

Study on mating ecology and sex ratio of three internally ovipositing fig wasps of *Ficus curtipes*

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Abstract

Studies on mating ecology and sex allocation in fig-parasitizing wasps ovipositing from outside the fig have given valuable insights into known factors that are responsible for the theory of sex ratio. Similarly, internally ovipositing fig-parasitizing wasps and fig-pollinating wasps provide interesting models for comparative analysis. In addition to the fig-pollinating wasp *Eupristina* sp., we found that *Ficus curtipes* hosts two species of internally ovipositing fig-parasitizing wasps: *D. yangi* and *Lipothymus* sp. *Eupristina* sp. males showed less aggression. *Eupristina* sp. has wingless males that mate only within the natal patch, providing excellent examples of full local-mate competition. *D. yangi* males showed high levels of aggression and lethal combat. *D. yangi* has winged males but mate mostly within the natal patch. Only a few matings occur after male dispersal. Its sex ratio was lower than the prediction of partial local mate competition theory. Wingless male *Lipothymus* sp., which mate partly after dispersal, did not present fatal fight. Therefore, the mating behaviour of *D. yangi* and *Lipothymus* sp. did not follow predicted patterns, based on wing morph. The mating pattern of *D. yangi* and *Lipothymus* sp. should follow the partial local mate competition theory. Furthermore, there was not a significant correlation between the proportion of males and the proportion of fruit parasitized in both winged *D. yangi* males and wingless *Lipothymus* sp. males.

Keywords: fig wasps, sex ratio, local mate competition, wing morphology

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Introduction

Hamilton (1967) first demonstrated the case in which a single foundress colonises a patch, where her progeny then develops and mates amongst themselves before dispersing,

that a female biased sex ratio is favoured. This has been termed local mate competition (LMC). LMC theory has been tested in a number of studies (Frank, 1985; Herre, 1985, 1987; Herre *et al.*, 1997; Molbo *et al.*, 2003). These studies have provided strong support for the main predictions of LMC theory. All fig-pollinating wasps have wingless males and most mate only within the natal patch. The biology of the fig-pollinating wasps conforms to the classical example of LMC.

However, fig-parasitizing wasps are much more morphologically different and include species with wingless males, species with winged males and species with both types of male (Cook *et al.*, 1997). A fraction of matings in fig-parasitizing wasps occurs after dispersal from the local

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patch, and this mixture of local and non-local mating has been termed partial LMC (Hardy, 1994). Since wingless males mate only in the natal patch and winged males commonly mate only after dispersal from the natal patch, it is difficult to study fig-parasitizing wasps because the degree of pre- and post-dispersal mating is not easy to estimate.

West & Herre (1998) proposed that the proportion of figs parasitized in a crop is a good measure of the average foundress number present in a fig. They found that as the proportion of fruit parasitized increases, progeny sex ratio tends to rise in both wingless male and winged male species in Panama. However, Fellowes *et al.* (1999) found a positive correlation across wingless male species but not across winged male species.

Most fig-parasitizing wasps oviposit through the wall of the fig from the outside. However, some species enter figs and oviposit in the female flowers, just as the fig-pollinating wasps do (Galil & Eisikowitch, 1969; van Noort & Compton, 1996). Indeed, sometimes they can affect passive pollination (Jousselin *et al.*, 2001; Zhang *et al.*, 2008). But these internally ovipositing fig wasps do not belong to the Agaonid lineage. The mating ecology of these wasps and their sex allocation are poorly known. So, these internally ovipositing fig-parasitizing wasps and fig-pollinating wasps should be very interesting models for comparative analysis (van Noort & Compton, 1996).

Most foundresses of the typical pollinating fig wasp are trapped inside the fruit and are constrained to lay all their eggs in one fig (Moore *et al.*, 2003). Males eclose first from their galls into the cavity inside the fig, males search for galls containing receptive females, chew mating holes and mate with females. Thus, wingless males show high levels of aggression and lethal aggression for the sake of more mating opportunities (Murray, 1987; Cook *et al.*, 1997). Hamilton proposed that the variation in the level of fighting across fig wasp species reflects different levels of the relatedness; specifically, the small number of foundresses characteristic of most species of the pollinating wasps implies that rival males are likely to be brothers and are less likely to fight because of their high degree of relatedness (Hamilton, 1979). In a comparative study, West *et al.* (2001) showed that the level of fighting between males shows no correlation with the estimated relatedness of interacting males but is negatively correlated with future mating opportunities. However, the presence of internally ovipositing non-agaonid fig wasps, as well as the discovery of male dispersal and fighting, suggests that this issue should be revisited.

Ficus curtipes Corner is pollinated by an undescribed *Eupristina* sp. in Xishuangbanna tropical area. *Diaziella yangi* and *Lipothymus* sp. were also observed to enter the figs and oviposit in the female flowers. *Eupristina* sp. have wingless males and mate only within the natal patch (within a fig); *D. yangi* have winged males. Most matings occur in the local patch, a fraction of matings occur after dispersal from the local patch. *Lipothymus* sp. have wingless males and mate in both inside and outside fig. *Diaziella yangi* and *Lipothymus* sp. have foundresses that enter the figs and oviposit in the female flowers at the same time as the agaonid wasp, *Eupristina* sp., and emerge from figs at the same time as the agaonid wasp, *Eupristina* sp. Hence, the natural history of *D. yangi* and *Lipothymus* sp. is very similar to that of the agaonid wasp, *Eupristina* sp. West & Herre (1998) proposed that the proportion of figs parasitized in a crop is a good measure of the average foundress number of non-agaonid

fig wasps present in a fig. Also, they showed that as the proportion of fig parasitized increases, progeny sex ratio tends to rise. *Diaziella yangi* and *Lipothymus* sp. do not belong to the Agaonid lineage. The relationship between progeny sex ratio and the prevalence in *D. yangi* and *Lipothymus* sp. is unknown. In this study, we compared the mating ecology and sex allocation of these internally ovipositing fig-parasitizing wasps and the fig-pollinating wasps. Specifically, we tested the following predictions: (i) the mating ecology of *D. yangi* and *Lipothymus* sp. should be similar to that of *Eupristina* sp.; and (ii) Progeny sex ratio should increase with the prevalence of *D. yangi* and *Lipothymus* sp. Our results show that the sex allocation of *D. yangi* and *Lipothymus* sp. from *F. curtipes* differs considerably from that of previously studied species.

Materials and methods

Study site

The study was carried out in the Xishuangbanna Tropical Botanical Garden (101°15'E, 21°55'N), located in southwest China and the northern margin of tropical Southeast Asia.

Study species

Ficus curtipes belongs to subsection *Conoscycea*. This species is widely distributed in Asia (China, India, Malaysia and Thailand). Trees grow up to 5–10 m tall and are epiphytic when young. At Xishuangbanna, this species occurs naturally in tropical forest and is also cultivated commonly in cities and villages as an ornamental tree. *Ficus curtipes* produces figs in synchronous crops with different trees flowering at different times throughout the year. At the female-flower phase, when the female flowers reach maturity and the stigmas become receptive, the figs are 11.76 ± 0.42 mm (SD, $n=30$) in diameter. The figs become dark red to purplish red when mature and are 15.52 ± 0.75 mm (SD, $n=47$) in diameter. The pollinating wasp (an undescribed species *Eupristina* sp.) and two internally ovipositing fig-parasitizing wasps (*D. yangi* and *Lipothymus* sp.) were studied. *Eupristina* sp. males are wingless; *D. yangi* males are winged; *Lipothymus* sp. males are wingless.

Observations of mating behaviour

We randomly selected figs containing wasps that were about to emerge; figs were bisected lengthways, from the stalk to the ostiole, to reveal the lumen. A piece of dark-red perspex was taped to the open side, covering the lumen and forming an artificial wall. The perspex had a hole drilled through it which had the same diameter as the ostiole. Then the figs were placed under a dissecting microscope and eclosion, fight and mating behaviours of each type of fig wasp were recorded; 150 figs containing fig wasps were observed.

Sex allocation of three species of wasps

Just before wasp emergence on each of five trees, 134 figs were collected randomly. Every fig was placed individually in a fine-mesh bag (200 × 200 mm), and the fig wasps allowed to emerge. All fig wasps, including the wingless

Table 1. Biological character of three fig wasps.

Fig wasps	Oviposition pattern	Male morph	Mating behaviour
<i>Eupristina</i> sp.	Ovipositing inside the fig	Wingless	Mate in fig cavity
<i>Diaziella yangi</i>	Ovipositing inside the fig	Winged	Mate mostly inside, only a few outside fig
<i>Lipothymus</i> sp.	Ovipositing inside the fig	Wingless	Mate both inside and outside fig

Table 2. The average number of three internally ovipositing fig wasps offspring.

Fig wasps	No. of fig crops sampled	No. of figs sampled	The average number of offspring (mean \pm SE)
<i>Eupristina</i> sp.	3	113	55.48 \pm 4.38
<i>Diaziella yangi</i>	3	49	63.37 \pm 3.57
<i>Lipothymus</i> sp.	4	15	41.13 \pm 6.91

males, were carefully collected and preserved in 70% ethanol. Each wasp was identified to species and counted.

Statistical analyses

One-way ANOVA was used to test the statistical differences in the mean sex ration of each type of fig wasps. A general linear regression model was used to analyze the relationship between the sex ratio and the proportion of fruit parasitized of *D. yangi* and *Lipothymus* sp. All analyses were performed using SPSS (13.0).

Results

Mating behaviour

The male morph and mating behaviour of three internally ovipositing fig wasps were shown in table 1. When fig wasps are adults and the fig softens and enlarges, the flowers become loose, allowing fig wasps to move within the lumen. *Eupristina* sp., *D. yangi* and *Lipothymus* sp. males all emerged from their galls and searched for females to mate. However, their mating behaviour varied. For *Eupristina* sp., males usually ignored one another and showed little aggression. Males cut a hole in the galls containing females and mated by introducing their telescopic mating organ. After mating, females enlarged the mating hole and emerged freely. The females may wait for males to cut an exit hole through the ostiole or wait for it to loosen and then emerge rapidly. For *D. yangi*, males showed high levels of aggression and lethal combat. On emergence into the lumen, males typically moved around until they found the galls where females were about to emerge. As the female emerged, she was grabbed and mated. Some males sometimes succeeded in mating with more than one female. Males interacted frequently with one another and often the bigger males killed smaller males. After mating, females immediately left the figs. For *Lipothymus* sp., after emerging males searched for the galls where females were ready to emerge and cut holes in the gall. As the female emerged through the hole, she was grabbed and

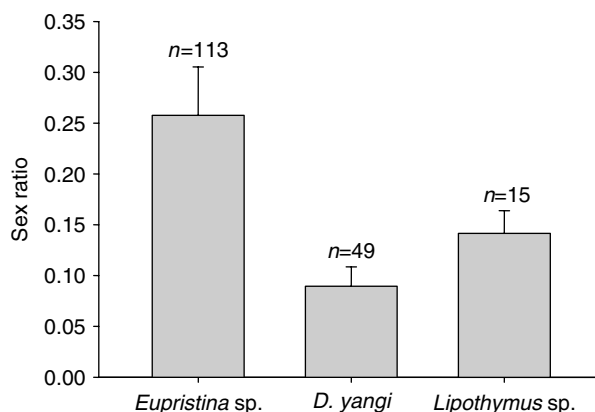


Fig. 1. Sex ratio of three internally ovipositing fig wasps.

mated. A fraction of matings of *D. yangi* and *Lipothymus* sp. occur after dispersal from the local patch; specifically, after *D. yangi* and *Lipothymus* sp. males leave the fig, they mate with females outside the fig.

Sex allocation of three species of fig wasps

By collecting natural figs, the average number of male and female offspring of three internally ovipositing non-agaonid fig wasps was obtained (*Eupristina* sp.: 55.48 \pm 4.38 (SE, $n = 113$); *D. yangi*: 63.37 \pm 3.57 (SE, $n = 49$); *Lipothymus* sp.: 41.13 \pm 6.91 (SE, $n = 15$)) (table 2). In nature, the sex ratio of *Eupristina* sp. (0.26 \pm 0.05, $n = 113$) was higher than male winged *D. yangi* (LSD, $P < 0.05$), but there is no significant difference with the sex ratio of male wingless *Lipothymus* sp. (LSD, $P > 0.05$). Also, there is no significant difference between the sex ratio of male winged *D. yangi* (0.09 \pm 0.02 (SE, $n = 49$)) and the sex ratio of male wingless *Lipothymus* sp. (0.14 \pm 0.09 (SE, $n = 15$)) (LSD, $P > 0.05$) (fig. 1). However, there were no significant correlations between the proportion of males and the proportion of fruit parasitized for *Lipothymus* sp. ($F_{1,3} = 0.07$, $P = 0.82$) and *D. yangi* ($F_{1,3} = 0.06$, $P = 0.83$) (fig. 2).

Discussion

Most Agaonid female wasps (called foundresses) are trapped inside the fig and are constrained to lay all their eggs in one fig. Each egg is laid in an individual flower, each forming a distinct gall inside the inflorescence. *Diaziella yangi* (Pteromalidae; Sycoecinae) and *Lipothymus* sp. (Pteromalidae; Otitesellinae) do not belong to agaonid lineages. These non-agaonid fig wasps (*D. yangi* and *Lipothymus* sp.)

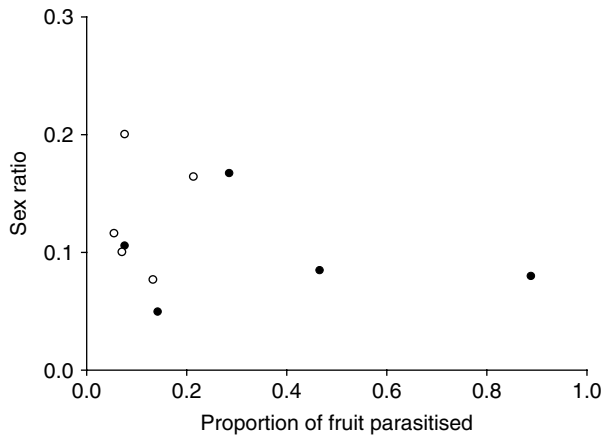


Fig. 2. The relationship between the sex ratio and the proportion of fruit parasitised.

enter figs and oviposit in the female flowers, just as the agaonid fig wasps do. In general, the behaviour of non-agaonid wasps has been extrapolated from the better studied pollinating fig wasps. However, the mating patterns of the non-agaonid female wasps *D. yangi* and *Lipothymus* sp. of *F. curtipes* are very different from that of pollinating species.

The fact that the winged male *D. yangi* and wingless male *Lipothymus* sp. can leave figs to mate is very important. Firstly, mating patterns are not as local as was previously thought. The extent of local mating determines the extent to which brothers compete locally for mates. This, in turn, is integral in causing females to bias the sex ratios of clutches towards females (Hamilton, 1967). In the case of *D. yangi* and *Lipothymus* sp., a female cannot anticipate the degree to which her male progeny will be involved in local mate competition since some of these progeny may disperse. Therefore, *D. yangi* and *Lipothymus* sp. fit to partial local mate competition (partial LMC) theory (Hardy, 1994). Partial LMC theory predicts that in winged non-agaonid wasps mating is also likely to occur away from the natal patch. In this case, the degree of LMC is reduced, and theory predicts a relatively less female biased sex ratio (West & Herre, 1998). Winged male *D. yangi* mate mostly within the natal patch (within a fig); only a few mates occur after dispersal. Wingless male *Lipothymus* sp. mate partly after dispersal. Greeff & Ferguson (1999) proposed that the mating opportunity may increase after dispersal from the natal patch. The mating behaviour of *D. yangi* and *Lipothymus* sp. did not follow predicted patterns which are based on wing morph. Therefore, it is insufficient to state that the mating behaviour and location are predicted only by the male of wing morph.

The mating activities of wingless parasitic fig wasp males are constrained to their natal figs. Thus, wingless males show high levels of aggression and lethal aggression for the sake of more mating opportunities (Murray, 1987; Cook *et al.*, 1997). Hamilton proposed that the small number of foundresses characteristic of most species of the pollinating wasps implies that rival males are likely to be brothers. The pollinating fig wasp males are less likely to

fight because of their high degree of relatedness. In comparison, the non-agaonid wasps can potentially oviposit in some figs, each laying only a few eggs before moving on. Consequently, the degree of relatedness is reduced, leading to lethal fights (Hamilton, 1972, 1979). *D. yangi* have foundresses that enter figs and oviposit in female flowers; the number of female *D. yangi* that enters figs was generally one. However, *D. yangi* males showed high levels of aggression and lethal combat. Vincent (1991) has shown that the relatedness of interacting males, male density and mating site are all associated with fighting. *D. yangi* sex ratio was lower than the prediction of partial local mate competition theory. The possible reason is that *D. yangi* foundresses possess different oviposition patterns and they have different population sizes. *D. yangi* have foundresses that enter the figs to oviposit, like agaonids, though *D. yangi* does not belong to the pollinating lineage Agaonidae. Although *D. yangi* males have wings, most matings occur in the local patch, while only a fraction of matings occur after dispersal from the local patch. So, the sex ratio of *D. yangi* is not consistent with the prediction. Thus, unlike non-agaonid wasps studied by Fellowes *et al.* (1999), their data showed that the non-agaonid wasp's sex ratio was close to 0.5. Although *Lipothymus* sp. has wingless males, some of males mated after dispersal from figs. This suggests that individual females weakly adjust the sex ratio of their progeny in response to variable mating competition. It is necessary that new parameters like population structure, the number of females developing in a fig and site of mating, should be proposed to study the sex ratio in using LMC theory.

Fellowes *et al.* (1999) showed a positive correlation between the progeny sex ratio and the proportion of fruit parasitized across wingless male species, but not across winged male species. They proposed that the foundress number is difficult to estimate directly for externally ovipositing non-agaonid wasps, and hence foundress females can not exactly estimate the degree of mate intensity which their progeny males will face. West & Herre (1998), using independent contrasts, found such a positive correlation in both wingless male and winged male species in Panama. In comparison, our result showed that there was not a significant correlation between the proportion of males and the proportion of fruit parasitized in both winged *D. yangi* males and wingless *Lipothymus* sp. males. The discrepancy between these studies could be due to either limitations of the data or because of a real biological difference. The study of the relationship between the internally ovipositing parasitic wasps and the number of foundresses is scarce. It is necessary that more direct observations and field studies be carried out. In this way, the adjustment effect of local mate competition or partial local mate competition to the fig-parasitizing wasps could be reflected objectively.

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