

# Brood size: a major factor influencing male dimorphism in the non-pollinating fig wasp *Sycobia* sp.

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**Abstract.** 1. Male polymorphisms have been described in some non-pollinating fig wasps, as well as in other animals. The proximal basis and the maintenance of alternative male reproductive strategies are either genetic or environmental.

2. Here we studied male dimorphism in the non-pollinating fig wasp *Sycobia* sp. We conducted experimental manipulations to study the factors influencing offspring male morph allocations and explore a possible basis for the determination and maintenance of male dimorphism in *Sycobia* sp.

3. The results showed that brood size was the major and underlying factor influencing the male morph ratio. When the brood size increases, the wingless male ratio also increases.

4. Also, our results indicated that there was no direct maternal control on offspring male morph allocation.

5. Male dimorphism in *Sycobia* sp. probably represents an environmentally determined conditional strategy, which responded to offspring population density at the level of the individual fig.

**Key words.** Alternative reproductive tactics, brood size, conditional strategy, male dimorphism, non-pollinating fig wasp.

## Introduction

Male polymorphisms have been described for a number of animal species and the male morphs exhibit differences in morphology, behaviour, physiology as well as life history (Gadgil, 1972; Gross, 1996). Social interaction is assumed to be a major evolutionary selection force in generating male polymorphisms. Three different kinds of strategy have been proposed to explain why and how selection favours alternative phenotypes. These include alternative strategies (genetic polymorphism with equal fitness resulting from frequency-dependent selection), mixed strategy (genetic monomorphism with equal fitness between alternative phenotypes resulting

from frequency-dependent selection), and conditional strategy (genetic monomorphism with unequal fitness between alternative phenotypes resulting from status-dependent selection), among which no case of mixed strategy is yet known (Gross, 1996).

The determination and maintenance of these alternative reproductive strategies may have either a genetic (Shuster & Wade, 1991; Ryan *et al.*, 1992; Lank *et al.*, 1995; Caillaud *et al.*, 2002;) or environmental basis (Emlen, 1994; Moczek, 1998; Josepha & Knowles, 2002; Cremer & Heinze, 2003). For genetically determined alternative strategies, the average fitness among male morphs must be equivalent, because the relative fitness of alternative phenotypes is dependent on their frequencies in the population, not on the competitive ability or *state* of individuals in the population (Gross, 1996). However, for a conditional strategy determined by environmental factors, fitness need not be equal among male morphs (Gross, 1996).

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Based on relatively few empirical studies, conditional rather than genetic determination of male morphs appears to be more common (Gross, 1996).

A number of genera among the non-pollinating fig wasps (Hymenoptera, Chalcidoidea) exhibit male polymorphisms with the different morphs displaying different mating behaviours (Hamilton, 1979; Vincent & Compton, 1992; Fellowes *et al.*, 1999; Greeff & Ferguson, 1999; Bean & Cook, 2001; Jusselin *et al.*, 2004; Cook & Bean, 2006). These organisms provide excellent model systems for testing theoretical predictions concerning male polymorphisms and their underlying mechanisms.

*Sycobia* sp. (Hymenoptera, Chalcidoidea, Epichrysoallinae) is one of approximately 15 non-pollinating fig wasp species hosted in the *Ficus benjamina* inflorescence. As a gall inducer, females of *Sycobia* sp. use their long ovipositors to penetrate the fig wall without entering the fig fruit and lay their eggs into the fig ovaries. Fertilized eggs develop into female wasps and unfertilized eggs into one of two alternative winged or wingless male morphs. Winged males are characterized by fully developed wings and larger compound eyes, and wingless males show features including large mandibles, wing and eye reductions, which are believed to be adaptations to the closed confines of the fig fruit (Hamilton, 1979). Winged males generally leave their natal figs and mate elsewhere with females that have dispersed from their own natal figs. In contrast, wingless males mate with females in the lumen of their natal fig, which they usually never leave.

Hamilton's (1979) model showed that in male-dimorphic non-pollinating fig wasps, the proportion of each male morph should be equal to the proportion of females that they can expect to mate with. However, his model includes the assumption that females lay only one egg per fig, which is highly unlikely in nature. Greeff (1995) and Cook *et al.* (1997) incorporated the effects of local mate competition (LMC) and concluded that the proportion of winged males should either equal (without LMC) or exceed (under LMC) the proportion of females that develop in figs without wingless males. This would lead to the fitness of one male morph being equal, or at least comparable with that of the other male morph, generating the conditions necessary for the alternative mating strategies in male-dimorphic non-pollinating fig wasps to be genetically determined. However, Pienaar and Greeff (2003a) tested the genetic polymorphism assumption in the male dimorphic species *Otitesella pseudoserrata* and argued that the two male morphs have equal fitness, but are not determined by different alleles. Instead, their model (Pienaar & Greeff, 2003b) showed that the alternative mating tactics in three species of male-dimorphic non-pollinating *Otitesella* fig wasps were probably a result of maternal control. To distinguish between these hypotheses, we conducted experimental manipulations in the male-dimorphic non-pollinating fig wasp *Sycobia* sp. to study the factors influencing offspring male morph proportions, and explore a possible basis for the determination and maintenance of male dimorphism.

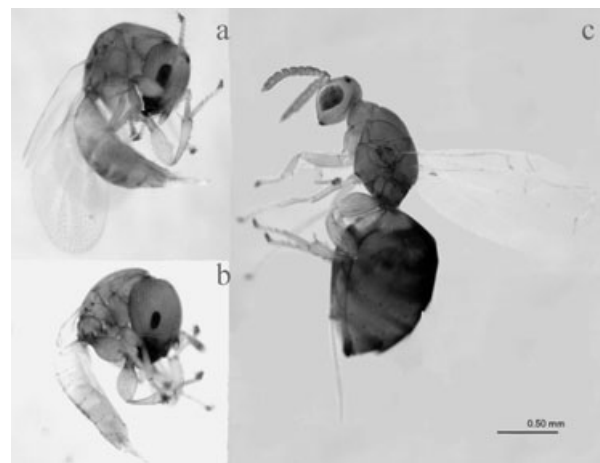
## Materials and methods

### Study site and species

Experiments were conducted in the Chinese Academy of Tropical Agricultural Sciences (CATAS), Danzhou City, Hainan province (Hainan Island), China (19°30.410'N, 109°29.340'E). Hainan Island lies south of the China mainland, separated by the 40-km wide Qiongzhou Straits. Its climate includes well-defined dry (November to April) and rainy (May to October) seasons. The annual mean temperature is 24.3°C, with the lowest in February (18.2°C) and the highest in July (29.6°C). The mean temperatures in the dry and rainy season are 16–24°C and 25–29°C, respectively. The annual mean rainfall is 1800 mm, of which 70–90% falls during the rainy season. *Ficus benjamina* L. is a monoecious, usually free-standing fig tree, grows up to 20 m in height with figs axillary on leafy branchlets (Zhou & Gilbert, 2003). Crops of *F. benjamina* usually synchronize within one tree, but not between trees. Being one of the 15 non-pollinating fig wasp species hosted in the *F. benjamina* inflorescence, *Sycobia* sp. was selected for experimental manipulations because of its typical male dimorphism (see Figure 1), easy handling and rearing, relatively large populations and long duration of oviposition, which usually lasts for 3–4 days (our personal observations).

### Experimental manipulations

Experiments were done as follows: when figs started to grow on a *F. benjamina*, we bagged figs to keep them free from any fig wasps with one fig fruit per bag on randomly chosen branchlets using specially-made nylon bags. The bags are columniform with wire netting rings bracing inside to ensure enough space for oviposition by *Sycobia* females, as they oviposit from the outside instead of entering the figs. We bagged a total of 213 figs on four trees in the dry season and three trees in the rainy season from August 2006 to September 2007. When



**Fig. 1.** Male dimorphism in *Sycobia* sp. (a, winged male; b, wingless male; c, female; a, b and c share the same size bar).

*Sycobia* females were observed approaching and ovipositing on unbaggged figs in the pre-receptive phase (phase A) on each tree, we collected fig fruits ready to mature from another tree, put them in large nylon bags and waited for fig wasps to emerge. Thus we obtained *Sycobia* females that had mated, but without ovipositing. Each bagged fig was assigned to one of the three experimental treatments: for the first treatment, we introduced one *Sycobia* female (foundress) into each bag ( $f = 1$ ), for the second, three foundresses were introduced into each bag simultaneously ( $f = 3$ ), and for the third, five foundresses were introduced into each bag simultaneously ( $f = 5$ ). Females in the bags oviposited and behaved just like females outside the bags on the same tree (our unpublished data). After introducing foundresses into bags, we resealed the bag and waited for the fig fruit to mature. When the fig fruits had matured and the offspring had emerged, we collected all the bags and counted offspring numbers under a dissecting microscope. We counted all the wasps that had emerged from the fruits and that remained in the lumens or ovaries of the figs. The numbers of females, winged males and wingless males were recorded respectively.

All data were processed using GLM (General Linear Model) in spss 11.5 (SPSS Inc., Chicago, IL) (Voelkl & Gerber, 1999; Zhang, 2002). The wingless male ratio (which we defined as the proportions of wingless males out of all males) were used as a response variable, season and foundress number as explanatory factors. As brood size (the number of all conspecific individuals in a patch, but not always the offspring of the same mother) has a strong influence on male dimorphism across fig wasps (Hamilton, 1979; Herre *et al.*, 1997), brood size was used as a covariate in the GLM. Arcsine transformation was carried out on the wingless male ratio to conform to the normal distribution and homogeneity of variance. Also, we studied the relationships between sex ratio, foundress number and brood size using GLM. Square root transformation was carried out on the sex ratio.

To explore possible maternal influences on offspring male dimorphism, we conducted another experiment. As above, we also bagged figs with one fig per bag using specially-made nylon bags and introduced single *Sycobia* foundress into each bag ( $f = 1$ ). We used one of the three types of *Sycobia* foundresses: foundresses only mated with winged males ( $n = 10$ ), foundresses only mated with wingless males ( $n = 25$ ), and unmated foundresses ( $n = 31$ ). We obtained females mated to the different types of males using the following methods: before the emergence of offspring, we opened the figs, collected two types of males respectively and put them into small nylon bags. Females were still in the ovaries at this time. After females emerged, we collected the females and put them into the nylon bags to make them mate with the two types of males. After observed mating, we introduced them into nylon bags. We got unmated females by directly collecting females still in the ovaries. As above, when the fig fruits had matured and the offspring had emerged, we collected all the bags and counted offspring numbers under a dissecting microscope. The numbers of females, winged males and wingless males were recorded respectively.

**Table 1.** Brood sizes, sex ratios and male ratios from foundress numbers in the manipulated experiments.

	$F = 1$	$F = 3$	$F = 5$
N	122	22	69
Brood size (mean $\pm$ SE)	87.55 $\pm$ 5.25	157.55 $\pm$ 13.17	180.41 $\pm$ 22.34
Sex ratio (mean $\pm$ SE)	0.29 $\pm$ 0.01	0.36 $\pm$ 0.02	0.38 $\pm$ 0.02
Winged male ratio (mean $\pm$ SE)	0.36 $\pm$ 0.02	0.26 $\pm$ 0.03	0.16 $\pm$ 0.04
Wingless male ratio (mean $\pm$ SE)	0.64 $\pm$ 0.02	0.74 $\pm$ 0.03	0.84 $\pm$ 0.04

F, foundress number; N, sample size.

This experiment was carried out on two trees during the rainy season from June to September 2007. All the data, together with the data from single foundress introductions in the rainy season ( $N = 52$ ) from the previous experiment detailed above, were analysed using GLM in spss 11.5. Likewise, the wingless male ratio was used as a response variable and brood size as covariate. Arcsine transformation was also done on the wingless male ratio.

## Results

In our manipulated experiments, the brood sizes, sex ratios and male ratios are listed in Table 1. Univariate ANOVA was carried out on the relationships between sex ratio and foundress number. The result showed that sex ratio increased significantly with foundress number ( $F = 17.123$ ,  $P < 0.001$ ). The relationship between sex ratio and brood size of single foundress was also studied. The result showed that the sex ratio decreased significantly with brood size of single foundress ( $F = 5.437$ ,  $P < 0.05$ ). These results on sex ratio in the non-pollinating *Sycobia* were consistent with those in pollinating fig wasps, which indicated that the LMC (Local Mate Competition) theory also played key roles on sex allocation in the non-pollinating *Sycobia*.

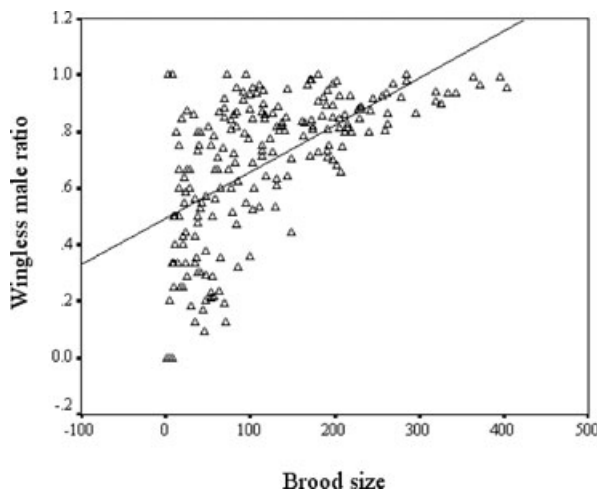
Univariate ANOVA showed that the wingless male ratio varied with both season ( $F = 4.842$ ,  $P = 0.029$ ) and foundress number ( $F = 9.346$ ,  $P < 0.001$ ). When brood size was included as a covariate, neither season ( $F = 1.601$ ,  $P = 0.207$ ) nor foundress number ( $F = 1.513$ ,  $P = 0.318$ ) significantly influenced the wingless male ratio and only brood size ( $F = 16.319$ ,  $P < 0.001$ ) was significant (see Table 2). When brood size increases, the wingless male ratio also increases (see Figure 2). Brood sizes of *Sycobia* sp. are usually larger in the rainy than in the dry season (brood size mean  $\pm$  SE in the manipulated experiments: dry season = 91.76  $\pm$  7.91, rainy season = 157.43  $\pm$  8.63; Kruskal–Wallis test:  $H = 36.123$ , d.f. = 1,  $P < 0.001$ ) and brood sizes of multiple foundresses are also usually larger than those of single foundress (brood size mean  $\pm$  SE in the manipulated experiments: one foundress = 87.55  $\pm$  5.26, five foundresses = 157.55  $\pm$  13.17; Kruskal–Wallis test:  $H = 16.363$ , d.f. = 1,  $P < 0.001$ ). Thus, the significant effects of season and

**Table 2.** Effects of season, foundress number and brood size on the wingless male ratio.

Source	Variable	SS	d.f.	F	<i>p</i>
Season	WLM ratio	0.591	1	4.842	0.029*
Foundress number	WLM ratio	2.283	2	9.346	0.000***
Season × foundress number	WLM ratio	0.030	2	0.123	0.885
Brood size as covariate					
Season	WLM ratio	0.148	1	1.601	0.207
Foundress number	WLM ratio	0.214	2	1.153	0.318
Season × foundress number	WLM ratio	0.089	2	0.478	0.621
Brood size	WLM ratio	1.513	1	16.319	0.000***

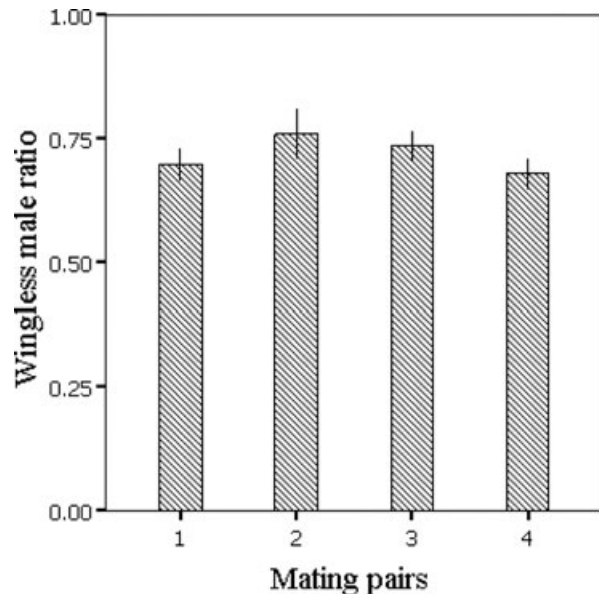
Univariate ANOVA was performed with alpha 0.05 using GLM in spss 11.5. WLM ratio, wingless male ratio, wingless male ratios were arcsine transformed; SS, sum of squares.

\* $P < 0.05$ , \*\*\* $P < 0.001$ .

**Fig. 2.** Linear regression between wingless male ratios and brood sizes (wingless male:  $N = 213$ ,  $R^2 = 0.329$ ,  $P = 0.000$ ).

foundress number that were detected by ANOVA appear to be as a result of differences in brood size.

To investigate possible maternal influences on offspring male morph ratio, ANOVAs were performed between offspring male morph ratios of foundresses from different mating pairs: foundresses only mated with winged males, foundresses only mated with wingless males, unmated foundresses and foundresses naturally mated as controls. The results showed that wingless male ratios in the offspring of foundresses from different mating pairs had no significant differences ( $F_{3,114} = 0.691$ ,  $P = 0.560$ , see Figure 3). These results, together with the results above that foundress number had no significant influence on the winged and the wingless male ratio, indicated that perhaps there were no significant direct maternal influences on offspring male morph allocations.

**Fig. 3.** Wingless male ratios (mean  $\pm$  SE) in offspring of foundresses from different mating pairs (1: foundresses naturally mated as controls,  $n = 52$ ; 2: foundresses only mated with winged males,  $n = 10$ ; 3: foundresses only mated with wingless males,  $n = 25$ ; 4: unmated foundresses,  $n = 31$ ).

## Discussion

Hamilton (1979) and Cook *et al.* (1997) suggested that brood size strongly influences the balance of mating opportunities for the two male morphs, such that winged males are favoured at low brood sizes, wingless males at high brood sizes and dimorphic males at intermediate brood sizes across fig wasps. Pienaar and Greeff (2003b) found this pattern between crops of one species and between figs with different foundress numbers. Our results were consistent with Pienaar and Greeff (2003b) and showed further that in *Sycobia* sp., winglessness increased with brood size, not only between crops of one species and between figs with different foundress numbers, but also between figs with one foundress or identical foundress numbers.

Our results also showed that the two factors (season and foundress number) influence male morph ratios via their effect on brood size. Thus, we can conclude that brood size, or population density should be the underlying factor influencing male morph ratio. The effects of population density on male polymorphism have also been demonstrated in other taxa. Tomkins and Brown (2004) showed that population density was likely to be the key determinant of the relative fitness of the alternative tactics and could drive the local evolution of a threshold dimorphism.

Pienaar and Greeff (2003b) argued that females could respond to population densities at the level of individual figs, and could be in a position to perceive what other females are doing. Hence, they may decide what type of offspring, be it female, non-dispersing male or dispersing male, to lay based on the optimization of their fitness returns. Our results do not

seem to support this. In *Sycobia* sp., at similar brood sizes, male morph ratios are not significantly different between offspring of one foundress and five foundresses. Also, at similar brood sizes, male morph ratios are not significantly different between offspring of mated and unmated foundresses. If foundresses could perceive the likely environmental conditions and decide what type of male offspring to lay, then a female should lay more wingless male offspring to compete with other males for mating opportunities, with females in the lumen of the fig when she perceives other females laying on the same fig. When a female is a virgin, she should lay more or even all winged male offspring to disperse outside to mate with females from other figs. In our view, in *Sycobia* sp., females can directly adjust offspring sex ratio (foundress number has a significant influence on sex ratio and sex ratio increases with foundress number) by fertilizing the eggs or not, just as pollinating (Frank, 1985; Herre, 1985; Herre *et al.*, 1997) and other non-pollinating fig wasps (West & Herre, 1998) do. Winged and wingless males all develop from unfertilized eggs and we found no evidence for control of the two male morphs by ovipositing *Sycobia* females. Ovipositing females may influence the nature of their offspring by manipulating developmental programmes of their offspring (Fox *et al.*, 1999). Such a mechanism may exist in the non-pollinating *Otitesella* fig wasps (Pienaar & Greeff, 2003a; b).

The difference in maternal strategies of these two genera may result from differences in ecological habits between the *Sycobia* sp. studied and non-pollinating *Otitesella* fig wasps (Pienaar & Greeff, 2003a, b). *Otitesella* females do not lay large clutches on single figs. Rather, they disperse their egg load over a number of figs and spend short time periods ovipositing on a single fig before moving on to another one. Numerous figs contain only a single offspring and among the figs containing *Otitesella* offspring, only 50% of them contain digitata males (one of the two male morphs). Hence, sib mating and LMC are not important factors in *Otitesella* species (Pienaar & Greeff, 2003b). Contrary to these, *Sycobia* sp. females may spend longer time periods laying large clutches on single figs. Among the figs containing *Sycobia* sp. offspring, most figs contain both male morphs [93.55% (58 out of 62 figs) in natural crops and 96.06% (268 out of 279 figs) in experimental crops, our unpublished data]. LMC and sib mating may play important roles in *Sycobia* sp. Thus different selection pressures may exist in *Otitesella* and *Sycobia* and may favour different reproductive tactics. More investigations into other male-polymorphic fig wasps will be helpful for understanding selection pressures on male polymorphisms and reproductive tactics in fig wasps.

Our results have proved that brood size is the underlying factor influencing the male morph ratio. With male morph ratios changing significantly between different brood sizes, we do not think the male dimorphism in *Sycobia* sp. has a genetic basis (also see Pienaar & Greeff, 2003a). Also, no direct maternal manipulations of offspring male morph allocations seem to exist in *Sycobia* sp. Thus, there is a high probability that environmental factors determine the male dimorphism in *Sycobia* sp., where larval developmental pathways can be easily changed through environmental cues. Consequently

through what environmental cues, can brood size, or population density influence male morph ratio? A possible cue is larval nutrition. Larval diet and nutrition have been proven to determine male dimorphism in some male-dimorphic animals, in which larger morphs develop under high quality and quantity of diets, and the alternative morphs develop under low quality and quantity of diets (Harvell, 1994; Josepha & Moczek, 1998; Knowles, 2002). It is possible that there are different nutritional demands between the two male morphs: winged males may need more nutrition for developing full wings and larger compound eyes as well as an energy store for dispersing, but wingless males may need less nutrition and energy than winged males for their confined activities. Ovaries near the centre of the fig are less space constrained and may contain more resources for larval development (Moore *et al.*, 2004). In fig wasps, unfertilized eggs grow into males. Unfertilized eggs of *Sycobia* sp. laid in different layers of ovaries may develop into winged or wingless males based on resources they obtained during development. Further investigations on the positions of unfertilized eggs in layers of ovaries will be needed.

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