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Why do fig wasps actively pollinate monoecious figs?

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Abstract Active pollination, although rare, has been documented in a few pollination mutualisms. Such behaviour can only evolve if it benefits the pollinator in some way. The wasps that pollinate *Ficus* inflorescences can be active or passive pollinators. They lay their eggs in fig flowers, so that a proportion of flowers will host a wasp larva instead of a seed. We show in an actively pollinated monoecious fig that lack of pollination does not induce fig abortion or affect wasp offspring size but results in smaller numbers of offspring. Hence, conversely to other active pollination systems, seed formation is not obligatory to sustain developing pollinator larvae; however there is a direct fitness cost to active pollinators not to pollinate. We then compared the locations of eggs and fertilised flowers of three actively pollinated *Ficus* species and one passively pollinated species. We found that more flowers containing wasp eggs were fertilised in the actively pollinated species relative to those of the passively pollinated one. These results along with comparison with similar studies on dioecious figs, support the hypothesis that active pollination has evolved in fig wasps to ensure that more flowers containing wasp eggs are fertilised as this may increase the chances of successful gall development. The stigmatic platform characterising actively pollinated figs is probably an adaptation to increase pollen dispersion within the fig.

Keywords Coevolution · Mutualism · Pollination behaviour · Stigma

Introduction

In most plant-pollinator interactions, pollination is a by-product of insect visitations; it occurs via morphological adaptations of flowers that favour pollen deposition on and removal from the pollinator body. In a few cases, however, insects display morphological and/or behavioural traits which allow them to load, transport and deposit pollen (Pellmyr 1997; Thompson 1989). Such a syndrome is known as active pollination. To date, it has been reported in three pollination mutualisms: the yucca/yucca moth interaction (Riley 1892), the *Ficus*/agaonid wasp association (Galil and Eisikowitch 1969) and the senita cactus/senita moth interaction (Fleming and Holland 1998). Active pollination could not have evolved in the absence of any benefit of the behaviour to the pollinator itself. In the case of the yucca and senita cactus moths, the selective advantage of active pollination is quite evident. Pollinator larvae feed on developing seeds and they face starvation in their absence (Pellmyr 1997; Holland and Fleming 1999). Furthermore, it has been shown that some *Yucca* species abort poorly pollinated fruits (Huth and Pellmyr 2000). This implies that active pollination also results from selection imposed by the host plant on the insects to increase seed set beyond what is needed for the nutrition of their larvae (Huth and Pellmyr 2000). Hypotheses concerning the selective pressures leading to the evolution of active pollination in fig pollinating wasps are not as clear (Kjellberg et al. 1987; Herre 1999). There is no evidence that pollination is obligatory for wasp development. In addition, while two-thirds of pollinating fig wasp species are active pollinators, other wasp species transfer pollen passively (Galil and Neeman 1977; Kjellberg et al. 2001). Molecular phylogenies show that there have been numerous transitions from active to passive pollination (Yokoyama 1995; Machado et al. 2001). This suggests that the selective

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factors maintaining active pollination in fig wasps can be relaxed.

Each of the 700 species of *Ficus* (Berg 1989) is pollinated by one (or several: Rasplus 1996; Molbo, personal communication) species-specific wasp(s) (Agaonidae; Chalcidoidea; Hymenoptera). The enclosed *Ficus* inflorescence (the fig) contains uniovulate female flowers. In monoecious *Ficus* species (approximately half of all species), each inflorescence produces seeds, fig wasps (pollen vectors) and pollen. When the fig is receptive, i.e., ready to be pollinated, female wasps (foundresses) enter the fig cavity, oviposit through the styles of individual flowers, and pollinate (Galil and Eisikowitch 1969). Agaonid wasp eggs are deposited between the integument and the nucellus of the ovule (Verkerke 1989). Ovules that have received an egg are transformed into a gall in which wasp larvae complete their development, while ovules that have been fertilised and did not receive an egg produce a seed. Thus each wasp larva develops at the detriment of a seed. When the figs are mature, male offspring wasps emerge from their galls, mate with female offspring wasps, after which female wasps get loaded with pollen and leave their natal fig.

When pollination is active, the wasps collect pollen from the anthers within their natal fig and store it in thoracic structures known as pollen pockets. Upon arrival at a receptive fig, each time a wasp deposits an egg in a flower, it picks some pollen grains from the pockets and deposits them on the stigmas (Galil and Eisikowitch 1969; Frank 1984; Greeff and Compton 1996). When pollination is passive, agaonid wasps do not exhibit such behaviour and the associated figs produce numerous stamens that dehisce at wasp emergence such that the wasps get covered with pollen when leaving their natal fig (Galil and Neeman 1977; Galil and Meiri 1981; Ramirez 1969, Kjellberg et al. 2001). These wasps transfer the pollen that adheres to their body.

Why do some agaonid wasps actively pollinate fig flowers?

What is the reproductive interest of agaonid wasps in pollinating fig flowers? In monoecious figs, whether individual flowers become a seed or a gall will yield different benefits for the mutualistic partners: figs benefit from both wasp production (their only pollen vectors) and seed production whereas wasps reproductive success is only directly linked to the number of flowers hosting their offspring (Janzen 1979; Herre 1989). Hence, the evolution of active pollination raises the following question: do fig wasps directly benefit from pollinating the flowers into which their offspring develop or indirectly benefit from enhancing seed production? In this framework we can propose three hypotheses concerning the adaptive significance of active pollination for the wasps.

1. According to early papers, fertilised flowers provide a better feeding substrate for wasp larvae (Kjellberg et al

1987; Verkerke 1989). Under this hypothesis, the function of active pollination for the wasps is to ensure the fertilisation of the flowers into which they oviposit.

2. Janzen (1979) suggested that unpollinated figs abort; this would constitute a retaliatory strategy by the fig against pollinators that defect (Axelrod and Hamilton 1981). Under this hypothesis, active pollination has evolved, as is conjectured for *Yucca* moths, under selection to increase seed set and limit fig abortion.
3. In monoecious figs, wasps might benefit indirectly from seed production. An increase in seed production might improve fruit development and allow more resources to be allocated to gall development (Herre and West 1997). Under this hypothesis, the function of active pollination for the wasps is to increase seed set.

In this study, we explore the relevance of each hypothesis concerning the selective advantage of active pollination in monoecious figs. We addressed two questions: (1) How does lack of pollination affect wasp development and (2) which flowers are fertilised: the flowers containing a wasp egg or the entire inflorescence? To address the first question, we interfered with the pollen loading process of one actively pollinated fig species (*F. microcarpa*), and measured the fitness consequences to the wasp in terms of the number and size of offspring. Regarding the second question, we looked at the flowers of three actively pollinated species (*F. microcarpa*, *F. salicifolia*, *F. sur*) and one passively pollinated fig (*F. maxima*), in order to determine whether they were fertilised and/or had received a wasp egg. The following predictions were derived from hypotheses 1, 2 and 3 and then compared with our results:

Under the first hypothesis (wasps benefit from developing in fertilised flowers): (1) lack of pollination affects number and size of offspring, and (2) active pollinators preferentially fertilise flowers into which they lay their eggs.

Under the second hypothesis (figs abort unpollinated fruit): (1) lack of pollination induces fig abortion, and (2) active pollinators preferentially fertilise flowers into which they do not lay an egg in order to produce seeds.

Under the third hypothesis (wasps benefit from seed development within the fig): (1) lack of pollination affects number and/or size of offspring, and (2) active pollinators preferentially fertilise flowers into which they do not lay an egg.

Materials and methods

Study species

Ficus microcarpa Linn. f. (subgenus *Urostigma*, section *Conosycea*) is pollinated by *Eupristina verticillata* Waterston). Experiments on this species were conducted in Seria, Brunei Darussalam, Borneo, in January 2000. *F. salicifolia* Vahl (subgenus *Urostigma*, section *Urostigma*) is pollinated by *Platyscapa awekei* Wiebes. *F. sur* Forssk. (subgenus *Sycomorus*, section *Sycomorus*) is pollinated

in South Africa by *Ceratosolen capensis* Grandi. *F. salicifolia* and *F. sur* were collected in South Africa in November 1999. According to the phylogeny of *Ficus* (Weiblen 2000), these three actively pollinating species belong to three lineages of monoecious figs. *F. maxima* (section *Pharmacosycea*) is pollinated by *Tetrapus costaricanus*, it belongs to the basal, passively pollinated, lineage of *Ficus*. *F. maxima* figs were collected in Panama in May 2001.

Interestingly, the stigma morphology of the study species was strikingly different. Though stigmas of *F. sur* were tubular and stigmas of *F. salicifolia* and *F. microcarpa* were elongate, in the three species, their global arrangement in the fig cavity resulted in the formation of a cohesive platform. They all reached the same level in the fig cavity and adhered to each other via their papillae. Such a stigmatic platform was not observed in *F. maxima*. In this species, stigmas were well individualised and some of them were elongate, projecting into the fig cavity. In addition, we regularly observed lateral pollen tube growth in *F. sur* and *F. microcarpa*: pollen grains that were deposited on one stigma often grew into a neighbouring style. Such a phenomenon was never observed in *F. maxima* and seems to be morphologically impossible because stigmas reach variable heights in the fig cavity.

Effect of lack of pollination on wasp development

We introduced pollen-free female wasps into receptive figs of *F. microcarpa*. To obtain pollen-free wasps, we collected figs in which wasps were about to emerge, on a single tree. We opened some of these figs and removed their few stamens with fine forceps. To be certain that female wasps that were going to be used were inseminated, we selected figs in which males had already emerged and were mating females that were still in their galls. Wasps were then allowed to emerge in a fine-mesh bag. Pollen-loaded foundresses (control) were obtained by allowing wasps to emerge normally from figs collected from the same tree. To control for the successful procurement of pollen-free wasps, a subsample of 20 females used in each treatment were squashed on a slide and the presence of pollen grains in their pollen pockets was checked for under a light microscope. The 20 wasps from the subsample of the control experiment carried numerous pollen grains in their pockets (>80 pollen grains per wasp) whereas the 20 wasps in the sample of the "pollen free" experiment had empty pollen pockets.

A total of 25 figs spread across five branches of a single tree were selected when figs were at the pre-pollination stage and enclosed in fine-mesh bags to prevent wasps access. When figs reached the receptive stage, on the same day, we introduced either a pollen-free wasp or a pollen-loaded wasp into randomly chosen figs among the preselected ones. Controlled pollinator introductions were achieved by placing pollinators near the ostiole, using a camel-hair brush. We successfully introduced a pollen-free wasp into 11 figs and a pollen-loaded wasp into 12 figs. A drop of non-toxic glue was applied on the ostiole after pollinator introduction to prevent any foundress from exiting a fig and entering another fig (Gibernau et al. 1996).

Figs were collected 3 weeks later when offspring wasps were about to emerge. Each fig was placed in a mesh-covered vial and the wasps were allowed to emerge over the next 24 h. In each fig, the numbers of male wasps, female wasps, empty flowers and bladders (flowers with swollen ovary but no wasps) were counted. We also measured the effect of lack of pollen on body size of the new emerging wasps. The tibia length of wasps (estimate of wasp body size: J-Y. Rasplus, personal communication) emerging from pollinated and unpollinated flowers was measured to the nearest micrometre under a stereoscopic microscope for about ten wasps per fig.

Statistical analysis

The number of wasps produced in a fig is positively correlated with the number of female flowers of the fig (Herre and West 1997). Thus to analyse the effect of lack of pollen on production within a

fig (wasps, bladders, undeveloped female flowers), we used an analysis of covariance with number of female flowers in the fig as a covariate. The treatment effect on wasp body size was tested using a mixed model analysis of variance, with the fig effect nested in the treatment effect. We used the GLM procedure of the SAS statistical package (SAS 1999).

Do pollinating fig wasp larvae only develop in fertilised flowers?

We performed pollination experiments on *F. microcarpa*, following the protocol used in experiment 1. On the same day, either a single pollen-loaded foundress or three pollen-loaded foundresses were introduced into randomly chosen receptive figs (the foundress number found for this species ranges from one to two; Gibernau et al. 1996). All foundresses had emerged from the same tree. We successfully introduced three foundresses into each of 14 figs and a single foundress into 12 other figs. Figs were collected 24 h after wasp introduction and preserved in FAA (1:1:8, formalin: acetic acid: 70% ethanol) in order to fix tissues.

We could not perform a pollination experiment on *F. sur*, *F. salicifolia* and *F. maxima* because trees in the appropriate stage could not be found during the period of the study. We therefore studied naturally pollinated figs. Figs of *F. salicifolia* and *F. sur* were collected 2 days after pollinator visits. We could thus not assess with certainty the number of foundresses that had visited the figs, as some might have escaped before figs were collected. Figs of *F. maxima* were collected when pollinators were seen entering the figs. Each fig was preserved in a vial for a day until wasps had died to ensure that foundresses had had time to deposit the pollen they transported. The number of foundresses that had visited each fig was recorded. Collected figs were preserved in FAA.

For each fig of the three species, 10–50 flowers per fig (depending on the species) randomly chosen within the inflorescence were removed with fine forceps. The flowers were soaked for 48 h in a solution of aniline blue (0.01%) in 0.1 M K_3PO_4 and NaOH (20%) in order to soften the tissues. Flowers were then placed on a microscope slide and gently squashed under a coverslip. They were examined for the presence of pollen tubes within the style and wasp egg presence in the ovule under a compound microscope with ultraviolet epifluorescence illumination. UV light excites the aniline blue taken up by the callose of pollen tubes, making the latter clearly visible (Kearns and Inoué 1993).

Statistical analysis

Data obtained for *F. microcarpa* and *F. maxima* were classified in a contingency table according to four factors: foundress number, fig, presence/ absence of egg, presence/ absence of pollen tube. The contingency tables used for *F. sur* and *F. salicifolia* data were similar except that they did not include the factor number of foundresses. We then fitted a log-linear model to the data, assuming Poisson error and using log link in the Glim statistical package (GLIM 1985). Initially, a full model was fitted to the data, including all factors and their two-way and three-way interactions. We then tested for the significance of interactions between the factors (non-significant interactions between factors mean that the factors are independent), by removing interaction terms from the full model by stepwise deletion. The difference in deviance between two nested models follows a χ^2 distribution with number of degrees of freedom equal to the difference in number of parameters in the two models (Crawley 1993). When overdispersion occurred, we used Pearson's χ^2 to adjust the scale parameters.

Results

Effect of lack of pollination on wasp development

Rates of fig abortion did not differ between *F. microcarpa* figs that had been visited by pollen-bearing and pollen-free wasps. Four figs of 11 unpollinated figs aborted, and 4 of 12 pollinated figs aborted. Figs in which "pollen-free" wasps had been introduced did not produce any seeds, confirming that the wasps introduced did not carry pollen. Both types of figs produced viable wasps. The number of wasps produced per fig was explained by total number of flowers within the fig ($F_{1,12}=6.37$, $P=0.026$) but also partly by treatment ($F_{1,12}=4.07$, $P=0.066$). Agaonid wasps reproducing in unpollinated figs tended to have fewer offspring (mean=32, SD=27, $n=7$) as compared with agaonid wasps reproducing in pollinated figs (mean=63, SD=36, $n=8$) (Fig. 1). The number of bladders was low in both treatments, but was slightly higher in unpollinated figs (pollinated figs: mean=5, SD=3; unpollinated figs: mean=8, SD=3; $F_{1,12}=4.92$, $P=0.04$). Tibia size of emerging wasps was the same for both treatments (pollinated figs; mean=0.134 mm; SD=0.006, $n=84$; unpollinated figs; mean=0.132 mm, SD=0.005, $n=41$; $F_{1,112}=0.34$, $P=0.56$) but varied among figs ($F_{10, 112}=2.05$, $P=0.034$). There was no correlation between the number of wasps produced in a fig and their average size ($F_{1,11}=3.71$, $P=0.08$), suggesting that figs producing less wasps did not produce bigger wasps. Wasp sex ratio did not differ between treatments (unpollinated figs male/male + female=0.08±0.05; pollinated figs male/male + female=0.09±0.08, Mann and Whitney *U* test, NS), suggesting a lack of differential larval mortality between sexes.

Do fig wasp larvae only develop in fertilised flowers?

In all three actively pollinated species, the interactions between presence of an egg in a flower and fertilisation of the flower were non-significant (Table 1): oviposition in a flower and fertilisation of a flower were independent events. For all analyses the residual scaled deviance was approximately equal to the residual degrees of freedom, showing that the data were not overdispersed.

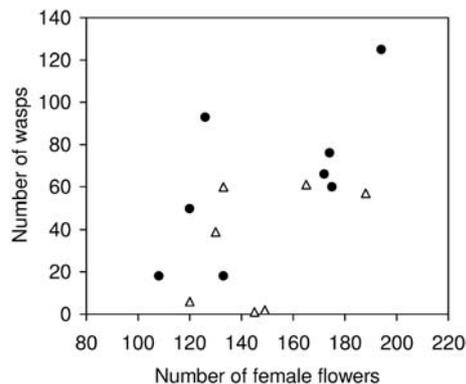


Fig. 1 Number of wasps produced in pollinated (●) and unpollinated figs (△)

For *F. microcarpa* the three-way interaction between number of foundresses, oviposition and fertilisation was non-significant: it implies that whatever the number of foundresses, the degree of association between oviposition and pollination did not change (Table 1). For *F. microcarpa*, the interaction between treatment and pollination was significant (Table 1): the probability of a flower being fertilised decreased with number of foundresses (Fig. 2). As a result, in single foundress figs, about half the eggs were deposited in non fertilised flowers whereas, in three-foundress figs most wasp eggs were deposited in fertilised flowers (73%) (Fig. 2).

In *F. salicifolia* figs, as only half of the flowers were fertilised, half of the wasp eggs were deposited in unfertilised flowers. In *F. sur* figs as most flowers were fertilised, most wasp eggs (82%) were deposited in fertilised flowers (Fig. 2).

The interaction between oviposition and pollination was significant in *F. maxima* (Table 1). Whatever the number of foundresses (range=1–5, mean=2.5) almost all wasp eggs were deposited in unfertilised flowers (86%) (Fig. 2), hence the probability of a flower receiving a wasp egg was negatively correlated with its probability of being fertilised.

Table 1 Results of Log Linear Models Analysis. The complete model includes all factors (fig, egg, pollen tubes) and all relevant two-way and three-way interactions between factors, χ^2 value corresponds to change of deviance resulting from the removal of the interaction term

<i>Ficus</i> species	Number of figs (number of flowers)	Interactions modelled	χ^2 (1 df)	Test
<i>F. salicifolia</i>	3 (60)	Pollen tube × egg	0.52	$P = 0.47$
<i>F. sur</i>	3 (160)	Pollen tube × egg	0.46	$P = 0.49$
<i>F. microcarpa</i>	26 (350)	Foundress number × pollen tube × egg	0.79	$P = 0.37$
		Pollen tube × egg	0.32	$P = 0.57$
		Foundress number × pollen tubes	37.1	$P < 0.001$
		Foundress number × pollen tube × egg	1.04	$P = 0.30$
<i>F. maxima</i>	15 (336)	Pollen tube × egg	24.4	$P < 0.001$

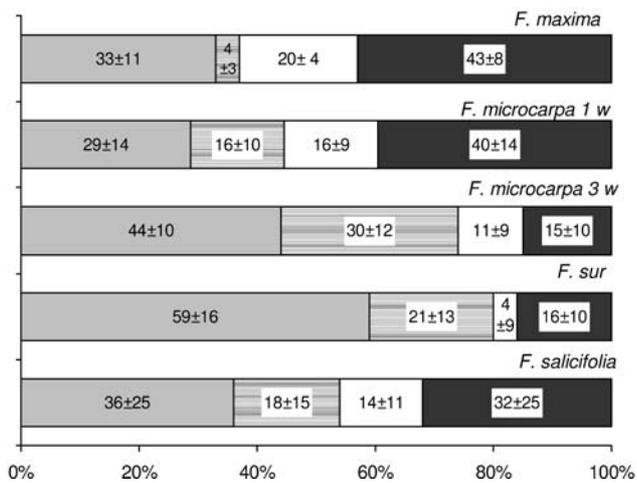


Fig. 2 Percentage of: pollinated flowers with no pollinator egg □, pollinated flowers with a pollinator egg ▨, non-pollinated flowers with a pollinator egg □, vacant flowers ■

Discussion

Effect of lack of pollination on wasp development

There are three hypotheses on the selective advantage of active pollination for agaonid wasps: (1) wasp larvae benefit from developing in fertilised flowers, (2) unpollinated figs abort, or (3) wasps benefit from the development of seeds. We show that unpollinated figs of *F. microcarpa* did not abort and produced viable pollinators. These results are consistent with similar experiments on the monoecious *F. religiosa* (Galil and Eisikowitch 1971), *F. sur* and *F. burtt-davyii* (Nefdt 1989) and the dioecious *F. condensa* (Jousselin and Kjellberg 2001). In addition, *Ceratosolen galili*, a parasitic agaonid wasp of the monoecious *F. sycomorus*, and the passive pollinator of the dioecious *F. carica*, reproduce in unpollinated figs in nature (respectively Compton et al. 1991, Kjellberg et al. 1987). Thus, there is now evidence on seven species (five active pollinators, one passive and one non-pollinator) that pollination is not obligatory for agaonid wasp's reproduction. Hypothesis 2 is therefore clearly contradicted by all published data: figs do not retaliate against wasps that do not pollinate by aborting fruit. This idea was initially based on a paper by Galil and Eisikowitch (1968) in which they conducted a pollen-free experiment which led to the abortion of the manipulated figs. However in a later paper, the same authors repeated the experiment and reported that the results of the first study were an artefact due to the use of opaque bags to enclose the figs (Galil and Eisikowitch 1971). The fact that agaonid wasps complete their development in unpollinated figs also implies that agaonid wasps are not obligate seed eaters; they can develop in unfertilised ovules, as do many parasitic galling wasps associated with figs (West et al. 1996). However, in *F. microcarpa*, similarly to results obtained on *F. sur* and *F. burtt-davyii* (Nefdt 1989), lack of pollen tended to decrease brood size. This is in line

with the observation by Herre and West (1997) that, in actively pollinated monoecious fig species, the number of wasps produced per fig is correlated to the proportion of flowers that develop. Hence, to the degree to which flower development is linked to pollination, greater pollination levels seem to benefit the wasps (Herre and West 1997). This is consistent with both hypotheses 1 (fertilised ovules provide better nourishment for wasp larvae) and 3 (figs producing seeds provide more resources for wasp larvae).

In agreement with hypothesis 1, Galil and Eisikowitch (1971) proposed that in the absence of ovule fertilisation, wasp larvae feed on nucellar tissue and the parthenogenetic endosperm instead of the fertilised endosperm, which might be relatively poor food sources and consequently increase larval mortality. On the other hand, lack of seed production might also decrease the quantity of resources allocated to the fig and induce the starvation of wasp larvae. However, our observation that wasps developing in non-pollinated figs reached on average similar body size as wasps developing in pollinated figs suggests that in the absence of pollination, wasp larvae did not suffer from lack of nutrition at the flower level. Hence, lowered nutrition is not an adequate explanation for smaller brood sizes. This is inconsistent with the predictions of both hypotheses 1 and 3. As our data are based on a single species and a small sample of figs, these results will need to be confirmed. Nevertheless, they suggest a possible fourth hypothesis: the fertilisation of the flowers in which wasps deposit an egg might increase the chance of successful gall formation, but not the quantity of food available for the larva. This would explain why larvae survivorship increases with pollination, but not their body size. This hypothesis is in line with the fact that the critical phase in galler development is the induction of the gall (Rohsfrichts 1992). It also implies that the function of active pollination for the wasps is the fertilisation of flowers into which they oviposit.

Which hypothesis do the data on distribution of fertilised flowers sustain?

In the three actively pollinated monoecious species studied, fertilisation of a flower and oviposition are independent events: wasps do not solely fertilise flowers into which they oviposit, they do not either preferentially fertilise flowers which do not receive an egg. These results are inconsistent with the predictions of both hypotheses 1 and 3. They also differ from what has been observed in male figs of functionally dioecious fig species in which active pollinators almost solely fertilise flowers into which they oviposit (Jousselin and Kjellberg 2001, see Table 2). However in dioecious species, the conflicts of interest between the partners of the mutualism are very different. In dioecious figs, the reproductive functions are separated onto different trees. Female trees bear figs within which wasps cannot oviposit, due to the exces-

Table 2 Summary of results on the distribution of fertilised flowers and stigma morphology according to the fig sexual function and the mode of pollination of the associated Agaonidea

	Active pollination	Passive pollination
Monoecious		
Produce wasps and seed	Independence between flower fertilisation and egg deposition (3 species) Stigmas form a platform	Flowers containing a wasp egg are almost never fertilised (1 species) Stigmas individualised
Dioecious (Jousselin and Kjellberg 2001)		
Male figs: produce wasps	Flowers containing a wasp egg are almost always fertilised (2 species) Stigmas individualised	Independence between flower fertilisation and oviposition attempt (2 species) Stigmas individualised
Female figs: produce seeds	Flower fertilisation is slightly dependent of oviposition attempt (2 species) Stigmas form a platform	Independence between flower fertilisation and oviposition attempt (2 species) Stigmas individualised

sively long styles of female flowers. As a result, female figs produce seeds but no wasps. Male trees bear figs that present both male and short-styled female flowers as a result, figs produce pollen and its vectors (wasps) (Valdeyron and Lloyd 1979). Hence, in male figs of dioecious species, deposition of pollen solely on flowers into which insects lay their eggs by ensuring that all fertilised flowers will host a larva, converge with figs reproductive interest, i.e. wasp production. Conversely, in monoecious figs, the sole fertilisation of flowers containing an egg is counter-adaptive, as it would result in absence of seed production. So, an alternative explanation of our results is that active pollination is aimed at the fertilisation of the flowers containing a wasp egg and fig traits favour the pollination of the entire inflorescence. Comparative analyses actually show that stigma structure is directly related to the mode of pollination of the pollinator (Jousselin 2001). During the receptive stage, stigmas of actively pollinated monoecious figs and female figs (which also produce seeds, Table 2) form a cohesive platform. This structure results in lateral pollen tube growth and probably equalises the chances of fertilisation for all flowers. Such a synstigma is absent in actively pollinated male figs and passively pollinated species (dioecious and monoecious) (Verkerke 1989; Jousselin and Kjellberg 2001; Jousselin 2001).

We also show that in the passively pollinated *F. maxima*, it is mostly flowers that do not receive a wasp egg that are fertilised. In this species, as in other monoecious figs, wasps develop preferentially in the shorter styled flowers (Ganeshiah et al. 1995; Nefdt and Compton 1996; Jousselin et al. 2001; Anstett 2001; E.J., E.A.H., F.K., unpublished data on *F. maxima*). Thus, it seems that the stigma morphology of *F. maxima* leads to more frequent fertilisation of long-styled flowers. If this result is general to passively pollinated monoecious fig species then, this also suggests that active pollination might have evolved to overcome this fig trait and allow the fertilisation of flowers in which wasps lay their eggs.

Conclusion

We show that, conversely to other active pollination systems, seed production is not obligatory to sustain wasp development, however we show a direct fitness cost for an active pollinator not to pollinate. From a previous study on dioecious figs and our observations of the distribution of fertilised flowers in monoecious figs, we suggest that active pollination might have evolved as a way to increase the rate of fertilisation of flowers in which wasp offspring develop. To properly test this hypothesis, comparative analyses of larval development in fertilised and unfertilised flowers are necessary. In this context, to the extent that the fertilisation of flowers containing a wasp egg also results in an increase in the level of fertilisation of the whole inflorescence, active pollination could benefit both partners of the mutualism. The stigmatic platform characterising actively pollinated monoecious fig species may actually ensure the pollination of the whole inflorescence.

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