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Why do fig wasps pollinate female figs?

Shazia Raja, Nazia Suleman, and Stephen G. Compton*

Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK, Tel. +44-113-3432858, Fax. +44-113-343, Emails. bgysra@leeds.ac.uk, bgyns@leeds.ac.uk, s.g.a.compton@leeds.ac.uk

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Abstract

The relationship between fig trees and their pollinator fig wasps is one of the best known examples of a highly-specific plant-insect mutualism, involving pollen-carrying foundress female fig wasps that enter the figs to lay their eggs. In functionally dioecious figs, the fig wasp larvae can only develop in the figs of male plants. Female plants have 'tomb blossoms' where the pollinators fail to reproduce and only seeds develop. Some foundress fig wasps can nonetheless re-emerge from figs after entry, so why do they not rapidly leave female figs without pollinating them? Selection on fig wasp behaviour generated on male fig trees, but expressed on both male and female plants, may provide an explanation. Wasps that re-emerge from female figs have no wings and will never reproduce. Consequently, natural selection cannot influence wasp behaviour once they enter a female fig and their behaviour should reflect what is optimal in male figs. Consistent with this explanation, pollinator behaviour in female figs was found to be largely similar to that in male figs. Thus pollination may be achieved because pollinators are doing what would have been best for them, if they had reached a fig where reproduction was possible.

Keywords: Dioecy, *Ficus*, *Liporrhopalum*, pollination, vicarious selection

1. Introduction

Quantification of the factors affecting the reproductive success of pollinators and the plants they pollinate is central to the study of pollination biology (Herre et al., 1999; Pellmyr, 2003). Pollinating fig wasps (Hymenoptera, Agaonidae) are associated with fig trees (*Ficus*, Moraceae), which are characterised by their unique inflorescence. The fig (syconium) has the form of a hollow sphere, lined on the inside by numerous tiny flowers. Each species of fig tree is pollinated by females of one, or sometimes 2–3, species of fig wasp that are not associated with any other *Ficus* species. How pollination is achieved depends upon the species of fig wasp. In some species pollen is transported passively, trapped on the body of foundresses and deposited incidentally in the figs. In other species, foundresses actively gather pollen from their natal figs into special structures on their bodies called pollen pockets and deliberately deposit the pollen directly on the stigmas of female flowers while ovipositing (Ramirez, 1974).

Such active pollination has evolved rarely, possibly in only four groups, yucca moths, senita moths, *Epicephala* moths and fig wasps (Cook et al., 2004). There are two breeding systems present in *Ficus*, monoecy and dioecy. The figs of monoecious species contain both female and male flowers and they produce seeds, wasps, and pollen within a single fig. The foundress females enter the figs through the ostiole, pollinate the female flowers and oviposit in some of them. The adult offspring mate inside the fig and the females exit carrying pollen in search of other figs to lay their eggs. Functionally dioecious fig species have two different types of figs, occurring on separate plants. Female figs contain only female flowers. Male figs contain modified female (gall) flowers and some male flowers. The male fig trees produce pollen and the fig wasps to transport it, whereas female figs only produce seeds. In dioecious fig trees those fig wasps that enter figs on male plants can reproduce, just as in monoecious figs, but no seeds are produced, whereas foundresses entering female figs cannot lay eggs, and only act as pollinators. Until relatively recently it was assumed that once a foundress enters a fig it could never re-emerge, but

*The author to whom correspondence should be sent.

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Gibernau et al. (1996) showed that some species can re-emerge and then subsequently oviposit in other figs. Increasing numbers of fig wasps are now known to re-emerge and Moore et al. (2003a) suggested that such re-emergence may be more common amongst the pollinators of dioecious than monoecious fig trees.

The mating system in dioecious fig species results in an evolutionary conflict between these fig trees and their pollinators. For fig seeds to be produced, the fig wasps must be attracted to enter and pollinate female figs. Even though foundresses may re-emerge from female figs (Gibernau et al., 1996) they always fail to reproduce and natural selection should favour foundresses that are able to discriminate between male and female figs. Despite this, experimental studies have usually found that foundress fig wasps fail to distinguish between male and female figs and are equally willing to enter figs of either sex (though there are technical difficulties with many such experiments, because the ages of the figs has rarely been standardised (S. Raja and S.G. Compton, unpublished). *Blastophaga psenes*, the pollinator of *Ficus carica*, is apparently exceptional in that foundresses prefer to enter male figs if given a choice (Anstett et al., 1998), but in this temperate species the sexes largely flower at different times, so most emerging wasps have no opportunity to exhibit this preference.

The general inability of pollinators to distinguish between male and female figs has been attributed to vicarious selection on the male plants leading to inter-sexual mimicry (Grafen and Godfray, 1991). Host fig choice in fig wasps is based largely on species specific volatile blends released from the figs at the time that they are receptive (Ware et al., 1993) and the volatiles released from receptive male and female figs appear to be sufficiently similar for the pollinators to fail to distinguish between them (Grison-Pige et al., 2001). A second factor reinforcing inter-sexual volatile mimicry may be 'selection to rush'. This favours foundresses that simply enter the first fig they encounter, regardless of the sex, because the probability of finding another receptive fig before dying is very slim (Patel et al., 1995; Moore et al., 2003a).

These factors explain why foundresses enter female figs, but they do not explain why pollination takes place in those species that depend on active pollination by the fig wasps. One explanation is that once they enter into a female fig, foundresses are in a situation where they have no further opportunity to reproduce, whatever their behaviour, and therefore natural selection can no longer operate on them. If this is the case, the behaviour they exhibit in female figs should reflect what would have been appropriate if they had entered a male fig, because each wasp entering a female fig comes from a long line of ancestors that have only ever entered male figs and thus experienced selection for behaviour inside male figs only. Furthermore, the relative rates at which pollen is dispersed and oviposition is achieved in male figs will determine the numbers of female flowers expected to be pollinated in female figs. To examine these questions we compared the frequency and timing of re-emergence from male and female figs, and, as an indirect measure for how active they are inside the figs (direct observations are problematic because behaviour is disrupted once the figs are opened), we recorded the rates at which they pollinate or attempt to oviposit after entry.

2. Materials and Methods

We used a glasshouse population of the small shrubby fig tree *Ficus montana* (section Sycidium) and its pollinator, *Liporrhopalum tentacularis*. The plant and pollinator populations were maintained at the Experimental Gardens of the University of Leeds, U.K. The plants originated from seed collected at CIFOR HQ, Bogor, Indonesia and from the Krakatau islands, Indonesia in 1995. The insects were collected from the same locations the following year. A parasitoid of the pollinator (*Sycoscapter* sp.) is also present in the culture.

Ficus montana is a functionally dioecious species that, despite its name, is found in lowland forest edges, and disturbed areas in South East Asia. Male figs are yellow to green in colour throughout their development, whereas female figs are deep red when mature. Mature figs of both sexes are 8–12 mm in diameter. The numbers of gall or female flowers inside the figs is highly variable, ranging from less than 50 to over 250 and does not vary significantly between male and female figs. Flowering within plants is typically asynchronous, with all stages of fig development often present on the same plant throughout the year. Adult female *L. tentacularis* are diurnal. On entry into figs, foundress females lose their wings and most of their antennae while passing through the ostiole, but nonetheless routinely re-emerge and can enter other figs close by. Foundresses that re–emerge and enter subsequent female figs can also pollinate them, indicating that individual *L. tentacularis* foundresses carry more pollen than they disperse in any one single fig (Moore et al., 2003a).

Liporrhopalum tentacularis females are active pollinators that always lose their antennae and wings at the ostioles of the first figs they enter. Experiments with *L. tentacularis* foundresses that lack pollen (Tarachai et al., 2008, in this volume) suggest that male figs are more likely to abort if they are not pollinated, and in those figs where abortion does not take place, fewer pollinator larvae are produced. Male *F. montana* figs appear incapable of producing seeds, as none have been found within hundreds of mature figs that have had all their flowers examined.

Prior to experimental manipulations, mature male figs were placed in plastic containers with mesh lids, so that the wasps could emerge naturally. Foundresses that emerged

from these figs were introduced into receptive phase male and female figs with a fine paintbrush. The figs were then bagged to prevent other foundresses entering and also to catch re-emerging foundresses. Foundresses were introduced singly into 100 figs (50 male: 50 female) and were observed hourly to determine the frequency and timing of re-emergence. Vaseline, which traps the wasps, was applied around the figs to prevent the foundresses from re-entering the same fig once they had emerged.

Preliminary experiments with the natural insecticide pyrethrum (pre-diluted, Fisons Ltd, U.K., concentration unknown) showed that contact with one micro litre was sufficient to kill foundresses in less than one minute. They also confirmed that pyrethrum is effective inside the figs and that it does not affect the eggs and larvae produced by the foundresses. Foundresses were killed at different intervals after they entered either male or female figs (2, 2.5, 5, 15, 30, 60, 120 and 240 minutes, N = 25 foundresses per time interval). This was achieved by gently introducing a syringe through the ostioles and then injecting one micro litre of the pyrethrum. Figs in which foundresses emerged prior to poisoning were discounted. The figs were bagged afterwards to prevent the entry of other foundresses and oviposition by *Sycoscapter* sp. When mature, male figs were picked and placed singly into nylon mesh bags. The progeny present were sexed and counted. Mature female figs were opened and the seeds counted

3. Results

Thirty three (66%) of the foundresses emerged from the male figs and thirty seven (74%) emerged from the female figs (NS, $\chi^2 = 0.005$, df = 1, P = 0.94). Some foundresses emerged after about one hour from figs of both sexes, while others did not emerge until the next day. Most foundresses emerged 3–4 hours after entry in both sexes of figs (Fig. 1). Figs where foundresses were killed 2 and 2.5 minutes after entry were all aborted by the plants. In male figs, the brood sizes achieved varied from 6.4 ± 0.44 progeny (Mean ± SE) for foundresses killed five minutes after entry to 27.8 ± 1.90 progeny in foundresses killed after 240 minutes. The equivalent numbers of seeds produced in female figs after five and 240 minutes were 17.5 ± 2.70 and 64.5 ± 8.80, respectively. Pollination rates were significantly higher than oviposition rates (Two Way ANOVA, $F_{(1, 129)} = 139.98$, P<0.001), but both declined rapidly with pollination rates decreasing significantly more rapidly than oviposition rates (Two Way ANOVA, $F_{(5, 129)} = 6.35$, P<0.001). Oviposition rates in the first five minutes averaging 1.15 ± 0.05 progeny per minute, compared with 0.23 ± 0.01 progeny per minute in those females that were allowed to continue oviposition for 240 minutes (Fig. 2, dashed line). In female figs, the rate of pollination decreased from 3.51 ± 0.54 seeds produced per minute in the first five minutes to 0.26 ± 0.36 seeds per minute over the course of 240 minutes (Fig. 2, solid line).

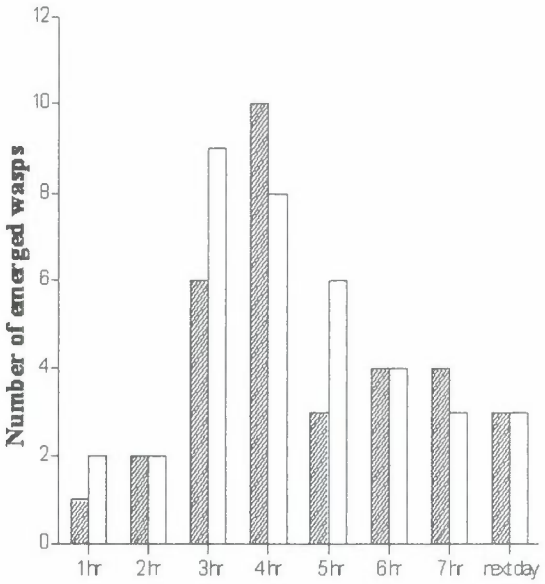

Figure 1. The timing of re-emergence of *Liporrhopalum tentacularis* foundresses from male (dashed bars) and female (open bars) figs of *Ficus montana*.

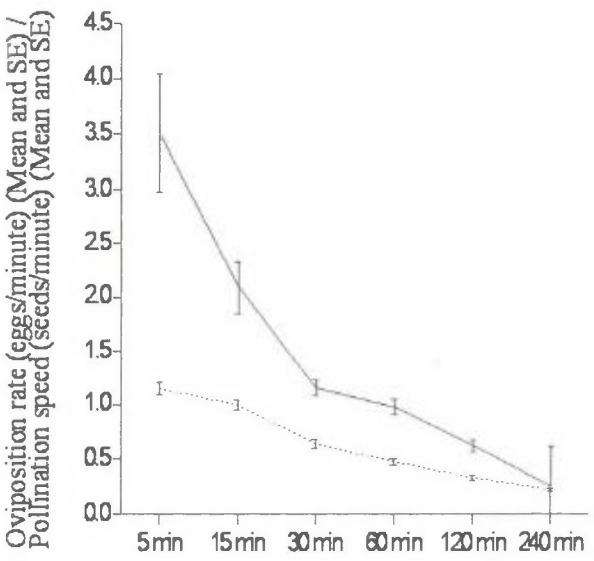

Figure 2. Changes in the estimated rates of oviposition (dashed line) and pollination of ovaries (solid line) by *Liporrhopalum tentacularis* during the time spent inside a fig of *Ficus montana*. Rates at each time interval are calculated over the entire period since they entered.

4. Discussion

Liporrhopalum tentacularis, the pollinator of *F. montana*, gains from its active pollination behaviour, because foundresses that enter male figs and pollinate produce more offspring than foundresses that lack pollen (Tarachai et al., 2008, in this volume). Furthermore, all the male figs in which foundresses were killed within five minutes of entry were subsequently aborted, indicating that there are strong disincentives against rapid re-emergence. A majority of the foundresses nonetheless do re-emerge eventually from male figs, and attempt to lay further eggs elsewhere. Foundresses that re-emerge from male figs can produce more progeny than those that do not, although the difference is slight even under relatively predator-free glasshouse conditions (Moore et al., 2003b). There may be additional gains through 'spreading the risk' across several figs or by laying small numbers of male eggs into figs where many female eggs were laid by other foundresses (Moore et al., 2003a).

Pollination and re-emergence behaviour are likely to be directly beneficial to male fig trees, because they can lead to the production of more pollen-carrying female fig wasps in the next generation (Tarachai et al., 2008 in this volume; Moore et al., 2003b), though the asynchronous all-year fruiting in *F. montana* means that they may rarely be foundress-limited. However, greater benefits accrue to male plants by the duplication of these behaviours amongst the wasp progeny that enter female figs. The frequency and timing of emergence of foundresses from male and female figs was similar, and because pollination rates (in female figs) were higher than oviposition rates (in male figs), this resulted in the generation of numerous seeds in the female figs. The observed foundress behaviour is beneficial for the wasps if they enter male figs, but is of no consequence for the wasps if they enter female figs, whereas both the female and male plants gain. Pollinator re-emergence means that female plants are likely to get more figs pollinated, and they may also gain through increased genetic diversity of the seeds produced and more opportunities for pollen competition, because more foundresses are likely to enter each fig.

Emergence behaviour appears to be more common amongst pollinators of dioecious than monoecious hosts (Moore et al., 2003b). Plant morphology clearly has a major impact on whether or not foundresses are 'allowed' to re-emerge, and the benefits for male dioecious trees of allowing re-emergence from their figs that are exhibited when foundresses they produce are present on female trees is a strong candidate for being the determinant of this pattern. For monoecious figs, an increase in the numbers of fig wasp progeny within a fig is achieved at the expense of producing fewer seeds, at least at higher foundress densities. Gains from allowing pollinator re-emergence may therefore be less than for dioecious fig species, especially as fig wasp sex ratio adjustment results in a higher proportion of male fig wasp progeny in figs with more foundresses and males are of little benefit to the plant (Anstett et al., 1998).

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REFERENCES

Anstett, M.C., Gibernau, M., and Kjellberg, F. 1998. Partial avoidance of female inflorescence of a dioecious fig by their mutualistic pollinating wasp. *Proceedings of the Royal Society of London Series B* **265**: 45–50.

Cook, J.M., Bean, D., Power, S.A., and Dixon, D. J. 2004. Evolution of a complex coevolved trait: active pollination in a genus of fig wasps. *Journal of Evolutionary Biology* **17**: 238–246.

Gibernau, M., Hossaert-McKey, M., Ansett, M.C., and Kjellberg, F. 1996. Consequences of protecting flowers in a fig: a one-way trip for pollinators? *Journal of Biogeography* **23**: 25–432.

Grafen, A. and Godfray, H.C.J. 1991. Vicarious selection explains some paradoxes in dioecious fig-pollinator systems. *Proceedings of the Royal Society of London Series B* **245**: 73–76.

Grison-Pige, L., Bessiere, J.M., Turlings, T.C.J., Kjellberg, F., Roy, J., and Hossaert-McKey, M. 2001. Limited intersex mimicry of floral odour in *Ficus carica*. *Functional Ecology* **15**: 551–558.

Herre, E.A., Knowlton, N., Mueller, U.G., and Rehner, S.A. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* **14**: 49–52.

Moore, J.C., Hatcher, M.J., Dunn, A., and Compton, S.G. 2003a. Fig choice by the pollinator of a gynodioecious fig: selection to rush, or intersexual mimicry? *Oikos* **101**: 180–186.

Moore, J.C., Dunn, A., Compton, S.G., and Hatcher, M.J. 2003b. Foundress re-emergence and fig permeability in fig tree-wasp mutualism. *Journal of Evolutionary Biology* **16**: 1186–1195.

Patel, A., Anstett, M.C., Hossaert-McKey, M., and Kjellberg, F. 1995. Pollinators entering female dioecious figs: why commit suicide? *Journal of Evolutionary Biology* **8**: 301–313.

Pellmyr, O. 2003. Yuccas, yucca moths and coevolution: a review. *Annals of the Missouri Botanical Gardens* **90**: 35–55.

Ramirez, B.W. 1974. Coevolution of *Ficus* and *Agaonidae*. *Annals of the Missouri Botanical Gardens* **61**: 770–780.

Tarachai, Y., Compton, S.G., and Trisonthi, C. 2008. The benefits of pollination for a fig wasp. *Symbiosis* **45:** 29–32.

Ware, A.B., Kaye, P.T., Compton, S.G., and Van Noort, S. 1993. Fig volatiles: Their role in attracting pollinators and maintaining pollinator specificity. *Plant Systematics and Evolution* **186**: 147–156.