Floral ratios in the figs of *Ficus montana* span the range from actively to passively pollinated fig trees

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A R T I C L E   I N F O

A B S T R A C T

Fig trees (*Ficus* spp., Moraceae) and their associated obligate pollinator fig wasps (Agaonidae) are partners in what is often a pair-wise species−specific association. Their interaction centres on the unique enclosed inflorescence of *Ficus* species − the fig. Among dioecious fig tree species, only pollinated ovules in figs on female trees develop into seeds. On male trees, galled ovules support development of the fig wasp offspring that will transport their pollen, but no seeds develop. Some fig wasp species actively collect and disperse pollen, whereas others are typical insect pollinators in that pollen is transferred passively. Active pollination is associated with improved larval survivorship in pollinated figs. Because active pollination is much more efficient, their host figs need to contain far fewer male flowers and across numerous *Ficus* species anther-ovule ratios are a good predictor of pollination mode. We examined variation in inflorescence size and floral ratios among male figs of the Asian *Ficus montana* and its consequences for the amounts of pollen that would be available for each pollinator to collect. Inflorescence size (total flower number) was highly variable, and female pollinator offspring production was higher in figs with more female flowers. Pollinator offspring numbers and anther-ovule ratios were also highly variable, and encompassed the range typical of both actively and passively pollinated fig tree species. In combination, this variation resulted in large differences in the extent to which pollinators were competing for access to pollen, with potential fitness consequences for both partners in the mutualism.

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1. Introduction

Flowering plants display a wide range of sexual systems. The majority of species are monoecious, with individuals that reproduce via a combination of seed and pollen production, but others exhibit gynodioecy or androdioecy and around five percent of the species are dioecious, with separate male and female sexes (Dellaporta and Calderon-Urrea, 1993). To explain this diversity of sexual systems requires an understanding of their pollination ecology. Pollen to ovule ratios indicate selfing versus outcrossing rates (Cruden, 1977). Reflecting this, the flowers of self-incompatible and other out-crossing plants typically produce more pollen than closely related self-compatible species (Cruden, 1977). More broadly, adequacy of pollen receipt and the potential for pollen competition and plant-pollinator co-evolution are all expected to influence selection on a variety of floral traits related to male and female function (Kevan and Baker, 1983; Bell, 1985).

Obligate mutualisms between plants and their pollinators provide some of the most clear-cut examples of co-evolution (Herre, 1999). These specialized plant-pollinator associations include the *Senita* cactus and Senita moths (Fleming and Holland, 1998), *Yucca* and Yucca moths (Pellmyr et al., 1996), *Colocasiomyia* flies and various species of Araceae (Yafuso, 1993), globeflowers and *Chiastochaeta* flies (Bratteler and Widmer, 1998), *Epicephala* moths and Phyllanthaceae (Kato et al., 2003) and figs and fig wasps. Pollination by *Colocasiomyia* and *Chiastochaeta* is achieved through passive transfer of pollen by the flies, without any specialized pollen collection and dispersal behaviour. This mode of pollination is typical of less co-adapted pollination systems and contrasts with the active pollination behaviour, and associated morphological adaptations, displayed by just four groups of insects: Senita moths, Yucca moths, Epicephala moths and some fig wasps.

Female fig wasp pollinators (Agaonidae) are attracted to species- and developmental stage-specific figs by volatiles released by receptive figs (van Noort et al., 1989; Grison et al., 1999). Each fig is a hollow, enclosed structure, lined on the inner surface by numerous

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tiny flowers. Female wasps enter through a narrow ostiole, attempt to lay their eggs in ovules that they also gall, and pollinate some of the flowers. When fig wasps of the next generation become adults, the females leave their natal figs to seek out receptive figs elsewhere, which are usually on other trees.

Around half of all *Ficus* species are monoecious, with trees that all produce figs that can support both seeds and pollinator fig wasp larvae, and the remainder are functionally dioecious, with male plants that have figs that support only fig wasp offspring and female trees with figs that only produce seeds. Most plants display male-biased pre-zygotic biomass allocation in their inflorescences (Cruden and Lyon, 1985), but *Ficus* species contain more female than male flowers (Compton and Nefdt, 1990; Berg and Corner, 2005) and their pre-zygotic biomass allocation is female-biased. This reflects the dual role played by female flowers inside figs. Pollinator fig wasp larvae develop inside galled female flowers and develop into adults that (if they are female) can transport pollen to other figs. Consequently female fig flowers contribute to both male and female reproductive functions. In monoecious *Ficus* species, whether a female flower contributes to male or female reproductive function (or neither) depends largely on the actions of the fig wasp foundresses that enter each fig, whereas in dioecious species post-zygotic development of the female flowers is constrained by sex specific morphological differences in floral structure and physiological capabilities that determine whether they can develop seeds or pollinator offspring.

Ficus is only rivaled by the genus *Phyllanthus* in terms of the number of included species that are involved in nursery mutualisms (Hoffmann et al., 2006; Kawakita, 2010). Some species of fig wasps pollinate actively, others are passive pollinators (Kjellberg et al., 2001). As well as the behaviours associated with pollen collection and deposition, actively pollinating species possess coxal combs of setae to help manipulate the pollen, and sternal pollen pockets to store the pollen during flight (Galil and Eisikowitch, 1969; Frank, 1984). Active pollination evolved early in the mutualism between fig trees and fig wasps (Compton et al., 2010) and phylogenetic studies suggest that active pollination may have evolved and also been lost several times in different lineages of fig wasps (Jousselin et al., 2003b; Cook et al., 2004; Cruaud et al., 2012). An estimated two thirds of extant fig wasp species display active pollination behaviour (Cook et al., 2004).

Active pollination is thought to have evolved to help ensure that the flowers that a fig wasp lays eggs into have also been pollinated. Among actively-pollinating species, larval survivorship has been shown to be higher if the figs are pollinated (Jousselin and Kjellberg, 2001; Tarachai et al., 2008), probably because the larvae feed on endosperm (Jansen-González et al., 2012), but there is no evidence that pollination is essential for fig wasp development (Zhao et al., 2014) and some passively-pollinating species have larvae that routinely develop in figs that are un-pollinated (Kjellberg et al., 1987; Jousselin et al., 2003a). This suggests that the fitness gains from developing in pollinated flowers vary between species, and that the loss of active pollination behaviour may be a response to having hosts that generate negligible fitness losses from failing to pollinate (Jandér et al., 2012). Situations where two or more species of sympatric agaonids share the same host, and pollination is active, provide an opportunity for the evolution of 'cheating' species that possess pollen pockets, but consistently fail to pollinate their hosts. The rarity of cheats (only two examples are documented) suggests that there are costs associated with failure to pollinate *Ficus* species that are routinely actively pollinated, though the nature of these costs is not always evident (Compton et al., 1991; Zhao et al., 2014). The difference in reproductive success of fig wasp individuals with offspring that develop in pollinated and un-pollinated figs has been described as reflecting a 'sanction' applied by the host plants (Jandér et al., 2012).

When pollination is passive, the female fig wasps do not show any specific pollen collection or deposition behaviour and their natal host figs produce large quantities of pollen grains that are released by anther dehiscence, so that the wasps become covered with pollen (Galil and Neeman, 1977; Kjellberg et al., 2001). As a consequence, passively-pollinated *Ficus* species need to produce far more pollen than actively pollinated species. This is reflected in the relative numbers of anthers and ovules inside their figs (Galil and Meiri, 1981; Compton and Nefdt, 1990; Kjellberg et al., 2001) with fig tree species that are pollinated actively having much lower anther/ovule ratios (0.01–0.15) than those species where passive pollination takes place (0.29–0.92, Kjellberg et al., 2001). Passively pollinated figs are more likely to have male flowers scattered throughout, whereas the smaller number of male flowers in actively-pollinated figs are often concentrated around the ostiole, though this relationship does vary between different *Ficus* lineages (Verkerke, 1989; Kjellberg et al., 2001).

Previous large scale inter-specific comparisons of the numbers of male and female flowers in figs (Kjellberg et al., 2001) were based mainly on samples obtained from one or a small number of plants, and consequently could not adequately assess the range of variation within most species. Here we describe intraspecific variation in inflorescence size and floral sex ratios in the male figs of a single species, *F. montana*, using individuals grown under uniform conditions. We then assess the reproductive consequences for individual plants of variation in female and male flower numbers in their figs by comparing male flower numbers and the numbers pollen-carrying adult offspring that develop in the figs. The results are discussed in terms of their implications for the evolution and loss of active pollination.

2. Study site and species

We studied glasshouse populations of the dioecious fig tree *F. montana* Blume, its pollinator *Kradibia* (=*Liporrhopalum*) *tentacularis* (Grandi) and the undescribed parasitoid *Sycoscapter* sp. The populations had been maintained at the experimental gardens of Leeds University since 1995 (Raja et al., 2008a). All the organisms originated from two sources: the Centre for International Forestry Research (CIFOR) plantation, Bogor West Java, Indonesia, and Rakata, Krakatau Islands, Indonesia. All the plants were grown from wild-collected seeds and were kept under similar conditions. Day lengths were maintained at a minimum of 16L:8D throughout the experimental period by providing artificial lights during periods when natural daylight hours were lower. Mean monthly minimum temperatures ranged from 17.9 °C to 21.8 °C and mean maximum temperatures ranged from 22.1 °C to 32.4 °C.

In its natural habitat, *F. montana* (*Ficus* Subgenus *Sycidium*) is found in the understory of forests or in secondary growth, at altitudes up to 1500 m, from Myanmar to Borneo (Berg and Corner, 2005). There is an asynchronous all-year fruiting pattern on individual plants of both sexes (Suleman et al., 2011a). In *F. montana*, significant variation in flower number among figs has been observed by different individuals growing under uniform conditions (Suleman et al., 2013a). Foundress females of *K. tentacularis* routinely re-emerge from the first figs they enter, after losing their wings and part of their antennae, and are capable of utilising up to four additional figs nearby (Suleman, 2007; Suleman et al., 2013b). In male figs, only one egg is normally laid per ovule, which is also galled (Ghana et al., 2012). The male flowers in these figs are functionally unisexual. Once the next generation of wasps has completed its development the anthers of the male flowers split open, but do not dehisce, forcing the recently-eclosed and mated adult females to actively collect the pollen into baskets located on the thorax (Compton et al., 2010). At this time, male pollinators