

# Body size in a pollinating fig wasp and implications for stability in a fig-pollinator mutualism

Cong Liu<sup>1,2</sup>, Da-Rong Yang<sup>1</sup> & Yan-Qiong Peng<sup>1\*</sup>

<sup>1</sup>Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, China, and <sup>2</sup>Graduate School of the Chinese Academy of Sciences, Beijing, China

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## Abstract

The fig–fig pollinator association is a classic case of an obligate mutualism. Fig-pollinating wasps often have to fly long distances from their natal syconia to a receptive syconium and then must enter the narrow ostiole of the syconium to reproduce. Large wasps are expected to have a greater chance of reaching a receptive syconium. In this study, we tested this hypothesis and then examined whether the ostiole selectively prevented larger pollinators from entering the syconial cavity. In Xishuangbanna, China, *Ceratosolen solmsi marchali* Mayr (Hymenoptera: Agaonidae) pollinates the dioecious syconia of *Ficus hispida* L. (Moraceae). The body size of newly emerged wasps and wasps arriving at receptive syconia were compared. Wasps arriving at receptive syconia were significantly larger than newly emerged wasps. We also compared the size of wasps trapped in the ostiole with those in the cavity. Wasps trapped in the ostiole were significantly larger than those in the syconial cavity. Thus, in the case of *F. hispida*, large wasps were more likely to reach receptive syconia, but the ostiole limited maximum fig wasp size. This indicates that the ostiole, as a selective filter to pollinators, stabilizes pollinator size. Hence, it helps to maintain stability in the fig–fig pollinator mutualism.

## Introduction

Fig trees [*Ficus* spp. (Moraceae)] have enclosed inflorescences. Enclosure is an effective protection of the flowers against non-specialist predators and harsh environmental conditions. Syconia (fig fruits; the almost completely enclosed fig inflorescences) are urn-shaped floral receptacles that can only be entered by the pollinator wasps through a pore, the ostiole, which is closed by bracts (Verkerke, 1989). Pollinating fig wasps can force their way through the ostiole to gain access to the flower, which they pollinate, and they simultaneously oviposit in some ovules (Bronstein et al., 1998; Wiebes, 1979). Thus, pollinating fig wasps are seed predator-pollinators and have coevolved with fig trees in this unique mutualism for at least 60 million years (Rønsted et al., 2005).

As the pollinator wasps born within a syconium will be the only pollen vectors of that syconium, they are an essen-

tial component of the male function of the tree (Gibernau et al., 1996; Herre, 1989). As adult female wasps live for just 1–3 days (Kjellberg et al., 1988; Dunn et al., 2008), they must rapidly locate a receptive syconium to reproduce. These tiny wasps can travel over 160 km in <48 h, by flying up into the air column and being carried by the wind until they detect the host-specific chemical cues of receptive syconia (Ahmed et al., 2009; Proffit et al., 2009). Only a few individuals out of thousands successfully reach receptive syconia (Anstett et al., 1997). A study in Panama reported that the mean size of wasps leaving syconia is smaller than the mean size of the foundress mothers. This pattern suggests that of the wasps that are born, the larger ones have a greater chance of reaching a receptive syconium (Herre, 1989). However, this prediction has not been examined explicitly.

Once the pollinator has located a receptive syconium, she needs to pass through the ostiole, which is not easy. Fig-pollinating wasps have evolved many adaptive characteristics for entering the ostiole, including a flattened head and thorax, teeth on the third segment of the antennae and tibia, and a unique mandibular appendage, a flap-like structure attached to the underside of the mandibles with

\*Correspondence: Yan-Qiong Peng, Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 88 Xuefu Road, Kunming 650223, China. E-mail: pengyq@xtbg.ac.cn

multiple rows of teeth pointing backward (van Noort & Compton, 1996; Weiblen, 2002). The shape of the ostiole is also correlated with the morphology of the wasp, and four ostiolar types with considerable differences in accessibility of the syconial cavity can be distinguished (Ramirez, 1974). Receptive syconia even delay the ostiole closure to facilitate encounter with the pollinator (Khadari et al., 1995). Nevertheless, many prospective pollinating fig wasps do not manage to pass through the ostiole. For example, about 48–65% of the pollinators may be trapped in the ostiole, meaning that only 35–52% of the pollinators reach the syconial cavity of *Ficus hispida* L. (Peng et al., 2005). Larger wasps are supposed to become more easily trapped in the ostiole, but these wasps are the ones that are expected to have a greater chance to reach a receptive syconium than smaller wasps. Therefore, the structures found in the syconia and those found in the bodies of their symbiotic agaonids are the result of mutual adaptations which favoured their symbiotic association.

In this study, we measured the body size of (1) emerging pollinators, (2) wasps arriving naturally at receptive syconia, (3) wasps that died when stuck in ostioles, and (4) those that reached the syconial cavity. Using these data, we ask whether larger wasps have a greater probability of reaching a receptive syconium, and whether larger wasps are more likely to be trapped in the ostiole.

## Materials and methods

### Study site and species

The study site was located in the Xishuangbanna tropical area (21°55'N, 101°15'E, at about 555 m a.s.l.), southwestern China. *Ficus hispida* is a dioecious, small to medium-sized free-standing tree, bearing syconia all year round on leafless branchlets hanging down from the trunk and bigger branches. The trees generally produced synchronous crops with asynchrony between trees. Sometimes, syconia were produced in asynchronous crops, but the overlap of phases of receptive syconia with wasps emerging from syconia on the same tree is limited. Thus, the pollinator, *Ceratosolen solmsi marchali*, usually has to fly to another fig tree to continue to reproduce.

In the natural community, a male syconium is oviposited by  $2.08 \pm 0.12$  (mean  $\pm$  SE;  $n = 182$ ) foundresses, and a female syconium by  $2.72 \pm 0.13$  ( $n = 246$ ) (Peng et al., 2005). The ostiole consists of  $39.15 \pm 0.43$  ( $n = 20$ ) bracts, with all bracts interlocking to form a helicoidal passage into the syconium cavity. Some foundresses were trapped in the ostiole when entering to syconia. About 7–9% of wasps trapped in the ostiole pointed outwards, and these wasps were mostly distributed in the lower ostiolar bracts; no wasp was found to escape successfully (C Liu, unpubl.).

Therefore, wasps of this species may try to re-emerge (Hu et al., 2009), but they cannot exit the syconium after oviposition.

### Collection of emerging wasps

From July to August 2009, three male trees located in Xishuangbanna Tropical Botanical Garden were selected, and five syconia at the wasp-emerging stage per tree were picked. Each syconium was stored in a nylon bag (20  $\times$  25 cm). When all fig wasps had freely emerged, they were killed quickly using ethyl acetate and 10 female pollinators per syconium were collected randomly. In total, 50 wasps were collected per tree.

### Collection of wasps arriving at receptive trees

From July to August 2009, four trees having plenty of receptive syconia (>100 syconia) were selected and if there were syconia in the wasp-emerging phase these were removed to ensure that the pollinators arrived from other trees. In this season, many pollinating fig wasps could be found flying around the syconia on receptive trees between 09:00 and 10:00 hours. During this period, we collected the flying wasps arriving at the experimental trees using nylon bags, and quickly killed them with ethyl acetate. Thirty newly arriving pollinators per tree were collected; in total 120 wasps were obtained from two male and two female trees.

### Collection of wasps trapped in the ostiole and in the cavity

Once the pollinators had entered the receptive syconia, the wings were clearly visible in the external bracts of the ostiole. Per tree, we marked about 30 syconia into which the wasps had entered and the next day the marked syconia were picked. Each syconium was opened to check whether the pollinators in the cavity had died. If all pollinators were dead, this suggested that the entering of pollinators had ended. Syconia with only dead wasps in the cavity were selected for analysis. We also collected 30 pollinators trapped in the ostiole and in the cavity from 20 to 30 syconia per tree. Pollinators trapped in the ostiole were collected by removing the bracts. We did not distinguish whether the wasps pointed inwards or outwards because those wasps trapped in the upper ostiole were difficult to judge head's direction. In total we collected 240 wasps from two male and two female trees.

### Measurement of wasp body size

We measured the length of nine characteristics representing wasp body size to the nearest 0.0025 mm using an eyepiece graticule mounted on a binocular stereoscope (Olympus SZX12–3141, Tokyo, Japan). Traits measured

were: distance between the eyes, head width, and length of head, thorax, pronotum, front femur, hind femur, abdomen, and ovipositor (Figure 1). The ovipositor was drawn from the abdomen and removed from its sheath for measurement.

#### Data analysis

Factor analysis was used to obtain correlation coefficients between the nine characteristics representing wasp body size. Principle component analysis then identified which characteristics were the strongest indicators of wasp body size (using SPSS, version 16.0, Chicago, IL, USA). With two selected characteristics, we employed a linear model (LM) with a priori contrasts to compare body size between four groups of wasps, using 'tree' as a random factor. All analyses were performed in R version 2.11.0 (R development Core Team, 2010).

## Results

#### Indicators of overall wasp body size

In the resulting correlation matrix, each of nine characteristics showed a significant correlation with most others (Table 1). Of the first two principal components (PC), PC1 related to head characteristics, including head length and width and the distance between two eyes, whereas PC2 related to femur characteristics, including the length of the front and hind femur. Head width and front femur length had the highest scores of the first and second components, respectively, and thus were selected to represent wasp body size (Table 2).

#### Are the larger wasps more likely to reach receptive syconia?

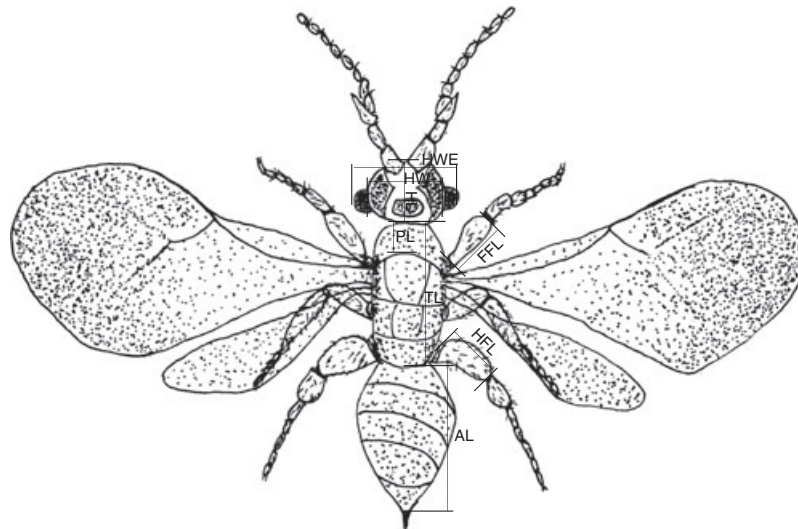
When comparing the body sizes of emerging wasps with those arriving at receptive syconia, the latter were significantly larger (LM: head width: slope = 0.013,  $t = 6.211$ ,  $P < 0.001$ ; front femur length: slope = 0.006,  $t = 4.61$ ,  $P < 0.001$ ), notwithstanding the obvious variation among trees (Figure 2). Therefore, large pollinators have a greater chance of reaching a receptive syconium.

#### Are larger wasps more likely to become trapped in the ostiole?

Wasps trapped in the ostiole were the largest on four selected trees (Figure 3). They were significantly larger than the wasps arriving at receptive syconia (LM: head width: slope = 0.010,  $t = 4.114$ ,  $P < 0.001$ ; front femur length: slope = 0.004,  $t = 2.667$ ,  $P < 0.01$ ) and those trapped in the syconial cavity (LM: head width: slope = 0.011,  $t = 5.278$ ,  $P < 0.001$ ; front femur length: slope = 0.006,  $t = 4.106$ ,  $P < 0.001$ ). Thus, larger wasps were easily trapped in the ostiole.

## Discussion

Body size is one of the most important life history traits of animals (Jervis et al., 2003; Bezemer et al., 2005). Head length, head area (length  $\times$  width), and mandible length have often been used as indicators for body size of fig wasps (Herre, 1989; Moore et al., 2004; Dunn et al., 2008). In this study, we compared nine characteristics related to wasp body size. The correlation between body size characteristics was positive in all cases and significant in most. We further compared the nine characteristics using princi-



**Figure 1** Characteristics representing *Ceratosolen solmsi marchali* body size. HWE, distance between eyes; HW, head width; HL, head length; PL, pronotum length; TL, thorax length; FFL, front femur length; HFL, hind femur length; AL, abdomen length.

**Table 1** Spearman correlation matrix about nine characteristics representing *Ceratosolen solmsi marchali* body size

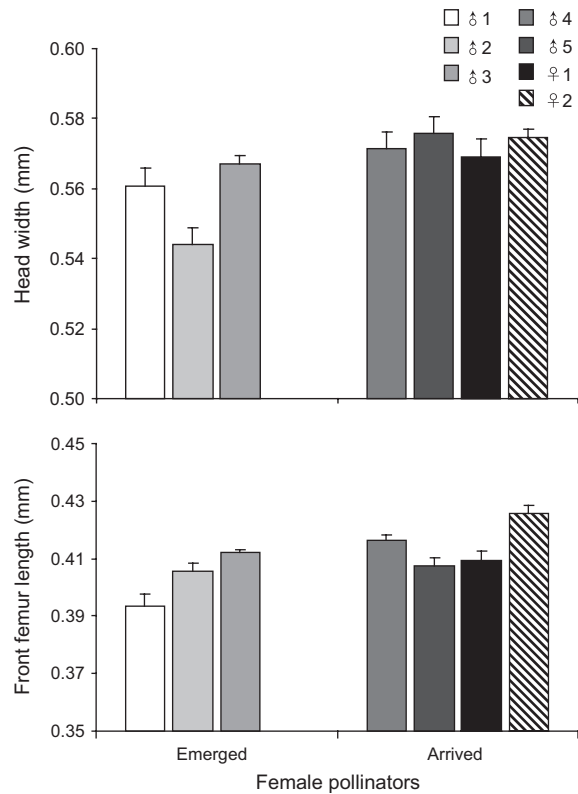
	Head length	Head width	Distance between eyes	Thorax length	Pronotum length	Front femur length	Hind femur length	Ovipositor length	Abdomen length
Head length	-								
Head width	0.517**	-							
Distance between eyes	0.530**	0.885**	-						
Thorax length	0.393**	0.428**	0.412**	-					
Pronotum length	0.269**	0.311**	0.329**	0.679**	-				
Front femur length	0.475**	0.406**	0.410**	0.423**	0.468**	-			
Hind femur length	0.388**	0.334**	0.360**	0.407**	0.455**	0.739**	-		
Ovipositor length	0.183**	0.254**	0.207**	0.136**	0.016	0.201**	0.046	-	
Abdomen length	0.240**	0.282**	0.305**	0.401**	0.365**	0.285**	0.306**	0.007	-

\*\*P<0.01.

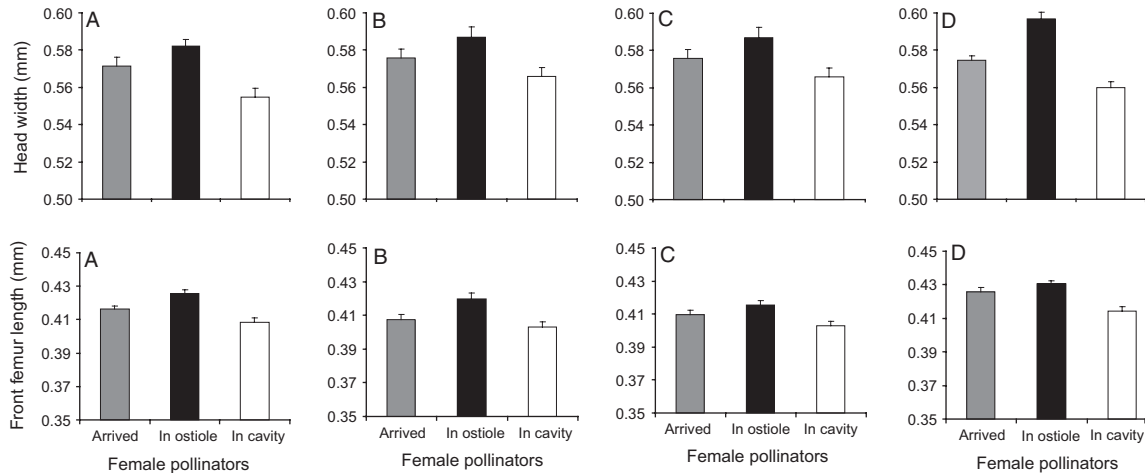
**Table 2** Rotated component matrix

	Component		
	1	2	3
Head length	0.615	0.388	0.092
Head width	0.905	0.125	0.200
Distance between eyes	0.889	0.130	0.237
Thorax length	0.341	0.401	0.603
Pronotum length	0.134	0.492	0.652
Front femur length	0.265	0.869	0.111
Hind femur length	0.136	0.842	0.224
Ovipositor length	0.461	0.273	-0.500
Abdomen length	0.231	0.127	0.675

Principle component 1 reflects head characteristics vs. body size. Principle component 2 reflects femur characters vs. body size. Principle component 3 reflects thorax and abdomen characters vs. body size. The thorax and abdomen are easily anamorphic and are not selected. The highest scores on the first and second components show that head width and front femur length were the best indicators of wasp body size.



**Figure 2** Mean (+ SE) body size of emerged and arriving *Ceratosolen solmsi marchali*. A total of 150 emerged wasps were collected from three male trees (1–3) and 120 arriving wasps were collected from four receptive trees, including two male and two female trees (4, 5, 1, and 2).



**Figure 3** Mean (+ SE) body size of three kinds of *Ceratosolen solmsi marchali*. Gray column, body size of wasps collected when arriving at receptive figs; black column, body size of wasps stuck in the ostiole; and white column, body size of wasps trapped in the cavity. A and B represent trees ♂1 and ♂2, C and D represent trees ♀1 and ♀2.

pal component analysis and found that head width and front femur length were the best indicators of wasp body size. We then used the two indicators to compare the size of newly emerged wasps with those arriving at syconia. Wasps arriving at syconia were significantly larger than newly emerged wasps, but the larger wasps were more easily trapped in the ostiole.

In most insects, adult size depends on larval development and is influenced by the duration of the feeding period as well as by food quantity and quality (Slansky & Scriber, 1985). In non-pollinating fig wasps (*Otitessella* spp.), larval development time and natal ovary position affected wasp body size (Moore et al., 2004), and it has also been shown that large fig-pollinating wasps produce more offspring and a more female-biased sex ratio (Herre & West, 1997). However, the factors that generate body size variation in pollinating fig wasps are still poorly understood.

Syconia are often produced in synchronous crops. At the population level, flowering asynchrony among trees enables wasps emerging from the crop on one tree to find receptive syconia on another. Any substantial gap in flowering at the population level would lead to extinction of the pollinator population (Harrison, 2000). In some species, asynchrony is also maintained at the individual level, but usually overlap between wasp emergent and receptive phases on the same tree is limited. Consequently, fig wasps have to fly from their native syconia to a receptive syconium, during their short adult life span (just 1–3 days) (Harrison & Rasplus, 2006). Herre (1989) suggested that larger wasps had a greater chance of reaching a receptive

syconium through comparing the body sizes of offspring and foundresses. In *Ficus* species of the subgenus *Sycomorus*, such as *F. hispida*, the pollinators that enter the syconia quickly disintegrate. Therefore, we compared the body size of emerging wasps and those arriving at receptive trees. The result confirmed Herre's (1989) prediction that wasps arriving at syconia were significantly larger than newly emerged wasps. However, there was obvious variation among trees, and the wasps arriving at syconia were sometimes small. An explanation could be that the wasps reaching a receptive tree may have flown very different distances depending on the relative location of trees with emerging wasps and receptive syconia. Moreover, wasp size could be correlated with the size of the syconia: a small syconium could produce small wasps so that some wasps arriving at a receptive tree are small too. This prediction needs to be tested in future research. In the case of *Sycomorus* figs, which are mostly pioneers, high density and high flowering frequency may imply that most wasps fly relatively short distances (Harrison & Shanahan, 2005; Harrison & Rasplus, 2006). Thus, compared with the wasps that fly long distances, dispersal may have less effect on wasp body size.

Ostioles play a key role in balancing foundress numbers, as well as maintaining pollinator–host specificity, and blocking non-pollinating fig wasps or other insects (Gibernau et al., 1996; van Noort & Compton, 1996). Fig-pollinating wasps have evolved many adaptive characteristics for entering the ostiole, but the process of gaining access to the fig cavity is so difficult that many prospective pollinating fig wasps do not manage to pass through the



ostiole (Peng et al., 2005). Our results showed that larger wasps were easily trapped in the ostiole. Obviously, wasps being large may be good for dispersal but bad for passing through the ostiole. With regards to mutualism stability, large wasps may be more fecund than small wasps. The tree may thus control the number of eggs laid in its syconia by controlling the size of the wasps through the selective filter of the ostiole. However, in dioecious species a fully exploited male syconium is a good thing for maximum pollen dispersal; in a female syconium it makes little difference to the syconium as the wasps do not develop unless their probing nullifies the ability of a flower to develop into a seed. In a monoecious species this may, however, be the case because wasps directly destroy potential seeds and more eggs coming into the syconium than is optimal for the syconium will be costly. Moreover, larger wasps may be able to carry more pollen than smaller wasps. Finally, the ostiole as a selective filter to pollinators stabilizes pollinator size, which will benefit the stability in fig–fig pollinator mutualism.

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