Causes of fighting in male pollinating fig wasps

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"Thou shalt not covet, but tradition approves all forms of competition."

Arthur Hugh Clough

Summary

A striking variation in the behaviour of pollinating fig wasps (Agonidae) is the occurrence of male fighting in some species while in others it is completely absent. Fighting behaviour was investigated at two levels.

Firstly, the variation in fighting behaviour between the species was used to examine factors that might cause the evolution thereof. Comparisons across species were done using phylogenetic regression. This method takes similarity due to phylogenetic constraints into account when data are compared. Kin selection theory implies that fighting is barred by the high degree of relatedness in competing males. We however find that the relatedness of the males do not have an influence on the evolution of fighting and this finding supports models suggesting that high LMC cancels benefits due to relatedness. Rather, that the only factor having a significant correlation with fighting is the release sex ratio. The release sex ratio and dispersal is also associated. Fighting and dispersal are not expected to have direct influence on each other and the association of both with the release sex ratio imply that this may be an indirect link between these two behaviours. A syndrome where fighting and dispersal is found together is in part explained by the release sex ratio. We conclude that the release sex ratio is the most likely cause of the evolution of fighting behaviour in pollinating fig wasps.

The second part of this study deals with the proximal determinants causing fighting, in the males of the species *Platyscapa awekei.* We show that the sex ratio which, is less female biased than non-fighting pollinator species, rapidly becomes even less female biased as soon as both sexes becomes active. Numerous fights are fought by the males in the female limited environment. The activity of the wasps is shown to be regulated by the gaseous environment, which change from a high to a low CO_2

concentration with the construction of an exit hole from the fig. The males of the species *P. awekei* are inactive, and do not engage in mating or fighting activities, in high CO_2, contrasted to males of other species, which are active in this environment. *P. awekei* females rapidly release once the CO_2 level is lowered and mating behaviour is only observed in this environment. The number of female to male encounters of every male decrease as the operational sex ratio becomes less female biased. Male fighting in this species is therefore expected due to the high sex ratio, which is enforced by the increase thereof. We conclude that the physical environment, in this species, affects the mating environment. The resultant reduction in the number of potential mating opportunities therefore escalates fighting between the males.

To Jaco: for keeping me on the scientific track

Acknowledgments

Preface

This study is inspired by various unique aspects of fig wasp life history (brought to my attention by my supervisor). Pollinator fig wasps are easily obtainable and many of their novel characteristics are measurable, including the skewed sex ratios and their fighting ability. This enables us to ask questions and test theories mating systems, sexual selection and altruism. The work contained in this study is therefore part of ongoing research on fig wasps, as model organisms, to answer questions related to many aspects of evolution.

Fighting, in some species, of male pollinating fig wasp was recently described, providing an opportunity for novel research in this area. The material in this study is consequently, largely concerned with this behaviour, the evolution thereof and other aspects connected with it. Each chapter is written as a journal article, which therefore has its own introduction, materials and methods, results and discussion sections. An introduction that precedes the two chapters introduces the reader to the general issues. This study ends with a conclusion section, highlighting the key findings.

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Introduction

Sexual selection, first described by Darwin (1859), is used to explain why males will adopt risky morphological and behavioural characteristics to obtain females. Many different types of competition have evolved to maximise the fitness of each male in the population who adopts the strategy (Maynard Smith & Price, 1973; Maynard Smith, 1974). A great number of theoretical and empirical studies have been published, advancing our understanding of how competition between and within the sexes, are influenced by the life history and environmental factors in which they find themselves (Maynard Smith & Price, 1973; Clutton-Brock & Parker, 1992; Andersson, 1994; Andersson & Iwasa, 1996; Reinholds, 1996). However, many questions remain on how and why particular strategies prevail in certain conditions. Empirical studies quantifying life histories and environmental factors as well as the type and degree of competition are needed to test theories regarding the evolution of competitive behaviour. This will enable us to improve our knowledge on the dynamics of sexual selection and the evolution of male conflict.

The question, "why does male fighting behaviour evolve?" focuses on the ultimate (*sensu* Tinbergen, 1963) determinants (selective pressures) of fighting behaviour. These factors are expected to drive the evolution of male fighting in successive generations. In addition, a correlation between fighting behaviour and these determinants are expected between different species. Comparative studies (see below) are therefore well suited to elucidate the selective pressures that promote the evolution of a specific behaviour such as male fighting. Theory emphasises the role played by the value of females, the operational sex ratio (OSR), the reproductive rate (PPR) and female choice on the

evolution of male fighting behaviour (Emlen & Oring, 1977; Clutton-Brock & Vincent, 1991; Andersson, 1994).

Contests between animals are largely affected by the value of the resources relative to the cost of fighting (Murray & Gerrard, 1985; Enquist & Leimar, 1987; Enquist & Leimar, 1990; Andersson, 1994). In male contests, access to receptive females is often the resource fought over. Here an increase in the relative value of the females will increase the probability of fighting between the males (Murray & Gerrard, 1985; Enquist & Leimar, 1987).

A very important factor, which may influence the evolution of male contests, is the OSR. Defined by Emlen and Oring (1977), as the ratio of receptive females to sexually active males, the OSR encapsulates a number of factors believed to be involved in the evolution of fighting. If the males can readily access a number of females without encountering one another (i.e. a female biased OSR), they have less chance or cause to compete and the evolution of fighting is less likely (Emlen & Oring, 1977). In a male biased OSR, where males encounter each other more often than they encounter receptive females, contests for females might ensue which may favour the evolution of fighting (Emlen & Oring, 1977). Several authors have explained how factors such as the temporal and spatial distribution of resources, the search time, extraction rate and challenge frequency determine the OSR (Emlen & Oring, 1977; Andersson, 1994), and thus affect the evolution of fighting behaviour (Murray & Gerrard, 1985; Enquist & Leimar, 1987; Enquist & Leimar, 1990). In short, we would expect fighting between males where: females are clumped but defendable (facilitating polygyny), males have long search times for females or long mating rituals, and males often encounter and challenge each other. It is important to remember however, that the OSR is only one of the factors affecting the evolution of fighting (Clutton-Brock & Parker, 1992).

Another factor that may increase the value of females relative to males is the usual skew found in the potential reproductive rate PRR of males and females (Clutton-Brock & Vincent, 1991). Bateman (1948) was the first to show empirically that the relationship between mating success and offspring reproduction, which is usually different between the sexes, has an effect on the strength and direction of sexual selection. Males normally have much steeper relationship between mating success and fecundity than females (Bateman, 1948; Andersson, 1994; Arnold & Duvall, 1994). Simply stated, males can usually sire more offspring when they mate with many females while females will not be able to produce more offspring if they mate with many males. The sex with the steeper fecundity-mating success slope has the lower relative value. In addition, females normally invest more per reproductive event than the males (Bateman, 1948; Clutton-Brock & Vincent, 1991). The PRR is largely affected by the proportion of time or energy each sex invests in their progeny (Bateman, 1948; Clutton-Brock & Vincent, 1991; Clutton-Brock & Parker, 1992). The sex, which invests more per reproductive event, is able to reproduce less frequently and this will also bias the OSR towards the other sex (Clutton-Brock & Parker, 1992). In species where the females can reproduce only once (although they can be mated several times), while the males are able to sire more than one brood, the PPR for the females are very low compared to that of the males. Under these circumstances the females have a higher value than the males and sexual selection will be stronger on the males, which in turn will favour the evolution of male fighting.

An additional factor that may cause the evolution of competitive behaviour, is female choice (or the lack thereof in some cases). Many theories explain the dynamics of male fighting when females are choosy (Andersson, 1994; Reinholds, 1996; Brown *et al.*, 1997). The general trend is that the absence of female choice increase male competition (Andersson, 1994; Alexander *et al.*, 1997; Brown *et al.*, 1997). Hence, in

species where the females are mated in a coercive fashion, the females cannot reject males and the direct competition between the males, for access to females, is escalated.

Fighting behaviour can also be looked at from a proximal view by asking the question: "why do males fight?", while we examine the sensory cues and the resulting mechanistic reactions. It is therefore important to look at the information that trigger fighting between individuals (Tinbergen, 1963). Decisions to fight are therefore based on the assessment on relative strengths (Enquist & Leimar, 1983), costs of fighting and the value of the reward (Maynard Smith & Price, 1973; Maynard Smith, 1974). For example, an individual may decide to fight if his opponent is smaller, weaker or slower and the value of the reward is high.

In the study of competitive behaviour it is very important to take into account the effect that relatedness may have. Intuitively, we expect less severe competition between related than unrelated individuals. Theory developed by Hamilton confirms that relatives should show less aggression towards each other due to inclusive fitness effects (Hamilton, 1963; Hamilton, 1964). Inclusive fitness includes both the fitness components obtained through personal reproduction of an individual and reproduction of his relatives (Hamilton, 1964). If however, the inclusive fitness of an individual is not maximised by altruism (or less than normal aggression) towards kin, competition between relatives may arise. These situations arise when relatives have high levels of local competition (Grafen, 1984; Murray, 1984; West *et al.*, 2001; Griffin & West, 2002). Local mate competition (LMC) implies that local competition takes place as a result of limited or no dispersal. Consequently, many individuals in these populations will be related. Studies that focussed on the relationships between the degree of competition, the scale of competition (local or global) and the relatedness of the competing individuals, indicated that the conflict limiting effect cancels when competition is mostly local (Grafen, 1984; Murray, 1984; West *et al.*, 2001; Griffin & West, 2002). Consequently, in circumstances

where there is no or little dispersal, fighting between relatives and non-relatives should be indistinguishable.

Comparisons of similar and different characteristics between species are a powerful way to reveal selective pressures (ultimate determinants) that may cause complex traits, such as male fighting. Correlations between a response variable (e.g. fighting) and an explanatory variable (e.g. agility) are tested for in different species through comparative methods. The main obstacle with comparisons across species is phylogenetic non-independence (Felsenstein, 1985; Grafen, 1989). Each species cannot be assumed to be a statistically independent data point as they share a common ancestry and some species will be more related than other. Phylogenetic inertia may therefore play a roll in correlations found between the response and explanatory variables and type 2 errors may occur. A number of suitable methods have been developed to deal with phylogenetic non-independence while comparing discrete variables (Grafen & Ridley, 1996; Ridley & Grafen, 1996). Grafen (1989) however, developed the phylogenetic regression, which is able to test both continuous and categorical variables (Grafen & Ridley, 1996), in a generalised linear model context, while taking the phylogenetic constraints into account. In this method, the phylogeny is incorporated and the branch lengths are distorted by expanding or compressing different regions of the tree. Depending on this distortion, weighting will emphasise changes closer to the tips or closer to the roots, indicating late or early evolution respectively, as specified by the data. This is taken into account during the linear regression (Grafen, 1989). Factors influencing the evolution of male fighting behaviour may therefore be elucidated by using this method when characters are compared between fighting and non-fighting species.

Pollinating fig wasps have been utilized in various empirical studies to test theoretical issues and a great deal is known about their; mating ecology (Godfray, 1988;

Herre *et al.*, 1997; Greeff, 2002; Moore *et al.*, 2002), phylogenetic background (Wiebes, 1982; Machado *et al.*, 1996; Machado *et al.*, 2001), interaction with fig trees (Ramirez, 1970; Bronstein, 1988b; Bronstein, 1988a; Compton *et al.*, 1996; Herre *et al.*, 1996; Anstett *et al.*, 1997; Cook & Lopez-Vaamonde, 2001; Cook & Rasplus, 2003) and life history in general (Hamilton, 1979; Herre *et al.*, 1997; Zammit & Schwarz, 2000; Greeff *et al.*, 2003). One or a few females, known as foundresses, crawl into a receptive fig and lay their eggs within the flowers. After development the wingless males eclose first and mate with the females while still in their respective galls (Hamilton, 1979). Mating is mostly between siblings, because of the low foundress number (see below; (Hamilton, 1979; Janzen, 1979). As a result the foundress females produce extreme female biased sex ratio to limit the high level of LMC between the males (Hamilton, 1967; Hamilton, 1979). After mating, females disperse from the fig, through a hole chewed by the males, to new receptive figs and start the cycle anew. Pollinator fig wasps species are mostly associated in a one to one relationship with specific host fig tree species (Ramirez, 1970; Janzen, 1979; Wiebes, 1979; Corner, 1985; Cook & Rasplus, 2003). Recent discoveries about the type and degree of competition between pollinator males (Greeff *et al.*, 2003), provides new opportunities to test established theories but also provide data for new theoretical work.

A number of species of pollinator male fig wasps engage in contest competition and severe fighting is seen, while in other species it is completely absent (Greeff *et al.*, 2003). Fighting in male pollinating fig wasps is unexpected according to earlier kin selection models (Hamilton, 1963; Hamilton, 1964; Hamilton, 1972; Grafen, 1984). The extreme female biased sex ratio is another factor which should bar male contests (Hamilton, 1967; Emlen & Oring, 1977). Furthermore, it is known that some species of pollinator males disperse actively (Greeff *et al.*, 2003). These observations led the way

to questions such as: Why do fighting evolve in some species but not in others? And: How do the mating ecology influence the fighting behaviour and *vice versa*?

In chapter one we compare data from fighting and non-fighting species to test theories on the evolution of fighting behaviour. These include the relatedness of the competing individuals (Hamilton, 1964; Grafen, 1984; Murray, 1984; West *et al.*, 2001), the sex ratio (Hamilton, 1967; Emlen & Oring, 1977; Frank, 1985; Enquist & Leimar, 1987), the number of female offspring (Emlen & Oring, 1977; Enquist & Leimar, 1987; West *et al.*, 2001) and the internal structure of the fig (Vincent, 1991; Greeff *et al.*, 2003). Each of these could potentially affect the competition between males.

In chapter two we quantify fighting and mating behaviour of the pollinator fig wasp species, *Platyscapa awekei.* This ecological data enabled us to determine that the sex ratio is the major cause of fighting in this species. The effect of the environment on the wasp's behaviour is in turn revealed.

Chapter 1

The evolution of fighting in male pollinating fig wasps

Abstract

Male contest competition is found in some species of pollinating fig wasps. Fighting and dispersal by males in this family is an uncommon behaviour because competition is mostly between brothers in an extremely female biased mating environment. In support of the new generation of kin selection models, we find that relatedness of competing males does not have an influence on the evolution of fighting since the local scale of competition leads to the cancelling of inclusive fitness benefits. Comparisons between species were done using phylogenetic regression. We found that the only factor having a significant relationship with fighting is the release sex ratio. The release sex ratio and male dispersal are also associated. Theoretically, fighting and dispersal are not expected to have a direct influence on each other and the association of both with the release sex ratio imply that this may be an indirect link between these two behaviours. Sex ratio may be the causal link between fighting and dispersal. We conclude that an increased ratio of males per female led to the evolution of fighting in pollinating fig wasps.

Introduction

A striking variation in the behaviour of pollinating fig wasps is the occurrence of male fighting in some species while it is completely absent in others (Michaloud, 1988; Greeff *et al.*, 2003). This is curious because, firstly, individuals involved in the contests are often brothers and secondly, the sex ratios of pollinating fig wasps are, as a rule, extremely female biased. Mothers try to limit unnecessary rivalry between brothers by producing more female biased sex ratios, when foundress densities are low, and conversely, less female biased sex ratios when foundress densities are high (Hamilton, 1967). The OSR, would be an indication of the probability that a male may encounter a receptive female, or another male (Emlen & Oring, 1977). Extremely skewed sex ratios should lead to a female biased sex ratio which would, in turn, prevent, or at least reduce the evolution of fighting (Emlen & Oring, 1977).

Various factors that may play an important role in the evolution of fighting, in addition to the relatedness of competing males, have been put forward. These include the sex ratio (Hamilton, 1967; Emlen & Oring, 1977; Frank, 1985; Enquist & Leimar, 1987), the number of female offspring (Emlen & Oring, 1977; Enquist & Leimar, 1987; West *et al.*, 2001), the risk of entrapment in a fig with too few males to construct an exit tunnel (Hamilton, 1979; Godfray, 1988) and the internal structure of the fig (Vincent, 1991; Greeff *et al.*, 2003). Theories on how these factors may influence each other are summarised in figure 1.1, and we test these to identify factors influencing fighting.

When close relatives are constrained to compete locally against each other, it is expected that the conflict limiting effects of relatedness are cancelled (Grafen, 1984; Murray, 1984; Griffin & West, 2002). West *el al.* (2001) recently showed empirically, that fighting could evolve in populations with highly related individuals competing. They found
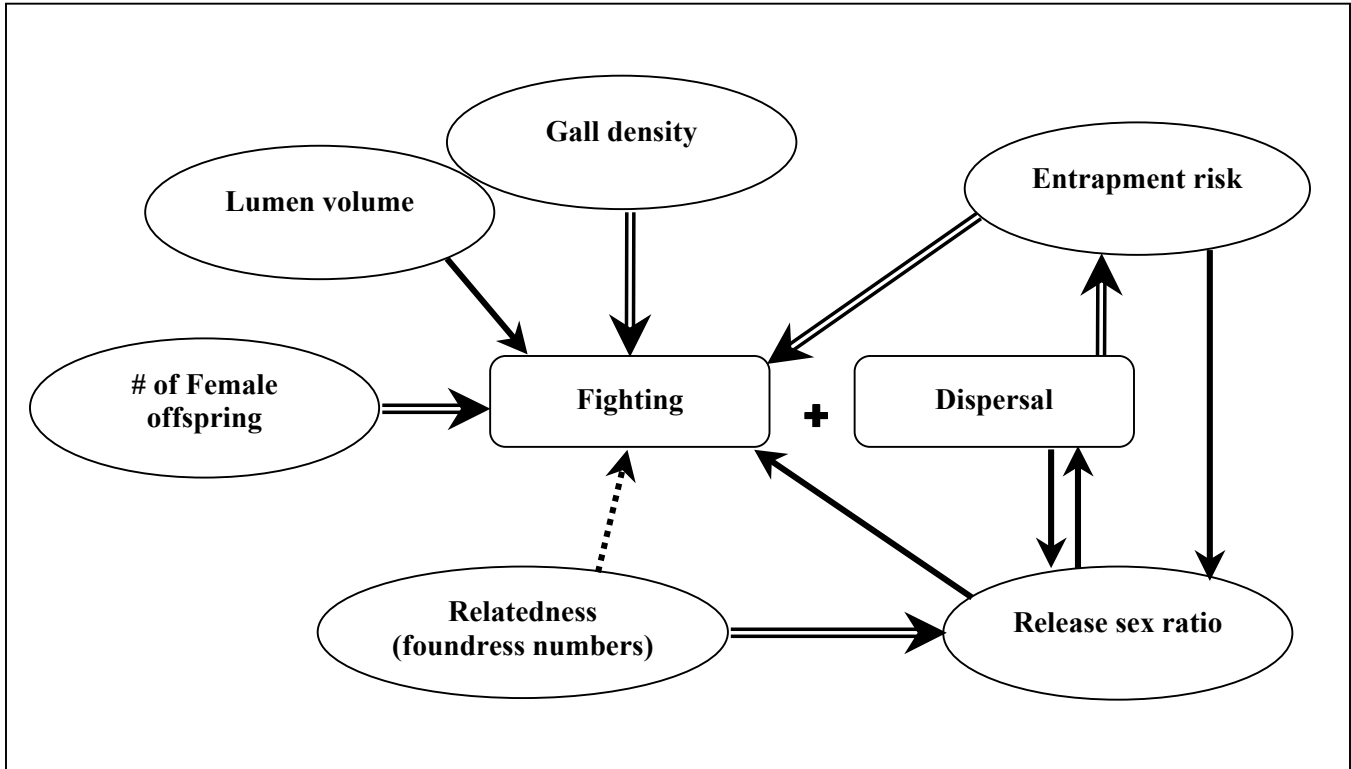
Figure 1.1. A representation of the possible interactions between the factors hypothesised to be involved in the evolution of fighting and dispersal. --- previously suggested, theoretically incorrect and not expected; = negative effect if increased; − enhancing effect if increased. See text for more detailed explanations.

no correlation between relatedness and the level of aggression in male fighting fig wasps, which suggest that the conflict limiting effect of relatedness is indeed cancelled. Their estimate of relatedness is however based on the release sex ratio (West *et al.*, 2001), which is not a very accurate measure of relatedness (Antolin, 1999; Greeff, 2002; Kjellberg *et al.,* in press). In the case of pollinating wasps a more direct method of gauging relatedness between competing males would be to count the number of foundresses per fig (Herre *et al.*, 1997).

The OSR, defined as the ratio of sexually active males to receptive females (Emlen & Oring, 1977), is an important factor that may play a role in the evolution of

male fighting. When males can access multiple females without encountering each other (i.e. a female biased OSR), they will be less likely to compete. If however, males frequently encounter females in the presence of other males (i.e. a less female biased OSR), competition may ensue as each male tries to mate with the contested females (Emlen & Oring, 1977).

Connected to the argument of sex ratio influencing fighting, is the total number of females in the fig. If there are many females, the relative value of each female will be low and male competition will be less likely to evolve (Emlen & Oring, 1977; Murray & Gerrard, 1985; Enquist & Leimar, 1987). If there are only a few females, the value of this resource increases and the probability of fighting increases (Enquist & Leimar, 1987). Resource value is determined by the spatial and temporal dispersion thereof (Emlen & Oring, 1977). West *el al.* (2001) found the number of female offspring to have a significant effect on the evolution of fighting in fig wasps. They argued and showed that the severity of fighting increased as the importance of any particular female increased.

The evolution of fighting behaviour is reduced by the risk of entrapment (Hamilton, 1979; Godfray, 1988). Males disabled or exhausted by fighting males may not be able to construct an exit hole. This would entrap the females and no progeny from any of the males or females within the fig, many of them sisters and brothers, will be produced by the entire kin group. Therefore, if fighting increases the risk of entrapment, the evolution of contest competition may be barred.

The physical environment may also play a role in explaining the evolution of fighting in pollinating fig wasps. It is expected that males will evolve fighting equipment only if the environment permits its use. Support of this is seen in the work done on non-pollinating fig wasp species where mating site plays a role in male morphology and mating behaviour (Vincent, 1991; Bean & Cook, 2001). The same constraints may be important in pollinating fig wasps, with bulky fighting morphologies only evolving if males

encounter each other in an environment spacious enough for fighting. A large lumen may serve as an arena where fighting can take place. However, female pollinating fig wasps are receptive only whilst still in their galls (Galil, 1977) and males need to crawl between the galls to get to the females. The tightness of the galls, or space between the galls, may thus influence the evolution of fighting morphologies and fighting.

All the fighting species studied by West *et al.* (2001) were non-pollinators where several females each lay one or a few eggs per fig. All the pollinator species they looked at were non-fighting. Their estimates of the severity of fighting, based on the injuries obtained during the wasps' lifetime (West *et al.*, 2001), is influenced by the fact that armoury differs markedly between groups potentially leading to confounding characters. By looking at a more homogeneous group, this is controlled for.

Since a phylogeny of fighting pollinators and their relatives have only been completed recently (Erasmus, submitted), this allowed us to do the first comparative study on fighting, considering pollinating fig wasps only. Additionally, the life history of pollinating fig wasps allows a more accurate estimate of relatedness of the competitor males: One or more pollinating female fig wasps (known as foundresses) enter a receptive host fig and lay their eggs in the flowers. After development, the wingless males emerge from their galls and mate with the females. The males then chew a tunnel out of the fig from which the females disperse to a new receptive host (Hamilton, 1979; Janzen, 1979). Since there is often only one female per fig, competition between rival males is mainly between brothers. This high level of LMC induces extreme female biased sex ratios, which are seen in pollinator fig wasps (Hamilton, 1979; Wiebes, 1979; Herre *et al.*, 1997). Nonetheless, fighting between related males, and dispersal to non-natal figs, have been documented in pollinating fig wasps (Greeff *et al.*, 2003). With dispersal however, the proportion of sib mating (*p*) should decrease. This will have an effect on the expected sex ratio produced by the females (*s*) which can be determined

as: $s = (1 - p)(2 - p) / (4 - p)$ and would therefore be less female biased as sib mating decrease (Taylor, 1993).

In this study, we used phylogenetic regression (Grafen, 1989) as a comparative method. The phylogenetic regression is well suited to test discrete as well as continuous parameters (Grafen & Ridley, 1996). Analyses are performed within the context of Generalised Linear Models while taking phylogenetic non-independence into account (Grafen, 1989). Through this analysis we are able to confirm that foundress numbers and therefore relatedness does not have an effect on the evolution of male fighting. We furthermore show that the absolute number of female offspring and the internal environment of the fig do not have an effect on male contests. The main factor found to cause fighting is the sex ratio. We also show that sex ratio and dispersal are associated.

Materials and Methods

We looked at 11 species of pollinating wasps and their associated host *Ficus* (table 1.1; appendix A). Although some of the host tree species may have more than one pollinating wasp we sampled in areas where only one pollinator species occur (table 1.2) and confirmed the species type using a binocular microscope.

Foundress numbers and relatedness

Figs from different fig-tree species, in the interfloral stage or C-phase (Galil & Eisikowitch, 1967; Galil, 1977), were collected from their indigenous habitats in South-

Table 1.1. Male pollinator wasps' fighting and dispersing status investigated in this study, and their associated host *Ficus* (adopted from Greeff *et al*., 2003).

Pollinator wasp species	Associated host *Ficus*	Fight	Disperse
Alfonsiella binghami	*Ficus stuhlmannii*	Yes	Yes
Alfonsiella species 1	*Ficus craterostoma*	Yes	Yes
Alfonsiella species 2	*Ficus petersii*	Yes	Yes
Allotriozoon heterandoromorphum	*Ficus lutea*	Yes	No
Courtella armata	*Ficus sansibarica*	No	No
Elisabethiella bergi breviceps	*Ficus trichopoda*	No	No
Elisabethiella comptoni	*Ficus abutilifolia*	No	No
Elisabethiella glumosae	*Ficus glumosa*	No	No
Elisabethiella stuckenbergi	*Ficus burkei*	No	No
Platyscapa awekei	*Ficus salicifolia*	Yes	Yes
Platyscapa soraria	*Ficus ingens*	No	No

Africa, during the period 1997 to 2004 (table 1.2). The figs were opened and the foundress pollinator wasps were identified and counted using a binocular dissecting microscope. The bodies of the foundress females were usually undamaged and the numbers of foundress females in a fig could be determined very accurately.

The foundress numbers were used to calculate the proportion of sib mating (*p*) as the inverse of the arithmetic ($\overline{X}_a$) and the harmonic ($\overline{X}_h$) means. The inverse of the arithmetic mean assumes that all females produce the same number of daughters (Greeff, 2002), whereas the inverse of the harmonic mean assumes that the number of females eclosing per fig are the same (Herre, 1985), regardless of the number of

Table 1.2. Fig tree collection table.

Fig species	Province in South Africa	Collection site	Collection Period
F. abutilifolia	Gauteng	Wonderboom reserve, Pretoria	From Oct-97 to Oct-04
	Gauteng	National Botanical Gardens, Pretoria	From Jun-01 to May-03
	Mpumalanga	Abel Erasmus pass	Jan-04
F. burkei	Gauteng	Hartbeespoort dam	Sep-02
	Gauteng	Wonderboom reserve, Pretoria	From Jun-01 to Jan-04
	Limpopo	Legalemeetse Reserve	Jan-03
	Mpumalanga	Nelspruit town	Sep-97
	Mpumalanga	The Downs	2003
F. craterostoma	Gauteng	Campus of University of Pretoria	Mar-03
	KwaZulu-Natal	Ubombo	Jan-04
F. glumosa	Mpumalanga	National Botanical Gardens, Nelspruit	From Oct-97 to Jan-04
	Mpumalanga	Nelspruit town	From Jan-04 to Oct-04
	Mpumalanga	Schoemanskloof Road	Jan-04
F. ingens	Gauteng	Catharina Road, Pretoria	Oct-04
	Gauteng	National Botanical Gardens, Pretoria	Jan-02
	Gauteng	Wonderboom reserve, Pretoria	Oct-03
	Mpumalanga	Nelspruit town	From May-01 to Jun-04
F. lutea	Gauteng	Campus of University of Pretoria	Feb-04
	KwaZulu-Natal	Near Richards bay	Jan-03
	Mpumalanga	Nelspruit town	From Nov-03 to Jan-04
F. petersii	Mpumalanga	Nelspruit town	From Feb-98 to Mar-04

F. salicifolia	Gauteng	Campus of University of Pretoria	From Sept-98 to Nov-03
	Gauteng	National Botanical Gardens, Pretoria	From Apr-02 to Feb-04
	Gauteng	Wonderboom reserve, Pretoria	From May-01 to Jun-03
F. sansibarica	Limpopo	Legalemeetse Reserve	Sep-04
	Mpumalanga	Crocodile Gorge, Nelspruit	Feb-04
F. stuhlmannii	Mpumalanga	Nelspruit town	Aug-04 to Oct-04
F. trichopoda	KwaZulu-Natal	Cosi-bay	Jan-04
	KwaZulu-Natal	Durban	Jun-04

foundresses per fig. A true estimate of the proportion of sib mating should be somewhere between the inverse of the arithmetic and harmonic means. Fifteen percent of mating in *P. awekei* involves a male that mate in their non-native figs (Janse van Vuuren in preparation). In *Alfonsiella* species, from *Ficus craterostoma,* this figure was estimated as 6% (Greeff, 2002). We used 15% as an estimate for the proportion of males that disperse in all the dispersing species. The proportion sib mating with a proportion, k, of matings between a disperser and a female was calculated as: $p = (1 - k)(1 / \overline{X}) + k \times 0$ with the arithmetic and harmonic means respectively . Using the proportion of sib mating (either with or without dispersal) we calculated the inbreeding coefficient $F = p / (4 - 3 \times p)$ (Suzuki & Iwasa, 1980) and the relatedness of the competing brothers as $r_b = (1 + F)/2$ and between competing individuals as: $r_c = (1/ \overline{X}) \times r_b \times (1 - k)$ (Hamilton, 1972).

Release sex ratio

Figs from the different fig-tree species listed in table 1.2 were collected while in early D-phase (Galil & Eisikowitch, 1967; Galil, 1977) during the period 1997 to 2004. Figs were examined to ensure no exit tunnel has been chewed. The figs were opened and individually placed in vials sealed with fine mesh gauze. The wasps normally started to release within a few minutes of opening and were completely released within 24 hours, before desiccation of the fig could constrict them to their respective galls. The number of male and female pollinator wasps, as well as the clutch size for each fig was determined after the wasps died. Wasps from within and outside the fig were taken into account but only if completely released from their respective galls (almost all of the wasps did release from their galls and we assumed that the few individual's inability to escape from their galls were not due to the artificial opening of the figs). From this the release sex ratio was determined as the proportion of males in the clutch for each fig. Figs containing male only broods were removed from analysis as they represent virgin foundress females with no choice in sex allocation and do not contribute to the mating environment, as no females are present.

We also used the proportion of sib mating, calculated from the harmonic mean and including 15% dispersal, to calculate the expected sex ratio (s) as: $s = (1 – p) \times (2 – p) / (4 – p)$ (Taylor, 1993).

Gall density

Fig species listed in table 1.2 were collected while in D-phase during the period 2003 to 2004. The internal volume of each fig was determined as follows: Water was

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injected into the fig using a syringe, thus displacing the air. This was done while the fig was held under water and the bubbles escaping from the ostiole were captured in a funnel attached to another syringe. The volume (in mm^3) of air released by the fig was then read from the syringe into which the displaced air was drawn. The displaced air represented the combined volume of the lumen (figure 1.2, part c) and the space between the galls (figure 1.2, air in part b). The water used to fill the fig as well as the water in the beaker, contained common dishwashing liquid (2-3 drops/500ml, LILD dishwashing liquid, Diversey Lever) to break the surface tension.

After the volume of displaced air was determined each fig was cut vertically, resulting in half a fig as is shown in figure 1.2. The following measurements were taken, with a calliper to the nearest 0.02 mm (figure 1.2): The diameter of the fig (x), the diameter of the lumen and galls (y), the diameter of the lumen only (z). Figs are not
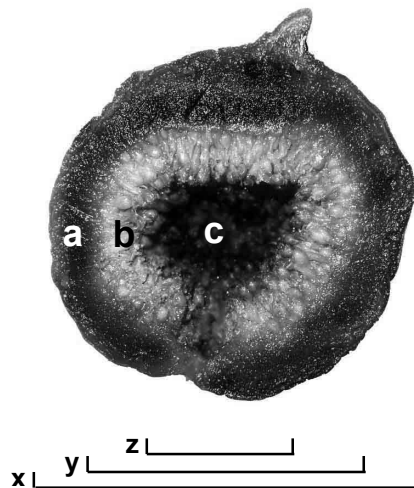
Figure 1.2. A longitudinal section through a fig (*F. sansibarica*). The total volume of the fig represented by *a* (this include the fig wall volume, the gall volume and the lumen volume), the volume of the gall and lumen represented by *b*, and the lumen volume represented by *c*. Two measurements of each *x, y* and *z* (one horizontally and one vertical) were taken to determine the respective volumes.

completely spherical and two measurements for each diameter (x, y and z) were taken. The first was horizontal as indicated in figure 1.2, while the second was taken at a right angle to the first (from the stalk to the ostiole). The average radius for each pair of measurements was determined and using the formula $\frac{3}{4}\Pi r^{3}$, the volume in mm^3 could be determined for, the total fig volume (a), the volume of the galls and the lumen (b + c) and the volume of the lumen only (c). From this the total volume occupied by the gall-layer (b) could be determined by subtracting the volume of the lumen only (c) from that of the combined gall and lumen volume (b + c). The volume of air between the galls was determined as: *(Total volume of released air) – (determined volume of lumen (c)).* From this, the percentage air between the galls could be determined as follows: *(air between the galls)/(total volume occupied by gall layer) x 100*. The gall density was calculated as: (*1 - the percentage space between the galls).* The gall densities for the different ficus species were used as a measurement of the tightness of galls while mating takes place .

Statistical analysis

Data for 7 different parameters for the 11 species of pollinating fig wasps and their hosts were obtained (tables 1.1 and 1.2).

Data comparisons were done using phylogenetic regression (Grafen, 1989). Dispersal, the number of foundresses (table 1.3), the release sex ratio (table 1.4), total number of female offspring (table 1.4), the lumen volume and the gall density (table 1.5) were all tested against fighting to see which had a significant effect. The effect of dispersal and the number of foundresses on the release sex ratio was also tested. These variables were tested without any transformation. All the data were treated as continuous except the occurrence of fighting and dispersal. We used the program

Phylogenetic Regression, version 0.5 (Grafen, 1989), implemented in the SAS statistical package. The phylogeny used (figure 1.3) is based on a consensus tree drawn from phylogenies based on ITS2 and 28S sequence data from the nuclear DNA and *COI* sequence data from the mitochondrial DNA (Erasmus, submitted). Although the sequence data show that Alfonsiella species 1 and species 2 are different it also suggests that *Alfonsiella* species 2 (from *F. petersii*) and *Alfonsiella binghami* (from *F. stuhlmannii*) are the same species (Erasmus, personal communication). Tentatively treating them as a sibling species will not bias our samples as they collapse into one contrast anyway. The phylogeny used here is fairly robust with well-supported nodes (Erasmus, submitted). Path segment lengths were derived from the default "Figure 2" method as described by Grafen (1989).

To determine if there is a significant difference between the gall-densities, during D-phase, for the different fig-tree species we did a one-way ANOVA.
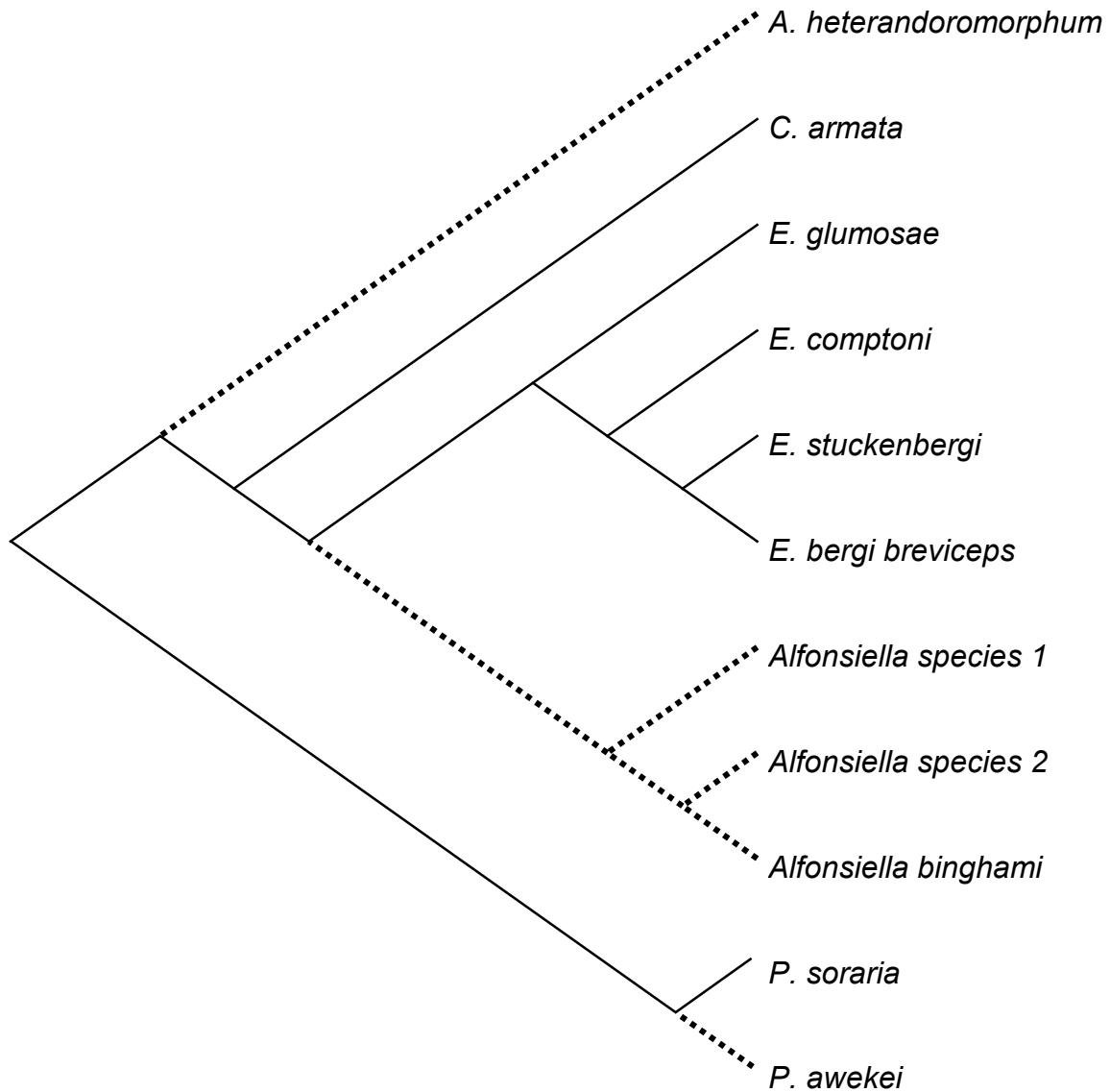
Figure 1.3. Working phylogeny, constructed as consensus tree from ITS2, 28S and COI phylogenies (Erasmus submitted), used for phylogenetic regression. The solid lines represent non-fighting lineages while the broken lines represent fighting lineages. From this we can see that fighting evolved at least three times independently.

Results

Foundress numbers and relatedness

The mean number of foundresses for each of the species we investigated is shown in table 1.3. We used all the relatedness estimates calculated in table 1.3 (with and without 15% dispersal calculated with the arithmetic and the harmonic means), but the results were quantitatively the same and we will report only those of the relatedness calculated from the harmonic mean including 15% dispersal (tables 1.6 and 1.7).

Release sex ratio

The mean sex ratio, the number of females and the expected sex ratio are given in table 1.4. The means for each was calculated using the mean of each crop (average crop size of 17 figs per crop). The standard deviation represents the variation between the crops for the release sex ratio or number of females for each species respectively.

Gall density

The size of the lumen (in mm^3) and the gall density are given in table 1.5. We found that there is a very significant variation between the gall densities, during release, for the different fig-tree species ($F_{10,284} = 12.06$, $P < 0.0005$).

Table 1.3. Foundress means, proportion of sib mating and the relatedness estimates, without (-*k*) and with (+*k*) dispersal, for the different pollinator species.

Pollinator wasp	N (crop)	$\overline{X}_a$	Arithmetic mean estimates				$\overline{X}_h$	Harmonic mean estimates			
			p (-k)	*p (+k)*	r_c *(-k)*	r_c *(+k)*		*p (-k)*	*p (+k)*	r_c *(-k)*	r_c *(+k)*
A. heterandoromorphum	2	2.35	0.43	0.43	0.58	0.58	1.58	0.63	0.63	0.65	0.65
Alfonsiella binghami	12	1.02	0.98	0.83	0.96	0.78	1.01	0.99	0.84	0.98	0.79
Alfonsiella species 1	3	1.09	0.92	0.78	0.87	0.73	1.04	0.96	0.82	0.93	0.76
Alfonsiella species 2	2	1.30	0.77	0.65	0.73	0.66	1.14	0.88	0.75	0.82	0.71
C. armata	3	2.23	0.45	0.45	0.58	0.58	1.61	0.62	0.62	0.65	0.65
E. bergi breviceps	2	2.96	0.34	0.34	0.56	0.56	2.08	0.48	0.48	0.59	0.59
E. comptoni	2	1.88	0.53	0.53	0.61	0.61	1.35	0.74	0.74	0.71	0.71
E. glumosae	3	1.26	0.79	0.79	0.75	0.75	1.13	0.88	0.88	0.83	0.83
E. stuckenbergi	2	1.04	0.96	0.96	0.93	0.93	1.02	0.98	0.98	0.96	0.96
P. awekei	6	1.69	0.59	0.50	0.63	0.60	1.44	0.69	0.59	0.68	0.63
P. soraria	4	1.16	0.86	0.86	0.80	0.80	1.07	0.93	0.93	0.89	0.89

Table 1.4. Average offspring sex ratio and total number of female offspring for the different pollinator species.

Pollinator wasp	N (crop)	Sex ratio ± SD	Females ± SD	Expected sex ratio
A. heterandoromorphum	3	0.22 ± 0.15	20.37 ± 19.07	0.06
Alfonsiella binghami	1	0.28	2.82	0.07
Alfonsiella species 1	2	0.32 ± 0.16	10.81 ± 0.97	0.10
Alfonsiella species 2	7	0.31 ± 0.19	11.52 ± 11.12	0.15
C. armata	1	0.22	83	0.15
E. bergi breviceps	2	0.15 ± 0.04	29.17 ± 31.64	0.10
E. comptoni	6	0.15 ± 0.11	67.78 ± 38.86	0.04
E. glumosae	5	0.24 ± 0.12	21.45 ± 16.81	0.01
E. stuckenbergi	8	0.19 ± 0.09	27.11 ± 18.81	0.22
P. awekei	16	0.27 ± 0.11	18.72 ± 10.83	0.17
P. soraria	6	0.14 ± 0.07	38.95 ± 19.9	0.02

Table 1.5. Data on the internal structure of different fig species (only one crop per species was used).

Ficus	N	gall density (%) ± SD	lumen vol (mm^3) ± SD
F. abutilifolia	16	58.88 ± 20.80	0.19 ± 0.14
F. burkei	30	49.63 ± 11.18	0.02 ± 0.01
F. craterostoma	30	62.36 ± 10.51	0.04 ± 0.02
F. glumosa	21	65.79 ± 13.56	0.01 ± 0.01
F. ingens	30	54.25 ± 14.35	0.01 ± 0.01
F. lutea	18	40.60 ± 23.28	0.24 ± 0.19
F. petersii	30	49.41 ± 14.66	0.00 ± 0.00
F. salicifolia	30	47.85 ± 13.79	0.01 ± 0.01
F. sansibarica	30	67.17 ± 15.40	3.54 ± 1.48
F. stuhlmannii	30	74.77 ± 12.54	0.12 ± 0.05
F. trichopoda	30	58.46 ± 14.32	0.04 ± 0.03

Phylogenetic regression

In the phylogenetic regression our phylogeny was reduced to 6 independent contrasts from 11 species when fighting was fitted to the phylogeny. All the species in the *Alfonsiella* genus grouped together while all the species in the *Elisabethiella* genus grouped together. We confirmed that fighting and dispersal are strongly associated ($F_{1,4}$ = 10.766, P = 0.030), even when we control for release sex ratio. None of the factors speculated to have an influence on fighting (figure 1.1) were significant, except the release sex ratio ($F_{1,8}$ = 7.462, P = 0.026; table 1.6).

We were also able to test the effect of parameters on the release sex ratio (figure 1.1; table 1.7). The influence of foundress numbers was not significant ($F_{1,9}$ = 0.682, P = 0.430). When the expected sex ratio based on the degree of sib mating and dispersal was fitted to the release sex ratio (tables 1.3 and 1.4), dispersal was significantly associated with the release sex ratio ($F_{1,8}$ = 18.857, P = 0.002), but the expected sex ratio ($F_{1,8}$ = 0.403, P = 0.542) was not (table 1.7).

Table 1.6. Results of the phylogenetic regression against fighting, values in italics were controlled for.

Parameters	Control	df	Estimate	Constant	F	P
Dispersal		4	-0.887	0.492	10.766	**0.030**
Lumen volume		4	-0.153	0.577	0.384	0.569
Gall density		4	-0.012	1.159	0.392	0.549
Foundress		4	0.024	0.451	0.019	0.897
Relatedness		4	-0.656	0.855	2.984	0.159
Females		8	-0.005	-0.503	0.716	0.422
	Release sex		*5.169*			
Release sex		8	5.169	-0.503	7.462	**0.026**
	Females		*-0.005*			

Table 1.7. Results of the phylogenetic regression against the release sex ratio, values in italics were controlled for.

Parameters	Control	df	Estimate	Constant	F	P
Foundress		9	-0.026	0.269	0.682	0.430
Dispersal		8	-0.166	0.219	19.060	**0.002**
	Expected sex ratio		*0.053*			
Expected sex ratio		8	-0.110	0.325	0.403	0.542
	Dispersal		*-0.130*			

Discussion

By using phylogenetic regression (Grafen, 1989) in our analysis, we controlled for similarities due to ancestry in the evolution of a specific behaviour (Felsenstein, 1985). Using this type of analysis we confirmed that sex ratio and fighting are strongly associated (see also table 1.1). The proportion of males to females (but not the absolute number of females) may be the main factor influencing the evolution of male contest behaviour. The relatedness, number of female offspring and internal structure of the fig had no correlation with fighting behaviour. The only other factor that seems to associate with fighting was dispersal.

Theory however suggests that fighting and dispersal should not have a positive effect on the evolution of the other. Hamilton (1979), stated that more severe fighting occur when males are not able to disperse and mate with other females (see also Enquist & Leimar, 1990). Conflict limiting effects, stemming from relatedness, can only persist if there is competition with unrelated individuals (Griffin & West, 2002). Therefore, individuals competing locally should compete less severely to maximize the fitness of their relatives, if there is an option to disperse (West *et al.*, 2001; Griffin & West, 2002). Limited dispersal, as in the case with male pollinating fig wasps, should therefore have a conflict-limiting effect, if any.

Fighting and dispersal may therefore be linked indirectly, by some other factor driving the evolution of both and appear as a syndrome, not directly affecting each other but always appearing together. We found that the release sex ratio had a significant effect on both and may be one of the factors that indirectly link dispersal and fighting. Other factors which may link fighting and dispersal indirectly is the similarity of fighting and dispersing morphologies (Greeff, 2002). There is a tight link between the morphology for fighting and dispersing for both the pollinating (Greeff *et al.*, 2003) and

non-pollinating fig wasps (Bean & Cook, 2001). The only fighting species that does not disperse is *A. heterandromorphum* (table 1.1). A possible explanation for the absence in dispersing behaviour in *A. heterandromorphum* is their lack of eyes (Greeff *et al.*, 2003; appendix A). Although this does not inhibit their fighting ability while inside the fig, they have a disadvantage when trying to disperse. The effect of the fighting, dispersing syndrome is probably lost in this species due to an irreversible phenotypic change, such as the loss of eyes.

The number of foundresses was used from which we determined the relatedness of the competing individuals. Our data confirm that relatedness does not play a role in the evolution of fighting (table 1.6). Relatedness is possibly cancelled in fighting and non-fighting species with high levels of LMC, removing the conflict limiting effect thereof (Grafen, 1984; Murray, 1984). Our result is one of the few experimental proofs that entirely local competition can cancel the effect of relatedness on inclusive fitness.

The number of foundresses are also assumed to have an effect on the release sex ratio (Hamilton, 1979; West & Herre, 1998a; West *et al.*, 2001), but this was not the case in our study. Nor was it the case with expected sex ratio based on the degree of sib mating. Our data support other studies which cautions the use of sex ratio to determine mating structure (Antolin, 1999; Greeff, 2002), as other factors such as the clutch size (West & Herre, 1998b; Kjellberg *et al.* in press), and male dispersal (Greeff, 2002) are known to affect the sex ratio.

West *et al.* (2001) found a significant association between the severity of fighting and the number of female offspring. In contrast, we find that the number of female offspring has no significant effect on the presence of fighting behaviour. This suggests that the value of the contested resource may be important in determining the severity of fighting, rather than the evolution of fighting in this group of wasps.

The internal structure of the fig did not have an effect on fighting behaviour. Even though the variation in gall density between the different species of *Ficus* is very significant ($F_{10,284}$ = 12.06, P < 0.0005) during the release of the wasps, this does not have a significant effect on the evolution of fighting. We also measured the lumen volume of the different fig species as a small lumen may constrain the evolution of fighting. No effect between the lumen volume and fighting were found. If the internal structure of the fig has any effect on the evolution of fighting it is very limited and does not involve gall density or lumen volume.

We found that the release sex ratio has the greatest influence on the evolution of fighting of all the parameters we tested ($F_{1,8}$ = 7.462, P = 0.026). If the sex ratio is less female biased, and the female offspring a limited resource, fighting may evolve as a consequence of the escalated competition between competing males for mating opportunities. Males will only invest in costly fighting gear when the reward of more matings than any other male outweighs the penalty of such investments. When there are enough females for each male, fighting will not be rewarded by a higher mating success. It is however important to note that an increase in the number of females will not affect fighting if the same proportional increase in the number of males is seen. The male to female relationship is therefore important and not only the number of females (or the number of males). Note that in theory, increases in non-lethal fighting should not lead to an increase in optimal sex ratios. High sex ratios are therefore the most likely cause of fighting behaviour and not *vice versa.*

In the situation illustrated above we assume that the OSR and the release sex ratio are correlated. This assumed correlation are on the conservative side since most fighting species possesses behavioural characteristics that actually increase the OSR. In *Allotriozoon* and the *Alfonsiella* species a female is removed from her gall after she has been mated by pulling her out by her antennae (Greeff *et al.*, 2003). A similar effect is

seen in *P. awekei* where the OSR rapidly becomes less female biased than the release sex ratio (Chapter 2). Thus, in fighting species the behavioural patterns can rapidly make the OSR even more male biased. When we look at the mating behaviour of non-fighting species we see that the OSR and release sex ratio are more similar than it is in the fighting species. This is because no removal of the females is seen and additional matings by the females are not precluded. Females can be mated several times before the exit hole is completed, as is reported in other non-fighting species (Murray, 1989; Murray, 1990).

Although release sex ratio and dispersal are very significantly associated, the directionality of this association is not clear. Release sex ratio may have an effect on the evolution of dispersal since equal sex ratio will increase LMC and dispersal could evolve to avoid LMC (Hamilton, 1967; Hamilton & May, 1977). Conversely, in dispersing species there is a more open breeding system in the population and the optimal sex ratio will be less female biased (Hamilton, 1967; Taylor, 1993; Greeff, 1995; West & Herre, 1998b).

Murray *et al.* (1985) suggested that the challenge frequency is inversely related to the operational ratio of the resource number (females) to competitor number (males). The sex ratio in pollinator fig wasps is less female biased in fighting than in non-fighting species. We therefore expect more challenges per female in the fighting species. The high sex ratio are expected to lead to the evolution of male contest behaviour and we empirically show that there is significant association between the sex ratio and fighting behaviour in pollinating fig wasps.

Chapter 2

The environmental context of male fighting in the pollinating fig wasp *Platyscapa awekei*

Abstract

The males of the pollinating fig wasp species *P. awekei* fight. The proximal cues inducing this behaviour may be the relative encounter rate of other males to females. This is directly dependant on the sex ratio. The sex ratio of pollinator species, in which the males fighting is observed, is known to be less female biased than those with non-fighting males. In this study we show that the change in the level of CO_2, due to the construction of an exit hole by the males, triggers the release of female pollinator fig wasps from their figs. In the species *P. awekei* mating is triggered by the same change and only start as the females leave. The females rapidly become a limited resource and the OSR becomes male biased. Escalated fighting between these males is therefore expected. We observe that the males of the species *P. awekei* readily engage in contest competition once the exit hole is constructed. This is the first study where male fighting, in pollinator fig wasps, is quantified and the regulation thereof explained in terms of the environment. We show how the physical environment affects the mating environment, which together with the produced sex ratio may be the major causes of male fighting in pollinator male fig wasps*.

Introduction

Comparisons between different species of pollinating fig wasps show that less female biased sex ratios are the ultimate cause of fighting between males (chapter 1). To elucidate the proximal causes of male fighting the emphasis need to be on the sensory cues and the mechanistic reaction (Tinbergen, 1963). The sex ratio can also be a proximate determinant of fighting, as males will encounter each other readily in less female biased sex ratios (Emlen & Oring, 1977). The result may be to fight as soon as females become receptive, as this may be the only way to ensure mating opportunities. Detailed observation on the mating and fighting behaviour as well as how the sex ratio affects this may allow us to identify proximate determinants of fighting.

Non-fighting pollinating fig wasps have, in general, extremely female biased sex ratios and much is known about their life history (Galil & Eisikowitch, 1974; Hamilton, 1979; Godfray, 1988; Murray, 1989; Zammit & Schwarz, 2000; see also chapter 1), which can be shortly described as follows: A female fig wasp crawls into a receptive host fig and lays her eggs and dies. The male offspring eclose first, determine which galls contain the females of the same species (Godfray, 1988; Murray, 1990), chews holes into the females' galls, and mate with the trapped female offspring (Galil & Eisikowitch, 1974; Hamilton, 1979; West *et al.*, 1998; Zammit & Schwarz, 2000). After mating the males would chew an exit hole out of the fig from which the females escapes (Galil & Eisikowitch, 1974; Hamilton, 1979; Godfray, 1988; Murray, 1989; West *et al.*, 1998; Zammit & Schwarz, 2000).

There are however some discrepancies between different species of pollinators in these events. After mating, the females of the species *Ceratosolen dentifer* eclose from their galls and accumulate in the fig lumen before an exit hole from the fig is constructed by the males (Godfray, 1988). In the species *Pleistodontes imperialis* the females are

mated in a scramble type competition (Zammit & Schwarz, 2000). The males construct a single insemination hole per gall and mate with the female. These holes are later expanded cooperatively by the males from which the females escape (Zammit & Schwarz, 2000). Multiple matings is seen in the species *Ceratosolen solmsi* (Murray, 1989; Murray, 1990). Here, a number of males will each construct a new insemination hole in female containing galls and mate with the female. These galls are reported to often contain five to six insemination holes (Murray, 1990). The females are seen to be less receptive after the first mating, resisting the males by whirling around within the gall or biting the tip of his abdomen (Murray, 1990). Yet, the coercive mating attempts by the males are reported to be largely successful (Murray, 1990). Conflict between the males in the above mentioned species are trivial since non fighting pollinator wasps have extreme female biased sex ratios (Hamilton, 1979; Herre *et al.*, 1997 chapter 1) which limits LMC (Hamilton, 1967; Taylor & Bulmer, 1980). Fighting may also be limited as the males would not want any of their sisters to be unmated, especially if the mating is done by their brothers (Hamilton, 1979). The males also need to construct the exit hole and severe fighting may disable them to do so, which will have no fitness advantage for any of them (Hamilton, 1979; Godfray, 1988).

Recent studies have however shown that some species of male pollinating fig wasps do engage in contest competition (Michaloud, 1988; Greeff *et al*., 2003). In these species the sex ratio is less female biased than that of the non-fighting species (chapter 1). In addition, in several of these species the males actively change the OSR (see below) by removing the mated females from the mating population. This is done in the *Alfonsiella, Nigeriella* and *Allotriozoon* species where the males pull the females from their galls directly after mating and thereby precluding any additional matings (Greeff *et al*., 2003). (Females are only mated whilst still within their galls (Greeff *et al*., 2003)). The result is: an already less female biased sex ratio becoming even less female biased (in

some cases even male biased), an increase in the value of the remaining females and an increase in the number of male encounters at every female; all these factors are expected to escalate male fighting (Emlen & Oring, 1977; Murray & Gerrard, 1985; Enquist & Leimar, 1987; Enquist & Leimar, 1990). These species show a female defence polygyny mating system, where the males compete for, and defend mated females (Emlen & Oring, 1977).

Galil *et al.* (1973) showed that the environment could have an important effect on the behaviour of pollinating fig wasps. They demonstrated that female pollinator wasps (*Platyscapa quadraticeps*) are inactive until the exit hole is constructed thereafter they emerge from their galls and leave the fig. They also demonstrated that the atmosphere within a fig contains 10% CO_2 (more than 300 times the CO_2 concentration of the normal atmosphere (Glueckauf, 1951)) before the exit hole is constructed (Galil *et al.*, 1973). When the exit hole is completed the level of CO_2 within the fig is considerably reduced as the internal and external atmosphere equilibrates. The change in the atmosphere has the additional effect of inhibiting the activities of the male wasps (Galil *et al.*, 1973). Mating therefore takes place at a 10% CO_2 level when the males are active and the females are inactive but receptive (Galil *et al.*, 1973). As the males are unable to hoard females, and the sex ratio is very female biased, the pressure to engage in contest competition is minimal (Emlen & Oring, 1977; Hamilton, 1979; Godfray, 1988; Murray, 1989; Herre *et al.*, 1997; Zammit & Schwarz, 2000). The gaseous environment therefore regulates the sequence of activities within the fig.

Hamilton (1967) showed that LMC could skew the population sex ratio. Populations are expected to have equal sex ratios under natural selection, in panmictic populations (Fisher, 1930). However, panmictic populations do not always occur in nature. Several fitness advantages are achieved by individuals producing skewed sex ratios in local mating populations, including less severe competition between related

offspring for matings. Therefore, in less female biased sex ratios, contest competition between males are favoured in a polygynous mating system (Emlen & Oring, 1977; Andersson, 1994). The mating system, OSR, and the behaviour regarding inter- and intra sexual conflict, are therefore seen to influence each other and form complex, yet predictable outcomes. The OSR, defined as the number of receptive females to the number of sexually active males (Emlen & Oring, 1977), is determined by the spatial and temporal distribution of the two sexes, which is to a large degree, influenced by the environment (Emlen & Oring, 1977).

In this study we show that the sex ratios may be the proximate cause for male fighting behaviour in the pollinator wasp species *Platyscapa awekei*. Males are seen to contest at every opportunity when a female is in the vicinity once the exit hole is completed. We also demonstrate the significant role of the environment (i.e. the CO_2 concentration) in generating the observed male biased OSR from a female biased population sex ratio. The female release rate as well as the life span of the males was determined and were used to quantify the change in the OSR for the species *P. awekei.* A less female biased sex ratio is obtained in a short time, after mating activities begin, causing a female defence polygyny mating system. To confirm that our CO_2 treatment is not unrealistic and that the effects observed are not due to CO_2 narcosis we looked at the effect of CO_2 on the non-fighting species *Platyscapa soraria* where the males and females behave similarly to the species *P. quadraticeps,* observed by Galil (1973). This is a unique study where the male fighting in pollinators are quantified and the regulation of the behaviour explained in terms of the environment.

Materials and Methods

Ficus salicifolia and *Ficus ingens* figs were harvested from trees growing in Pretoria from March 2003 to June 2004. The figs were determined to be in the developmental stage, just before or after the males eclosed from their galls (before and during D phase; *sensu* Galil & Eisikowitch, 1967; Galil, 1977) by looking at the colour (slightly yellow) and firmness (softer than the figs in C phase) of the fig. The figs were examined for exit holes and only those without any were used in further observations. The pollinator wasps for *F. salicifolia* and *F. ingens* are *Platyscapa awekei* and *Platyscapa soraria* respectively, and can easily be identified under a binocular microscope (see also appendix A). The behaviour of the wasps in high and low CO_2 conditions (see below) was quantified and the male life span was determined. All data were analysed, using the statistical package SPSS version 12.0.

Quantification of mating and fighting

Observation of the wasp's behaviour was continuous. Observations started directly after the fig was opened and lasted between one and three hours at a time. The duration of mating was taken from the time the male inserted his aedeagus into the females' gall until he removed it. The following criteria was used to denote fighting behaviour: that the two interacting males faced each other; and repeatedly bit each other at short intervals or bit and held for more than 2 seconds; and that both males participated simultaneously in biting each other. Males displayed several other types of aggressive behaviour which were not recorded as fighting and included: biting from the side or back, pushing each other without biting, a single bite (mostly seen when they were passing each other at

close proximity to a female), and when more than two males engaged in aggressive behaviour. We also recorded the time of female departure.

Experiment 1 (mating and fighting in high CO_2)

To determine the behaviour of the wasps, within an unopened fig, a controlled-atmosphere chamber, similar to the one used by Galil *et al*. (1973), was constructed. The chamber consisted of a 25-litre Perspex box with a red see through Perspex lid. Gloves protruded into the chamber from the front with which the samples were manipulated. The chamber was connected to two gas cylinders, one containing CO_2 (Afrox, purity of 99.0% carbon dioxide) and one containing compressed air (Afrox, 79% nitrogen, 21% oxygen, 0.9% argon and 0.03% carbon dioxide). The flow rate of each was controlled by rotameters (Platon, glass variable area flow meters) and the gas mixture was bubbled through water to prevent dehydration of the figs. The CO_2 percentage range to which the atmosphere could be adjusted was 0% CO_2, and from (3.5%) to (100%). A dissecting microscope was placed on the Perspex lid through which the behaviour could be observed. Before each observation the atmosphere was equilibrated to 10% CO_2 for 10 minutes. The figs were placed in the chamber before they were opened. The figs were opened by slicing them in half (from the pedicel to the ostiole). The behaviour of the wasps (*P. awekei* and *P. soraria*) was recorded from which the mating and fighting could be quantified.

Experiment 2 (mating and fighting in low CO_2)

The figs were opened and viewed under a binocular dissecting microscope placed within a darkened chamber but with a normal atmosphere (the CO_2 level in a normal atmosphere is 0.03% (Glueckauf, 1951), where normal atmosphere is defined as the atmosphere outside the fig or within the fig after the exit hole is completed. We will refer to an atmosphere of 0.03% CO_2 as a normal atmosphere or a low CO_2 atmosphere). The figs were opened by slicing them in half (from the pedestal to the ostiole). The opened fig simulated the effect of an exit hole. The light source was fitted with red filters, which enabled us to observe the wasps while simulating the internal environment of the fig. The behaviour of the wasps (*P. awekei* and *P. soraria*) was noted, without adding or removing wasps from the half fig being observed. From this, the mating and fighting could be quantified.

Experiment 3 (effect of CO_2 concentration on the release of females)

F. salicifolia figs in early D-phase (before the exit tunnel has been chewed; (Galil & Eisikowitch, 1967) were collected from trees growing in Pretoria. Twenty-six figs were opened in either low or high CO_2 and the number of released females was recorded every 5 minutes for 300 minutes. The experiment was repeated with 9 *F. ingens* figs (observed for 40 minutes), which were also collected in D-phase, before an exit hole was dug. The number of females that released in experiment 1 (high CO_2) and experiment 2 (low CO_2) was added. The number of females releasing in high and low CO_2 was compared for *P. awekei* and *P. soraria.*

We also did a paired sample test with 10 *F. salicifolia* figs, where each fig was opened in high CO_2 concentration. We removed one half of the fig from the high CO_2 environment and recorded the number of females that released every 5 minutes for both halves. The number of females releasing in high and low CO_2 was compared.

Experiment 4 (physical effect of CO_2 on pollinator activity)

The activity of male and female *P. awekei* were tested to see if high levels of CO_2 had any negative effect on the wasp's behaviour due to physiological constraints. Males and females were collected from figs opened in a low CO_2 atmosphere; thereafter they were placed into a 10% CO_2 atmosphere, on a flat paper with a reference grid drawn on it. We could easily determine the distance they moved from the number of blocks they crossed. The distance they ran per measured time was used to estimate their speed. The experiment was repeated with a control group in a low CO_2 atmosphere. We also opened figs in a 10% CO_2 atmosphere and directly placed the males on the paper to estimate their walking speed (females could not be used as they do not readily release in a 10% atmosphere, see discussion).

Experiment 5 (determination of male life span)

Galls containing male *P. awekei* wasps were removed from early D-phase *F. salicifolia* figs. Fifteen galls were placed in separate 2 ml eppendorf tubes and sealed. Fifteen galls were placed in Eppendorf tubes with a hole in the lid, which was covered with fine mesh gauze. We were therefore able to determine the life span of males in a

humid (closed tubes) and dry (tubes closed with mesh) environment. The males were observed until they expired, which was easily recognised as they stopped moving and fell over. Life span was measured from the time a male emerged from his gall until he expired.

Results

Experiment 1 (behaviour of P. awekei in high CO_2)

We observed the behaviour of *P. awekei* from 4 syconia, after the figs were opened and kept in a 10% CO_2 atmosphere (total observation time of 243 minutes). The males were very docile and rarely moved except when threatened. (Pollinator males were occasionally threatened by males from the species *Otitesella pseudoserata,* which were commonly found within the figs we sampled. These males are larger than the pollinator males and are able to sever them with their jaws (personal observation). The pollinator males would quickly retreat between the galls if they were brushed against). Although the females were seen to create and enlarge holes in their natal galls no matings were recorded in the high CO_2 atmosphere. No fighting between the pollinator males was recorded. Only two females released while the behaviour was observed in 10% CO_2.

Experiment 2 (behaviour of P. awekei in low CO$_2$)

We observed the behaviour of *P. awekei* from 18 syconia, while they were in a normal atmosphere (total observation time of 1128 minutes). The effect of the low levels of CO$_2$ in the normal atmosphere simulated the conditions within a fig after the exit-hole has been completed.

We observed 18 matings with an average length ± SD of 10.47 ± 6.66 minutes. The holes in the natal galls of the females were usually created and enlarged by the females themselves. Males would search between the galls for a receptive female. Once found, the male would insert his aedeagus into the gall. No male was seen to be displaced by a rival male once his aedeagus was inserted into the gall in any of the fights where one male was already mating (for 19 challenges during 7 mating events) irrespective of the relative sizes of either male. During mating, males would sometimes chew and expand the hole, but would move away after removal of his aedeagus. In all but two cases, females were seen to be mated only once. In the remaining two, another male discovered a mated female before she could emerge from the gall and was re-mated. Females emerged within 5 minutes after mating stopped, without assistance by the males. (In the two cases where the females were re-mated they were respectively discovered by a second male, 3 minutes, and in less than 1 minute, after the first male left). No matings were observed once the females were out of their galls. A Mann-Whitney U rank test was used to analyse the difference in the number of matings. In high CO$_2$ conditions, significantly less matings occurred than in low CO$_2$, in the first 45 minutes after a fig was opened (Mann-Whitney U rank test: Z = -2.225, N = 12, two-tailed P = 0.026; table 2.1). This is an indication that pollinator wasps do not mate in high CO$_2$

Table 2.1. Total number of times a mating or fighting event was observed in either high or low CO_2 for *P. awekei* (number of observation given in brackets). The time for each observation was the first 45 minutes for all the observations (we excluded the observations of the matings after 45 minutes to have a standardised time of each treatment to compare).

	Experiment 1 (High CO_2)	Experiment 2 (Low CO_2)
Mating events	0 (4)	8 (8)
Fighting events	0 (4)	52 (16)

(simulating figs without an exit hole), while mating in low CO2 do take place (simulating figs with an exit hole). Interactions between males were only recorded as fights if the males were facing each other and both showed aggressive behaviour. Thirty-two fights had an average length ± SD of 70.69 ± 79.38 seconds. The large standard deviation confirms the variability in the length of fights with the shortest fight lasting only 2 seconds while the longest lasted 255 seconds (see figure 2.1). Two types of contests, or a mixture of the two were commonly seen. The first being two males repeatedly biting each other at short intervals and secondly, two males biting and holding onto the other's head and jaws (they would often push quite vigorously during this jaw-locking contest but never incurred any visible external damage). Fighting was only ever observed when a female was present in a nearby gall. One of the males involved in the contest would usually mate with the female if they did not move too far away during the fight for a third male to have access to the female. No male was seen to displace a male who had his aedeagus inserted into a gall containing a female (for 19 challenges during 7 mating events), irrespective of the relative sizes of either male. A significant increase in fighting was found, in the first 45 minutes after a fig was exposed to low CO2 (Mann-Whiney U

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rank test: Z = -3.068, N = 20, two-tailed P = 0.002; table 2.1). This indicated that the change from high to low CO_2 might prompt fighting.
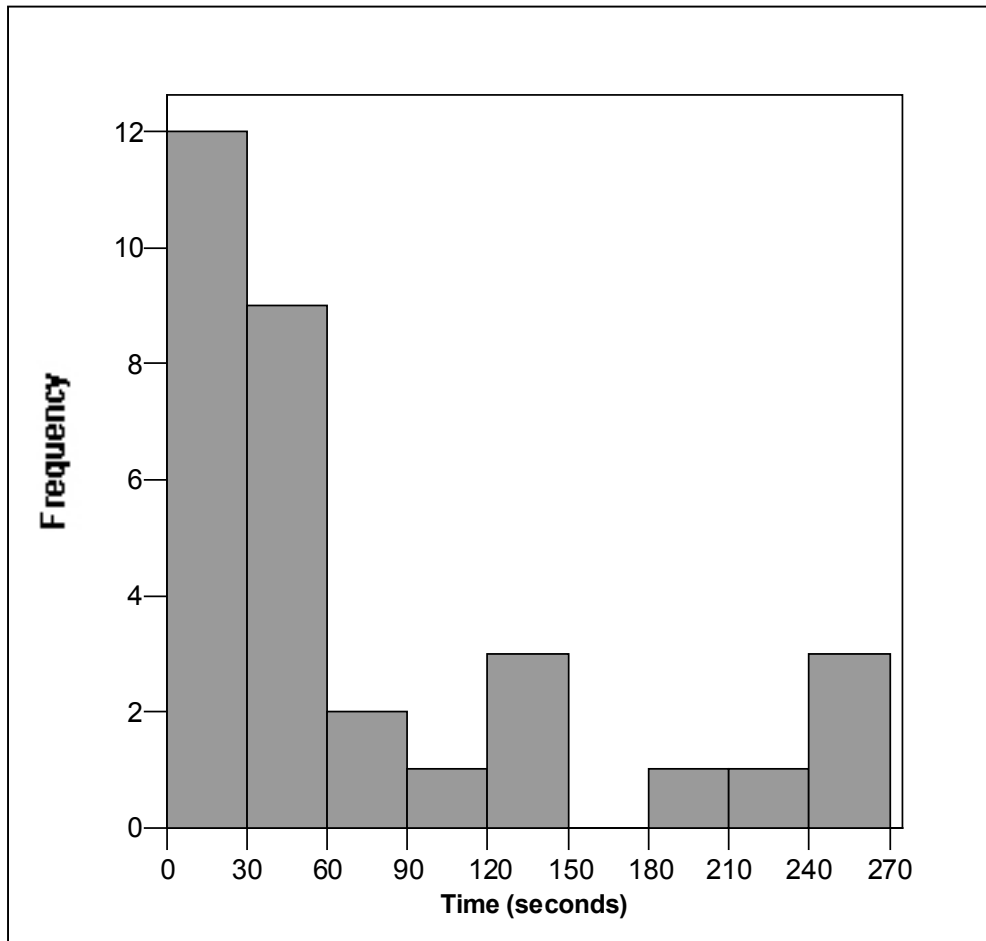
Figure 2.1. Frequency distribution of the length of fights for the 32 observed male fighting events.

Experiment 3 (effect of CO_2 concentration on the release of P. awekei females)

P. awekei females readily released when the CO_2 level was low. A paired sample t-test was done to determine the effect of high and low CO_2 on the number of released females. No females released in the high CO_2 atmosphere while 24 females released in

the low CO_2 atmosphere. A significant increase in the number of released females was therefore seen when the CO_2 was low (t_9 = -3.582, two-tailed P = 0.006). All the female release data from the scanning and continuous observations (experiments 1, 2 and 3) for the first 45 minutes after the fig was opened (in high and low CO_2) was combined, and we found a significant increase in the number of released females when the CO_2 was low (Mann-Whitney U rank test: Z = -6.420, N = 76, P < 0.001; table 2.2). The low level of CO_2 did indeed increase the chance of release of the females.

The rate of release per fig was calculated in 20-minute intervals (figure 2.2). The average number ± SD of females and males in a fig is 18.72 ± 10.83 and 5.91 ± 3.25 respectively (calculated for 366 figs from 16 crops, chapter 1). We determined the number of females present within a fig by subtracting the average number of females that released in 20 minutes from the average number of females within a fig. Males disperse from the fig at a rate of 15% per two hours, for the first four hours and at 10% between four and six hours after the exit hole is completed (Moore, personal communication). We calculated the average number of males within a fig, taking into account the proportion of dispersers for the first 320 minutes after the exposure to low CO_2 (see also male life span below). Assuming that all the individuals in the fig are sexually active we were able to determine the change in the OSR over the first 300 minutes after the fig was opened (figure 2.2).

Table 2.2. The total number of females released in either high or low CO_2 (number of observations given in brackets). The time for each observation was the first 45 minutes for all the observations on *P. awekei* and the first 40 minutes for *P. soraria.*

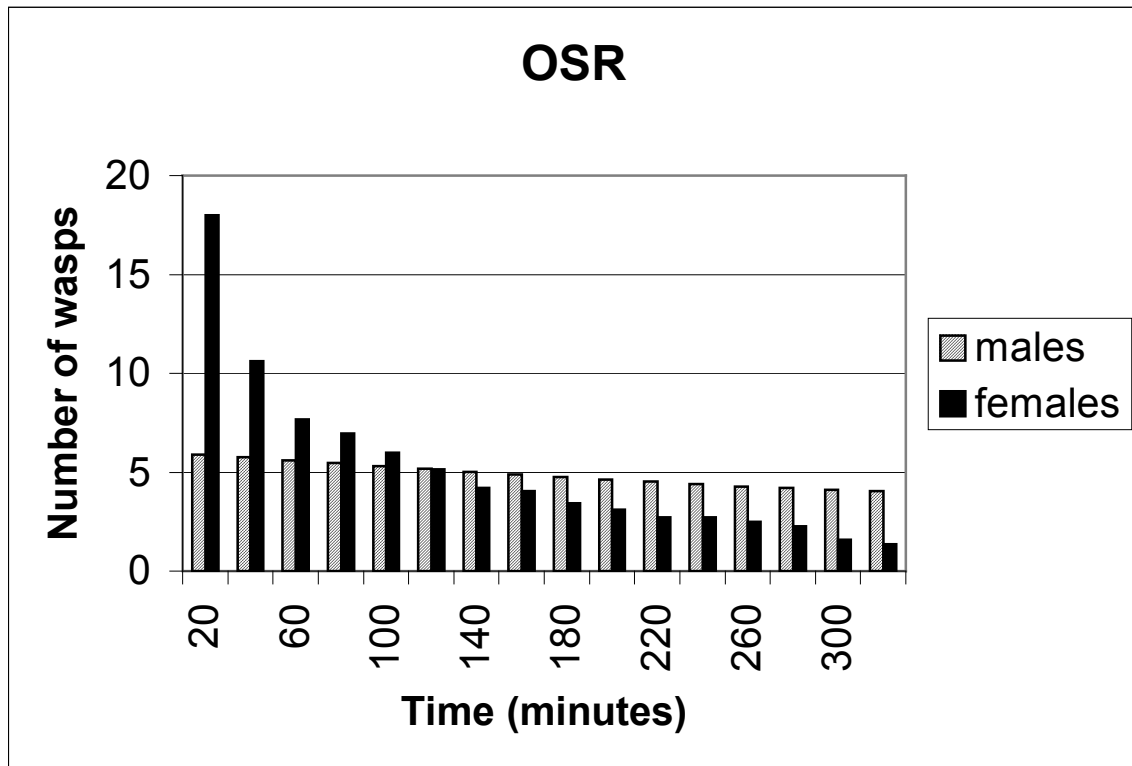|	High CO_2	Low CO_2
P. awekei	1 (22)	333 (54)
P. soraria	1 (4)	38 (4)

Figure 2.2. The number of males and females present in a fig over 320 minutes after the CO_2 is lowered. Estimated from the release rate of the females and dispersal rate of the males (Moore, personal communication), and the average number *P. awekei* males and females occurring within a fig (chapter 1).

Behaviour of P. soraria (experiment 1, 2 and 3)

We observed 5 mating events in high CO_2 in *P. soraria* (2 observation periods, with a total observation time of 130 minutes). The aim of the observations was not to quantify their mating behaviour, but to ascertain that they do mate in high CO_2 levels. Although mating does take place in high CO_2 in this species, significantly more females released in low CO_2 than in high CO_2, in the first 40 minutes after the figs were opened (Mann-Whitney U rank test, $Z = -2.535$, $N = 9$, $P = 0.011$; table 2.2). No fighting between the males were observed in the high or low CO_2 (total continuous observation time 186 minutes)

Experiment 4 (physical constraints of CO_2)

Males in a high CO_2 environment showed less activity than males in a low CO_2 environment. This is true for males placed in a high CO_2 environment regardless of whether they came from a high or a low CO_2 environment. We measured the walking speed for these three treatments (1. males in high CO_2 only, 2. males in low CO_2 only and 3. males placed in high CO_2 after they were exposed to low CO_2 see figure 2.3). We did a power transformation on our data (to the power 2) and found a significant difference between the three treatments when we did a one-way-ANOVA ($F_{2, 55} = 9.768$, $P < 0.001$). A Post Hoc test revealed that there is only an increase in walking speed when the CO_2 is lowered (Tukey $P = 0.001$ for the difference between treatment, 1 and 2 as well as 3 and 2) but that the difference between treatment 1 and 3 showed no significant difference (Tukey $P = 0.999$). The effect of the males moving faster is therefore clearly due to the lowering of the CO_2 in the atmosphere.

We did a power transformation (to the power 2), on the walking speed data for the females in high and low CO_2. No significant difference ($F_{1, 37}$ = 2.211, P = 0.146; figure 2.3) in the walking speed of the females in the two treatments was found when we did a one-way ANOVA.

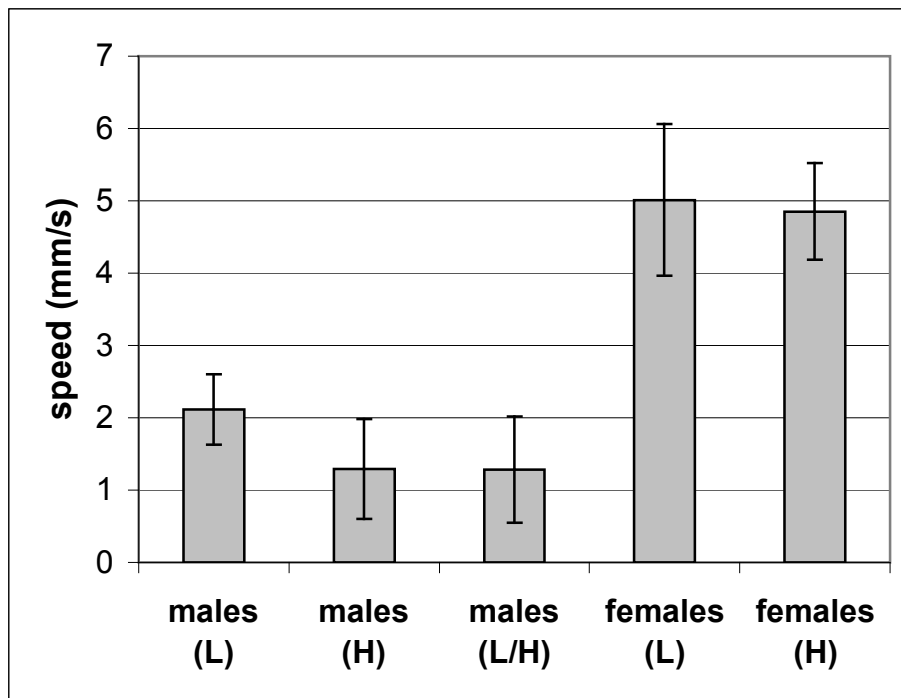
Figure 2.3. The effect of CO_2 on walking speed ± SD (mm/s) of the males and females in high (H) or low (L) CO_2. The walking speed of males that were first exposed to low, and then to high (L/H) CO_2 levels were also investigated.

Male life span

The average life span ± SD of males within closed tubes was calculated to be 20.50 ± 9.48 hours, while the males within the aerated tubes had an average life span ± SD of 14.33 ± 10.48 hours. This shows that even after the exit hole is completed and the air less humid within the fig that most males will live longer than two hours when the OSR becomes male biased (figure 2.2).

Discussion

Compared to other pollinator wasps, the sequence of events in *P. awekei* is very different. In this species mating is observed to take place only after the exit hole is constructed and fighting between the males are readily observed. While searching, the males would ignored each other if they came into close contact when no female were in the vicinity. When a receptive female was present, males continually contested over her by biting each other repeatedly. Two males would often bite and hold each other's head and mandibles, while trying to push one another away. Contests lasted between a few seconds to just under five minutes (figure 2.1) and would start before or during mating by one of the males. Additionally, more than two males were sometimes observed to compete for a female where groups of three to four males pushed and bit each other. No visible external damage was ever observed on any part of the male's bodies or appendages. No takeovers were seen once a male had his aedeagus inserted into the gall, even when repeatedly contested by a number of other males.

In the observation chamber there is a drastic change in the activity of female pollinator wasps when the CO_2 level is lowered from 10% to 0.03%. This change in CO_2

level simulates the fig wall being perforated by the males and the females are able to escape from the fig through this hole. The females are seen to rapidly release from their galls when the internal and external atmosphere equilibrates. Both species (*P. awekei* and *P. soraria*) showed a significant change in the release rate when the CO_2 level is changed. A similar result found by Galil (1973) where the females of the pollinating species, *P. quadraticeps,* released only in low CO_2 environments. An explanation for the delay in release from the galls, until the exit hole is dug could be the potential of physical damage, which might occur to the females, if they were to accumulate in the small lumen.

The pollinator males, of the two species, had different behaviour in high CO_2 atmosphere. The *P. soraria* males were active and several matings were seen. The females did not leave their galls in the high CO_2 atmosphere. Males did not fight when they encountered each other but rather continued their search for receptive females, which were readily available.

In contrast to *P. soraria* males that follow the common fig wasp behaviour, *P. awekei* males differ in certain key aspects. They were clearly inactive in the high CO_2 atmosphere and no matings were seen. They moved significantly slower in the high CO_2 atmosphere (figure 2.3), than in the low CO_2 atmosphere (they did however move away quickly when we tried to remove them with a soft bristle paintbrush). No fights were seen between the males while they were in a high CO_2 atmosphere. When the CO_2 levels are lowered the males became very active and there is a significant increase in the number of matings and fights (table 2.1). There is also a significant increase in their walking speed relative to that in a high CO_2 atmosphere.

There may be a number of reasons why the *P. awekei* males are inactive in a high CO_2 environment. Firstly, it could be some physiological constraint on the males where they are unable to function optimally in a high CO_2 environment. *P. awekei* males are

known to disperse actively (Greeff *et al*., 2003), and must be adapted to a life in low CO_2 atmosphere as they move between figs. *P. quadraticeps* males were seen to be active only in high and not a low CO_2 atmosphere (Galil *et al*., 1973), and may be physiologically adapted to function only in one type of atmosphere. There are however evidence to the contrary which suggest that there is a limited, if any, physiological constraint on *P. awekei* males. The males need to eclose from their respective galls while in a high CO_2 atmosphere after which an exit hole out of the fig must be dug. The males are also seen to be able to move away quickly from *Ottiseline* males when threatened as well as being able to run about when prodded by a brush. When we measured the speed of the *P. awekei* females we found no difference between their mobility in a high or a low CO_2 atmosphere (figure 2.3). They are also the only species recorded, where the females construct the exit hole from their galls. The females are therefore able to function equally well in either atmosphere and this is possibly true for the males too (at least over a short time span).

An alternative explanation for the low activity of *P. awekei* males in high CO_2 could be because the females are not receptive in this atmosphere. The males do not waste energy on searching for females or on fighting until the atmosphere change and the females becomes receptive. By waiting, the females force the males to spend energy only on digging an exit hole before they mate and fight, which could reduce their ability to dig later.

Regardless of the cause for the change in behaviour of *P. awekei* males and females, the effect is a sudden decrease in the number of females to a relative small decrease in the number of males (Moore, personal communication). The OSR therefore becomes male biased in less than two hours (figure 2.2) after the exit hole is completed and the associated atmospheric changes took place (assuming that the same proportion of males and females are sexually active). We observed some of the females not being

mated when males passed their galls, even when the CO_2 level was low, indicating that not all the females are receptive as soon as the CO_2 level is lowered. The OSR may therefore be even more male biased than estimated.

The mating system is also influenced by the change in the environment when the males become sexually active. Both a female and male biased OSR exists during the sexually active phase of the males, although the male biased OSR is present for a greater part of the male's life span. This has the following implications for the males and for male-male conflict. The number of possible future matings will decrease with the decreasing number of females. The males need to search each gall individually for a female and cannot easily and directly assess the number of females left in the population. This will give rise to contest rather than scramble competition, as the males do not know if a mating opportunity is their only or last.

The sex ratio in this species is less female biased than in other pollinator species and if males are not distributed in an ideal free distribution this effect is enhanced. In addition, the OSR rapidly becomes male biased, which also lead to an increase in the frequency of challenges between the males per female. The mating system is also, in part, defined by the spatial and temporal distribution of the receptive females (Emlen & Oring, 1977). *P. awekei* females are receptive for a short, asynchronous period, relative to the males, increasing the environmental potential for polygamy (Emlen & Oring, 1977). These factors as well as the fact that males contests for the females (albeit one at a time) make this a female defence polygyny mating system.

In conclusion, fighting between the males of the species *P. awekei* is probably triggered by the sex ratio. Males frequently encounter each other, as the sex ratio is less female biased than in other pollinator species that quickly turns into a male biased OSR. The behaviour of *P. awekei* are seen to regulated by the gaseous environment and are different from other pollinator fig wasps. Low levels of CO_2 trigger the release of females

of both species. The males of *P. soraria* are active in high levels of CO_2 and this may produce a female biased OSR during mating while males of *P. awekei* are active only in low CO_2. This study also confirms the importance of controlling the gaseous environment in which fig wasp behaviour is observed

Conclusion

Fighting in male pollinating fig wasps appears to be driven mainly by the sex ratio. Chapter one of this dissertation examines how small variations in the life histories and the environment may cause male fighting in some pollinator species but not in others. In chapter two, the mating system of the species *P. awekei* is investigated and the influence of the sex ratio thereon revealed. The effect of the environment on the OSR is also elucidated. The main findings of this study therefore are:

The level of relatedness of the competing individuals does not play a role in the evolution of fighting behaviour. This supports of a number of theoretical studies (Grafen, 1984; Murray, 1984; Griffin & West, 2002). The environment (i.e. the gall density and the lumen volume of the fig), as well as the number of female offspring per fig also does not have a significant effect on the evolution of male fighting in pollinator fig wasps. The sex ratio is however seen to have a significant effect on the evolution of fighting and there is a strong correlation between species with a less female biased sex ratio and males fighting. Less female biased sex ratios will increase the interactions between males with other males and simultaneously decrease the interactions of males with females. Males will therefore need to compete more to obtain matings, and as the sex ratio becomes more equal fighting behaviour evolve. Fighting between males will however not directly influence the sex ratio as the foundress females would always try to limit LMC. Dispersal is also significantly linked with less female biased sex ratios but no directionality can be inferred as dispersal can drive the sex ratio to be less female biased and *vice versa.* The association of fighting behaviour and dispersal is not supported by theory. Fighting and dispersal may therefore be driven by some other factor (such as the sex ratio) and still have antagonistic effects on each other.

An improvement on this study would be to obtain more species from different genera, which have different fighting behaviours rather than many species in one subfamily having the same behaviour. An example would be to include data form the species *Courtella michaloudi* that fight and are known to group with the non-fighting *Courtella armata* (Erasmus, submitted), which would bar the collapse of *Courtella armata* with the *Elisabethiella genera.* This would increase the number of independent data points, as all the species that lie adjacent to each other on the phylogeny and have the same behavioural characteristic collapse to form a single data point during the phylogenetic regression. Careful selection of species from completed phylogenies is important in planning a comparative study.

In *P. awekei* the males are inhibited by the high CO_2 found within a fig. No mating therefore takes place until the exit hole is constructed. When the exit hole is completed, the CO_2 level in the fig changes. This triggers the males to start mating with the females and the females to disperse from the fig. The female biased sex ratio, originally produced to limit LMC, rapidly changes and becomes male biased. A similar effect of lowered CO_2 is seen on the number of releasing females of *P. soraria,* but the non-fighting males are active in the high CO_2 environment. Fighting is readily seen between the males of *P. awekei* in the low CO_2 atmosphere if a female was present. Males repeatedly bit and pushed each other to be first to engage mating. This behaviour was also observed while mating took place although no takeovers were recorded. The sex ratio of *P. awekei* is less female biased than non-fighting pollinator wasps and the rapid increase thereof could be the main determinant of fighting in this species.

An additional experiment which could have been done, would be to increase or decrease the number of males per fig. From this we could see if there is an association of the sex ratio with the number and frequency of fights per fig.

The key roll of the sex ratio in the evolution of male fighting is revealed in this study. The proximate role of the sex ratio on males is also illustrated. Male fighting, both proximal and ultimate is therefore to a large degree driven by the sex ratio. Further studies for a number of fighting and non-fighting pollinator wasp species are however required to determine how the produced sex ratio and the OSR are related. From this better assumptions on the mating system and the evolution of male conflict could be made.

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Appendix A

Dorsal and side view of the pollinator males and their associated females used in this study, with the fighting status and host *Ficus* indicated. Note the size difference between the mandibles and legs of the fighting and non-fighting species

Alfonsiella binghami (fighting; from *Ficus stuhlmannii*)

Alfonsiella species 1 (fighting; from *Ficus craterostoma*)
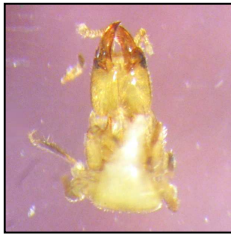
Alfonsiella species 2 (fighting; from *Ficus petersii*)

Allotriozoon heterandoromorphum (fighting; from *Ficus lutea*)

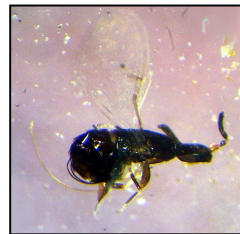*Courtella armata* (non-fighting; from *Ficus sansibarica*)

Elisabethiella bergi breviceps (non-fighting; from *Ficus trichopoda*)

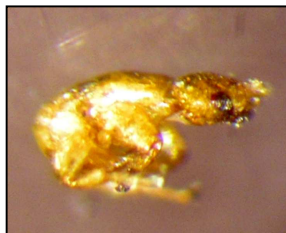*Elisabethiella comptoni* (non-fighting; from *Ficus abutilifolia*)

Elisabethiella glumosae (non-fighting; from *Ficus glumosa*)

Elisabethiella stuckenbergi (non-fighting; from *Ficus burkei*)

Platyscapa awekei (fighting; from *Ficus salicifolia*)

Platyscapa soraria (non-fighting; from *Ficus ingens*)