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The style–length of the female florets and their fate in two dioecious species of Xishuangbanna, China

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Abstract Constraints and evolution are central for the resolution of conflicts between mutualism species and for the stability of mutualisms. Dioecious fig species and their specific pollinators are also in conflict on the use of fig ovaries. Here, our experiments provided some data on the female florets allocation in two dioecious fig trees. The results showed that: (1) there is a bimodal distribution in the style-length of two fig trees' female florets, moreover, the style-lengths are fairly similar and narrowly distributed in gall figs and more variation seems to occur in seed figs; (2) the styles in seed figs are a little longer than those in gall figs; (3) the pollinator's ovipositor lengths are shorter than the style-lengths in seed figs, but they are very similar to those in gall figs so that pollinators can only lay their eggs into the ovaries of gall figs, but not in seed figs; (4) the stigmas stick together, and the style is curly and flexible in seed syconia of the two fig species studied, so it is very difficult for the pollinators to find suitable ovipositing sites and lay their eggs in seed figs; (5) the variations of stylelengths are bigger in seed figs than gall figs, but they are smaller in dioecious figs than monoecious figs; (6) for Ficus cyrtophylla, about 10% styles are shorter in seed figs than those in gall figs, even shorter than ovipositor. In contrast, about 2% styles in gall figs of Ficus hispida are longer

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Present address: D.-R. Yang Xishuangbanna Tropical Botanical Garden, Kunming Division, Chinese Academy of Sciences, 88 Xuefu Road, Kunming, Yunnan 650223, People's Republic of China than its corresponding pollinator's ovipositor. In a word, our study suggests that the female floret's fate in these two fig species is mainly dependent on its style–length, but not all. The stigma shape and the floral organization can both also attribute to their fate in the two fig species studied.

Keywords Dioecious · *Ficus* · Ovipositor length · Style–length · Mutualism

Introduction

One of the most interesting symbiotic interactions involves the pollination of the genus Ficus. This mutualism is obligatory for figs (Ficus species, Moraceae) and their pollinator fig wasps (Agaonidae: Hymenoptera). Ficus species have a peculiar inflorescence, the syconium or fig, which is lined with unisexual flowers that are male and female florets, respectively. The only connection to the exterior is the ostiole, located at the tip of the fig and protected by a cluster of bracteoles. Fig trees and fig wasps are involved in a very intimate mutualism in which they depend completely on each other in the long-term for pollination of the figs and the completion of the life cycle of the wasps. Fig trees are pollinated by species-specific wasps, and each wasp larva develops at the expense of an ovary (Ramirez 1970, 1974; Janzen 1979; Wiebes 1979; Michaloud et al. 1986, 1996; Herre et al. 1996b; Ma et al. 1997; Yang et al. 2001, 2002; Li et al. 2002). Moreover, this intimate relationship has presented in the Cretaceous (Galil 1977), and it is widely regarded as a model system for the study of coevolved mutualism.

In the world's 750 known *Ficus* species, approximately half of them are monoecious and others are functionally dioecious (Berg 1989; Weiblen 2000). In monoecious species, each syconium produces seeds, pollen and fig wasps (Yang et al. 2001; Weiblen 2002). The syconia in the functionally dioecious species are born on separate plants, which are respectively known as gall figs and seed figs. Hermaphrodite (functionally male) trees produce wasps

and pollen, while female trees produce seeds only. Female wasps pollinate and attempt to lay eggs in both type of figs but their offspring only develop in gall figs (King 1887; Weiblen et al. 2001; Yang et al. 2002). Until now, numerous authors are interested in the question as to why the fig wasps' offspring do not develop in the seed figs but only in the gall figs. The search for mechanisms that can restrain oviposition and stabilize the wasp-seed conflict has spanned more than three decades.

Symbiotic partners conflict with each other because their reproduction both depend on the female florets in the figs and the fitness of one partner often increases by reducing the fitness of the other. In other words, if any partner in the mutualism excessively exploits the female floret resources in syconia, it would result in the extinction of this mutualism. Therefore, the mechanisms that can restrain oviposition are the key to sustain this intimate mutualism. Compared with dioecious species, the conflict in monoecious species is more outstanding. Thus, previous studies have identified the factors that can stabilize the seed-pollinator conflict in monoecious fig species. Their results suggested that the great and continuous variation in style-length of the female flowers was a typical characteristic of monoecious figs, moreover, both seeds and wasps might develop in any flower (Ramirez 1969; Janzen 1979; Bronstein 1988; Verkerke 1989; West and Herre 1994; Ganeshaiah et al. 1995; Nefdt and Compton 1996; Herre and West 1997; Kathuria et al. 1995; Kathuria 1999). Recent studies on some monoeicous species showed that the style-length was not a key factor for a female flower's fate but its position (Anstett 2001). Only a few studies on the seed-pollinator conflict in dioecious species exist which were mostly performed on species from Neotropical areas and Africa.

In this paper, we present our work on two typical dioecious species *Ficus hispida* and *Ficus cyrtophylla* in Xishuangbanna to identify the factors of mutualism stability. Furthermore, we answered the following questions: (1) Does the fate of a female floret in the syconium only depends on its style–length? (2) Is there a bimodal distribution in these two dioecious species?

Materials and methods

Study species

Ficus cyrtophylla Wall. ex Miq. (section Sycidium) and *Ficus hispida* L. (section Sycocarpus) are both small- to medium-sized free-standing trees and dioecious species (Wu et al. 2003). The inflorescences of *F. hispida* are pollinated by *Ceratosolen solmsi marchali* Mayr (Yang et al. 2002), while those of *F. cyrtophylla* are pollinated by *Blastophaga* sp.

Methods

Because pollinating fig wasps only oviposit into the figs that are in the female-floral phase, we measured the style–

length of female florets in these female-floral phase figs. For F. cyrtophylla, we collected 34 gall figs from 10 functionally male trees and 34 seed figs from nine female trees; While for F. hispida, 27 gall figs were collected from eight functionally male trees and 28 seed figs were from eight female trees. We equally divided one fig into four parts and selected 5-6 florets per quarter at random to measure style-length resulting in a total of 20-30 florets per fig and at least in 20 florets per tree. At the same time, we collected some male-phase gall figs to get pollinators from various fig trees of the two species before the wasps exited, and placed into nylon bags until the wasps exited. Finally, we selected 40-50 female pollinating wasps to measure ovipositor length. Both style-lengths and ovipositor lengths were measured at $1.0 \times$ ocular micrometer fitted to an OLYMPUS (SZX12), a dissecting microscope. Simultaneously, the style and synstigma morphology in two kinds of figs were also compared.

A one-way ANOVA was used to determine if there were significant differences in the style–length among syconia and trees. An independent sample *t*-test was used to test if style–lengths had significant differences between gall figs and seed figs. For statistical treatment, oneway ANOVA and *t*-test were performed using SPSS 11.5 software.

Results

The style–length and corresponding pollinator's ovipositor-length distribution in each species

For *F. cyrtophylla* and *F. hispida*, there were significant differences in the style–length among trees and figs (Table 1). These results indicated that great style–length variations existed among different trees and figs. However, the style–length of gall figs always occurred in the reach of their corresponding pollinators, which was not the case for seed figs (Figs. 1 and 2).

There were significant differences in the style–length between gall figs and seed figs of the two species (*F. cyrtophylla*: t = -61.541, p < 0.001; *F. hispida*: t = -100.086, p < 0.001). The pollinators' ovipositor lengths were shorter than the style–lengths in seed figs but very similar to those in gall figs (Table 2).

There was a bimodal distribution in the style–length of two species (Figs. 1 and 2). However, the ovipositor-length distribution of the corresponding pollinators was very similar to the style–length distribution in gall figs. In *F. hispida*'s gall figs, about 2% of the style–lengths were longer than its pollinator's ovipositor length (Fig. 1). And about 10% of the style–lengths in *F. cyrtophylla*'s seed figs were shorter than its pollinator's ovipositor length (Fig. 2).

For *F. cyrtophylla*, the coefficients of variability (CV) in gall figs and seed figs were 0.166 and 0.209, respectively, suggesting a higher variation of the style–length in seed figs than in gall figs. For *F. hispida*, the coefficients of variability (CV) in gall figs and seed figs were more similar with 0.150 and 0.138, respectively (Table 2).

Table 1	One-way	ANOVA	on the sty	yle-length	variations among	g trees and f	ìgs.
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				Among trees		Among figs	
Ficus species	Trees sampled (n)	Figs sampled (n)	Florets sampled (n)	F	р	F	р
F. cyrtophylla							
Gall figs	10	34	651	34.092	< 0.001	13.561	< 0.001
Seed figs	9	34	536	12.185	< 0.001	5.839	< 0.001
F. hispida							
Gall figs	8	27	534	103.704	< 0.001	29.700	< 0.001
Seed figs	8	28	582	27.831	< 0.001	12.864	< 0.001

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Fig. 1 The ovipositor or style–length distribution in *F. hispida*



cyrtophylla

Fig. 2 The ovipositor or

style-length distribution in F.

□ Gall fig ■ Seed fig □ Ovipositor

The characteristics of the style morphology and floral organization in two kinds of figs

Through dissecting 61 gall figs and 62 seed figs from different trees of the two species studied, we found the differences between the two figs were not only in the style morphology but also in the floral organization. The female floret's styles in gall figs were short and straight, but those in seed figs were long and curly (Fig. 3A and B), the more short the styles in seed figs are, the more curly they are. Furthermore, the longer styles in seed figs were narrower and more flexible, Moreover, the stigma shape also differed between the two kinds of figs. In the two species' gall figs, every stigma looked like a bugle. The stigma surface in gall figs was larger than that in seed Fig. 3A and B).

In *F. cyrtophylla*, the male florets were scattered within the cavity among the female flowers in gall figs., all stigmas were well individualised; while for *F. hispida*, the male florets were lined around the ostiole, for the female ones in the whole gall fig, all stigmas were well individualised. Because of interlocking of the hairs on the style, there were some cohesive stigmas in seed figs of the two dioecious species studied (Fig. 3C and D).

Table 2Mean style–lengthsof two dioecious fig species andthe ovipositor lengths of theirpollinating fig wasps

	style–length (mm)				Oviposit	tor length (mm)		
Ficus species	n	Mean	SD	CV	n	Mean	SD	CV
F. cyrtophylla								
Gall figs	651	0.584	0.097	0.166	45	0.852	0.0988	0.116
Seed figs	536	1.385	0.289	0.209				
F. hispida								
Gall figs	534	0.620	0.093	0.150	50	0.751	0.094	0.125
Seed figs	582	1.649	0.228	0.138				

Fig. 3 Female florets of receptive figs from gall figs (*upper row*) and seed figs of *F. hispida* **A** and *F. cyrtophylla* **B**. Cohesive stigma of *F. hispida* **C** and *F. cyrtophylla* **D**



Discussion

Fig trees and wasps are in conflict over the fate of female flowers, and numerous authors have paid more attention to this conflict. For monoecious and dioecious species, the settlement mechanism of this conflict is different from each other. In monoecious species, the fig tree is selected to allocate one part of its reproductive effort to seed production and another to the male function. While in dioecious species, the whole functionally male tree is selected to the male function but the female tree to seed production. For pollinators, it may be beneficial to probe all female flowers in the syconium. Why the pollinators do not occupy all female florets in seed figs?

Our results showed that there was a bimodal distribution in the style–length of two dioecious species and the style–lengths in seed figs were much longer than those in gall figs. and than the ovipositor length. Because the larvae of wasps feed on developing endosperm, only the eggs laid between the ovule and integument in the ovary can develop (Cunningham 1888; Grover and Chopra 1971). Female wasps attempted to lay eggs in seed figs, too. But because the ovipositor length is shorter than the style–length in seed figs, they cannot completely pierce thoroughly the style to lay eggs into the fig ovaries. Therefore, their offspring only could develop in the gall figs. The fate of female flowers in the two dioecious species studied was mainly depended on the style–length, that is, the two dioecious species could resolve the seed-wasp conflict by the variations in style–length between gall and seed figs. This conclusion is also consistent with previous reports on some reported dioecious species (Ganeshaiah et al. 1995; Weiblen 2002; Weiblen et al. 2001).

Nefdt and Compton (1996) have reported that the observed foundresses often probed down a number of styles before ovipositing into one of them (distinguished by a pumping action of the gaster) and they seemed more likely to oviposit when the style was short. We also found similar phenomena. Because the pollinator's life is very short, only 1–2 days outside the figs, each wasp must budget her time between searching (probing floral styles for egg-free ovules) and handling (pushing eggs down styles) (Douglas et al. 2004). The coefficient of variability (CV) in seed figs. was much bigger than that in gall figs, which indicated little variation occurred in gall figs. Furthermore, the coefficient of variability of ovipositor was respectively 0.116 in F. cyrtophylla and 0.125 in F. hispida, which were very adjacent to that in style-length in gall figs. The little variation in gall figs could decrease the time the pollinator spends on probing floral styles for appropriate reproductive sites, so as to lay more eggs in her limited life span. The coefficients of variability (CV) in the two dioecious species studied were smaller than those in several monoecious fig. species in India (Kathuria et al. 1995). These results imply that dioecious species might have relatively perfected the style–length's variation for providing the gall figs. for wasp reproduction. Whether this is all-pervading in all the dioecious species or not need to be widely tested in future.

Compared with some previous reports on the seed-wasp conflict in some monoecious species (Galil and Eisikowitch 1968; Bronstein 1988; Kathuria et al. 1995; Anstett 1996, 2001; Nefdt and Compton 1996; Herre 1999; Otero and James 2002; Douglas et al. 2004; Jousselin et al. 2004), we found that dioecious species and monoecious species may adopt different mechanisms to resolve the conflict. The monoecious species control seed loss through increasing the style-length variation which can increase the probing and handling time for the pollinator's oviposition, but the dioecious ones decrease the style-length variation and only produce short-styled flowers in gall figs to facilitate the pollinator's oviposition, and the seed figs mainly inhibit the pollinator's oviposition through almost only bearing longstyled florets. Kjellberg and Maurice (1989) presented seasonal conditions might favor the evolution towards functional dioecy in *Ficus* through changing the variation in the style–length, which resulted in the occurrence peak of seed set and wasp production in different time year-round. Douglas et al. (2004) deduced that any evolutionary transition to monoecy from dioecy would necessarily be accompanied by an increase in the style-length variation from their models. Our results agreed with this conclusion, but is it ubiquitous in the whole fig world? This should be tested through dissecting a number of monoecious and dioecious fig species.

That style–length variations and the significant differences among the fig trees and syconia in the two fig species studied indicated there were some strong reproductive races between wasp production and seed production in the evolutionary scale. Each partner would like to complete its biggest reproductive benefits. These also showed that the wasp-seed conflict could be well resolved through the style–length variations. Our results also implied that the two dioecious species might be the transitional type in the evolutionary course from monoecy to dioecy.

In addition, about 2% style–lengths in gall figs of *F*. *hispida* were longer than the ovipositor length, but why no accidental seeds do produce in these gall figs, which is a very interesting question, too. This may be because its pollinator, *C. solmsi marchali* Mayr, is an active pollinator,

it always manages to precisely deposit pollen on the stigmas of the flowers into which they have oviposited and very little pollen ends up on the stigmas of non-oviposited flowers, and the separation of the stigmas forbids growth of a pollen tube from one flower into another.

In F. cyrtophylla, about 10% of the style–lengths in seed figs were shorter than those in gall figs and the ovipositor length. But why did the pollinating fig wasps not explore these florets in seed figs? Through dissecting a number of two types of figs, we found that the stigmas in gall figs were well individualised but those in seed figs existed as cohesive stigma, which could conduct growing pollen tubes to all conjoint female flowers and contribute to improve the seed set efficiency (Verkerke 1989; Jousselin and Kjellberg 2001). But the cohesive stigma would make it very difficult for pollinators to find appropriate oviposition sites. Except that, the styles in seed figs were often more narrower and flexible. All of these may hamper the pollinator's oviposition. In addition, the life of the pollinator is very short, only 1–2 days. Therefore, the cohesive stigma could decrease the oviposition chances for the pollinators to guarantee the seed set efficiency in female trees, and the pollinators cannot probe these short-styled flowers to reproduce in seed figs; This also suggested that the floral organization in seed figs could play a very important role on the female flower's fate as well.

In the two dioecious species, every stigma in gall figs looked like a bugle and well individualized, which were very helpful for wasp oviposition. The stigma surface in gall figs was much bigger, but not the case for seed figs. Consequently, except for the style–length of a female flower, the characteristics of the female floret's morphology also contributed to their fate. In a word, the fig wasp's oviposition was not only affected by the ovipositor length but also by the stigma shape and the floral organization, that is, the dioecious species can resolve the seed-wasp conflict not only through the style-length but also the stigma morphology and floral organization. Hence, it seems that the gall figs of dioecious species present some adaptations to facilitate wasp oviposition. These results also suggested that dioecious species and their corresponding pollinator had some high coadaptations on the female flower allocation.

In summary, our results suggest that the female floret's fate in these two fig species is mainly dependent on its style– length, but not all. The stigma shape, the cohesive stigma and the flower organization can all also attribute to their fate, which implies that host plants can set up a competitive landscape that favors reciprocal behavior. Compared with other reports on the wasp-seed conflict (Bronstein 1988; Bronstein and Bernays 1992; West and Herre 1994; Nefdt and Compton 1996; Herre 1999; Anstett 2001; Weiblen et al. 2001; Otero and James 2002; Jousselin et al. 2004), all the results indicate that different host-pollinator species combinations can achieve the mutualism stability through different mechanisms.

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