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Measuring the discrepancy between fecundity and lifetime reproductive success in a pollinating fig wasp

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Abstract

Lifetime reproductive success in female insects is often egg- or time-limited. For instance in pro-ovigenic species, when oviposition sites are abundant, females may quickly become devoid of eggs. Conversely, in the absence of suitable oviposition sites, females may die before laying all of their eggs. In pollinating fig wasps (Hymenoptera: Agaonidae), each species has an obligate mutualism with its host fig tree species [*Ficus* spp. (Moraceae)]. These pro-ovigenic wasps oviposit in individual ovaries within the inflorescences of monoecious *Ficus* (syconia, or 'figs'), which contain many flowers. Each female flower can thus become a seed or be converted into a wasp gall. The mystery is that the wasps never oviposit in all fig ovaries, even when a fig contains enough wasp females with enough eggs to do so. The failure of all wasps to translate all of their eggs into offspring clearly contributes to mutualism persistence, but the underlying causal mechanisms are unclear. We found in an undescribed Brazilian *Pegoscapus* wasp population that the lifetime reproductive success of lone foundresses was relatively unaffected by constraints on oviposition. The number of offspring produced by lone foundresses experimentally introduced into receptive figs was generally lower than the numbers of eggs carried, despite the fact that the wasps were able to lay all or most of their eggs. Because we excluded any effects of intraspecific competitors and parasitic non-pollinating wasps, our data suggest that some pollinators produce few offspring because some of their eggs or larvae are unviable or are victims of plant defences.

Introduction

Lifetime reproductive success in female herbivorous insects and parasitoids can be determined by various factors. These include egg- and time limitation (Heimpel et al., 1998; Rosenheim et al., 2008), larval nutrition, restricted access to hosts, competition for oviposition sites, predation, and environmental stochasticity (Godfray, 1994; Rosenheim et al., 2008). All can prevent a female from translating her lifetime production of eggs, her fecundity, into viable offspring. For instance in pro-ovigenic species, in which individuals carry their lifetime's egg load upon eclosion, achieved fecundity is considered to be time constrained if females die in the presence of oviposition sites whilst they still contain viable eggs, or egg-limited, if females run out of eggs whilst still in the presence of suitable oviposition sites (Driessen & Hemerik, 1992; Heimpel et al., 1998; Rosenheim et al., 2008).

Pollinating fig wasps (Hymenoptera: Agaonidae) are important insect models for a broad range of ecological and evolutionary topics (Cook & Rasplus, 2003; Herre et al., 2008). These include mutualisms, sex ratios, coevolution, life history evolution, host–parasite interactions, dispersal, pollination ecology, and niche separation (reviewed by Herre et al., 2008). These studies often require counts of the offspring individual females produce,

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their lifetime reproductive success. Fig wasps are pro-ovigenic (Copland et al., 1973; Nefdt & Compton, 1996; Kathuria et al., 1999; Ghara & Borges, 2010). However, the number of eggs females carry usually exceeds their offspring production, which has important implications. For example, the shortfall between fecundity and lifetime reproductive success can explain discrepancies between predicted and realized offspring sex ratios (e.g., Kathuria et al., 1999) and promotes evolutionary stability of the fig–pollinator mutualism (Nefdt & Compton, 1996; Yu et al., 2004; Herre et al., 2008).

The fig–pollinator mutualism is obligate because neither partner can reproduce without the other. Fig trees [*Ficus* spp. (Moraceae)] produce enclosed, spherical inflorescences (syconia, colloquially 'figs'), each of which contains from <100 to more than 1 000 flowers, depending on the species. Each tree species is pollinated only by its own wasp species (sometimes more than one wasp species, e.g., Haine et al., 2006), and the mean number of female wasps entering each syconium (known as foundresses) varies both across fig species and across syconia within species (Herre, 1989). Each wasp larva feeds on a single *Ficus* ovule. An ovule can thus become a seed, when pollinated, or a wasp gall, when an egg is also deposited in it, but not both. The foundresses often lay eggs in many ovules, but they never exploit all ovules, even when the collective fecundity of all foundresses in a syconium would enable them to do so (Nefdt & Compton, 1996).

The factors preventing all pollinator foundresses from realizing their reproductive potentials are not completely understood, but multiple mechanisms have been suggested. For example, Kathuria et al. (1999) showed that single foundresses lay all or almost all of their eggs, but in syconia in which multiple wasps are present, variance in the number of eggs laid increases. Consistent with this, Wang et al. (2009) experimentally demonstrated that interference competition among foundresses in *F. racemosa* reduces individual reproductive success. In other words, if a single foundress contains N eggs, K foundresses always realize <KN offspring. More generally, Yu et al. (2004) argued that in at least some fig species, restricted pollinator longevity (time limitation) (Dunn et al., 2008a; Wang et al., 2009; Ghara & Borges, 2010) as a result of, for instance, increased mortality rates caused by interference competition, high temperatures (Wang et al., 2009), and/or low humidity (Dunn et al., 2008a) can result in foundresses dying before all fig ovules are exploited. This time constraint means that optimally foraging foundresses should therefore initially oviposit into inner ovules (Jousselin et al., 2001; Yu et al., 2004), either because inner ovules have shorter handling times (their styles are short, and foundresses oviposit down the styles to reach the ovaries) and/or because inner ovules are more valuable to the foundresses. There are many reasons for a quality gradient in ovules. For instance, outer ovules are compacted more tightly than inner ovules, which impedes mating and offspring eclosion (Anstett, 2001; Dunn et al., 2008a). Moreover, pollinator offspring in outer ovules are more vulnerable to attack from externally ovipositing parasitic wasps than those in inner ovules (Dunn et al., 2008b), and finally, some outer ovules may also be defended by the tree from wasps and 'reserved' for seeds via unknown mechanisms (West & Herre, 1994). For some combination of these reasons in any particular fig species, wasps will therefore fail to exploit most outer, long-styled ovules, which subsequently become seeds (Yu et al., 2004; Dunn et al., 2008b). This explains the ubiquitous spatial segregation of pollinator wasp galls and seeds within the mature syconia of monoecious *Ficus* species; galls cluster near the centre of the syconium, whereas seeds develop near the outer wall.

Surprisingly, given its importance in explaining fig–wasp mutualism persistence, to date no published study has reported a direct comparison of concurrent measurements of individual foundress fecundity and lifetime reproductive success for the same wasp population. Estimates of fecundity (counts of the total eggs within foundresses that have yet to lay a single egg) are also rare in the literature, possibly because of a perceived difficulty of dissecting the usually very small wasps. Moreover, no publication has presented a detailed description of the methods used to count eggs. Nefdt & Compton (1996) describe squashing wasps between microscope slides, then carefully moving body parts to reveal the eggs. Kathuria et al. (1999) report staining the ovaries of individual wasps with aceto-carmine but provide little information as to how the eggs were subsequently counted. More recently, Ghara & Borges (2010) also reported removing ovaries, but did not provide a detailed account on how egg counts were achieved, because they used different methods for pollinating and non-pollinating fig wasps (R Borges, pers. comm.).

In contrast, estimates of the lifetime reproductive success of individual foundresses (number of adult wasp offspring produced) are more common in the literature and are typically based on counts of offspring wasps that emerge from syconia containing only a single foundress body (e.g., Herre, 1989). This method introduces three possible sources of error in counts of foundresses and/or their offspring: (1) in some *Ficus* species, foundresses are known to exit syconia, possibly after oviposition (Moore et al., 2003); (2) during development, the flowers within the syconia of some *Ficus* species become very tightly packed. This leads to the dismembering and partial destruction of foundress bodies, which may also result in

inaccurate foundress counts; and (3) many non-pollinating fig wasps (NPFWs) are parasitoids or inquilines that kill and directly replace pollinator larvae in their galls, and these 'missing wasps' need to be accounted for. An experimental approach that eliminates these errors is to introduce a single foundress into a receptive syconium that has been bagged to exclude both NPFWs and other pollinators.

The purpose of this paper is twofold. First, to compare directly and concurrently for the first time the fecundity and lifetime reproductive success of individual female pollinating fig wasps from the same population. We exclude the effects of intraspecific competition and parasitic NPFWs. Second, to describe in detail, with micrographs, a simple method that we have devised to count the eggs of pollinating fig wasps.

Materials and methods

General fig–wasp biology

As they grow, the syconia of monoecious *Ficus* go through a series of predictable developmental stages (Galil & Eisikowitch, 1968). Relatively early in their development, syconia become receptive to foundresses (B-stage) whereby the entrance to the ostiole (the tunnel by which the wasps enter the syconium) becomes visible. Depending on the species, from one to several fully fecund foundresses will then enter the syconium (typically, having flown from other fig trees at a later stage of the developmental cycle). Each foundress will deposit each of its eggs into the ovary of a single female flower whilst simultaneously spreading the pollen it carried from its natal tree (Cook & Rasplus, 2003). Foundresses have limited life spans (<48 h; Dunn et al., 2008a; Wang et al., 2009) and usually die within the syconium in which they have oviposited. An ovary containing an egg becomes a gall in which a single wasp larva consumes the plant tissue that would otherwise have become a seed. When the pollinators are developing in some of the ovaries, known as the interfloral C-phase (Galil & Eisikowitch, 1968), NPFWs that are parasites (inquilines or parasitoids) of the pollinators often attack with their long ovipositors from outside the syconium. Parasitic NPFWs develop at a faster rate to the pollinators so that when the syconium reaches the male flower phase (D-phase), all wasps are adult. Male pollinators are the first to emerge from their galls because the females lack chewing mouthparts. The males chew holes into the galls containing females and mate with the females. The males later return and enlarge the mating holes to enable the females to emerge from their galls. At the same time, some males also chew tunnels in the syconium wall to enable the females to disperse to another, receptive tree after the females first collect pollen from male fig flowers (Zammit & Schwarz, 2000). NPFWs also tend to use the exit tunnels produced by pollinators for dispersal. Male pollinators are blind and flightless and do not leave their natal syconium unless they fall out of the exit tunnel they have chewed. After wasp dispersal, the syconium enlarges as it rapidly ripens (E-stage), and those of many *Ficus* species are often brightly coloured to attract frugivorous vertebrates that act as seed dispersers.

Study species

The study was performed during March and April 2009, with additional fecundity measurements taken in April 2010. In both years, we used two *Ficus citrifolia* (Miller) trees that were growing in the grounds of the University of São Paulo campus at Ribeirão Preto, Brazil. *Ficus citrifolia* is a monoecious, medium-sized fig tree, which in this part of Brazil is pollinated by an undescribed species of *Pegoscapus*. Voucher specimens have been deposited in the fig wasp collection at the faculty of Philosophy, Sciences and Letters, University of São Paulo campus at Ribeirão Preto, Brazil.

Fecundity

In both years, we collected 10 figs at the male flower phase (D-phase; Galil & Eisikowitch, 1968) from a single tree. The pollinating wasps that had developed in these syconia were all about to emerge naturally, so the syconia were placed together into a small-mesh bag to prevent the wasps from escaping. On return to the laboratory, the bag was left at room temperature (22 °C) for 24 h for the wasps to emerge. We then used a fine paintbrush moistened with tap water to haphazardly and sequentially select 30 live wasps, which we then placed into a 50-mm Petri dish containing tap water that had been cooled to 5 °C to semi-anesthetize the insects.

Each wasp was sequentially removed from the dish and placed into a small droplet of cold, distilled water on an upturned 110-mm glass Petri dish that had also been cooled to 5 °C. The wasp was placed under a stereomicroscope at 40× magnification and was killed by decapitation with two fine entomological pins mounted on small metal handles. The gaster was carefully separated from the mesosoma using the same fine needles and placed into a small droplet of clean phosphate-buffered saline (PBS) solution (Figure 1A).

Because the wasps are pro-ovigenic, the full ovaries exert pressure on the gaster causing distension. When this pressure is released by the separation of the gaster and mesosoma, the ovaries expand and protrude from the opening (Figure 1A and B). This aids their removal from the gaster, which is initiated by the careful pressing down of the joint

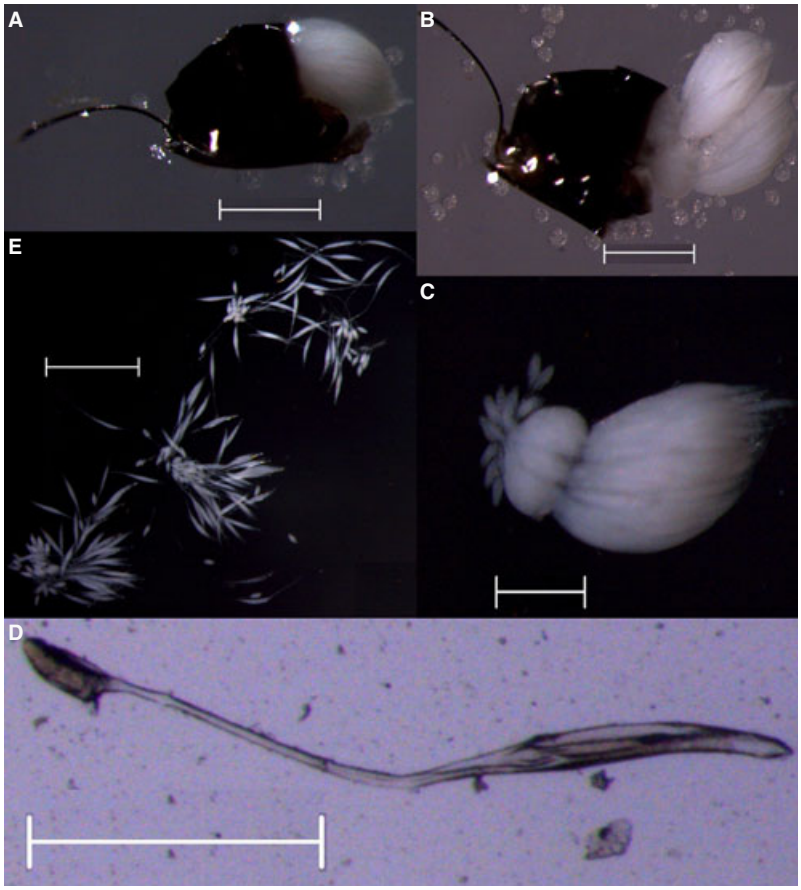

Figure 1 Micrographs showing different stages of pollinating fig wasp dissection, ovary removal, and egg morphology. (A) The wasp gaster immediately after separation from the mesosoma. Note that the ovaries now protrude from the open gap at the top, facilitating removal. Scale bar = 0.5 mm. (B) The ovaries as they are removed from the gaster. Scale bar = 0.5 mm. (C) The ovaries after removal from the gaster. Note that some ova have separated from the main mass of closely packed ova, but each is still joined to its ovariole by a fine peduncle. Scale bar = 0.2 mm. (D) An individual fig wasp egg, the morphology of which can be clearly seen to consist of an ovum connected to an ovariole with a fine peduncle. Scale bar = 0.2 mm. (E) A single ovary after it has been carefully spread. Each ovariole can be clearly identified and counted. This particular ovary contains a total of 92 ovarioles and hence an equal number of ova. Scale bar = 0.2 mm.

between the tergites and the sternites with one fine needle. Because a common oviduct joins the two ovaries, they can then both be completely removed from the wasp body with the other fine needle. The common oviduct can then be broken carefully, again using the two fine needles. Each ovary can then be placed into its own droplet of clean PBS solution on a fresh glass Petri dish that has been cooled to 5 °C (Figure 1C).

The eggs are tightly packed in the ovary, especially the ova (Figure 1C; Copland et al., 1973). Each egg consists of three clear components: (1) the ovoid ovum, joined by (2) a long, thin peduncle to (3) a long, serpentine ovariole (Figure 1D). The ova cannot be separated without damage and can probably only be counted reliably by chemical staining, and/or specialist microscopy, e.g., confocal or fluorescent microscopy. We found, however, that even though the ovarioles are tightly packed, they can be readily separated and spread by the careful use of two fine entomological pins, especially if removed from a freshly killed wasp (Figure 1E). By the careful adjustment of a cold light source, the ovarioles of a spread ovary can be counted giving a direct and accurate measure of fecundity (Figure 1E, Appendix 1).

Because egg counts are destructive, we could not estimate both fecundity and lifetime reproductive success from the same individual female wasps. We therefore estimated three values from three different samples of wasps: (1) fecundity, (2) eggs remaining in foundresses after oviposition and foundress death, and (3) lifetime reproductive success, by counting the total number of offspring produced. To estimate how many eggs were successfully laid (4), we subtracted the number of eggs remaining in each dead foundress from mean fecundity.

To estimate (2), we collected, from another two receptive trees, a further 23 B-stage syconia [receptive to pollinators (Galil & Eisikowitch, 1968)] known to contain foundresses. We knew that at least one foundress was present because when a wasp enters a syconium its wings become detached and are left at the ostiole's entrance. These syconia were returned to the laboratory where each one was carefully bisected twice with a razor blade and two pairs of fine forceps. If a syconium contained a single, dead foundress, it was carefully removed with a fine paintbrush and then processed for egg counts as described earlier. Syconia that still contained live foundresses or contained more than a single foundress were not used. We did not

use experimental introductions of single foundresses owing to logistical constraints; the few receptive trees at our site at the time of the study that had been bagged to prevent natural wasp infestation were needed for lifetime reproductive success measurements.

Lifetime reproductive success

We selected haphazardly 50 pre-receptive A-phase syconia (Galil & Eisikowitch, 1968) on two branches on each of two *F. citrifolia* trees. Syconia at this developmental stage are small and are yet to be infested by any wasp species (Elias et al., 2008). A fine-mesh drawstring bag was placed over all of the syconia on each branch. After 20 days, all syconia were checked daily for receptivity to pollinating wasps. To do this, we first determined whether the entrance to the ostiole was visible. If it was, a haphazardly selected, freshly emerged female pollinator (from a syconium taken from another *F. citrifolia* tree nearby) was carefully placed at the ostiole's entrance using a fine, soft paintbrush. If the wasp entered that syconium, the syconium was marked with a fibre-tipped pen, and the process repeated for all syconia for a particular branch. This process was repeated daily until all syconia had received a single foundress. To control for foundress age, each wasp had emerged from its natal syconium within 24 h from when it was introduced into a syconium.

After foundress introduction and final bagging to prevent further wasp infestation, all syconia were checked on an ad hoc basis for a further 3 weeks to ensure that each bag remained secure, then daily to determine the stage when the wasps were about to emerge (male flower phase, D-stage; Galil & Eisikowitch, 1968). When each syconium reached D-stage, it was removed from the branch and placed into its own small cylindrical plastic vial (40 × 20 mm), which had a fine-mesh lid to provide ventilation and to prevent the wasps from escaping. Each vial was then returned to the laboratory and left for 48 h to allow the female wasps to emerge. Each vial was then placed into a freezer at −25°C for 2 h to kill the wasps, which were then counted. Each syconium was then carefully split with a razor blade to confirm that only a single foundress was present, to count the male wasps, and to count any female wasps still in their galls or loose in the syconium cavity.

Results

In our 2009 data, egg loads within freshly emerged female wasps were significantly greater than the numbers of offspring produced by single foundresses (t-test corrected for unequal variances: $t = 3.10$, d.f. = 69.7, $P = 0.003$; Table 1; differences in offspring produced between branches and trees were not significant). In short, fecundity was greater than lifetime reproductive success. The coefficient of variation (CV) for reproductive success was also considerably higher than for fecundity (Table 1). Relatively few eggs remained in dead single foundresses (Table 1; difference in eggs remaining in wasps between trees sampled was not significant). The fecundity of the 2010 wasps was significantly lower than the 2009 wasps (t-test: $t = 10.63$, d.f. = 56, $P<0.001$), but the coefficient of variation for the number of eggs across females was similar for both years (Table 1).

Table 1 Descriptive data of fecundity (eggs per fully gravid wasp), the numbers of eggs left after oviposition per wasp in dead single foundresses, and the lifetime reproductive success per experimentally introduced single foundress. CV (%) = (SD/mean) × 100.

	Mean ± SEM	Range	CV (%)
2009			
(1) Fecundity	212.29 ± 9.92	141–283	16.88
(2) Eggs in dead foundresses	23.83 ± 10.55	0–95	153.21
(3) Lifetime reproductive success	175.16 ± 6.69	5–284	37.99
(4) Eggs laid [(1)−(2)]	188.46 ± 7.61	117–212	19.37
2010			
Fecundity	128.10 ± 24.23	89–172	18.91

Row numbers correspond to the measurements described in the Fecundity section. See text for statistical analyses.

Discussion

By making concurrent measurements of fecundity and lifetime reproductive success in the same population, we show for the first time that even lone female pollinating fig wasp foundresses do not always attain their maximum reproductive potential in a fig. This appeared to be due mainly to (some) foundresses realizing few successful offspring after oviposition rather than to the foundresses failing to lay all of their eggs. Moreover, maximum estimates of fecundity and lifetime reproductive success were similar, whereas minimum reproductive success was clearly lower than was minimum fecundity (Table 1). This strongly suggests that lone foundresses typically lay all or most of their eggs, as reported by Kathuria et al. (1999) for *Eupristina belgaumensis* Joseph, the pollinator of *Ficus drupacea* Thunb. In other words, the wasps do not appear to be time-limited, presumably because a lone foundress in a syconium with abundant flowers of high profitability incurs few costs of oviposition (see Rosenheim et al., 2000).

The design of this study rules out some explanations for the failure of some females to translate all of their eggs into offspring, such as interference competition for oviposition sites among foundresses and the presence of non-pollinating fig wasps (Dunn et al., 2008b; Wang et al., 2009). Our data do not exclude the effects of experimental handling of foundresses and/or possible extended exposure to natural environmental conditions by wasps that had oviposited naturally. Overall handling differences between experimental foundresses and wasps used for fecundity measures were negligible. Additional exposure to potentially more hostile environmental conditions may have resulted in reduced egg loads via resorption in wasps that oviposited naturally. However, we think this was unlikely because resorption is most likely to occur in synovigenic insects, insects that are relatively long-lived as adults and can produce new eggs during their lifetime (Rosenheim et al., 2000).

Instead, we interpret our results to suggest egg or larval mortality after oviposition, as a result of some combination of genetic defects, disease, or the effects of plant defences against the presence of eggs or larvae. In addition, our data may reflect oviposition in flowers that received no viable pollen, as in *F. citrifolia* pollinator wasp larvae can only successfully develop in pollinated flowers, because they feed mainly on endosperm (Jansen-Gonzalez, 2009). To our knowledge, there are no data on rates and causes of variation in egg viability or larval mortality in any fig wasp, although this has been measured in other hymenopterans (e.g., Petters & Mettus, 1980; Hardy & Cook, 1995; Ueno, 1999; Pirk et al., 2004; Helanterä et al., 2006). Unfortunately, we think that such measurements will never be possible with fig wasps, because foundresses are highly unlikely to use any artificial oviposition substrate to allow the collection of individual eggs.

Plants use a variety of biochemical and physiological defences against insect herbivores (Howe & Jander, 2008), which includes chemically attacking eggs and larvae (Hilker & Meiners, 2010). Of particular relevance to our data is the hypothesis of West & Herre (1994), who suggested that outer, long-styled ovules within the *Ficus* syconium are immune to wasp attack through biochemical and/or physical defences and are 'reserved' by the tree for seed production. However, a single foundress is likely to oviposit mainly in inner, short-styled ovules (Jousselin et al., 2001; Yu et al., 2004). West & Herre's (1994) 'unbeatable seeds' hypothesis is thus unlikely to explain the discrepancy we found between fecundity and reproductive success, unless there are plant defences in some short-styled inner ovules, or lone foundresses did in fact use long-styled outer ovules in which to oviposit. The 'unbeatable seeds' hypothesis remains to be empirically tested, although in *F. citrifolia* individual ovaries do not show any obvious, immediate reaction to *Pegoscapus* eggs (Jansen-Gonzalez, 2009), and wasps in other fig species have been found to develop successfully in ovules of all lengths (Dunn et al., 2008b).

We emphasize that we have not ruled out the possibility of insufficient pollination in explaining the difference between fecundity and lifetime reproductive success. This is because in *F. citrifolia*, an ovary containing a wasp egg only begins to develop into a gall once the endosperm is well developed (Jansen-Gonzalez, 2009), so an egg laid into an unpollinated flower will not develop. The *Pegoscapus* pollinator of *F. citrifolia* actively pollinates the tree (Kjellberg et al., 2001). This is a more effective process than passive pollination (Kjellberg et al., 2001), and a single foundress may be able to pollinate all flowers within a syconium, especially in *Ficus* species with small syconia (Herre, 1989). The syconia of *F. citrifolia* are medium sized (ca. 325 flowers), and lone foundresses are common, with a mean number across syconia in a population typically <2 (e.g., Herre, 1989). Moreover, the foundresses in the syconia we used for this work all must have carried and deposited at least some pollen, because *F. citrifolia* trees will abort syconia in which wasps oviposit but do not receive pollen (Jander & Herre, 2010). Further work to clarify how rates of pollination may affect pollinator reproductive success will thus be useful.

The reasons for the difference in wasp fecundities between the two study years are unclear, but there may be among-tree and/or temporal variation in the mechanisms that result in overall variance in wasp fecundity, e.g., through differences in larval nutrition or egg resorption owing to environmental variation (see above; Rosenheim et al., 2000, 2008). More comprehensive work in the future capturing between host tree and temporal variation in fecundity and reproductive success is therefore warranted because this may reveal corresponding variation in the wasps' potential to overexploit their host; this will be the case when the average number of eggs entering syconia exceeds the average number of flowers within.

There is conflicting opinion on the fate of the ovarioles after oviposition, although they do not remain inside the wasp (DW Dunn, pers. obs.). Grandi (1920) states that in *Blastophaga psenes* (L.), the 'egg tail' is incorporated into the ovum during oviposition, presumably by atrophy. Abdurahiman & Joseph (1978) dissected *F. hispida* ovules and found that each egg of the pollinator *Ceratosolen solmsi marchali* (Mayr) still had attached part of the peduncle that joins the ovum to the ovariole. This suggests that Grandi (1920) was incorrect, or different mechanisms operate in different pollinator species. If the ovariole were absorbed into the ovum, the peduncle would first have to undergo the same process. However, the ovarioles may be

reabsorbed into the wasp during oviposition, which would have to be a rapid process because of the wasps' restricted life spans (Dunn et al., 2008a; Wang et al., 2009; Ghara & Borges, 2010). Copland et al. (1973) suggested that each ovariole passes through the ovipositor during oviposition and becomes lodged in the flower style. If true, foundresses could use ovarioles lodged in styles as cues to determine whether flowers are worth spending time probing with their ovipositors. Additionally, the lengths of the peduncles joining the ova and ovarioles within an ovary are highly variable (Figure 1D), with eggs on the curved outside being longer than those within. Short eggs may thus take less time to lay than long eggs. These two factors may affect wasp oviposition rates and patterns, which are known to contribute to mutualism stability (Yu et al., 2004; Wang et al., 2009). Clarifying post-oviposition ovariole fates in pollinating fig wasps will thus be a fruitful topic of future research.

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Appendix 1

Measuring fecundity

Our method of measuring fecundity (counting eggs) gives at least two advantages over previous, briefly described methods: (1) the eggs cannot be obscured by various body parts as often happens when squashing a wasp between two microscope slides (sensu Nefdt & Compton, 1996; DW Dunn & RAS Pereira, pers. obs.) and (2) chemical staining of the ova (sensu Kathuria et al., 1999; Ghara & Borges, 2010) or specialist microscopy is not required. We hope that our description of a simple method to count the eggs of pollinating fig wasps will facilitate and encourage future studies.

Measuring fig wasp fecundity has probably been neglected because of a perceived difficulty in dissecting these very small insects. Our micrographs (Figure 1) are of a medium-sized (body length ca. 2 mm) *Pegoscapus* species. Pollinating fig wasps vary considerably in body size, even within genera (Cook & Rasplus, 2003). Our egg counting method works well with pollinator species over a wide size range – e.g., from *Pleistodontes greenwoodi* (Grandi) (1 mm body length) to *Pleistodontes nigriventis* (Girault) (5 mm body length) (DW Dunn, J. Ridley, JM Cook & DW Yu, unpubl.). Moreover, this method works well on pollinating fig wasps from at least four genera (DW Dunn, J Ridley, JM Cook & DW Yu, unpubl.), making it generally applicable to all pollinating fig wasps.

There are several useful points for others who intend to use this method. First, the ovarioles of freshly killed wasps separate more readily, and are more robust, than those from wasps that have been dead for longer than 24 h. We acknowledge that some studies may require wasps other than those that have recently died (e.g., Kathuria et al., 1999). However, care is needed to avoid ovariole breakage when the ovaries are spread, which is more likely the longer the wasps have been dead, because broken ovarioles are likely to result in overestimated egg counts. Second, using ethanol to preserve wasps, even at low concentrations, desiccates the eggs so they quickly become invisible; the ovaries of freshly killed wasps kept in tap water at 5 °C remain in good condition for several days. Third, the use of a circular cold light source mounted above the stage of a stereomicroscope makes the ovarioles difficult to see. An angled light source from above gives better results, although the best results are obtained by adding an understage light. Fourth, throughout the process, the eggs must be kept hydrated with either water or PBS solution. If they dry out, they cannot be counted, even after attempted re-hydration. Using glass instead of plastic on which to perform dissections minimizes desiccation, as glass takes longer to warm-up. Additionally, glass is less likely to be scratched by dissecting instruments, which makes maintaining optimal light settings easier. The use of a cover slip also slows the rate of desiccation.