Secondary galling: a novel feeding strategy among 'non-pollinating' fig wasps from *Ficus curtipes*

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Abstract. 1. The interaction between pollinator fig wasps (Agaonidae) and their host fig trees (*Ficus*) is a striking example of an obligate plant–insect mutualism, but figs also support numerous 'parasites' of the mutualism. Female agaonids (foundresses) lay their eggs in shorter-styled flowers, whereas longer-styled flowers produce seeds. A few 'non-pollinating' fig wasps (NPFWs) can also enter figs to oviposit

2. Fig wasp oviposition site choice and larval biology in figs of an Asian monoecious species, *Ficus curtipes* Corner, were recorded where two NPFW species oviposit inside the figs, such as the agaonid.

3. *Eupristina* sp. agaonids chose flowers in proportion to their availability, rather than preferring to oviposit in shorter-styled flowers. *Diaziella yangi* van Noort & Rasplus and *Lipothymus* sp. (Pteromalidae) foundresses followed *Eupristina* sp. into receptive figs and laid their eggs entirely in flowers that already contained pollinator eggs. This indicates that both NPFWs are inquilines under the widely-used terminology in the fig wasp literature, because they utilise galls generated by the pollinators. However, their adult bodies and galls were larger than those of the pollinators, showing that they independently stimulate ovule growth. These species are better described as secondary gallers that modify galls previously generated by the pollinators and kill these primary gallers.

4. Use of the term 'inquiline' among NPFWs inadequately and often inappropriately describes their biology. No known NPFWs are inquilines in the strict sense that they do not harm their hosts. 'Primary gallers', 'secondary gallers', 'seed predators', and 'parasitoids' describe their biology more accurately.

Key words. Agaonidae, coexistence, *Ficus*, inquiline, oviposition, Pteromalidae

Introduction

Mutualisms, where individuals of different species interact to their mutual benefit, are subject to invasion by non-mutualists that take advantage of the resources or rewards that the partners in the mutualism provide and there is an evolutionary continuum between mutualists and parasites (Bronstein, 1994; Pellmyr *et al.*, 1996). The interaction between fig trees (*Ficus* species, Moraceae) and their pollinating fig wasps (Chalcidoidea, Agaonidae) is a striking example of an obligate plant–insect mutualism (Janzen, 1979; Weiblen, 2002).

The inflorescences of fig trees (figs, also known as syconia) are urn-shaped, enclosed structures lined by many tiny flowers that are normally pollinated only by agaonids. Host specificity is highly developed, with each species of pollinator typically associated with a particular species of fig tree and each species of fig tree pollinated by one or a small number of agaonid species.

About half of 800 described *Ficus* species have a monoecious breeding system, in which seeds and wasps develop within the same figs (Wiebes, 1979). Foundress female agaonids enter the figs through a narrow opening, the ostiole. Once inside they attempt to lay their eggs inside the numerous ovules that are available, which they reach by probing with their ovipositors along the length of the styles. At the same time they pollinate some of the flowers. The agaonid larvae feed on

galled ovules and therefore destroy a proportion of the flowers that might otherwise have produced seeds. The next generation of adult female agaonids that emerge from the galled ovules reflect the male reproductive success of the trees, because they can carry pollen to figs on other trees. Seed production reflects the tree's female reproductive success. Agaonid foundresses often lay their eggs in many of the ovules, but large numbers of seeds are also produced. The balance between the numbers of seeds and pollinator offspring may be achieved in several ways (Nefdt & Compton, 1996) and it seems unlikely that a single mechanism can explain the stability in all fig–pollinator mutualisms (Herre *et al.*, 2008; Dunn *et al.*, 2011). Early studies assumed that there were distinct 'short'- and 'long'-styled female flowers in the figs, with the former specialising in wasp production and the latter in seed production. This separation of flower types based on style lengths has been discounted among Old World fig tree species, and most agaonids have ovipositors that can reach all the ovules in their associated figs (Nefdt & Compton, 1996). It has nonetheless been found consistently that shorter-styled flowers are more likely to contain agaonid offspring and longer-styled flowers are more likely to contain seeds (Dunn *et al.*, 2008), with more of the longer-styled flowers utilised as more foundresses enter a fig (Compton *et al.*, 1994). One reason that shorter-styled flowers may be preferred by ovipositing agaonid foundresses is that their ovules are more centrally located and may be less prone to attack by non-pollinating fig wasps (NPFWs) (Al-Beidh *et al.*, 2012a).

There are numerous species of chalcidoid NPFWs that develop inside figs and exploit the mutualism (Compton & Hawkins, 1992; West *et al.*, 1996; Rasplus *et al.*, 1998; Segar & Cook, 2012). Most NPFWs have larvae that develop in the ovules, but some develop in galls located in the outer walls of the figs. NPFWs typically have long ovipositors that they use to penetrate the fig wall while standing on the outer surface of the figs. Almost always just a single adult agaonid or NPFW emerges from each ovule. NPFWs are typically characterised as being gallers, inquilines or parasitoids (Compton & van Noort, 1992; West & Herre, 1994), but the detailed biology of the vast majority of NPFW species is not known (Tzeng *et al.*, 2008). More recently, NPFWs that are specialist hyper-parasites and seed-feeding species have also been recorded (Pereira *et al.*, 2007; Compton *et al.*, 2009).

Agaonids are pro-ovigenic: the short-lived adult females emerge from their natal figs with all their eggs already mature, which allow them to lay all their eggs over a short period of time (Copland *et al.*, 1973; Nefdt & Compton, 1996; Kathuria *et al.*, 1999; Ghara & Borges, 2010). In contrast, some NPFWs have been reported to be synovigenic, with eggs that mature progressively during their longer adult life-spans (Compton, 1993; Ghara & Borges, 2010).

Several different lineages of NPFWs contain species with females that enter the figs to oviposit from the inside, in the same way as agaonid females. They share morphological adaptations for entering figs with agaonids, such as flattened heads, smooth bodies, and spiny legs (van Noort & Compton, 1996), and their behaviour makes it possible for them to potentially pollinate their host figs. Most are not pollinators, however, because their host fig trees depend on active pollen

collection, transport, and deposition by the agaonids to achieve effective pollination. Not all fig trees have agaonids that display active pollination and those without active pollinators produce large amounts of pollen that sticks to the surface of the fig wasps (Kjellberg *et al.*, 2001). Internally-ovipositing NPFWs associated with these fig tree species can be effective pollinators (Jousselin *et al.*, 2001; Zhang *et al.*, 2008). Internally-ovipositing fig wasps have been assumed to be independent gall-formers that compete with agaonids for oviposition sites, and this feeding strategy has been confirmed for those *Sycophaga* species (Sycophaginae) that enter figs to oviposit (Galil *et al.*, 1970). The egg maturation patterns of internally-ovipositing NPFWs have not been described.

Ficus curtipes Corner is a passively-pollinated fig tree species with two internally-ovipositing NPFWs that can pollinate its figs (Zhang *et al.*, 2008). Here we describe studies of the biology of these NPFWs and their relationship to the tree's agaonid pollinator. Specifically we asked: (i) Is egg maturation in these NPFWs pro-ovigenic, as in agaonids and do they contain the same number of eggs as the agaonid associated with *F. curtipes*? (ii) Are they of a similar body size and are their ovipositors of a similar length to those of the tree's agaonid pollinator and do they insert their ovipositors in a similar way? (iii) Do all three internally-ovipositing fig wasps prefer to oviposit in short-styled flowers? and (iv) What is the relationship between the NPFWs and the agaonid: are the NPFWs gallers, inquilines or parasitoids?

Materials and methods

Study site and species

The study was conducted at the Xishuangbanna Tropical Botanical Garden (101°15'E, 21°55'N, at about 555 m a.s.l.), located in SW China at the northern margin of tropical SE Asia.

Ficus curtipes belongs to the monoecious subgenus *Urostigma*, subsection *Conosycea*. This species is widely distributed in China, Malaysia, India, and Thailand. Growing up to 5–10 m, it occurs naturally in Xishuangbanna's tropical forests. Individual trees generally produced synchronous crops with asynchrony between trees in the timing of their crops. The figs are located in the leaf axils and reach about 15 mm in diameter at maturity. Each fig contains about 180 female and 140 male flowers (Gu *et al.*, 2012). An undescribed *Eupristina* species (Agaonidae), *Diaziella yangi* and *Lipothymus* sp. (two internally-ovipositing NPFW species belonging to Pteromalidae, subfamilies Sycoecinae and Otitesellinae, respectively) are associated with figs of *F. curtipes* at Xishuangbanna, together with at least 10 species of NPFWs that oviposit from the outside of the figs (Zhang *et al.*, 2008 & Y-Q. Peng, unpublished). All sycoecines have females that enter figs to oviposit, whereas the majority of otitesellinae species oviposit from the outside of figs. Unusually for *Eupristina*-pollinated figs, *F. curtipes* is pollinated passively (Kjellberg *et al.*, 2001), and the large amounts of pollen produced allow *D. yangi* and *Lipothymus* sp. foundresses to also pollinate the figs, although they are not independent of *Eupristina* sp. because their foundresses are unwilling to enter figs that

have not been entered by the agaonid and consequently they always develop in figs that also contain the typical pollinator (Zhang *et al*., 2008). Consequently, the two NPFWs are not able to replace the agaonid wasp and establish an independent mutualistic relationship with their host plants.

Reflecting their passive pollination, the stigmas of *F. curtipes* flowers are extremely long and do not form the flat platform (synstigma) typical of most figs (Galil, 1977). *Eupristina* sp. foundresses consistently insert their ovipositors at about one-quarter of the length of the stigmas, then down the styles (Zhang *et al*., 2009 and Fig. S1). Consequently, style length alone gives an underestimate of the length of the ovipositors needed to reach the ovules. Foundresses of all three species do not re-emerge from figs once they have entered through the ostiole.

Adult female body sizes, egg loads, and egg sizes

Freshly-emerged adult females were collected from > 20 D phase figs (also known as the male phase, when the next generation of wasps emerge from the figs) on one tree. The head lengths of 22 *Eupristina* sp., 30 *D. yangi*, and 26 *Lipothymus* sp. were measured to the nearest 0.01 mm using an eyepiece graticule mounted on a binocular microscope (Olympus SZX12–3141; Olympus, Tokyo, Japan). Their gasters were then opened to allow the number of eggs they contained to be counted and egg dimensions recorded. Ten eggs were measured from each of 10 females per species. The length (L) and the width (W) of each egg was measured and the volume (V) was calculated using the formula $V = 1/6\pi LW^2$ where L is length and W is width of the egg (Blackburn, 1991; Giron & Casas, 2003; Ghara & Borges, 2010). In total, 200 wasps per species were dried with silica gel and the dry weight per 20 wasps was measured to the nearest 0.0001 g using an electronic balance. Figs were also collected before the wasps had emerged to compare the sizes of galls containing adult female wasps. The lengths and widths of 30 galls containing *Eupristina* sp., 27 galls with *D. Yangi*, and 26 galls with *Lipothymus* sp. were measured.

Oviposition sites

B phase figs (also known as the female phase, when figs are entered and pollinated) were removed from the trees after *Eupristina* sp., *D. yangi* or *Lipothymus* sp. foundresses had been observed to enter the figs, and then split open to allow their oviposition behaviour to be observed. Afterwards, the ovules where eggs had been laid were dissected to find where the eggs were placed, and how many eggs were present.

Experimental introductions of internally-ovipositing fig wasps

Young (A phase) figs on one tree were enclosed within fine mesh nylon bags (200 × 200 mm) to prevent fig wasps from entering. When the figs became receptive (with more open ostioles that allowed the fig wasps to enter), we introduced females of the three internally ovipositing species into the figs. These wasps had been collected the same day from D phase figs on another tree. We performed the following introductions: (i) one *Eupristina* sp., (ii) one *D. yangi*, (iii) one *Lipothymus* sp., (iv) one *Eupristina* sp. plus one *D. yangi*, and (v) one *Eupristina* sp. plus one *Lipothymus* sp. Where two species were introduced, the *Eupristina* sp. was introduced first, and the NPFW was then introduced shortly after. The nylon bags were returned after the wasp(s) had entered the figs to prevent further fig wasp entry. After one day, the experimental figs were collected and stored in FAA solution. Sample sizes were 14 figs with one *Eupristina* sp., 12 figs with one *D. yangi*, 9 figs with one *Lipothymus* sp., 12 figs with one *Eupristina* sp. and one *D. yangi*, and 15 figs with one *Eupristina* sp. and one *Lipothymus* sp.

Each fig was divided into two halves through the ostiole, and female flowers from one half were selected to collect the following data: (i) style length, and combined pedicel plus ovule length (some ovules were sessile, with no visible pedicels) and (ii) the number of eggs in each ovule (the eggs were revealed by dissecting each ovule–no staining was required) We dissected between 45 and 100 flowers in 62 figs, with a total of 4060 flowers overall.

Data analyses

Gall size, body weights, body size, egg size, and egg load of three internally ovipositing wasps were compared by one-way analysis of variance (ANOVA) with *post hoc* multiple comparisons (LSD). Correlation coefficients between body size and egg load, between egg size and egg load, as well as between style lengths and pedicel lengths were obtained using Pearson's methods. The independent-samples *T*-test was used to compare the difference in style lengths between the flowers with zero, one and more than one egg(s), and also to compare differences in pedicle and ovary lengths.

All analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, IL, U.S.A.).

Results

Body and egg sizes of the three internally ovipositing wasps

Adult females of the two NPFWs were significantly larger than *Eupristina* females, based on their head sizes and body weights. They also emerged from larger galls (Table 1). Eggs in the recently emerged adults of all three species were mature, a pro-ovigenic oviposition pattern showing that the females are adapted for laying their eggs shortly after emergence, over a short period of time. *Eupristina* sp. had the largest egg load, with *D. yangi* females containing significantly fewer eggs and *Lipothymus* sp. females having far fewer eggs than either of the other two species (Table 1). *Eupristina* sp. eggs were yolky with a filamentous pedicel, and separated easily from the ovarioles. In *D. yangi* and *Lipothymus*, the eggs were firmly attached to long ovarioles (Fig. S2). They had long pedicels, with an ovoid ovum joined by a long, thin peduncle to a long,

Table 1. Body sizes, egg loads, and egg sizes of the three internally-ovipositing fig wasps associated with *Ficus curtipes*. Egg volumes are based on 100 eggs from 10 females per species. Wasp weights are collected from 200 wasps with groups of 20 wasps weighed together. Thirty galls with *Eupristina* sp., 27 galls with *D. Yangi*, and 26 galls with *Lipothymus* sp. were measured to obtain gall lengths and widths. ANOVA and LSD were used to compare between-pair differences between means.

Species	N (wasps)	Head lengths (mm) (mean ± SE)	Egg loads (mean ± SE)	Egg volumes ($mm^3 \times 10^{-4}$) (mean ± SE)	Wasp weights (g) (mean ± SE)	Gall sizes (mm) (mean ± SE)
Eupristina sp.	22	0.38 ± 0.005a	135.0 ± 2.6a	1.11 ± 0.040a	0.0100 ± 0.0003a	1.136 ± 0.049a
Diaziella yangi	30	0.43 ± 0.005b	121.3 ± 1.6b	1.33 ± 0.037b	0.0155 ± 0.0011b	1.287 ± 0.084b
Lipothymus sp.	26	0.42 ± 0.007b	89.3 ± 1.8c	1.16 ± 0.012c	0.0146 ± 0.0011b	1.264 ± 0.071b

Within columns, values with the same letters indicate non-significant variation between means; different letters represent significant difference between means ($P < 0.05$).

Table 2. Egg deposition patterns in figs where different combinations of internally-ovipositing fig wasps were introduced into figs of *Ficus curtipes* (one foundress of each species).

Treatments	Sample sizes (figs)	Female flowers (mean ± SE)	Flowers with 0 egg (mean ± SE)	Flowers with 1 egg (mean ± SE)	Flowers with 2 eggs (mean ± SE)	Flowers with 3 eggs (mean ± SE)
Eupristina sp.	13	69.15 ± 2.82	44.92 ± 2.64	24.23 ± 2.55	0	0
Diaziella yangi	12	73.83 ± 5.01	73.83 ± 5.01	0	0	0
Lipothymus sp.	9	73.78 ± 4.13	73.78 ± 4.13	0	0	0
Eupristina sp. + *Diaziella yangi*	12	70.00 ± 3.39	49.33 ± 3.16	5.00 ± 0.95	15.67 ± 2.64	0.50 ± 0.19
Eupristina sp. + *Lipothymus* sp.	16	67.50 ± 3.59	49.67 ± 2.82	11.00 ± 2.21	6.81 ± 1.51	0.63 ± 1.18

serpentine ovariole. Egg volume varied across species, with those of *Eupristina* sp. being the smallest.

Egg load and body size were positively correlated among individuals of *Eupristina* sp. ($r = 0.59$, $P < 0.01$) and *Lipothymus* sp. ($r = 0.65$, $P < 0.01$), but less strongly correlated in *D. yangi* ($r = 0.33$, $P = 0.07$). Within species, egg number and egg size were not correlated: in *Eupristina* sp. ($r = 0.44$, $P = 0.21$), in *D. yangi* ($r = 0.18$, $P = 0.63$) and in *Lipothymus* sp. ($r = -0.10$, $P = 0.79$), so there was no indication of a trade-off between egg number and egg size within species.

Oviposition sites

As with *Eupristina* sp., the two non-agaonids inserted their ovipositors at about one-quarter of the length of the stigmas, rather than from the tip of the stigmas or their junction with the styles (Fig. S1).

Ovules inside figs where a single *Eupristina* sp. foundress had been introduced contained either one egg or no eggs (Table 2). *Diaziella yangi* and *Lipothymus* sp. did not lay any eggs in those figs where they were the only species to be introduced. They did lay eggs if they were introduced together with a *Eupristina* sp. female, but only into ovules where pollinator eggs were already present, resulting in some ovules containing two (or rarely three) eggs (Table 2). In figs entered by both *Eupristina* sp. and *D. yangi*, 74% of the ovules with eggs contained two to three eggs, whereas in figs where *Eupristina* sp. and *Lipothymus* sp. were present, only 48% contained two to three eggs. Overall, 3 eggs were recorded

in 10 ovules when *Lipothymus* was introduced and 3 ovules where *D. yangi* was introduced. Both NPFWs placed their eggs adjacent to those of *Eupristina* sp. (Fig. 1).

The figs where foundresses of two species were introduced together contained a smaller total number of eggs than figs where only a single *Eupristina* sp. was introduced (Table 2). The single *Eupristina* sp. foundresses only laid eggs in 29.5% of the available female flowers when sharing a fig with *D. yangi*, and 25.7% when sharing with *Lipothymus* sp. This compares with 35.0% occupancy in figs where a *Eupristina* sp. foundress was introduced alone and suggests that interference between the ovipositing females reduced oviposition success of *Eupristina* sp. by 5.5% and 9.3%, respectively.
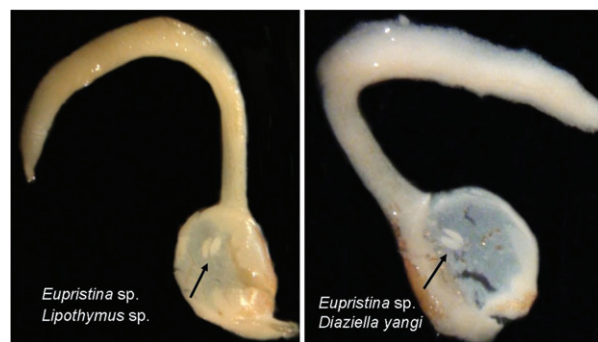

Fig. 1. Egg placements inside ovules, where pairs of eggs were present.

Style and ovipositor lengths

The styles of female flowers in receptive *F. curtipes* figs (excluding the stigmas) ranged in length from 0.40 to 1.42 mm (mean + SE = 0.895 ± 0.126 mm, $n = 4060$ styles from 62 figs; Fig. 2). The ovipositor of *Eupristina* sp. had a mean length of 1.81 ± 0.018 ($n = 22$) with a range of $1.65 - 2.00$ mm, and was longer than all the styles. The ovipositors of *D. yangi* and *Lipothymus* sp. were shorter than those of the pollinator [mean $\pm$ SE = 1.13 mm $\pm$ 0.0063, range = 1.06 – 1.18 mm ($n = 29$), and mean $\pm$ SE = 1.10 mm $\pm$ 0.0110, range = 0.97 – 1.19 mm ($n = 26$), respectively]. Because of the location where the ovipositors are inserted, style length underestimates the distance required to reach the ovules, where the eggs are laid. The stigmas of *F. curtipes* were 1.32 ± 0.01 mm long (mean $\pm$ SE, $n = 100$ stigmas from 10 figs) suggesting that an additional 0.33 mm had to be traversed by the ovipositors. A *Eupristina* sp. female with an ovipositor of average length should therefore be able to lay eggs in all female flowers, if it can fully insert its ovipositor. In contrast, *D. yangi* females could reach 38.2% of the ovules and *Lipothymus* sp. could reach only 32.0% of the ovules, again assuming that the full lengths of the ovipositors could be inserted down the styles.

Oviposition site choice

The lone females of all three species laid considerably fewer eggs in the figs than their egg loads suggest was possible. Flowers that contained eggs had similar mean style lengths to those that did not ($t = -1.64$, $P = 0.1$), showing that the *Eupristina* sp. foundresses did not prefer to lay their eggs in shorter-styled flowers. The distribution of flowers

containing two to three eggs was also independent of style length in the figs with *Eupristina* sp. and *Lipothymus* sp. ($t = -0.29$, $P = 0.78$), and in the figs with *Eupristina* sp. and *D. yangi* ($t = 1.08$, $P = 0.28$), showing that, in spite of their shorter ovipositors, neither NPFW displayed a preference for shorter-styled flowers (Fig. 3). These results suggest that all three internally ovipositing wasps laid eggs in the female flowers without being influenced by the lengths of their styles.

The combined lengths of pedicels and ovules were measured 24 h after the wasps had been allowed entry into the figs in order to record the locations of the ovules relative to the periphery of the figs. There was a significant negative correlation between style lengths and pedicel plus ovule lengths ($r = -0.276$, $P < 0.001$). Post-oviposition floral changes were already evident. In figs where a single *Eupristina* sp. foundress had been allowed entry, the flowers that contained eggs had pedicels that were beginning to elongate and as a result their ovules were becoming more centrally located than the ovules that did not contain eggs (Fig. 4). However, the mean length of pedicels and ovules had no significant difference in the figs with *Eupristina* sp. ($t = -1.22$, $P = 0.22$), with *Eupristina* sp. and *D. yangi* ($t = -1.45$, $P = 0.15$), and with *Eupristina* sp. and *Lipothymus* sp. ($t = -0.93$, $P = 0.35$).

Discussion

The internally-ovipositing NPFWs associated with *F. curtipes* are capable of carrying pollen into its figs, but the tree still requires the services of the typical agaonid pollinator (*Eupristina* sp.) because the NPFWs are reluctant to enter figs if the agaonid has not already entered and if they do enter agaonid-free figs they fail to reproduce (Zhang *et al.*, 2008).
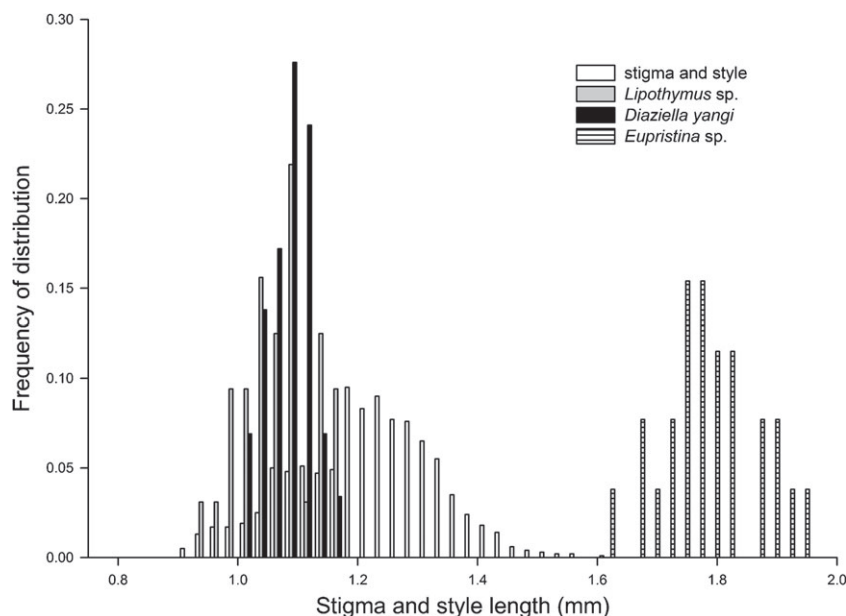

Fig. 2. Matching between the style and ovipositor lengths of three internally ovipositing fig wasps.
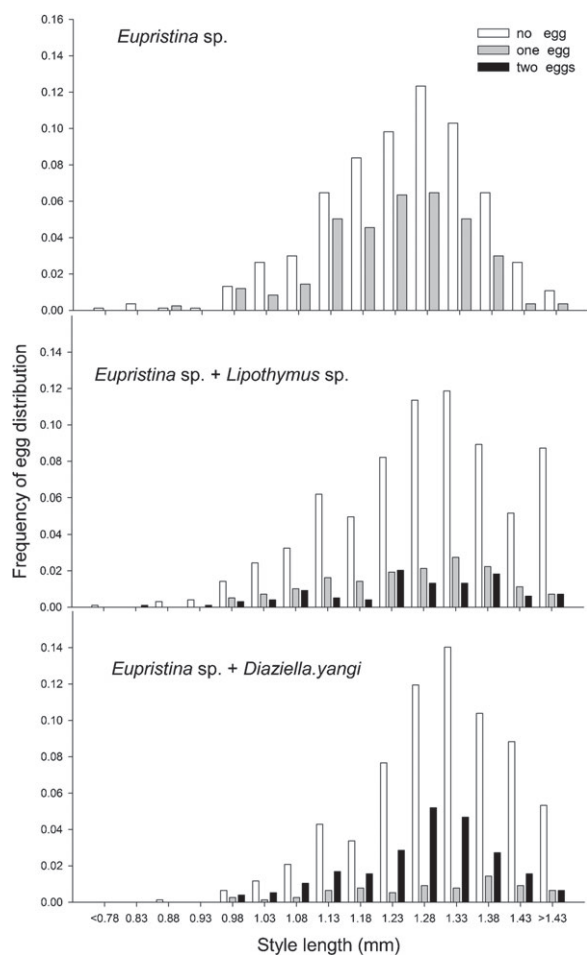
Fig. 3. Style lengths of flowers occupied by three internally ovipositing fig wasps.

Fig. 4. Pedicel plus ovary lengths of flowers occupied by three internally ovipositing fig wasps.

Our results clarify the reasons that the NPFWs depend on the agaonid. Females of both *D. yangi* and *Lipothymus* sp. targeted ovules that already contained *Eupristina* eggs to lay their own eggs: the proportion of flowers containing two eggs is far higher than if their females were simply ovipositing at random, irrespective of whether they contained an agaonid egg or not. We cannot exclude the possibility that the NPFWs also laid some additional eggs in ovules that lacked agaonid eggs, but this seems unlikely given that no eggs are laid when females are introduced into agaonid-free figs (Zhang *et al.*, 2008).

Their oviposition behaviour suggests that both internally-ovipositing NPFWs utilise ovules previously galled by *Eupristina* sp., rather than being independent gallers of the ovules. However, these species do not appear to be inquilines as typically understood in the fig wasp literature, because they do not depend solely on resources provided by the original galler. The galls where they develop eventually reach a larger size than those that contain *Eupristina* sp., and the adults are larger than the pollinators. This suggests that galls containing the NPFWs provide more resources than those galls containing
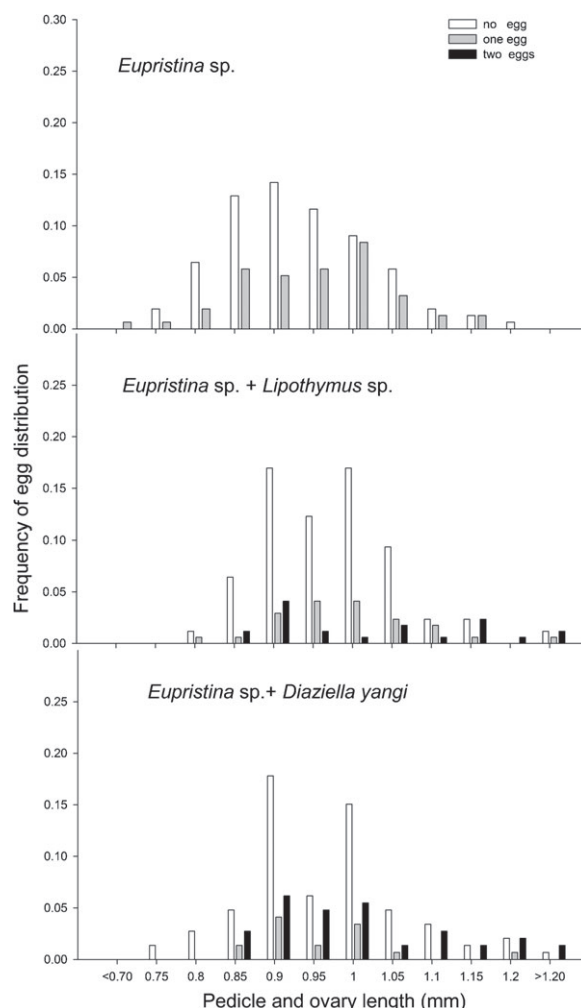
the agaonid alone, and that the NPFW foundresses (or their larvae) are able to manipulate their host plant to achieve this. They can be regarded as 'secondary gallers' that modify previously galled ovules, a life history not previously recognised among fig wasps. In oak galls, insects with a similar biology are referred to as 'lethal inquines' (Stone *et al.*, 2002).

No fig wasps that utilise galled ovules fit the strict definition of an inquilines: 'An animal that characteristically lives commensally in the nest, burrow, or dwelling place of an animal of another species' (http://www.thefreedictionary.com/inquiline) because although 'inquiline' fig wasps utilise ovules that have been galled by another species, they always kill the original occupant and therefore they are not commensal. The same extended use of the term inquiline is used to describe some of the cynipid species associated with gall wasps on oaks that also regularly kill the original gall causers (Askew, 1961).

Many, perhaps most 'inquiline' NPFWs can more accurately be described as parasitoids that also consume some plant material, and this is how they are routinely described in other plant–insect systems (as for example with *Eurytoma gigantea*

and the intensely studied *Eurosta* gall fly system on *Solidago, see* Weis *et al*., 1985). Whether other NPFWs species loosely described as inquilines are also secondary gallers remains to be seen, but the ability to increase the growth of host gall tissues is widespread amongst the 'inquilines' associated with galls of Cynipidae on oaks and other plants (Askew, 1961; Laszlo & Tothmeresz, 2006). Rather than using the catch-all term of 'inquilines', the biology of ovule and seed-feeding NPFW can be more precisely summarised using the terminology 'primary gallers', 'secondary gallers', 'seed predators', and 'parasitoids'. Some parasitoid NPFWs consume plant tissue (Peng *et al*., 2005, 2010; Pereira *et al*., 2007; Wang *et al*., 2010), and secondary gallers can destroy primary gallers (Zhang *et al*., 2008).

All sycoecines have previously been assumed to be independent gallers of fig ovules. Their independence from agaonids does not appear to have been confirmed experimentally, but African species are often reared from figs where no agaonids are present, which suggests this is the case (S. van Noort, pers. comm.). They nonetheless can have a negative impact on pollinator reproduction in shared figs (Al-beidh *et al*., 2012b). All otitesellines have also been assumed to be independent gallers, and many certainly are (for example Compton, 1993).

Adult females of *D. yangi* and *Lipothymus* sp. display convergence in external appearance with *Eupristina* sp. females as a result of their shared need to penetrate the ostioles of their host figs (van Noort & Compton, 1996). We found that they share additional features associated with internal oviposition. Egg maturation of *D. yangi* and *Lipothymus* sp. is pro-ovigenic, as in agaonid fig wasps. This adaptation allows the females to lay large numbers of eggs in a short time, a necessity for these internally-ovipositing species because they must lay all their eggs in one fig, and there is only a brief period after pollinator entry before the styles start to wither and become unsuitable. Some externally-ovipositing gall formers are also pro-ovigenic (Ghara & Borges, 2010).

All three species insert their ovipositors at a point about one-quarter of the length along the elongate stigmas. Sense organs present at the tips of NPFW ovipositors (Ghara *et al*., 2011) can facilitate the location of styles where the agaonid has previously oviposited, and the shared insertion point for the ovipositors suggests that the NPFWs follow the path cut by the agaonid's ovipositor, as seen in some other NPFWs that oviposit from outside the figs (Compton *et al*., 2009). Eggs of the three species are also deposited in the same place within the ovules, but although the NPFW eggs are slightly larger they are not readily distinguishable visually. Larger eggs may allow the NPFW larvae to emerge more quickly, and their shared location will facilitate killing of pollinator larvae.

In spite of their smaller body weights, *Eupristina* sp. females have significantly longer ovipositors than those of the NPFWs and also larger egg loads. Most agaonids preferentially deposit their eggs in shorter-styled flowers, but *Eupristina* sp. is an exception, because flowers with different style lengths were equally likely to have its eggs laid in them. The point of ovipositor insertion into the styles is also unusual in this species (Zhang *et al*., 2009) and may make detection of shorter-styled flowers more difficult. The preference for shorter-styled flowers shown by other agaonids may be linked to the these flowers having ovules that are more centrally located, and less likely to be attacked by externally-ovipositing fig wasps (Compton & Nefdt, 1990; Dunn *et al*., 2008; Al-beidh *et al*., 2012a), although there are alternative explanations (West & Herre, 1994; Anstett, 2001; Yu *et al*., 2004). Each ovule is attached to the fig wall by a pedicel of varying length. Style lengths of fig flowers are strongly negatively correlated with pedicel lengths (Jousselin *et al*., 2004) and the flowers with the longest styles are sessile, with ovules located next to the fig wall. In dioecious figs, where there is much less variation in style lengths than in monoecious figs such as *F. curtipes*, the pedicels of flowers that have been galled elongate rapidly to position their ovules as centrally as space will allow, in positions where attacks by externally-ovipositing fig wasps are less frequent (Yu & Compton, 2012). Just 24 h after entry by a *Eupristina* foundress there were already indications that the pedicels of those flowers that contained eggs were elongating and moving their ovules towards the centre of the figs, although the difference was not statistically significant.

The number of eggs laid by single foundresses of all three species was considerably less than the average egg loads of their respective species. The number of flowers containing one or more eggs fell when a NPFW foundress was introduced at the same time as a *Eupristina* sp., reflecting interference between the females as they moved in the small space available in the lumens of the figs. Inserting the NPFWs into the figs immediately after the agaonid female will have limited the number of ovules available to them, because they had to wait for the agaonid to oviposit before they themselves could do so.

The biology of most of the several thousand species of NPFWs can only be assumed by extrapolation from the small number of detailed studies of related species. The unexpected 'secondary galling' behaviour exhibited by *D. yangi* and *Lipothymus* sp. is difficult to detect and may turn out to also be present among NPFWs that oviposit from outside the figs.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12030

Figure S1. The site of insertion by ovipositors of three internally ovipositing fig wasps.

Figure S2. The pro-ovigenic eggs of *Diaziella yangi* and *Lipothymus* sp.

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