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Reproductive strategies of two forms of flightless males in a non-pollinating fig wasp under partial local mate competition

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Abstract. 1. The underlying basis of alternative male reproductive strategies is either genetic or environmental. Several non-pollinating fig wasp lineages have dimorphic males, typically with winged males that disperse from natal figs to mate and flightless males that seek mating opportunities in natal figs.

2. *Walkerella* sp. from *Ficus benjamina* has dark and pale wingless males. Observations and experiments in Xishuangbanna, Southern China found that (i) the sex ratio of *Walkerella* sp. did not vary with foundress number or brood size. (ii) The frequency of dark males increased with brood size and foundress number and they were absent from figs with a single foundress. This produced a higher proportion of dark males at higher densities. (iii) Males of both morphs fought, but injuries to dark males were more frequent. (iv) Dark males were more likely to disperse away from their natal figs and (v) they were more resistant to dehydration.

3. Responses to selection are constrained by the genetic options available. Consequently, selection pressures acting on different lineages can produce similar outcomes that are achieved in different ways. *Walkerella* species lack winged males, but dark males display some of their features, dispersing from natal figs and displaying appropriate physiological and behavioural adaptations. However, dark males also displayed increased levels of damage from fighting – a feature unlikely to be shared with the winged males of other species.

Key words. Dimorphism, dispersal, *Ficus*, fighting, sex ratio, *Walkerella.*

Introduction

Alternative reproductive tactics are often associated with polymorphisms. They have attracted extensive empirical and theoretical attention (Pienaar & Greeff, 2003a; Moore *et al*., 2004). Three different types of strategy are considered to determine such phenotypic diversity, namely (i) alternative strategies that are genetically based; (ii) mixed strategies that are also genetically based; (iii) conditional strategies that are maintained by status-dependent selection (Pienaar & Greeff, 2003a; Moore *et al*., 2004). Alternative strategies and conditional strategies are well documented, but there is no conclusive evidence for the existence of mixed strategies (Pienaar & Greeff, 2003a).

Numerous non-pollinating fig wasps (NPFW) exhibit male morphological dimorphisms (Cook *et al*., 1997; Greeff & Ferguson, 1999; Bean & Cook, 2001; Niu *et al*., 2009), or even trimorphisms (Vincent & Compton, 1992; Moore *et al*., 2004). Genetic determination of morph identity precludes ovipositing females from responding to local reproductive environments by adjusting their progeny morph ratios. Some studies have found that male morphs are environmentally rather than genetically determined (Pienaar & Greeff, 2003a,b). Offspring allocation decisions in these species therefore involve not only whether to lay male or female eggs, and where to lay them (Moore *et al*., 2004), but also the morph of male to produce (Hamilton, 1979; Greeff, 1997; Bean & Cook, 2001; Pienaar & Greeff, 2003a; Pienaar & Greeff, 2006). Dimorphic species usually have winged and wingless males, but species with more than

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one wingless morph have also been recorded (Hamilton, 1979; Murray, 1990; Vincent & Compton, 1992; West & Herre, 1998; Greeff & Ferguson, 1999; Bean & Cook, 2001). In some NPFW with wingless dimorphic males, one male morph engages in regular lethal fights, while the other morph rarely fights (Moore *et al*., 2004; Cook & Bean, 2006). The fighting males often have heavier cuticles (equivalent to body armour), and large scythe-like jaws (Vincent, 1991; Cook *et al*., 1999). In contrast, other less imposing forms of male are small and flattened, allowing them to slip between densely packed flowers and enter seeds to mate with un-emerged females (Cook *et al*., 1999). In at least one species of the NPFW *Otitesella*, the sexes and morphs are not randomly distributed relative to distance from the fig wall, and individuals that develop in galls closer to the centre are also larger (Moore *et al*., 2004).

According to local mate competition theory, female-biased sex ratios are favoured when one or a small number of foundresses contribute progeny to a combined brood, but as the number of mothers contributing offspring increases, the optimal progeny sex ratio becomes progressively less female-biased (Hamilton, 1967; Hardy & Mayhew, 1998). Reflecting this, the sex ratios of pollinators and internally ovipositing NPFW are typically female-biased and vary according to foundress number (Herre *et al*., 1997; Raja *et al*., 2008; Song *et al*., 2008). In contrast, females of externally ovipositing NPFW can potentially oviposit in many figs, and many more females can contribute to the total brood present in any one fig. Consequently, their sex ratios are typically much less female-biased, a characteristic that may favour the development of fighting (Nelson & Greeff, 2009).

It is generally assumed that winged NPFW males mainly disperse from their natal figs to search for mates, whereas wingless males mate in their natal figs, but there are exceptions (Greeff & Ferguson, 1999; Bean & Cook, 2001; Greeff *et al*., 2003; Moore *et al*., 2006). For example, the African NPFW *Otitesella longicauda* has two distinct types of flightless males, the religiosa and digitata morphs (Greeff & Ferguson, 1999). Most of the former disperse from their natal figs and most of the latter do not (Greeff & Ferguson, 1999; Moore *et al*., 2004). *Otitesella* is absent from Asia, where the subfamily Otitesellinae is represented by several other genera, including *Walkerella*. Species in this genus can also display male dimorphism. Like all species of Otitesellinae, only wingless males are present.

Here we compare the behaviour and physiology of male morphs in an undescribed species of *Walkerella* associated with *Ficus benjamina* in China. We address the following questions about offspring allocation in *Walkerella* sp. (i) Does offspring sex ratio change in response to offspring density or foundress number? (ii) Do male morph ratios also change? (iii) Do the male morphs differ in their willingness to disperse or fight and in their ability to survive outside the figs?

Materials and methods

Study site and species

The study was carried out in the Xishuangbanna Tropical Botanical Garden (XTBG) (101°15′E, 21°55′N), located in south-west China. In Xishuangbanna, *Ficus benjamina* and *Ficus benjamina* var. *nuda* coexist. Here, we only studied *Ficus benjamina. Ficus benjamina* L. (Section *Conosycea*) is a large free-standing monoecious fig tree that is native to Xishuangbanna (Bai *et al*., 2008). In Xishuangbanna, *F. benjamina* produces figs throughout the year in synchronous crops, with different trees fruiting at different times. Crops comprise several thousand figs. Mature figs are subglobose, yellow and measure 12–25 mm in diameter (Bai *et al*., 2008).

In addition to the pollinator fig wasp *Eupristina koningsbergeri*, *F. benjamina* supports 14 NPFW at XTBG, including *Walkerella* sp. *indesc*. (Pteromalidae, Otitesellinae). *Walkerella* sp. foundresses oviposit from the outside of the figs. Adult males emerge before the females and will often fight with conspecifics they encounter within the figs, using their large mandibles. The males of *Walkerella* sp. are wingless, with large mandibles. They can be either dark brown, or pale yellowish-brown, with no intermediates.

Natural populations of Walkerella sp.

Between 2002 and 2008, 374 late C and early D phase figs (the stages when young adults are present, but before there are any exit holes from the figs) were collected from five *Ficus benjamina* trees. The figs were sliced open and placed individually in cotton bags to allow the wasps to emerge. All the *Walkerella* sp. females, dark males, and pale males in each fig were then recorded. Males damage their mandibles during fights more frequently than other parts of the body (antennae and legs were occasionally damaged, but heads were never detached), and mandible condition was used as a quantitative measure of fighting frequency. All the *Walkerella* sp. males in 48 of the above figs were scored for the presence or absence of mandible damage. In order to investigate size differences between the two morphs we measured male head length and mandible length. These were convenient because they were usually still measurable even in the most badly damaged males (Moore *et al*., 2009).

Late C phase figs contain fig wasp pupae and adults that have not yet emerged from their galls. The position of 528 individual galls (from 30 figs) relative to the periphery of the figs was determined by measuring the distance from the inside of the fig wall to the innermost surface of the gall. The gall was then opened to allow the fig wasp inside to be identified.

Experimental manipulation of foundress numbers

Pre-female (B) phase figs were enclosed in fine mesh nylon bags to prevent any female fig wasps from ovipositing. We waited for *Walkerella* sp. females to start laying eggs in adjacent figs at the same developmental stage, then released one, three, or five foundresses, reared from figs collected on other trees, into each bag. At maturity, the figs were removed and their contents recorded. Sample sizes were 16 (single-foundress), three (three-foundress) and three (five-foundress) figs.

Male emergence from figs and subsequent longevity

Thirty late C phase figs were collected from a single tree in 2008 and placed individually in fine mesh bags. *Walkerella* sp. males were allowed to emerge naturally until all the wasps had died. The numbers of pale and dark males inside and outside the figs were then recorded. An additional 33 figs at the same developmental stage were collected from the tree. Each fig was sliced open and then placed in bags until *Walkerella* sp. males started emerging from their galls. Approximately equal numbers of dark and pale individuals were then placed individually in 100 ml beakers and maintained under laboratory conditions that ranged from 25 to 29 °C and relative humidities ranging from 55% to 68%. Survivorship was recorded every 2 h until all the males had died. Survival analysis was used to determine whether the morphs persisted for different lengths of time under these conditions.

Data analysis

We used General Linear Models (GLM) with binomial error structure to analyse relationships between sex ratios and brood size, sex ratios and foundress number, male morph ratios and male number within a fig, and male morph ratios and foundress number. Male mandibular damage number was analysed using GLM with a quasi-Poisson error structure. F-tests examined the relationship between brood size and foundress number. Paired t-tests analysed differences in mandibular damage and emergence rates between dark males and pale males. Linear models (LM) were used to analyse the relationship between mandible length and head length (Fig. 1). Survival analysis was carried out using the survival library, with the Cox proportional hazards regression model fitted with the coxph function (Dalgaard, 2004). Proportionality of hazards was confirmed with the cox.zph function. All analyses were carried out using R (version 2.5.1).

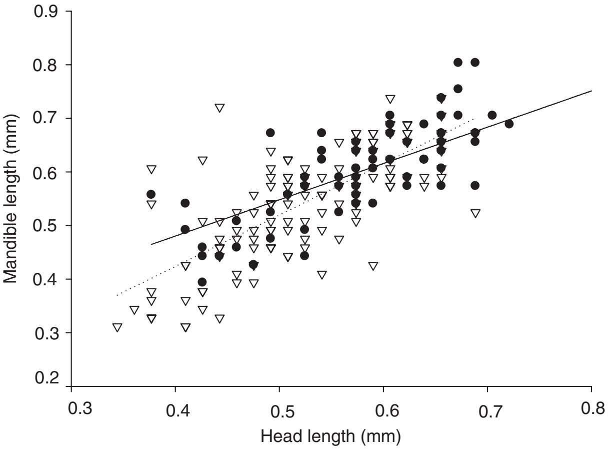

Fig. 1. The relationship between head length and mandible length of *Walkerella* sp. males. Circles and solid line, dark males; triangles and dotted line, pale males.

Results

Natural populations

Walkerella sp. was present in 92 of the 373 figs, from which we reared 893 individuals. Combined brood sizes within individual figs ranged from 1 to 33, but most occupied figs contained five or fewer individuals. Females were in a majority [overall sex ratio (proportion males) = 0.41]. Progeny sex ratios was not significantly correlated with wasp densities in individual figs (GLM: Slope = $\beta \pm \text{SE} = -0.01 \pm 0.01$, $P = 0.48$, $n = 92$).

Thirteen (14.13%) of the 92 figs contained only male *Walkerella* sp. that would have had no mating opportunities without emergence, and five (5.43%) of the figs contained lone females that could only have mated with males from other figs. The remaining 74 figs contained both males and females. Up to 18 males shared a fig, but over a quarter of the figs with males contained only a singleton (Fig. 2). Dark males were the less common morph irrespective of male density [based on 365 males of which 87 (23.83%) were dark and 278 (76.16%) were pale], and were present in only one of the 24 figs that contained a single male. Morph ratios varied significantly between figs with different densities of males (GLM: Slope = $\beta \pm \text{SE} = 0.58 \pm 0.28$, $P < 0.05$, $n = 92$), with a higher proportion of dark males in figs that contained three or more males (Fig. 3).

Mandibular damage was recorded in 48 of the 281 males examined (Fig. 4). The likelihood of being damaged varied with male density inside the figs (GLM: Slope = $\beta \pm \text{SE} = 0.20 \pm 0.02$, $P < 0.01$, $n = 48$), but not female density (GLM: Slope = $\beta \pm \text{SE} = 0.04 \pm 0.03$, $P = 0.15$, $n = 48$). No damaged males were recorded in figs containing three or fewer males (Fig. 4). Damaged males only appeared when four or more males shared a fig, but male damage rates overall marginally failed to increase significantly with male number within a fig (GLM: Slope = $\beta \pm \text{SE} = 0.07 \pm 0.04$, $P = 0.07$, $n = 33$). Overall, 30.6% of the damaged males were dark and 69.4% were pale, showing that males fight irrespective of their colour. However, the rate of injuries to

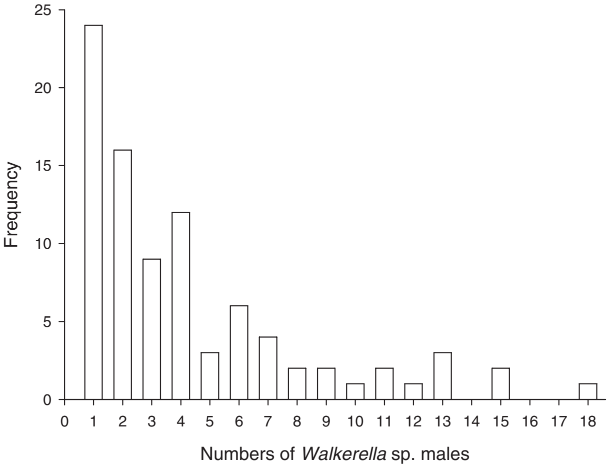

Fig. 2. The numbers of *Walkerella* sp. males present in the figs.

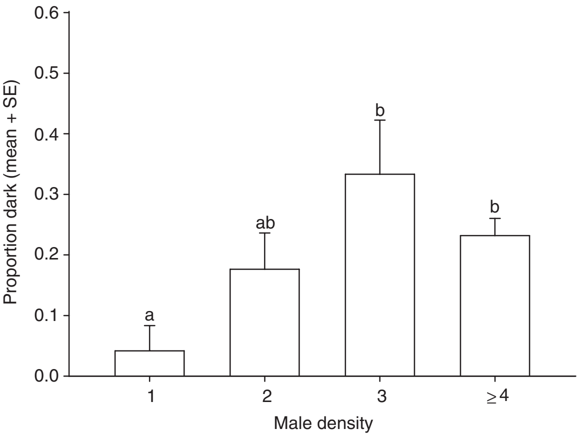

Fig. 3. The proportion of dark *Walkerella* sp. males in relation to the overall density of males in the figs. Bars with different letters are significantly different (ANOVA, $P < 0.05$).

(the less common) dark males was significantly higher than that among pale males (paired t-test: $t_{28} = 4.24$, $P < 0.01$) with $47.59 \pm 9.19\%$ (mean ± SE) of dark males damaged compared with $11.18 \pm 1.92\%$ of pale males. This difference was not simply the result of dark males tending to be in figs with high male densities as injury rates among dark males were higher irrespective of density (ANCOVA: $F_{3,76} = 9.39$, $P < 0.01$). In both forms, mandible length was significantly correlated with head length [regression of mandible length (y) and head length (x) in pale males: $y = 0.96x + 0.04$; dark males: $y = 0.68x + 0.21$] (Fig. 1). The slopes of the relationship were significantly different (ANCOVA, $P < 0.05$), but with no difference in intercepts ($P = 0.10$). Mandible length increased relatively more rapidly in pale males.

Dark and pale *Walkerella* males had similar mean distances to the periphery of the figs, but females were significantly closer to the periphery than the males (Table 1).

Experimental manipulation of foundress numbers

Progeny numbers (Table 2) increased with increasing numbers of foundresses (ANOVA: $F_{2,21} = 12.34$, $P < 0.01$). The average number of male offspring in figs with one foundress was also less than with three (ANOVA: $F_{1,18} = 33.85$, $P < 0.01$) or five foundresses (ANOVA: $F_{1,18} = 28.13$, $P < 0.01$). Progeny sex ratios were highly variable between figs and did not differ between foundress densities (GLM: Slope $= \beta \pm \text{SE} = 0.11 \pm 0.18$, $P = 0.53$, $n = 22$, Table 1). None of the 16 figs with a single foundress contained dark male offspring, which were only present in figs with multiple foundresses (Table 2). However, the proportion of dark males did not differ between figs with three and five foundresses (GLM: Slope $= \beta \pm \text{SE} = 0.14 \pm 0.90$, $P = 0.87$, $n = 22$, Table 1). Overall, the dark morph occurred at a frequency of 0.24. If dark males were determined by the presence of a single allele (they are haploid), then the chance of observing 16 figs that did not contain the allele would be less than 0.01. This suggests there is not a genetic basis for the dimorphism.

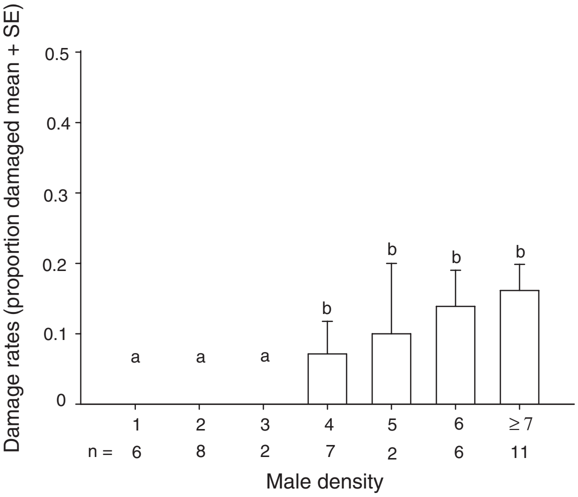

Fig. 4. The proportions of males with damaged mandibles in relation to male density. Bars with different letters are significantly different (ANOVA, $P < 0.05$). Both pale and dark males are included.

Male dispersal and survival outside the figs

Around one quarter of the 135 males emerged from the 30 figs ($28.6\% \pm 5.7$, mean ± SE). Both dark and pale males emerged, but dark males were more likely to do so ($36.0\% \pm 8.1$ dark males compared with $22.6\% \pm 6.4$ pale males, mean ± SE, paired t-test: $t_{21} = -2.28$, $P < 0.05$). Male *Walkerella* sp. only survived a few hours once they vacated the figs, with pale males surviving 9.1 ± 0.7 h compared with 10.84 ± 0.55 h for dark males (mean ± SE, $n = 31$ and 29). There was no overall difference in rates of survivorship, but pale males tended to die more rapidly during the period 7–10 h after removal from the figs (Fig. 5).

Discussion

Walkerella sp., like most NPFW, oviposits from the outside of the figs. This produces a quite different reproductive

Table 1. The location of galls occupied by *Walkerella* sp. and other fig wasps based on distances from the inner edges of galled ovules to the inner wall of the figs (pedicel length plus ovule length).

Fig wasps	*n*	Mean distance (mm) (SD)	Fig wasps	*n*	Mean distance (mm) (SD)	*t*	*P*
Walkerella females	278	2.48 (0.83)	*Walkerella* males	100	3.12 (0.98)	6.25	<0.01
Dark males	35	3.10 (0.98)	Pale males	65	3.14 (1.00)	1.02	>0.05

Table 2. Brood size and composition in figs with different numbers of *Walkerella* sp. foundresses.

	Foundress density	Mean ± SE	Range
Brood size	1	7.75 ± 0.77	2–12
	3	15.00 ± 2.52	10–18
	5	15.67 ± 1.45	13–18
Brood sex ratio	1	0.29 ± 0.05	0.13–1.00
	3	0.40 ± 0.03	0.35–0.44
	5	0.36 ± 0.16	0.25–0.54
Females	1	5.81 ± 0.68	2–9
	3	9.00 ± 1.53	6–11
	5	10.33 ± 2.19	6–13
Total males	1	1.94 ± 0.23	1–4
	3	6.00 ± 1.15	4–8
	5	5.33 ± 0.88	4–7
Pale males	1	1.94 ± 0.23	1–4
	3	5.00 ± 0.58	4–6
	5	4.33 ± 0.33	4–5
Dark males	1	0.00 ± 0.00	0
	3	1.00 ± 1.73	0–3
	5	1.00 ± 1.73	0–3
Proportion dark	1	0.00 ± 0.00	0–0
	3	0.13 ± 0.13	0–0.38
	5	0.14 ± 0.14	0–0.43

Sample sizes were 16, three, and three figs for one, three, and five foundresses, respectively.

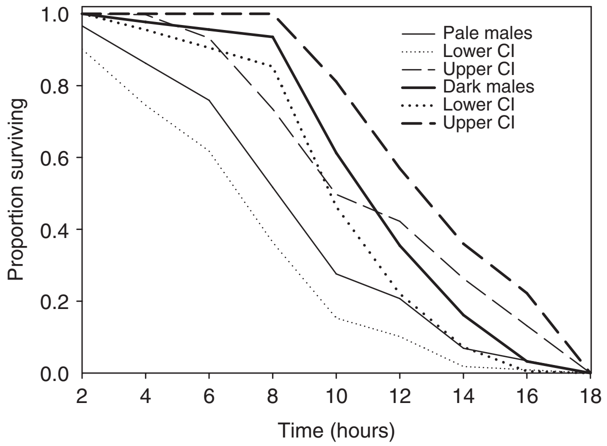

Fig. 5. Survivorship of dark and light *Walkerella* sp. males under laboratory conditions after being removed from their figs (means with 95% confidence intervals). Note that the confidence intervals only fail to overlap for a brief period from about 6–10 h after the males were removed from the figs.

environment from pollinator fig wasps because *Walkerella* sp. foundresses can disperse their eggs across many figs, laying fewer eggs in each. As a result, matings among their progeny are less likely to be between siblings, so males are less likely to be competing with their brothers for mating opportunities. Individuals are also more likely to find themselves without any conspecifics sharing their figs, forcing them to disperse to find a mate (Fellowes *et al*., 1999).

As is typical of NPFW, the sex ratio of *Walkerella* sp. was only slightly female-biased (Fellowes *et al*., 1999; Pereira & Prada, 2008). Unlike pollinator fig wasps, progeny sex ratios were not responsive to foundress numbers (even when females were forced to be on figs at the same time), nor the number of eggs that were laid (Raja *et al*., 2008). The sex ratio in *Walkerella* sp. therefore appears to be independent of local factors operating at the level of individual figs.

Males are present in almost all NPFW, the exception being certain specialist hyperparasitoid fig wasps, where males occur infrequently if at all (Compton *et al*., 2009). NPFW have been recorded with up to three or more distinct male morphs and those with a single form of male may have only winged or only wingless males. Analysis of the phylogeny of *Philotrypesis*, a group where male form is particularly labile, has shown that lineages are rapidly responsive to variation in the reproductive environments provided by different host fig species (Jousselin *et al*., 2004). Their results also suggest that winged males can re-appear in lineages where they had previously been lost. This is in contrast to the Otitesellinae, the subfamily that includes *Walkerella* sp., where the ability to produce winged males has apparently been totally lost.

Male dimorphism in *Walkerella* sp. provides an interesting contrast with species of the *digitata* group of *Otitesella*. The religiosa morphs that tend to stay within their figs are often specialised for fighting, although smaller individuals can be ‘sneaky’ (Moore *et al*., 2004). Differences in appearance in *Walkerella* were less dramatic, but there were allometric differences between the morphs, with mandible length increasing more rapidly in pale males. A further contrast with the *digitata* group of *Otitesella* is provided by the locations where the larvae develop inside the figs. Moore *et al*. (2004) found that their females and the more actively dispersing male morph of two *Otitesella* species were more likely to be found towards the centre of their figs, whereas in *Walkerella* sp. both male morphs were located more centrally than the females. Given that more peripheral ovules are more readily accessible to ovipositing foundresses, and may be galled first, this suggests that the sequence of oviposition of male and female eggs may be different in these two genera.

Walkerella sp. males fight using their mandibles and can kill each other. No damaged males were found in figs containing three or fewer males, showing that fighting is density-related (Murray, 1987; Pereira & Prada, 2008). Dark males were produced in figs where other males were likely to be present, which may favour a willingness to fight over females, despite the lack of any relationship between combined brood sizes and overall sex ratios in the figs (Nelson & Greeff, 2009). Their greater willingness to emerge from figs means that they are more likely to find themselves in situations where they will be fighting with unrelated males (Hamilton, 1979; Greeff, 1997; West *et al*., 2001), but evidence from pollinator fig wasps suggests that this is not linked to a greater likelihood of fighting (Nelson & Greeff, 2009).

The most obvious difference between the two male morphs of *Walkerella* sp. is in their colour, with one morph much darker than the other. Darkening of the insect cuticle follows a moult and results from sclerotisation and melanisation (Andersen *et al*., 1996). One of the major functions of the cuticle is to reduce water loss from the body (Schwarz &

Moussian, 2007) and darker coloured insects are typically more desiccation resistant, as demonstrated in *Blattella germanica* (Appel & Tanley, 1999) and several *Drosophila* species (Parkash *et al*., 2008; Singh *et al*., 2009). Melanin production requires amino acids (Blois, 1978), and life-history costs associated with increased melanin production have been identified (Windig, 1999). Within-species variation in the extent of melanisation has been linked to both genetic (Windig, 1999) and environmental factors, such as temperature (Stoehr & Goux, 2008) and rearing densities (Thompson *et al*., 2002). In *Walkerella* males, greater melanisation appears to be associated with an increased likelihood of exposure to sunlight among dark males.

Walkerella sp. morph ratios varied in response to local conditions. The dark males of *Walkerella* sp. were consistently in a minority, but a higher proportion of them were produced in figs where more offspring were present. Furthermore, no dark sons were produced by lone foundresses: they only appeared when several females were present on a fig at the same time. An alternative possibility is that dark sons might be an induced response to factors acting on *Walkerella* sp. larvae within the figs. Starvation can induce darker coloration amongst developing *Tenebrio molitor* larvae (Schawang & Janovy, 2001) and higher larval densities might increase competition for resources amongst *Walkerella* sp. larvae. However, the numbers of ovules galled by *Walkerella* sp. are only a small proportion of the total flowers inside the figs, which also typically contain numerous ovules galled by pollinators and other NPFW, as well as developing seeds, so phenotypic effects driven by food shortages seem unlikely. One possibility is that *Walkerella* sp. foundresses might respond to chemical markers released by earlier females during oviposition. Alternatively, dark males might be produced in response to the physical presence of other females at the time of oviposition.

Moving between figs in search of mates has inherent risks for the small, delicate and flightless males of *Walkerella* sp., but in figs where all the conspecific females have mated, or where no females are present, as is the case here within a minority of the figs, selection will clearly favour males that are willing and able to find mates in other figs. Both male morphs of *Walkerella* sp. have the ability to disperse and mate in other figs, but dark males were more likely to attempt to do so. Their colour may be an adaptation linked to their greater willingness for dispersal, and the heightened risks of dehydration that it presents. Dark males only survived longer than pale males during a period a few hours after exposure to desiccating conditions, a difference in survivorship that was much less than that detected between two different species of fig wasps (Warren *et al*., 2010). This brief period of greater survivorship among dark male *Walkerella* sp. may nonetheless still be important if it gives them a better chance of encountering unmated females.

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References

Andersen, S.O., Peter, M.G. & Roepstorff, P. (1996) Cuticular sclerotization in insects. *Comparative Biochemistry and Physiology*, **113**, 689–705.

Appel, A.G. & Tanley, M.J. (1999) Water composition and loss by body color and form mutants of the German cockroach (Dictyoptera: Blattellidae). *Comparative Biochemistry and Physiology A*, **122**, 415–420.

Bai, L.F., Yang, D.R. & Compton, S.G. (2008) A gall midge inhabiting the figs of *Ficus benjamina* in Xishuangbanna, south-western China. *Symbiosis*, **45**, 149–152.

Bean, D. & Cook, J.M. (2001) Male mating tactics and lethal combat in the nonpollinating fig wasp *Sycoscapter australis*. *Animal Behavior*, **62**, 535–542.

Blois, M.S. (1978) The melanins: their synthesis and structure. *Photochemical and Photobiological Reviews*, Vol. 3 (ed. by K. C. Smith), pp. 115–134. Plenum, New York, New York.

Compton, S.G., van Noort, S., Mcleish, M., Deeble, M. & Stone, V. (2009) Sneaky African fig wasps that oviposit through holes drilled by other species. *African Natural History*, **5**, 9–15.

Cook, J.M. & Bean, D. (2006) Cryptic male dimorphism and fighting in a fig wasp. *Animal Behaviour*, **71**, 1095–1101.

Cook, J.M., Compton, S.G., Herre, E.A. & West, S.A. (1997) Alternative mating tactics and extreme male dimorphism in fig wasps. *Proceedings of the Royal Society of London B*, **264**, 747–754.

Cook, J.M., Bean, D. & Power, S. (1999) Fatal fighting in fig wasps – GBH in time and space. *Trends in Ecology & Evolution*, **14**, 257–259.

Dalgaard, P. (2004) *Introductory Statistics with R. Survival Analysis.* Springer, New York, New York.

Fellowes, M.D.E., Compton, S.G. & Cook, J.M. (1999) Sex allocation and local mate competition in Old World non-pollinating fig wasps. *Behavioral Ecology and Sociobiology*, **46**, 95–102.

Greeff, J.M. (1997) Offspring allocation in externally ovipositing fig wasps with varying clutch size and sex ratio. *Behavioral Ecology*, **8**, 500–505.

Greeff, J.M. & Ferguson, J.W.H. (1999) Mating ecology of the nonpollinating fig wasps of *Ficus ingens*. *Animal Behaviour*, **57**, 215–222.

Greeff, J.M., van Noort, S., Rasplus, J.Y. & Kjellberg, F. (2003) Dispersal and fighting in male pollinating fig wasps. *Comptes Rendus Biologies*, **326**, 121–130.

Hamilton, W.D. (1967) Extraordinary sex ratios. *Science*, **156**, 477–488.

Hamilton, W.D. (1979) Wingless and fighting males in fig wasps and other insects. *Sexual Selection and Reproductive Competition in Insects* (ed. by M. S. Blum and N. A. Blum), pp. 167–220. Academic Press, New York, New York.

Hardy, I.C.W. & Mayhew, P.J. (1998) Partial local mating and the sex ratio: indirect comparative evidence. *Trends in Ecology & Evolution*, **11**, 431–432.

Herre, E.A., West, S.A., Cook, J.M., Compton, S.G. & Kjellberg, F. (1997) Fig-associated wasps: pollinators and parasites, sex ratio adjustment and male polymorphism, population structure and its consequences. *Insect Mating Systems* (ed. by J. Choe and B. Crespi), pp. 226–239. Cambridge University Press, Cambridge, U.K.

Jousselin, E., van Noort, S. & Greeff, J.M. (2004) Labile male morphology and intraspecific male polymorphism in the *Philotrypesis* fig wasps. *Molecular Phylogenetics and Evolution*, **33**, 706–718.

Moore, J.C., Pienaar, J. & Greeff, J.M. (2004) Male morphological variation and the determinants of body size in two Otiteselline fig wasps. *Behavioral Ecology*, **15**, 735–741.

Moore, J.C., Loggenberg, A. & Greeff, J.M. (2006) Kin competition promotes dispersal in a male pollinating fig wasp. *Biology Letters*, **2**, 17–19.

Moore, J.C., Obbard, D.J., Reuter, C., West, S.A. & Cook, J.M. (2009) Male morphology and dishonest signalling in a fig wasp. *Animal Behaviour*, **78**, 147–153.

Murray, M.G. (1987) The closed environment of the fig receptacle and its influence on male conflict in the Old World fig wasp, *Philotrypesis pilosa*. *Animal Behaviour*, **35**, 488–506.

Murray, M.G. (1990) Comparative morphology and mate competition of flightless male fig wasps. *Animal Behaviour*, **39**, 434–443.

Nelson, R.M. & Greeff, J.M. (2009) Evolution of the scale and manner of brother competition in pollinating fig wasps. *Animal Behaviour*, **77**, 693–700.

Niu, L.M., Hu, H.Y., Huang, D.W., Fu, Y.G. & Peng, Z.Q. (2009) Brood size: a major factor influencing male dimorphism in the non-pollinating fig wasp *Sycobia* sp. *Ecological Entomology*, **34**, 696–701.

Parkash, R., Rajpurohit, S. & Ramniwas, S. (2008) Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *D. melanogaster*. *Journal of Insect Physiology*, **54**, 1050–1056.

Pereira, R.A.S. & Prada, A.P. (2008) Mating strategies and aggressive combat in wingless Neotropical wasps. *Brazilian Archives of Biology and Technology*, **51**, 753–760.

Pienaar, J. & Greeff, J.M. (2003a) Maternal control of offspring sex and male morphology in the *Otitesella* fig wasps. *Journal of Evolutionary Biology*, **16**, 244–253.

Pienaar, J. & Greef, J.M. (2003b) Different male morphs of *Otitesella pseudoserrata* fig wasps have equal fitness but are not determined by different alleles. *Ecology Letters*, **6**, 286–289.

Pienaar, J. & Greeff, J.M. (2006) Adaptive non-Fisherian sex ratios in a patchily distributed population with outbreeding. *Evolutionary Ecology*, **20**, 431–445.

Raja, S., Suleman, N., Compton, S.G. & Moore, J.C. (2008) The mechanism of sex ratio adjustment in a pollinating fig wasp. *Proceedings of the Royal Society of London B*, **275**, 1603–1610.

Schawang, J.E. & Janovy, J.J. (2001) The response of *Gregarina niphandrodes* (Apicomplexa: Eugregarinida: Septatina) to host starvation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults. *Journal of Parasitology*, **87**, 600–605.

Schwarz, H. & Moussian, B. (2007) Electron-microscopic and genetic dissection of arthropod cuticle differentiation. *Modern Research and Educational Topics in Microscopy* (ed. by A. Mendéz-Vilas and J. Diaz), pp. 316–325. Formatex Research Center, Badajoz, Spain.

Singh, S., Ramniwas, S. & Parkash, R. (2009) Fitness consequences of body melanization in *Drosophila immigrans* from montane habitats. *Entomological Research*, **39**, 182–191.

Song, B., Peng, Y.Q. & Yang, D.R. (2008) The role of foundress relatedness in the offspring sex ratio of fig wasp *Diaziella yangi* (Hymenoptera:Pteromalidae). *Acta Entomologica Sinica*, **51**, 411–416.

Stoehr, A.M. & Goux, H. (2008) Seasonal phenotypic plasticity of wing melanisation in the cabbage white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae). *Ecological Entomology*, **33**, 137–143.

Thompson, J.J.W., Armitage, S.A.O. & Siva-Jothy, M.T. (2002) Cuticular colour change after imaginal eclosion is time-constrained: blacker beetles darken faster. *Physiological Entomology*, **27**, 136–141.

Vincent, S. (1991) *Polymorphism and fighting in male fig wasps*. Unpublished PhD thesis, Rhodes University, Grahamstown, South Africa.

Vincent, S. & Compton, S.G. (1992) A new polymorphic fig wasp from *Ficus ingens* (Moraceae). *African Journal of Zoology*, **104**, 363–370.

Warren, M., Robertson, M.P. & Greeff, J.M. (2010) A comparative approach to understanding factors limiting abundance patterns and distributions in a fig tree–fig wasp mutualism. *Ecography*, **33**, 148–158.

West, S.A. & Herre, E.A. (1998) Partial local mate competition and the sex ratio: a study on non-pollinating fig wasps. *Journal of Evolutionary Biology*, **11**, 531–548.

West, S.A., Murray, M.G., Machado, C.A., Griffin, A.S. & Herre, E.A. (2001) Testing Hamilton's rule with competition between relatives. *Nature*, **409**, 510–513.

Windig, J.L. (1999) Trade-offs between melanization, development time and adult size in *Inachis io* and *Araschnia levana* (Lepidoptera: Nymphalidae). *Heredity*, **82**, 57–68.

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