in situations where mate competition is at low levels. This is widely documented for the agaonid pollinating wasps (Moore et al., 2002). When only one foundress oviposited, we observed over 52% of female-only broods. As matings occur exclusively inside the natal fig in these *Philotrypesis* species, these extreme female-biased sex ratios originate from unmated females (Murray, 1987). In nature, multiple foundresses are often observed and female-only broods must seldom occur. Therefore, pattern in which multiple foundresses lay in a single fig may help to ensure copulation in the population.

Variation in infestation levels and brood sizes of both *Philotrypesis* species was greater from tree to tree than from month to month. This pattern of variation may result from differences in the size of the host trees and their fig crops. In the study area, each *F. hispida* male tree seems to produce crops with particular characteristics. For example, the synchrony level of fig development and the number of receptive figs in a given time period, varies between trees

of different age. These complex interacting factors may be favorable for maintaining the coexistence of this fig species and its associated fig wasps.

REFERENCES

Abdurahiman, U.C. 1986. Biology and behavior of *Philotrypesis pilosa* Mayr (Torymidae). *Bulletin of Entomology* 27: 121–127.

Anstett, M.C. 2001. Unbeatable strategy, constraint and coevolution, or how to resolve evolutionary conflicts: the case of the fig/wasp mutualism. *Oikos* 95: 476–484.

Boucek, Z. 1993. The genera of chalcidoid wasps from *Ficus* fruit in the new world. *Journal of Natural History* 27: 173–217.

Harrison, R.D. and Yamamura, N. 2003. A few more hypotheses for the evolution of dioecy in figs (*Ficus*, Moraceae). *Oikos* 100: 628–635.

Jousselin, E., Rasplus, J.Y., and Kjellberg, F. 2001. Shift to mutualism in parasitic lineages of the fig/fig wasp interaction. *Oikos* 94: 287–294.

Kjellberg, F., Jousselin, E., Bronstein, J.Y., Patel, A., Yokoyama, J., and Rasplus, J.Y. 2001. Pollination mode in fig wasps: the predictive power of correlated traits. *Proceedings of the Royal Society of London B* 268: 1113–1121.

Kerdelhué, C. and Rasplus, J.Y. 1996. The evolution of dioecy among *Ficus* (Moraceae): an alternate hypothesis involving non-pollinating fig wasp pressure on the fig-pollinator mutualism. *Oikos* 77: 163–166.

Kerdelhué, C., Rossi, J.P., and Rasplus, J.Y. 2000. Comparative community ecology studies on Old World figs and fig wasps. *Ecology* 81: 2832–2849.

Moore, J.C., Compton, S.G., Hatcher, M.J., and Dunn, A.M. 2002. Quantitative tests of sex ratio models in a pollinating fig wasp. *Animal Behaviour* 64: 23–32.

Murray, M.G. 1987. The closed environment of the fig receptacle and its influence on male conflict in the Old World fig wasp *Philotrypesis pilosa*. *Animal Behaviour* 35: 488–506.

Pacala, S.W., Hassell, M.P., and May, R.M. 1990. Host-parasitoid associations in patchy environments. *Nature* 344: 150–153.

Rønsted, N., Weiblen, G.D., Cook, J.M., Salamin, N., Machado, C.A., and Savolainen, V. 2005. 60 million years of co-divergence in the fig-wasp symbiosis. *Proceedings of the Royal Society of London B* 272: 2593–2599.

Weiblen, G.D., Douglas, W.Y., and West, S.A. 2001. Pollination and parasitism in functionally dioecious figs. *Proceedings of the Royal Society of London B* 268: 651–659.

West, S.A. and Herre, E.A. 1994. The ecology of the New World fig-parasitizing wasps Idarnes and implications for the evolution of the fig-pollinator mutualism. *Proceedings of the Royal Society of London B* 258: 67–72.

Wiebes, J.T. 1994. *The Indo-Australian Agaoninae (Pollinators of Figs)*. Amsterdam. North-Holland. 208 pp.