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Spatial variation in pollinator gall failure within figs of the gynodioecious *Ficus hirta*

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ABSTRACT

Figs, the inflorescences of *Ficus* species (Moraceae), contain numerous uni-ovulate flowers. Male trees of gynodioecious *Ficus* have figs that support development of pollinator fig wasp offspring (Agaonidae) and rarely produce seeds. Pollinator larvae develop inside galled ovules that expand rapidly after eggs are laid to fill the available space. Galls that fail to support successful larval development can be abundant and failures may influence oviposition behavior and modify realized offspring sex ratios. We examined pollinator reproductive success in figs of the Asian *Ficus hirta* where we had allowed entry by either one or two foundresses and prevented attack by parasitoids. At the developmental stage when adult offspring were about to emerge from their galls, we recorded where in the figs their galls were located, the distributions of sons and daughters in the galls and whether galls that developed closest to the periphery of the figs were more likely to fail. Foundress number had an effect on gall location, but not total offspring numbers. No spatial variation in the distribution of male and female adult offspring was detected. Overall, over 25% of the galled ovaries failed to support offspring development, and failure rates were independent of foundress number. More peripheral galls were more likely to fail in figs entered by two foundresses. Gall location in gynodioecious figs is determined largely by the extent to which their basal pedicels expand after galling. Competition for nutrients between galls, with those developing shorter pedicels being at a disadvantage, may explain the results. If pedicel length is related to timing of oviposition, then pollinator eggs laid later are less likely to survive.

Fig trees (*Ficus* spp., Moraceae), have a largely pan-tropical distribution, and form one of the largest genera of woody plants, with more than 750 species (Berg, 2003). The mutualism between fig trees and their pollinating fig wasps (Agaonidae) is one of the most intensively studied of plant-insect interactions. Fig trees have unique enclosed protogynous inflorescences (figs, also called syconia) that depend on fig wasps (Agaonidae) for pollination. They also support diverse communities of non-pollinating fig wasps (NPFW). From one to several adult female pollinator fig wasps (foundresses) enter the figs in order to lay their eggs inside the many ovules that line their inner surface. Once inside, the females lay their eggs by inserting their ovipositors down the styles and they also either actively or passively pollinate them using pollen that they had carried from their natal figs (Weiblen, 2002). The mutualism has persisted largely unchanged for at least tens of millions of years (Ronsted et al., 2005; Compton et al., 2010) and is a model system for studies of coevolution (Weiblen, 2002). The offspring sex ratios of pollinator fig wasps are female-biased and the extent of this bias often varies according to the number of foundress females that share a fig (Hamilton, 1967). As a consequence, fig wasps have also become a model system for studies of sex ratio evolution (Kathuria et al., 1999). Fig wasp offspring sex ratios often broadly correspond to predictions based on optimality theory (Herre et al., 1997) and a simple mechanism of sex ratio adjustment based on variable oviposition site limitation in combination with laying mostly male eggs at the start of an oviposition sequence has been demonstrated in some species (Raja et al., 2008), although this can be modified by interactions with other foundresses sharing a fig (Greeff and Newman, 2011).

Fig trees display two breeding systems: monoecy and functional dioecy. In monoecious fig trees, approximately half of the described species, the trees produce individual inflorescences that perform both female (seed production and dispersal) and male (pollen production and dispersal) functions. Style lengths in these figs are variable and longer-styled flowers are more likely to produce seeds and shorter-styled flowers are more likely to produce pollinator offspring (Compton and Nefdt, 1990; Ganeshaiah et al., 1995). The preference amongst

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foundresses for shorter-styled flowers means that most of their eggs tend to be laid in ovules that are initially more central, perhaps in response to selection to avoid NPFW, though other factors may also be important (Compton et al., 1994; Anstett et al., 1996; Jousselin et al., 2004; Yu et al., 2004).

In gynodioecious fig tree species there is specialization in sexual function, with the development of seeds and pollen-carrying fig wasps taking place on different individual trees. Trees that produce only seed-bearing fruits are functionally female, while others that support the development of pollen-carrying fig wasp progeny are functionally male. Female flowers in figs on male trees produce no seeds and have shorter styles, of relatively uniform length, and stigmas adapted to facilitate oviposition, rather than pollen capture (Verkerke, 1989). This allows oviposition to be rapid, especially in the first few minutes after entry (Raja et al., 2008). The ovules develop into galls that enlarge quickly to fill the available space. Early growth of the galls is likely to be induced by gland products released by females when they probe the ovules in search of oviposition sites, because it occurs before the larvae hatch (Ghana et al., 2015).

The eventual location of galled ovules within developing gynodioecious figs is determined by the degree of extension of the pedicels by which they are attached to the fig wall (Yu and Compton, 2012). Factors determining the extent of pedicel growth are poorly understood. Pedicel extension can be variable between the sexes, resulting in differences in average positions of galls containing male and female offspring. This can lead to sexual differences in mortality rates, because more peripheral galls are more likely to be attacked by parasitoids (Yu and Compton, 2012). More centrally-located galls can also produce larger pollinator offspring than those from more peripheral galls (Peng et al., 2014), which suggests that there are also nutritional advantages for larvae in more central galls, and that if gall failures are linked to nutritional factors they may be less frequent there. The relative ability of pollinators to stimulate pedicel growth inside male figs of gynodioecious species is therefore linked to their survival and can potentially influence their oviposition strategies. The ability to change the internal spatial structure of figs during their development may play a role at fig wasp population and community levels, and influence the stability of the fig and the fig wasp mutualism.

Some galled ovules in figs fail to support the successful development of pollinator offspring. They are referred to as 'failed' or 'empty' galls or 'bladders'. Whether eggs were laid inside ovules that developed into empty galls is usually unknown, but there is evidence from the gynodioecious *F. montana* that a single egg is laid inside each empty gall (Ghana et al., 2012). Empty galls reach about the same size as successful galls, but are typically hollow shells, with no evidence of dead larvae inside. Gall failure is a major source or indicator of larval mortality among pollinator fig wasps and can exceed losses due to parasitoids. These failures of larval development may be due to competition for resources within the figs involving other pollinator galls or gall-forming NPFW. A lack of pollination of the ovule, damage caused by probing parasitoids, or any other factors that lead to damage or insufficient resources being available are other possible causes (Suleman et al., 2012). The significance of nutrition for developing larvae has been demonstrated in experiments where figs were entered by pollen-free fig wasp female and this resulted in less female-biased offspring sex ratios (Nefdt, 1989). The larval mortalities reflected in empty galls can therefore modify realized sex ratios. Male hymenopterans are haploid, and can be more resistant to nutrient shortages than females (Grosch, 1948), which suggests a possible mechanism for this effect.

Here, we describe controlled experiments that examined the relationship between foundress number, pollinator gall location and gall contents in figs of the gynodioecious fig tree *F. hirta*. The following questions were addressed: in the absence of non-pollinators, how are the ovules containing male and female offspring of pollinator females distributed within the figs, does their position vary according to the number of foundresses, and are gall failures located evenly with respect to distance from the outside of the figs?

1. Materials and methods

1.1. Study site and species

Our studies were carried out at the South China Botanical Garden (SCBG) in Guangdong Province (N 23°10.246; E 113 °20.938′). The area has a tropical monsoon climate with short winter and a long warm and humid summer. The dry season extends from October to March, and the wet season from April to September. The annual mean temperature was 21.4˜21.9 °C (Guangzhou Meteorological Bureau). Maximum temperatures are in July and August and the minimum temperatures in January (Yu et al., 2006).

The development of figs was described by Galil and Eisikowitch (1968). A-phase describes young immature figs. B- (female) phase receptive figs attract foundresses and allow them to enter, oviposit and pollinate. C-phase is the longest period and is where fig wasp offspring and seeds are developing. D- (male) phase figs are when fig wasp adult offspring mate and females become loaded with pollen before emerging and flying away in search of receptive figs. Finally, E-phase male figs shrivel and eventually fall to the ground, whereas female figs become soft and fleshy and offer a food reward to seed dispersers. Not all galled ovules support the successful development of fig wasp offspring. We refer to galled ovules that failed to support adult offspring as 'empty galls'.

Ficus hirta Vahl. is a gynodioecious shrub or small tree, found commonly at the edges of forests and on cleared hillsides near habitations. Paired figs are borne along the branches. They are spherical or ellipsoid, with a diameter when mature of 10–20 mm. The production of young receptive figs is continuous throughout the year with figs produced both synchronously and asynchronously on individual plants and at the population level (Yu et al., 2006). Both male and female figs contain about 800 female flowers, and male figs also contain more than 100 male flowers (Yu et al., 2004, 2008). At maturity, the small, red and sweet female fruit are attractive to a variety of birds, which are the main seed dispersers (Corlett, 2006).

The recorded pollinator of *F. hirta* is *Valasia javana (*Hill) Mayr (Agaonidae, Agaonidae, Agaoninae) (Cruaud et al., 2010). They are possibly active pollinators (Finn Kjellberg; private communication). At SCBG, more than 70% of *F. hirta* figs are entered by a single foundress (Yu et al., 2008), but in male figs, foundress numbers can range from 1 to 9 (mean ± SE = 1.7 ± 1.6: Yu et al., 2008). Pollinator offspring sex ratios under natural conditions are female-biased, but highly variable, with a mean proportion of males of 0.25 (Yu and Compton, 2012). The foundresses are not known to re-emerge from the first fig they enter. Three species of NPFW have also been reported from figs of this species (Mayr, 1885; Nair et al., 1981). At SCBG, NPFW were present in 68% of a sample of 107 figs, with numbers ranging between zero and 298 (mean ± SD = 41.93 ± 55.66)(Yu and Compton, 2012). Adult females of all three species oviposit from outside the figs, and NPFW can have negative effects on both plant and pollinator reproduction (Yu and Compton, 2012).

1.2. The influence of foundress number on offspring characteristics

Between June and August 2011, A-phase figs were covered with organdy cloth to prevent access to pollinator and non-pollinator fig wasps. When they became receptive, one or two foundresses that had emerged the same day from D-phase figs that had been bagged earlier were allowed to enter the figs. When two foundresses were introduced they had been obtained from different figs and the second female was introduced at least 30 min after the first (Table S1; Supplementary data). The bags were then returned around the figs to prevent entry by additional pollinators and oviposition by non-pollinators.

The figs were removed from the plants and their contents examined