Longevity, early emergence and body size in a pollinating fig wasp – implications for stability in a fig–pollinator mutualism

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Summary

1. Fig trees (Ficus) are pollinated only by agaonid wasps, whose larvae also gall fig ovules. Each ovule develops into either a seed (when pollinated) or a wasp (when an egg is also laid inside) but not both.

2. Ovipositing wasps (foundresses) favour ovules near the centre of the enclosed inflorescence (syconium or ‘fig’), leaving ovules near the outer wall to develop into seeds. This spatial stratification of wasps and seeds ensures reproduction in both partners, and thereby enables mutualism persistence. However, the mechanism(s) responsible remain(s) unknown.

3. Theory shows that foundresses will search for increasingly rare inner ovules and ignore outer ovules, as long as ovipositing in outer ovules is sufficiently slow and/or if inner ovules confer greater fitness to wasps. The fig–pollinator mutualism can therefore be stabilized by strong time constraints on foundresses and by offspring fitness gradients over variation in ovule position.

4. Female fig wasps cannot leave their galls without male assistance. We found that females in outer ovules were unlikely to be released. Inner ovules thus have added value to foundresses, because their female offspring are more likely to mate and disperse.

5. For those offspring that did emerge, gall position (inner/outer) and body size did not influence the order in which female pollinators exited syconia, nor did early emerging wasps enjoy increased life spans.

6. We also found that the life spans of female wasps nearly doubled when given access to moisture. We suggest that conflict resolution in the fig–pollinator mutualism may thus be influenced by tropical seasonality, because wasps may be less able to over-exploit ovules in dry periods due to time constraints.

Key-words: cooperation, Ficus, fig wasp, mutualism, optimal foraging, Pleistodontes imperialis

Introduction

Mutualisms are interspecific interactions in which both partners benefit (Janzen 1985; Herre et al. 1999; West, Griffin & Gardner 2007a) and are important contributors to global biodiversity at multiple trophic levels (e.g. Christian 2001; Brundrett 2004; Harrison 2006). Although hosts and symbionts both profit from their association, benefits are usually obtained by imposing costs onto the other partner. Why, then, does one partner not destabilize the mutualism by exploiting the other unsustainably (Herre et al. 1999; Anstett 2001; Yu 2001; Frank 2003; Foster & Wenseleers 2006)? The diversity of mutualisms has meant that no all-encompassing explanation for their maintenance has been forthcoming until recently (see Foster & Wenseleers 2006; West, Griffin & Gardner 2007b). In some mutualisms hosts can often favour those symbionts that are cooperative and impose sanctions on cheats (e.g. Kiers, Rousseau, West & Denison 2003; Edwards et al. 2006). More generally, it has been shown that a high benefit : cost ratio of cooperation, high within-species relatedness and high between-species fidelity are important factors contributing to mutualism stability (Foster & Wenseleers 2006).

Fig trees (Ficus spp.) are important in maintaining diversity in tropical and subtropical forests (Janzen 1979; Cook & West 2006; Harrison 2006). Their association with pollinating wasps (Hymenoptera: Agaonidae) is an obligate mutualism.
because neither partner can reproduce without the other (Cook & Rasplus 2003). Most of the 750 + Ficus species have their pollination requirements fulfilled by a single species-specific wasp species (but see Machado et al. 2005), and thus show considerable between-species fidelity. The enclosed Ficus inflorescences (syncoria or ‘figs’) provide food for the wasps’ larvae. Approximately 50% of Ficus species are dioecious, in which wasps develop only in ‘male’ trees and thus do not inflict a significant reproductive cost to the plant (Cook & Rasplus 2003). However, in monoecious Ficus, both wasps and seeds develop in the same syconium. Ovipositing females (foundresses) lay only one egg per female flower and they do this by inserting their ovipositor down the style (Jousselin, Hossaert-McKey, Vernet & Kjellberg 2001). One wasp offspring thus costs the tree one seed, and this highlights the inherent conflict in the mutualism. To reproduce, monoecious Ficus need to produce both wasps (to disperse pollen) and seeds from the same syconium, but the wasps gain in the short term only by exploiting the tree’s ovules. Selection should favour increased ovule exploitation by wasps, which will eventually destabilize the mutualism (Anstett 2001). Despite nearly 40 years of research, however, the mechanism(s) preventing over-exploitation of monoecious figs by their pollinators remains unidentified (Jousselin, Kjellberg & Herre 2004; Yu et al. 2004).

The style lengths of flowers in the receptive syncoria of monoecious Ficus are highly variable (Janzen 1979; Ganeshiah et al. 1995; Ganeshiah, Kathuria & Shaankar 1999). Long-styled flowers (referred to hereafter as ‘outer ovules’) develop into wasp galls or seeds at the mature syconium’s outer wall, while short-styled flowers (referred to hereafter as ‘inner ovules’) become galls or seeds near to the syconium’s central cavity (the ‘lumen’) (Bronstein 1988; Nedft & Compton 1996). Most outer ovules remain unexploited by pollinator wasps and develop into seeds, whereas inner ovules are used by ovipositing wasps and most develop into galls, which suggests that foundresses favour inner ovules for oviposition (Herre 1989; West & Herre 1994; Nedft & Compton 1996; Jousselin et al. 2001, 2004). The four main hypotheses explaining why pollinators do not over-exploit figs are based on this spatial stratification of wasps and seeds: (1) unbeatable seeds – some (outer) ovules are protected physically or biochemically against wasp damage (West & Herre 1994). However, no empirical data support this notion. (2) Short ovipositors – pollinators’ ovipositors are too short to reach outer ovules (Galil & Eiskowitch 1968; Ganeshiah et al. 1999; Jousselin et al. 2004); however, this is not so in several wasp species (Bronstein 1988; Nedft & Compton 1996; Herre 1999). (3) Insufficient eggs – because wasps disperse long distances, too few foundresses enter receptive syconia to exploit all ovules (Nedft & Compton 1996); moreover, the ostiole (used by wasps to enter the receptive syconium) may close to prevent excess foundresses from entering (Herre 1989; Nedft & Compton 1996). However, in many Ficus more foundresses enter receptive syconia than are required to exploit all ovules, yet seeds are still set and the mutualism is maintained (Anstett, Bronstein & Hossaert-McKey 1996; Herre 1999).

Yu et al. (2004) provided a new hypothesis to resolve the fig–pollinator problem by focusing upon how foundresses ‘optimally forage’ for oviposition sites. If inner ovules are more profitable to ovipositing foundresses, through shorter handling times or because they convey higher fitness to wasp offspring, then foundresses should focus their oviposition initially on inner ovules. As search times for egg-free inner ovules increase foundresses will, at some point, expand their preference and also begin to utilize outer ovules. The more profitable that inner ovules are compared with outer ovules, the rarer egg-free inner ovules must be before foundresses begin to exploit outer ovules.

One potential cause of variation in ovule profitability is variation in handling time caused by variation in style lengths. Outer ovules have long, narrow styles (Jousselin et al. 2001, 2004), so foundresses should take longer to deposit an egg compared with inner ovules (Yu et al. 2004). The resulting gradient in profitability has an important consequence for the stability of fig-wasp mutualisms. Achieved fitness is predicted to decrease with each succeeding foundress because early foundresses are able to lay in inner ovules, which take less time, leaving mainly outer ovules for late foundresses. As a result, the number of ovules that receive an egg is less than a straight-line extrapolation of the first foundress’s fecundity (Yu et al. 2004).

Moreover, if a gradient in ovule profitability is caused by differences in offspring fitness, such that eggs laid in inner ovules have the highest value, foundress fecundity can become disconnected from foundress fitness. In the extreme, late-arriving foundresses can be selected to devote much of their short life spans (Kjellberg, Doumesche & Bronstein 1988) to searching for unexploited inner ovules, therefore laying fewer total eggs than if they had oviposited randomly (Yu et al. 2004). The fig host thus ‘controls’ its symbiont indirectly by preventing resource over-exploitation (Yu 2001; Kiers et al. 2003; Edwards et al. 2006). It does this by providing ovules of highly variable profitability to foundresses, which decreases the benefits to non-cooperative foundresses, i.e. those that exploit outer ovules (see Foster & Wenseleers 2006).

However, there have been few attempts (Anstett 2001; Dunn et al. 2008) to measure the relative value of inner and outer ovules to foundresses. There are also few data on pollinating fig wasp longevity, with only two published studies (Kjellberg et al. 1988; Compton, Rasplus & Ware 1994). Longevity may have added importance if the time available to pollinators to lay eggs in the syconium varies between the tropical wet and dry seasons, because moisture availability often affects insect longevity (e.g. Hicks, Hagenbuch & Meffert 2004). This paper has two aims: (1) to measure the effects of humidity on female pollinator longevity; and (2) to examine how ovule position affects two aspects of offspring fitness: (i) the likelihood of female offspring remaining trapped in the syconium and (ii) rapid emergence from the syconium. The highly specialized nature of fig-wasp behaviour within the syconium also suggests that female body size may be associated with rapid emergence. We therefore conducted an additional investigation.
into the influence of female body size on rapid emergence from the gall and/or syconium.

Materials and methods

STUDY SYSTEM

Our study species, *Pleistodontes imperialis* Saunders (Hymenoptera: Agaonidae), is the pollinator of *F. rubiginosa* Desf. Ex Vent. 1805 (Moraceae: Urostigma, section Malvanthera) (Lopez-Vaamonde et al. 2002; Haine, Martin & Cook 2006). The natural range of *F. rubiginosa* is the east coast of Australia, from southern New South Wales to northern Queensland (Dixon, Jackes & Bielig 2001; Haine et al. 2006). In tropical Queensland, which has distinct wet and dry seasons, most *F. rubiginosa* grow as small- to medium-sized lithophytes.

When *F. rubiginosa* syconia reach the male flower phase (Gahil & Eisikowitch 1968), a *P. imperialis* male emerges by biting a hole in the wall of its own gall. Each male then searches repeatedly for a gall containing a female, bites a hole in the gall, and then mates with the female by inserting his acedeagus through the hole. Males return later to galls containing mated females in order to enlarge the mating holes, thus enabling the females to emerge. It is not known whether males return only to the females with whom they originally mated or if the males open any ovule containing a mated female. Finally, a few of the males chew holes through the syconium wall to allow the females to escape and disperse. Like most species of pollinating fig wasp (*Kjellberg, Jousselin, Hossaert-McKey & Borges & Hossaert-McKey 2006*), female *P. imperialis* thus cannot escape from their galls or the syconium without male assistance (Zammit & Schwarz 2000).

As a result, in a receptive syconium, ovules that are difficult for males to access should be less valuable to ovipositing foundresses. Their daughters might escape male attention and will thus remain trapped in their galls. Moreover, even among females that remain and are released, males might take longer to release females from less accessible galls. Those females would lose part of their life span waiting to be released. More time spent in the gall would result in less time available for dispersing to and ovipositing in a receptive syconium, as well as leading to an increased risk of predation posed by frugivorous vertebrates or possibly ants (Schatz, Proffit, Rhaki, Borges & Hossaert-McKey 2006).

Finally, body size may be an important factor in giving females an intraspecific competitive advantage in early emergence from their galls, and/or the syconium. This is because small females may be able to emerge earlier than large females when males are chewing exit holes. Additionally, small females might also emerge earlier from the syconium by negotiating their way more quickly through the exit tunnels chewed by males in the syconium wall. Alternatively, if there is direct competition for access to exit tunnels chewed by males, large females may be at an advantage.

Experiment 1: wasp longevity

During June 2006 we collected 32 syconia, just prior to wasp emergence, from four wild *F. rubiginosa* trees in Townsville, northern Queensland. All syconia from each tree were from the same crop. Each syconium was placed into a cylindrical plastic tube 10 × 3 cm, which had a mesh lid for ventilation that also prevented emerging wasps from escaping. Each tube was allocated randomly to one of two treatments: (1) dry tightly packed cotton wool (controls) or (2) tightly packed cotton wool that had been moistened with tap water (moist treatment). The cotton wool was approximately 15 mm deep at the bottom of each tube.

All tubes were then placed into a laboratory at room temperature (approximately 25 °C) with a natural light/dark cycle, and were monitored every 30 min until the first female *P. imperialis* emerged. Then, at 2-h intervals, each syconium from which female pollinators had emerged was transferred to an identical fresh tube. Each cohort of emerged wasps was left in its original container. This process was repeated until no more female pollinators emerged from a syconium for 6 h.

Every 2 h we checked all cohorts of emerged wasps and recorded how many were dead. We repeated this process until all wasps for a particular emergence cohort for a syconium were dead. The dead wasps for each emergence cohort for each syconium were then transferred to a small 1.5 mL plastic vial and stored in 80% ethanol prior to measuring.

For measuring, all wasps from a particular emergence cohort were placed into a watch-glass that contained a small amount of 80% ethanol to enable all wasps to float. Each wasp was then removed systematically with a fine paintbrush and placed into one of six arbitrarily numbered identical watch-glasses until each one contained (as near as possible) an identical number of wasps. A dice was then thrown to determine from which watch-glass wasps were to be measured. Five wasps were then removed carefully blindly from the selected watch-glass using a fine paintbrush. If there were less than five wasps present, the dice was thrown again and the required number of wasps was removed from another watch-glass.

The head of each selected wasp was removed carefully with fine forceps to avoid distortion. The head area (length × width) of each wasp from a sample of five females from each emergence cohort was used to estimate body size. If five or fewer pollinating wasps for a particular cohort were available, they were all measured. Measurements were made to the nearest 0.0025 mm, using an eyepiece graticule mounted on a binocular microscope.

Experiment 2: likelihood of female pollinators remaining trapped in the syconium

During December 2005 we collected 18 syconia from two wild *F. rubiginosa* trees, each from a separate site in northern Queensland (Cardwell and Yungaburra). All syconia from a single tree were from the same crop. Immediately after picking, each syconium was placed into a cylindrical plastic container (10 × 3 cm) with fine mesh netting as a lid for ventilation. As soon as possible after collection, all containers were placed into a laboratory at room temperature (25 °C) with a natural light/dark cycle. Syconia were checked at 2-h intervals until the first pollinating wasps emerged. Then, from each syconium, wasps were left to emerge for a further 24 h. The contents of the container were then immersed in 80% ethanol and sealed for storage prior to dissection. All emerged wasps from a single syconium were stored in 80% ethanol in a 1.5 mL plastic vial.

In the laboratory, each syconium was sliced into eighths for dissection. All ovules were removed systematically, then allocated to one of five categories: (1) galls still containing a female *P. imperialis*; (2) galls containing a male *P. imperialis*; (3) galls that had been exited by a wasp (which can be determined from the exit hole bitten by males (Zammit & Schwarz 2000; Yao et al. 2005); (4) galls containing non-pollinating fig wasps; and (5) seeds. Categories of all galls other than those with exit holes could be determined visually and confidently after dissection with fine forceps. Galls still containing female *P. imperialis* were devoid of mating holes chewed by males, indicating that none had received any male attention (Zammit & Schwarz 2000; Yao et al. 2005).
All female *P. imperialis* that had emerged successfully from their syconium were counted, as were all male and female *P. imperialis*, and all non-pollinating fig wasps that were loose in the lumen.

For this experiment, we expected galls still containing female *P. imperialis* to be rare and exited galls to be common. Therefore, we measured the lengths of all ovules still containing a female *P. imperialis*, and a sample of five to 10 of those ovules exited by a wasp. To select an as near-to-random sample of exited galls as was practically possible, we placed all exited galls from a single syconium into a watch-glass and moistened them with 80% ethanol. We then placed each exited ovule systematically into one of six arbitrarily numbered watch glasses using a fine paintbrush, then threw a dice to determine from which watch-glass were to select our sample of exited galls. Five to 10 ovules were then taken blindly using a fine paintbrush. If there were insufficient ovules in a particular watch-glass, another was selected using the same methods.

Ovules were measured (pedicel length + gall length) to the nearest 0.0025 mm using an eyepiece graticule mounted on a binocular microscope. We did not measure pedicel length directly as an indication of a gall’s relative position to the outside of the syconium for two reasons. (1) In *F. rubiginosa*, there is no reliable landmark where the pedicel joins the gall to make accurate and repeatable measurements; and (2) galls at the very outside edge of the syconium do not have pedicels. This would result in a series of zeros in the data set and would present problems for statistical analysis (see Dunn et al. 2008). The size of each syconium was estimated by measuring the length and width to the nearest 0.05 mm with digital calipers, taking the mean of the two measurements, then calculating the volume of a sphere (Dunn et al. 2008).

**Experiment 3: early emergence from the syconium**

During July 2006 we collected 25 syconia from 11 *F. rubiginosa* trees in the Hervey’s Range area, 50 km west of Townsville, northern Queensland. All syconia were treated in exactly the same manner as syconia that were used for experiment 2, except: (1) each syconium was left for only 2 h after the first wasps were seen to have emerged prior to immersion in 80% ethanol; and (2) because we expected ovules that had been exited by a wasp and those that still contained a female *P. imperialis* to both be relatively common, we measured a sample of five to 10 of the ovules that contained a female *P. imperialis* and five to 10 of those exited by a wasp. We used the same methods as described in experiment 2 to select as near a random sample as possible for galls that still contained a female pollinating wasp or those that had been exited by a wasp. We did not differentiate between the very few galls still containing female *P. imperialis* that had mating holes chewed by males (D.W. Dunn, personal observation) and those that did not.

Syconia used in experiments 2 and 3 contained a mean of 424.25 ± 23.69 female flowers, and were 2.29 cm³ (SE = 0.09) in size.

**BODY SIZE AND EMERGENCE PATTERNS**

To test the hypothesis that body size affects early emergence from the gall and syconium in female *P. imperialis*, we took a sample of five individuals from each of three categories from all syconia used in experiments 2 and 3: (i) those still in their galls; (ii) loose in the lumen; or (iii) emerged successfully from the syconium. We used the same method as described in experiment 1 to select wasps from each category for each syconium. If there were five or fewer individuals for any particular category in a syconium, all wasps were measured for that category. We measured the total head length and width to the nearest 0.0025 mm using an eyepiece graticule mounted on a binocular microscope. We estimated female body size as head area (length × width).

**STATISTICAL ANALYSES**

**Experiment 1: wasp longevity**

To estimate the effects of moisture on wasp longevity, we used a Cox regression assuming proportional hazards between treatments. In addition to the humidity treatment, emergence cohort (in blocks of 2 h after the first wasps to emerge) was included as a factor in order to see if variance in wasp longevity could be attributed to time-to-emergence. Because most wasps emerged less than 10 h after the first cohort, all wasps that emerged after 10 h were put into the last cohort. ‘Tree’ was also included as a random factor to control for variance between trees (West & Herre 1994), and syconium volume was included as a continuous covariate. Censoring was not required because the total life spans of all wasps were measured.

Wasp size was not included in our survival analysis, because it was impractical to obtain both longevity and size measurements for all the 2577 wasps. Instead, to estimate the effects of wasp size on longevity, we therefore conducted a second analysis in which we took the mean life span for each emergence cohort for each syconium as the response variable in a general linear model. Humidity treatment was included as a fixed factor, and mean wasp size per cohort and syconium size were entered as covariates.

**Experiment 2: likelihood of female pollinators remaining trapped in the syconium**

We used a binary logistic regression to fit the probability of a female *P. imperialis* remaining trapped in her gall (because no male had released her). Ovules containing trapped female *P. imperialis* (1) and those from which a wasp had emerged (0) were used as the binary response variable. Ovule length and syconium volume were entered as continuous explanatory variables, and tree was entered as a random factor.

**Experiment 3: early emergence from the syconium**

We also used a binary logistic regression to estimate the probability of early emergence as a function of ovule length. Exited galls (1) and galls still containing a female *P. imperialis* (0) were used as the binary response variable. Similarly, we included syconium volume as a covariate and tree as a random factor.

It should be noted that galls exited by female *P. imperialis* cannot be differentiated from those vacated by pollinator males or parasitic non-pollinating wasps of both sexes (Yao et al. 2005). However, the total number of female pollinators in a syconium or those that had emerged from a syconium in both experiments 2 and 3 always greatly exceeded the total number of all other wasps (mean parasite load per syconium = 8.00 ± 1.3%), meaning that wasps other than female pollinators were unlikely to have affected our results to any great extent.

**BODY SIZE AND WASP EMERGENCE**

To test whether wasp size affects patterns of female pollinator emergence from galls and/or syconium we used a general linear model, using wasp measurements collated from the syconium used in...
experiments 2 and 3. Collection period (syconia left for 24 h or for 2 h for wasp emergence) and wasp emergence category (still in gall, emerged from the gall but still in the lumen and emerged successfully from the syconium) were entered as categorical factors. Syconium size was included as a covariate (West & Herre 1994; see also Dunn et al. 2008). All analyses were performed with spss version 11·0·2 for the Macintosh computer.

Results

EXPERIMENT 1: WASP LONGEVITY

Longevity and size

Control wasps had significantly higher mortality rates than those given access to moisture ($\beta \pm SE = 4.27 \pm 0.17$, Wald = 616·68, $P < 0.001$; Fig. 1a). There were also significant differences in mortality rates among emergence cohorts (Wald = 110·78, $P < 0.001$). However, there were no clear patterns of wasp life span that could be attributed to the order of emergence, other than the last cohort of wasps to emerge appearing to have the lowest expected life spans (Fig. 1b). We therefore recalculated a simplified model to compare the mortality rates of very late-emerging wasps (10 h + from the first wasps to emerge) with all other wasps. This showed that very late-emerging wasps had marginally non-significantly higher mortality rates than wasps that had emerged previously (Wald = 8·69, $P = 0·06$).

In addition, mean wasp size did not explain variation in cohort longevity, either as an interaction effect with the humidity treatment ($F_{1,103} = 1·45$, $P = 0·23$) or as a main effect ($F_{1,104} = 0·50$, $P = 0·48$), independent of the effects of moisture ($F_{1,104} = 65·03$, $P < 0·001$).

Emergence patterns

A mean of 82·03 (SE = 8·44) pollinators emerged from each syconium (mean size = 2·37 cm³; SE = 0·15). Most exited their syconium in the earlier emergence cohorts, with approximately 48% exiting successfully within only the first 2 h (Fig. 2). There were no significant differences in the mean sizes of wasps among the five different emergence cohorts ($F_{4,480} = 1·52$, $P = 0·12$).

EXPERIMENT 2: LIKELIHOOD OF FEMALE POLLINATORS REMAINING TRAPPED IN THE SYCONIUM

Female pollinators that remained trapped in their galls were significantly more likely to occupy outer ovules, compared with females that had been released successfully by males ($\beta \pm SE = -0·75 \pm 0·29$, Wald = 6·88, $P = 0·009$; Fig. 3). In addition, small syconia were more likely to contain trapped females than were larger syconia ($\beta \pm SE = -1·62 \pm 0·48$, Wald = 11·19, $P = 0·001$). The likelihood of females being trapped was also influenced significantly by unmeasured factors between the trees from which syconia were sampled (Wald = 9·09, $P = 0·005$). There was no significant effect of

Fig. 1. Survival plots showing (a) the mortality rates of female *Pleistodontes imperialis* given control (dry) or moist treatments (overall mean longevity (± standard error) = 34·32 ± 0·29 h, control treatment = 25·66 ± 0·25 h, moisture = 45·48 ± 0·37 h), and (b) the mortality rates of female *P. imperialis* from the five emergence cohorts.

Fig. 2. The emergence patterns from syconia exposed to control (dry) or moist treatments. Each cohort is relative to the first female pollinator wasps to have emerged. The percentages shown are for each of the two treatments.
logistic regression.

raw data. Solid points are calculated probabilities from a binary

length, from those syconia given 24 h for emergence. Open circles are

trapped in their galls in the natal syconium as a function of ovule

increases the longevity of female

There are three main findings from our experiments. (1) Moisture

Discussion

emergence (Fig. 3). The probability of female Pleistodontes imperialis remaining

trapped in their galls in the natal syconium as a function of ovule length, from those syconia given 24 h for emergence. Open circles are raw data. Solid points are calculated probabilities from a binary logistic regression.

the 14 syconia used in the study (Wald = 4·52, \( P = 0.98 \)), so this factor was removed for model simplification prior to refitting.

EXPERIMENT 3: EARLY EMERGENCE FROM THE SYCONIUM

Data from experiment 1 suggested that approximately half of wasps in a syconium would emerge within the first 2 h. We found no evidence to suggest that ovule length affects the probability of a wasp exiting its gall within this time-period (β ± SE = 0.33 ± 0.21, Wald = 0.02, \( P = 0.88 \)). There was no significant variation in early emergence from syconia that could be attributed to random factors between the trees sampled (Wald = 1·99, \( P = 0.97 \)) or syconium size (β ± SE = −0.06 ± 0.47, Wald = 0.02, \( P = 0.90 \)).

Body size and emergence

Female pollinators still in their galls were significantly smaller than released females in the lumen or those that had emerged successfully from their syconium (\( F_{1,462} = 15·07, P = 0.001 \); Fig. 4). Importantly, there was no significant interaction between the two time-periods and the three categories of emergence, suggesting that small wasps failed to exit their galls throughout the duration of the release process (\( F_{1,460} = 0.46, P = 0.63 \); Fig. 4). This interaction was therefore removed for model simplification prior to recalculation. Larger syconia contained larger female pollinators (\( F_{1,462} = 19·40, P < 0.001 \)). Wasp size did not differ between the two time-periods of wasp emergence (\( F_{1,462} = 3·07, P = 0.08 \)).

Discussion

There are three main findings from our experiments. (1) Moisture increases the longevity of female P. imperialis from a mean of 25·66 (± 0·25) h to 45·48 (± 0·37) h, an increase of approximately 77%. (2) Female P. imperialis developing in outer ovules at the syconium wall are less likely to be released by males. Because trapped offspring do not contribute to their mother’s fitness, outer ovules have reduced value to ovipositing foundresses. (3) Females still in their galls are smaller than those released by males. Males may thus avoid galls containing small females.

Our data suggest that female P. imperialis may live longer in the tropical rainy season compared with the dry season. When humidity is low, a wasp will clearly have less time in which to disperse and find a tree with receptive syconia, and to choose and enter a receptive syconium. Importantly, reduced longevity may also reduce the time available to foundresses for oviposition, thus lowering the number of exploited ovules and changing the relative proportions of exploited ovules across the overall size distribution (Yu et al. 2004; Dunn et al. 2008). Specifically, dry season first foundresses may be unable to exploit as many inner ovules as those in the wet season because of reduced time for oviposition, leaving more high-value inner ovules for subsequent foundresses. Therefore, we predict seasonal differences in foundress oviposition profiles within syconia. In the dry season, more foundresses may contribute to offspring in favoured inner ovules that develop into galls near to the lumen, and the overall number of ovules receiving eggs should be lower than in the rainy season. Conflict resolution in this mutualism may therefore be skewed more in the favour of the host, F. rubiginosa, in the dry season and towards the symbiont, P. imperialis, in the wet season.

These interpretations are clearly speculative, and assume that females arriving at a receptive syconium in the dry season will be already closer to death compared with those in the wet season. Additionally, seasonal variation in external moisture may correlate positively with moisture inside the receptive syconium. Although the transpiration rates inside receptive syconia under different degrees of external humidity have not been investigated previously, Patiño, Herre & Tyree (1996) examined the effects of temperature and syconium size on transpiration rates of mature syconia in several species of New World Ficus. Patiño et al. (1996) found that the cavities of larger syconia were kept relatively cool by transpiration: preventing transpiration led to wasp mortality due to an increase in temperature. If similar principles applied to receptive syconia, a reduction in water availability may increase wasp mortality further under more natural conditions than those used for our experiment, because reduced transpiration rates are likely to increase syconial cavity temperatures nearer to any wasp mortality threshold.

Our data show that the order of emergence had little effect on wasp longevity, although the last cohort of wasps to emerge had the highest mortality rates. This last cohort represents the few wasps (< 5%; Fig. 2) that, for unknown reasons, were likely to have spent a large proportion of their lives in the syconium prior to emergence.

Elsewhere, we have shown that P. imperialis larvae in outer ovules are more likely to be killed by parasitic wasps that oviposit from outside the syconium (Dunn et al. 2008). Here, we extend these results by showing that female offspring in outer ovules that remain unparasitized are none the less unlikely to be released by males. An ovule’s value to an ovipositing foundress is therefore lower the closer it is to the syconium wall, with potential knock-on effects on oviposition behaviour and mutualism stability (see Introduction; Yu et al. 2004; Dunn et al. 2008). Those wasps that choose to overexploit their hosts by ovipositing in outer ovules will incur greater costs than those that concentrate on inner ovules. The provision of ovules of variable profitability by the tree may thus reduce indirectly the costs of cooperation, a factor identified recently as being important in stabilizing mutualisms in general (Foster & Wenseleers 2006).

Our data provided no evidence to support our hypothesis that, in F. rubiginosa, wasps in inner ovules may be able to leave the syconium more quickly than those in outer ovules. There are few published data on detailed patterns of emergence in other pollinator species, although Greef & Ferguson (1999) showed similar rates of emergence in Platyscapa soraria Weibes (Hymenoptera: Agaonidae), the pollinator of the African fig F. ingens (Miquel 1867), to the probabilities of emergence in P. imperialis in this study. Similarly, Bean & Cook (2001) reported similar emergence patterns in P. froggatti Mayr (1909). However, the relative position within the syconia of trapped wasps was not reported in either paper. Anstett (2001) demonstrated that in Euspristina verticillata Waterston 1921 (Hymenoptera: Agonidae), the pollinator of F. microcarpa (Linn. f.), females emerging from inner galls left the syconium first. This may confer an additional advantage to E. verticillata foundresses selecting inner ovules for their offspring. F. microcarpa is taxonomically distant to F. rubiginosa. Although still in the subgenus Urostigma, F. microcarpa resides in the section Conoscecha, which diverged from the Malvaventhera approximately 40 million years ago (Rønsted et al. 2005). This gives additional credence to the suggestion that the mechanisms that contribute to the overall factors that maintain the fig-pollinator mutualism are likely to vary across such a speciose genus as Ficus (Herre 1999).

Pollinator females left in their galls were smaller than those released by males (Fig. 4). Females that did not emerge from their galls after 24 h were also more likely to be located in the outer ovules (Fig. 3), suggesting a possible effect of ovule position on wasp size due to space constraints on outer ovules (Anstett 2001). However, there is no clear evidence in P. imperialis that female body size correlates with ovule length, and hence gall position within the syconium (Dunn et al. 2008). Moreover, female wasps still in their galls early in the male release process, in our syconia allowed only 2 h for release, were still smaller than released wasps. These patterns are consistent with males avoiding galls containing small females throughout the release process. It may be that this behaviour has resulted from selection on males to avoid expending effort on ovules containing small females, which are likely to be of reduced reproductive value compared with large females (Bonduriansky 2001). Outer ovules may be packed more tightly than inner ovules (Anstett 2001), which would also increase the energetic cost required by male wasps needed to release females, or males may be reluctant to search widely within their natal syconium for unknown reasons (Frank 1985).

Within the females that emerged successfully from their galls and syconium, we found no evidence to suggest that size conferred an early emergence advantage; size patterns of emergence were the same for both time-periods (Fig. 4). Because variance in the size of female offspring is not determined by gall position in the syconium (Dunn et al. 2008; see also Anstett 2001; Moore et al. 2004), benefits associated with size are unlikely to affect ovule profitability to P. imperialis foundresses, and hence mutualism stability via influencing variation in foundress oviposition behaviour.

CONCLUDING REMARKS AND SUGGESTIONS FOR FUTURE RESEARCH

Yu et al. (2004) highlighted the potential importance that variation in foundress longevity may have on the conflict between pollinating fig wasps and fig trees. Our data show that moisture availability affects the longevity of female P. imperialis to such an extent that ovule exploitation patterns could differ seasonally. This should be tested in F. rubiginosa and other Ficus species by comparing ovule exploitation patterns by wasps in syconia in which foundresses have oviposited in the dry or wet seasons. Additionally, we find that the reduced probability of female offspring being released by males from outer ovules is likely to increase the value of inner ovules to foundresses. These findings add to the strong
offspring survivorship benefits that accrue to *P. imperialis* foundresses that select inner ovules (Dunn et al. 2008) and are likely to contribute to the maintenance of the fig–fig wasp mutualism (Yu et al. 2004; Dunn et al. 2008). Our data give credence to the notion that the larger, more sessile partner in the mutualism, the tree, has control over its wasp symbiont. The provision of larval resources of varying profitability to ovipositing foundresses thus contributes indirectly to factors preventing ovule over-exploitation by wasps, by increasing the costs to those wasps that choose to unsustainably exploit their hosts by utilizing outer ovules.

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**References**


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