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A comparison of pollinator fig wasp development in figs of Ficus montana and its hybrids with Ficus asperifolia

Salah Ghana[1#], Nazia Suleman[1]* & Stephen G. Compton[1,2]

[1]School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK, and [2]Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

[#]Present address: Zoology Department, Faculty of Sciences, Tripoli University, PO Box 13793, Tripoli, Libya

***Correspondence and present address:** Nazia Suleman, Plant Protection Division, Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad, Pakistan. E-mail: nazianasir2002@yahoo.co.in

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Abstract

Figs (Moraceae) and pollinator fig wasps (Hymenoptera: Agaonidae) have a highly specific mutualistic relationship but fig wasps occasionally enter atypical hosts, and this can lead to hybrid fig trees and the potential for gene flow between species. Many fig trees are dioecious, with fig wasp offspring developing in galled ovules inside figs on male trees, whereas seeds develop only in figs on female trees. We generated experimental hybrids between the Asian Ficus montana Blume and a closely related African species Ficus asperifolia Miquel. Male F1s were sterile if entered by Kradibia tentacularis (Grandi) (Hymenoptera: Agaonidae), the pollinator of F. montana, because its offspring always failed to develop, without ovule enlargement. As with the F1s, figs on most male backcross plants [F. montana × (F. montana × F. asperifolia)] also aborted shortly after pollinator entry, resulting in a higher turnover of figs than with F. montana, although the times taken for the figs to reach receptivity were similar. Pollinator larvae nonetheless consistently managed to develop inside the figs of one backcross plant and also occasionally in a few figs from another backcross individual. In these figs, galled ovules developed as normal, whereas in figs that aborted the galled ovules failed to enlarge. The sex ratio of K. tentacularis progeny in the backcross figs was female biased and did not differ from that in F. montana figs. Sycoscapter spec., a parasitoid of K. tentacularis, was able to lay eggs and developed normally inside male backcross figs where its host was present.

Introduction

The significance of hybridisation for plant evolution and speciation is increasingly recognised (Whitham et al., 1999; Whitney et al., 2010). The extent to which hybrids have been important in the evolutionary histories of different lineages depends in part on the fitness of individual hybrids relative to their parental species, which can vary from less fit to more fit than their parents (Arnold et al., 1999). Fitness depends on many factors such as compatibility of parental genomes (Kirk et al., 2005), the interaction between vegetative performance and environment (Campbell & Waser, 2001), and interactions with their pollinators (Gross & Rieseberg, 2005; Schiestl & Schluter, 2009). In plants with nursery (brood-place) pollination systems, where pollen is carried by individuals that had developed in or on their host plants, the ability of these insects to develop successfully will also determine the relative fitness of hybrid individuals.

The relationship between fig trees (Ficus spp., Moraceae) and their fig wasp pollinators (Hymenoptera: Agaonidae) is an obligatory mutualism that has existed for at least 34 million years and probably more than 60 million years (Compton et al., 2010). There are around 800 described fig tree species, each (with very few exceptions) pollinated by different species of fig wasps (Lopez-Vaamonde et al., 2009; Cruaud et al., 2010). Figs are unique spherical inflorescences lined with many small flowers. Fig trees depend on the tiny fig wasps to transport their pollen from tree to tree and they only breed inside the figs. The female pollinators are adapted in their morphology and behaviour to enter the figs (Frank, 1984). When a female wasp succeeds in entering a fig, it attempts to lay eggs inside the flowers by inserting the ovipositor along the styles, typically laying one egg into each ovule, which the female also galls, resulting in its rapid expansion (Ghana et al., 2012). Larvae complete their development a few weeks later. Adult males emerge first and mate with females before they have emerged from their galls. Loaded with pollen, the short-lived females then leave their natal figs and seek out receptive figs where they can lay eggs (Cook & Rasplus, 2003). Pollination of the figs can be passive or active. In passively pollinated trees, the fig wasps do not show any specialised behaviour for collecting pollen from their natal figs, and pollen covers their bodies as they emerge from the figs. The pollen is unloaded accidently in the new figs they enter. In species that display active pollination, the female fig wasps have pollen pockets where the pollen is stored and subsequently unloaded when the females are ovipositing (Jousselin & Kjellberg, 2001; Kjellberg et al., 2001). Monoecious fig trees produce figs that support the development of both seeds and pollinator offspring.

Populations of their pollinator fig wasps are maintained by asynchronous fruiting on different trees, which provides them with an all-year source of figs in which to oviposit (Smith & Bronstein, 1996). Fig production on the same tree is nonetheless usually synchronous, which forces the fig wasps to disperse and ensures outcrossing (Bronstein & Patel, 1992). Dioecious fig trees have more variable fruiting phenologies. They have distinct female and male trees that develop only seeds or pollinator offspring, respectively.

Receptive figs release olfactory signals that attract their pollinators (van Noort et al., 1989; Molbo et al., 2003; Chen et al., 2009; Proffit et al., 2009). It was widely believed that the relationship between fig trees and their pollinators was strictly one to one, with each fig wasp species pollinating only one fig species, which required the services of that one species, but exceptions are increasingly being reported, where one tree is pollinated by several species of fig wasps (Michaloud et al., 1996; Peng et al., 2008; Compton et al., 2009; Chen et al., 2012). Records of pollinators entering the figs of atypical hosts (Moe et al., 2011), of sharing the same host, and that host switching has taken place suggest that introgression and hybridization among fig species may also not be as rare as previously assumed (Compton, 1990; Ware & Compton, 1992; Parrish et al., 2003; Machado et al., 2005; Renoult et al., 2009). Molecular evidence supports this conclusion (Machado et al., 2005; Wei et al. 2014).

The relative performance of hybrid plants and their parents, as measured rates of seed germination, vegetative growth, and fruit production (Kirk et al., 2005; Kimball et al., 2008; Di et al., 2009), has illustrated that hybrid fitness and other character traits are not readily predictable and can vary depending on environmental conditions. In dioecious fig trees, the reproductive success of female plants can be measured in terms of their seed production. In male trees, female pollinator numbers provide a proxy for the plants' reproductive success. Introgression of genes from one fig tree to another is dependent on the movements of pollinators between trees and then also on their ability to reproduce inside the figs. Here, we compare the reproductive success of male Ficus montana Blume with that of experimentally-generated hybrids between F. montana and Ficus asperifolia Miquel, a closely-related, but not sympatric species, when only Kradibia (= Liporrhopalum) tentacularis (Grandi) (Hymenoptera: Agaonidae), the routine pollinator of F. montana, was available to enter the figs. Female K. tentacularis are attracted to receptive figs of F. montana and its hybrids, but not F. asperifolia and when pollinators are introduced into its figs they fail to reproduce (Ghana et al., 2015). Female F1 hybrids between these species that are pollinated by K. tentacularis produce as many viable seeds as their parents and appear to display similar fitness (Ghana, 2012). Here, the reproductive success of male plants was measured in terms of the number of figs they produced, the development times of their figs, and the numbers of pollinator offspring that developed to adults in their figs.

Materials and Methods

Ficus montana is a dioecious fig tree placed in subgenus Sycidium and section Sycidium and distributed in lower Myanmar, Thailand, Peninsular Malaysia, Sumatra, Java, and Borneo (Berg & Corner 2005). It is a shrub that reaches about 2 m high. Ficus asperifolia is a closely related and similar-sized species from Africa, but less branched and more scandent in habit (Berg & Wiebes, 1992). Populations of F. montana and its pollinator K. tentacularis originated from the Centre for International Forestry Research (CIFOR) plantation, West Java, and from Rakata, Krakatau Islands, both Indonesia, and had been in glasshouse culture in the Leeds University Experimental Gardens since 1996 (Suleman et al., 2014). The plant population comprised mainly cuttings from the source plants originally obtained from the wild population, supplemented by second-generation plants grown from seed. Kradibia tentacularis females actively pollinate the figs of F. montana and routinely re-emerge from both male and female figs, allowing them to pollinate and lay eggs in several figs (Suleman et al., 2013a).

The glasshouses were provided with a heating system to maintain minimum temperatures and in the summer they were covered with liquid shade (cool glass) to reduce light transmission and temperature. A 14-h minimum day length was maintained during the winter period using artificial lights. Plants were fed with liquid feed (Peters Excel®, Everris Limited, Ipswich, UK;18+10+18) every 6 weeks.

Only the green-leaved form of F. montana was utilized (Yaowanit et al., 2012). In the glasshouses this species displayed an asynchronous all-year fruiting pattern on individual plants of both sexes (Suleman et al., 2011), with significant variation in flower number among figs on plants grown under uniform conditions (Suleman et al., 2013c; Kjellberg et al., 2014). Ficus montana can reach maturity and start to produce figs less than 1 year after germination. The F. asperifolia population originated from seed collected in Kibale forest in Uganda. It also fruits asynchronously on individual plants, but few figs were produced during the winter months under our growing conditions.

In addition to their pollinators, figs also support numerous species of non-pollinating fig wasps (NPFW) that are parasitoids of pollinators or independently gall the ovules. An undescribed Sycoscapter spec. (Hymenoptera: Pteromalidae) originating from Indonesia was present in the Leeds culture. It is a parasitoid of K. tentacularis and the most widespread and abundant of the NPFW associated with F. montana in the plant’s native range (Zavodna, 2004; Raja et al., 2008b; Suleman et al., 2012, 2013b). Voucher specimens are maintained in the SG Compton collection, University of Leeds, Leeds, UK.Fig developmental stages were described by Galil & Eisikowitch (1968) and modified for dioecious species by Valdeyron & Lloyd (1979). Phase A is a pre-receptive phase where the ostiolar bracts are still closed and the female wasps cannot enter the figs. Phase B is the receptive phase where the female pollinator wasps can enter the figs to lay eggs and pollinate female flowers. Phase C is the longest phase, when the progeny of wasps and seeds are completing their development. Phase D is when male wasps mate with females and make exit holes, and female wasps then load with pollen and leave their natal figs to find other receptive figs and start a new cycle. Female fig trees lack this phase, as the wasps do not develop inside their figs and pass directly from phase C to E (the post-floral stage) when the figs ripen and become attractive to seed dispersers. E phase male figs wither and eventually fall as they do not need to attract pollinators.

We generated F1s in 2006 and backcrosses to F. montana [F. montana × (F. montana × F. asperifolia)] in 2008 from the parental material using K. tentacularis to transport pollen from D phase figs into receptive (B phase) figs. Fig wasps carrying pollen from known hosts (F. montana or F1 hybrids) were placed at the ostioles of receptive figs using a fine paint brush. Where necessary, entry was encouraged by rubbing the surface of receptive F. montana figs onto the ostiolar area of the figs (Ghana et al., 2015).Crosses such as F. asperifolia × F. asperifolia and others with F. asperifolia as the male parent could not be generated because its pollinator, Kradibia hilli Wiebes, was not available and K. tentacularis cannot reproduce in male figs of F. asperifolia.

The numbers of female flowers – and therefore potential oviposition sites – differ significantly among the figs of F. asperifolia (means ± SE = 340.47 ± 27.98) and F. montana (86.64 ±_4.63), with F1s (209.88 ±_14.54) and backcrosses (167.28 ±_6.61) containing intermediate numbers (Ghana, 2012).

Initially, fig development was monitored on all the male hybrid individuals in the general glasshouse environment, in which free-flying K. tentacularis had access to figs on all the plants, as well as to the parental species. This revealed that F1 individuals could not support development of K. tentacularis (see Results).

For a more detailed assessment of subsequent generations, six male F. montana and six male backcrosses were selected that were approximately the same size. The plants were positioned together in the same area of the glasshouse to minimize environmental variation. When new figs started developing, they were tagged and numbered. The plants were checked twice a week to record new figs and check the development of older figs. Whether pollinator wasps had entered was indicated by the presence of wings in the ostiolar bracts. Some figs will have been entered by wasps that had lost their wings entering other figs, and will have been entered earlier than recorded. Emergence of wasps from the figs and fig abortions were also recorded.

Five late C phase figs were collected from each plant (if they were available, which was often not the case with backcrosses) and kept in plastic containers covered by fine mesh lids until the adult wasps emerged. Next day the wasps were counted, sexed, and the contents of the female flowers in the figs were recorded. These comprised galls where wasps had exited, empty galls where wasp offspring had failed to complete their development (Ghana et al., 2012) and un-utilized flowers (female flowers that had not developed because the fig wasps had failed to gall them).

Statistical analysis

All data analyses were performed using R (2.12.2) R Development core team, 2008). A generalized linear model (GLM) with Poisson error determined the effect of months on fig production with the fig groups (male plants of F. montana and backcrosses) as fixed effects and fig production as a random effect. The models were compared with ANOVA. A Wilcoxon rank-sum test was used when the data were not normally distributed (Shapiro tests) and could not be transformed. Fisher's F test compared variances between response variables (Crawley, 2007).

Results

Fig wasp development in figs of hybrid plants

More than 80 F1 male offspring were reared to maturity from seeds collected from five F. asperifolia female plants that had been pollinated by K. tentacularis introduced onto the surface of the figs. New A phase figs on the F1 offspring were produced at about the same time and in about the same numbers as F. montana growing in the same glasshouses and, in contrast to figs of F. asperifolia, the figs were routinely entered by free-flying K. tentacularis females. However, all the 800+ figs entered by pollinators aborted within 2 weeks of pollinator entry and no F1 figs on any of the plants supported the development of pollinator offspring. Backcrosses were therefore necessarily generated using pollinators reared from F. montana figs.

Among the 30 male backcross plants where mature fig production was monitored, only five male plants were found to be suitable for any K. tentacularis reproduction. The remainder aborted all their figs. In figs entered by pollinators this occurred in the weeks following pollinator entry, in the same manner as figs on F1 plants. Fig development on two of the backcross plants where at least some figs reached maturity and four plants where successful pollinator reproduction was not observed were compared with F. montana fig development in further studies.

Fig production and abortion

The numbers of figs produced by the six male backcross plants between May and October 2008 ranged from 45 to 789, with a total of 2 067 figs recorded. This compares with 68-305 figs produced on the six F. montana, where a total of 1 208 figs were recorded. Fig production showed a clear seasonal pattern, with greater rates of initiation of new figs during the summer (Figure 1). The highest numbers of figs in both groups were found in August, with mean ±SE = 96.2 ± 27.6 figs on the backcrosses and 53.3 ± 12.0 on F. montana. Month had a significant effect on fig production of both the backcrosses and F. montana (Glm; d.f. = 65, P<0.001).

Backcrossed plants produced an average of about 350 figs each during the study period, compared with 200 figs on the male F. montana, but very large within-group variation meant there was no significant overall difference in the total fig production of the two groups (Wilcoxon test: W = 20, P = 0.82). In particular, backcross plant one, the only individual to be able to support large numbers of pollinator offspring, produced very few figs. High fig production on most of the backcross plants was therefore associated with a high turnover of figs – where more figs were aborted, more new figs were initiated. The variation in numbers of figs per plant of F. montana and backcrosses was significantly different (Fisher's Ftest, $F_{5,5}$ = 0.09, P = 0.02).

Almost all of the figs on the male backcrosses were aborted, except for those on one tree (tree 1) where only around 4.4% were aborted and the rest developed normally. Among F. montana, fig abortions were also frequent, and ranged from 39.8 to 86.8% on different trees (Table 1). Overall, male backcrosses nonetheless aborted many more figs than male F. montana (mean ± SE = 83.6 ± 16.3 and 59.9 ± 8.0%, respectively; Figure 2, Table 1), with a significant difference in the proportion of figs aborted (χ^2 = 706.09, d.f. = 1, P<0.001). The much higher rate of abortions among figs on backcrosses could have reflected their avoidance by pollinators or factors that occur after pollinator entry.

Pollinator entries into figs

Among a sub-sample of 21 F1 male figs, 19 (90.5%) had been entered by winged foundresses. The proportion of receptive phase male figs of F. montana entered by winged K. tentacularis foundresses was 81.5%, compared with 67.1% among male backcrosses (n = 1 208 and 2 067 figs, respectively). A significantly higher proportion of F. montana figs was entered compared with F2s ($\chi^2 = 277.8$, d.f. = 1, $P<0.001$). Note that these are figs entered by winged foundresses, and are underestimates of total pollination rates as additional figs will have been entered by foundresses that had lost their wings elsewhere. Ficus montana also had more figs that successfully produced fig wasp offspring: 41.2% compared with 2.1% in backcrosses ($\chi^2 = 712.3$, d.f. = 1, $P<0.001$).

Development rates and timing of abortion

The development times from when a fig was first recorded to receptivity were similar in male figs on F. montana and backcrosses (Figure 3A,B) with mean ± SE = 21.95 ± 0.17 days (n = 986 figs) and 22.19 ± 0.15 days (n = 1 387), respectively (Wilcoxon test: W = 677 343, P = 0.7). The variance in development times was also not significantly different ($F_{984,1\ 387} = 0.89$, P = 0.07). Among figs where development was completed, there was however a significant difference in development times of figs from receptivity to wasp emergence (as evidenced by exit holes through the ostioles), with a greater overall development time for figs from backcross plants: mean ± SE = 48.02 ± 0.37 days in figs on males of F. montana (n = 476 figs) and 53 ± 0.76 days in figs on backcrosses (n = 46) (Wilcoxon test: W = 5 154, P<0.001; Figure 4A,B). Variances in these development times were also significantly different ($F_{475,40} = 2.76$, $P<0.001$).

Among figs that fail to reach maturity, there was a significant difference in mean times from receptivity to abortion, with figs on backcross plants tending to abort sooner: mean ± SE = 33.58 ± 0.75 days for figs of F. montana and 9 ± 0.19 days for figs on backcrosses (Wilcoxon test: W = 613 277.5, P<0.001; Figure 5A,B). Their variances were also significantly different ($F_{509,1325} = 5.65$, $P<0.001$).

Fig wasp development

Kradibia tentacularis and its parasitoid Sycoscapter spec. developed routinely in figs of F.

montana. They also emerged from most of the figs on male backcross plant one and from a few figs on plant two. The figs failed entirely on the remaining backcross plants (Table 1). From the eight sampled backcross figs in which K. tentacularis completed development (five figs from plant one and three figs from plant two), fig wasp adult offspring emerged from around 40% of the female flowers present, compared with 35% of the female flowers in the F. montana figs (Table 2). The mean number (± SE) of K. tentacularis offspring that developed in figs of F. montana was 28.5 (± 2.7) compared with 56.1 ± 14.5 in figs on backcrosses (Table 3). One possible contributor to this was that the number of female flowers (potential oviposition sites) was significantly higher in backcrosses than in F. montana (170 ± 24.0 and 120.8 ± 7.8 flowers, respectively; Table 2), (Wilcoxon test: W = 65.5, P<0.05). In the F. montana figs 53% of the galled female flowers were empty, compared with 38% in figs on the backcrosses, suggesting that survival rates of developing larvae were higher in the former (χ^2 = 83.89, d.f. = 1, P<0.001). The proportion of female flowers that produced fig wasp adult offspring (0.40) was also significantly higher in the backcross figs than in the F. montana figs (0.35) (χ^2 = 13.83, d.f. = 1, P<0.001).

Offspring sex ratios provide an indirect means of assessing the numbers of offspring generated by individual foundresses, because larger clutches are more female biased. The pollinator offspring sex ratios (proportion males) in figs on backcrosses ranged from 0.20 to 0.54 and in figs of F. montana from 0 to 0.82. The figs that contained more male than female offspring had probably been entered by two or more foundresses, at least one of which was unmated and could only produce haploid male offspring (Table S1).

Sycoscapter spec.

As with F. montana figs, female parasitoids were able to insert their long ovipositors through the wall of backcross figs and lay their eggs in female flowers that contained larvae of its host. They were also able to complete their development and emerge from the figs. In F. montana figs, Sycoscapter spec. offspring numbers ranged from 0 to 39 with a mean ± SE per fig of 14.1 ± 2.1. In figs on backcrosses their numbers ranged from 0 to 33 (13.4 ± 4.7; Tables 2 and 3). Their sex ratio (proportion males) ranged in F. montana from 0 to 1 and in backcrosses from 0 to 0.76 (Table 4). Parasitism rates (the relative numbers of host and parasitoid offspring) were significantly different on the two plant groups (χ^2 = 35.41, d.f. = 1, P<0.001).

Discussion

Male F1 hybrids between F. montana (male) and F. asperifolia (female) were sterile in the presence of K. tentacularis (the pollinator of F. montana in South East Asia), because the insects consistently failed to reproduce in their figs, which aborted before any mature pollen (or vectors to transport it) were produced. This is in contrast with female F1 plants, which readily produced viable seeds when entered by K. tentacularis (Ghana, 2012). Male backcrosses to F. montana showed a more variable pattern. Although most backcrosses were unable to support pollinator development, some plants were capable of sustaining the development of small numbers of pollinators and one individual was highly successful. If they were sympatric, male F1s would therefore have no opportunity to contribute to gene flow between F. montana and F. asperifolia, but if backcrosses were generated via female F1s, some of these could do so.

We examined fig development in six backcross plants that covered the observed range from sterile to apparently fully fertile. As recorded in a previous study of glasshouse F. montana (Suleman, 2007), fig production in male F. montana and backcrosses was higher in the warmer summer months. Male F. montana and backcross figs also took the same time to develop receptivity. With the exception of the one backcross that regularly supported fig production, the backcross figs initiated more than 50% more figs that F. montana individuals, but most or all of these aborted at an early stage, despite being attractive to pollinators. Given that the one backcross that successfully supported the development of many figs was similar to F. montana in terms of the total number of figs it produced, it appears that the high abortion rate in male backcross plants was being compensated for by the production of many new figs. Female figs of dioecious Ficus species are traps for any pollinators that enter them, because their wings are removed on entry but they cannot lay any eggs (Raja et al., 2008a). Female figs entered by pollinators quickly cease to be attractive and remain on the plants for weeks or months. In contrast, figs on the backcross plants quickly aborted and new figs were initiated in their place. Microscopic examination of flowers found that K. tentacularis females lay similar numbers of eggs in F. montana, F1s, and backcross figs (Ghana, 2102). Because of the rapid turnover of attractive figs, male hybrid plants would represent much more of a potential drain on local K. tentacularis populations, because they trap so many more pollinator females.

The behavior of K. hilli, the pollinator of F. asperifolia, has not been investigated. It is a slightly larger species than K. tentacularis, associated with slightly larger figs than those of F. montana and this might make it more difficult to enter F. montana figs. The complete or partial inability of K. tentacularis to develop in F1 and backcross figs has parallels with the inability of some other insects such as the fruit flies Tephritis dilacerata (Loew) and Tephritis formosa (Loew) to develop atypical hosts because the buds abort or the larvae die before completing their development (Berube, 1978). Clearly most backcrosses are less capable than F. montana at supporting the development of the pollinator offspring, suggesting that the wasps are also less capable of preventing the plant from aborting its figs. The F1s, and backcrosses that could not support pollinator development, failed to form galls in response to oviposition (Ghana et al., 2015). Backcrosses will contain slightly different proportions of parental material and different gene combinations (Hill, 1993). Those backcross plants that could support pollinator development have some traits responsive to galling shared with F. montana, but understanding the inheritance of such traits must wait for a better understanding of the galling process in figs.

The fitness of hybrid plants and animals can vary from lower to higher than the fitness of both parents or one of them (Arnold, 1992). Female F1 hybrids between F. montana and F. asperifolia and backcrosses to F. montana produce many viable seeds and in the presence of K. tentacularis their fitness appears similar (Ghana, 2012). Although male F1 hybrids between the two species are entirely sterile, backcrosses with F. montana as the male parent can be generated and our results showed that some backcross males can be fully or partially fertile. Under natural conditions, pollinator females developing in these backcrosses could then enter female F. montana and F1 figs, and the introduction of F. asperifolia genes into F. montana populations would take place via both sexes of the plants.

The significance of hybridization and introgression in the relationship between fig trees and their insect pollinators, and how they relate to the tree-specific volatiles released by receptive figs, is still poorly understood (Profitt et al., 2009; Moe et al., 2011; Wei et al., 2014). Hybridization can lead to the production of new taxa (Abbott, 1992; Chapman & Burke, 2007) and our results are consistent with studies that suggest that hybridization has occurred in the evolutionary history of some Ficus species (Parrish et al., 2003; Machado et al., 2005; Renoult et al., 2009). No comparable experimental data on the ability of hybrids to support pollinator development is available, but field studies of putative hybrids between some closely-related species suggests that some can support fig wasp development (Wei et al., 2014). Low fitness in early generations of hybrids does not necessarily prevent gene flow and the establishment of new evolutionary lineages (Arnold et al., 1999), but clearly gene flow is more likely via female than male plants in the two fig tree species we studied (Ghana, 2012), and if the ability to gall does limit fig wasp development more generally (Ghana et al., 2015) then this may be the case among Ficus hybrids in general. Most plants are unlike fig trees, in that they do not have nursery pollination systems where the plant's male reproductive success depends on being able to support the development of pollinator offspring. Sexual differences in the fitness of hybrids may therefore be unusually strong in this system, but they are not unique (Pertl et al., 2002).

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Figure captions

Figure 1 Seasonal variation in the mean (± SE) numbers of new male figs of Ficus montana (diamonds) and backcrosses (squares) produced in 2008 under glasshouse conditions.

Figure 2 Mean (± SE) number of abortions of male figs of Ficus montana (diamonds) and backcrosses (squares) in 2008 under glasshouse conditions.

Figure 3 Development times of figs on (A) male Ficus montana and (B) male backcrosses, timed from their first appearance to receptivity.

Figure 4 Development times of figs on (A) male Ficus montana and (B) male backcrosses timed from wasp entry to emergence of the next generation of wasps.

Figure 5 Timing of abortions of figs on (A) male Ficus montana and (B) male backcrosses from the time the figs were first visible.

Table 1 Total number of figs produced during the study period, the number of figs aborted, and the number of figs that produced wasps, on male Ficus montana and backcrosses. Plant numbers are the same as in Table 2

Group	Plant no.	Fig production	Figs aborted	Figs produced wasps
F. montana	1	171	68	103
	2	305	248	57
	3	235	104	131
	4	156	82	74
	5	273	149	124
	6	68	59	9
Backcrosses	1	45	2	43
	2	602	599	3
	3	228	228	0
	4	233	233	0
	5	170	170	0
	6	789	789	0

Table 2 Contents of mature male figs of Ficus montana and backcrosses, including numbers of male and female Kradibia tentacularis and its parasitoid Sycoscapter spec. Only two of the backcross individuals produced any mature figs

Group	Plant	Fig no.	Female flowers			K. tentacularis		Sycoscapter spec.		Total no. wa
			Bladder	Un-utilized	Total	Male	Female	Male	Female	
F. montana	1	1	61	6	119	18	16	3	15	52
		2	38	5	109	3	26	8	29	66
		3	74	30	154	28	6	4	12	50
		4	73	50	177	4	29	4	17	54
		5	98	3	152	2	30	7	12	51
	2	1	2	3	69	5	57	1	1	64
		2	4	2	68	5	53	4	0	62
		3	6	5	60	3	42	4	0	49
		4	8	5	68	2	14	7	32	55
		5	26	6	99	4	31	11	21	67
	3	1	42	2	89	4	14	7	20	45
		2	66	0	85	1	12	1	5	19
		3	62	0	78	0	10	2	4	16
		4	69	0	113	2	20	13	9	44
		5	37	8	97	7	19	7	19	52
	4	1	47	0	81	2	14	4	14	34
		2	48	2	76	0	10	7	9	26
		3	52	1	80	0	4	10	13	27
		4	74	0	114	3	14	6	17	40
		5	51	14	98	8	7	9	9	33
	5	1	124	14	171	17	16	0	0	33

		2	120	21	170	4	23	1	1	29
		3	110	36	168	3	16	1	2	22
		4	94	17	152	2	19	7	13	41
		5	92	34	153	25	0	2	0	27
	6	1	89	48	194	4	53	0	0	57
		2	82	10	146	50	0	3	1	54
		3	76	5	114	32	0	1	0	33
		4	115	21	164	1	22	4	1	28
		5	89	69	207	5	35	7	2	49
Total			1929	417	3625	244	612	145	278	1279
Mean ± SE			64.3 ± 6.29	13.9 ± 3.22	120.83 ± 7.79	8.13 ± 2.11	20.4 ± 2.81	4.83 ± 0.62	9.27 ± 1.67	42.63 ±
Backcrosses	1	1	42	87	213	12	72	0	0	84
		2	117	18	260	56	47	4	18	125
		3	77	3	198	43	50	6	19	118
		4	100	4	217	11	69	8	25	113
		5	18	47	149	4	59	16	5	84
	2	1	50	34	88	1	3	0	0	4
		2	91	68	175	2	8	2	4	16
		3	30	18	60	3	9	0	0	12
Total			525	279	1360	132	317	36	71	556
Mean ± SE			65.63 ± 12.63	34.87 ± 10.77	170 ± 23.99	16.5 ± 7.44	39.62 ± 10.11	4.5 ± 1.95	8.87 ± 3.59	69.5 ± 1

Table 3 Mean (± SE) numbers of Krabidia tentacularis and its parasitoid Sycoscapter spec. in figs of Ficus montana and backcrosses. Only two of the backcross individuals produced any mature figs

Group	Plant	No. figs	K. tentacularis	Sycoscapter spec.
F. montana	1	5	32.4 ± 0.93	22.2 ± 3.79
	2	5	43.2 ± 8.32	16.2 ± 7.96
	3	5	17.8 ± 2.9	17.4 ± 4.92
	4	5	12.4 ± 2.42	19.6 ± 1.44
	5	5	25 ± 2.45	5.4 ± 3.68
	6	5	40.4 ± 6.09	3.8 ± 1.59
Total		25	28.53 ± 2.69	14.1 ± 2.09
Backcrosses	1	5	84.6 ± 6.7	20.2 ± 5.47
	2	3	8.67 ± 2.4	2 ± 2
Total		8	56.12 ± 14.48	13.37 ± 4.71

Table S1 Sex ratio (proportion males) of Kradibia tentacularis and its parasitoids Sycoscapter spec. in male figs of Ficus montana and backcrosses

Group	Plant	Fig no.	K. tentacularis	Sycoscapter spec.
F. montana	1	1	0.53	0.17
		2	0.10	0.22
		3	0.82	0.25
		4	0.12	0.19
		5	0.06	0.37
	2	1	0.08	0.50
		2	0.09	1
		3	0.07	1
		4	0.13	0.18
		5	0.11	0.34
	3	1	0.22	0.26
		2	0.08	0.17
		3	0	0.33
		4	0.09	0.59
		5	0.27	0.27
	4	1	0.13	0.22
		2	0.00	0.44
		3	0.00	0.43
		4	0.18	0.26
		5	0.53	0.50
	5	1	0.52	0.00
		2	0.15	0.50
		3	0.16	0.33
		4	0.10	0.35
		5	1	1
	6	1	0.07	0
		2	1	0.75
		3	1	1
		4	0.04	0.8
		5	0.13	0.78
Backcrosses	1	1	0.14	0
		2	0.54	0.18
		3	0.46	0.24
		4	0.14	0.24
		5	0.06	0.76

2	1	0.25	0
	2	0.2	0.33
	3	0.25	0

1