Social evolution in *Melittobia*

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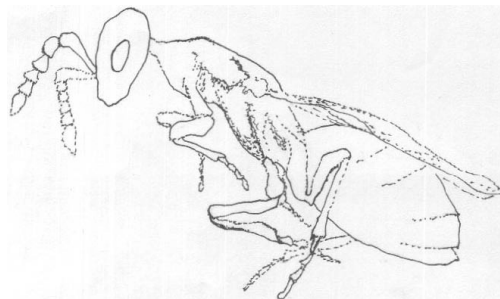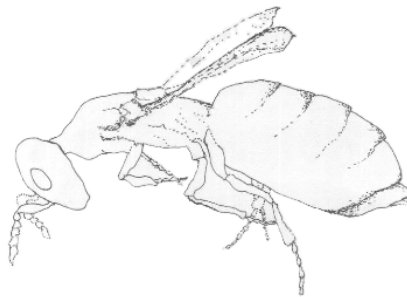
Abstract

Interactions between individuals can range from peaceful cooperation, through mediated contest, to escalated conflict. Understanding such diversity of interactions between individuals requires an understanding of the costs and benefits involved with these behaviours, and the influence of relatedness between interacting individuals. Species in the parasitoid wasp genus *Melittobia* display social behaviours at both extremes of this spectrum, from the potentially cooperative traits of the ratio of male to female offspring that they produce, and the dispersal of females to new habitats, to the extreme conflict of violent contests between males. In this thesis, I examine a number of aspects of social evolution in *Melittobia*. First, I consider the pattern of sex allocation – the division of resources between male and female offspring - where local mate competition theory predicts that females will adjust their offspring sex ratio (proportion of males) conditionally, with females laying increasingly female biased sex ratios as the number of other females laying eggs on the same patch increases. In Chapter 2, I show that *M. acasta* females always lay an extremely female biased sex ratio, and that this may be explained in part by the fact that male *Melittobia* engage in violent lethal combat in competition for mates. Early emerging males have a competitive advantage and thus there is no advantage for later laying females to produce a less female biased sex ratio. However, I also demonstrate that the advantage of early emergence can be reduced when we consider a males body size, which linked to fighting ability, suggesting that the occurrence of this extreme conflict does not fully explain the unusual pattern of sex allocation in *Mellitobia*. In Chapter 3, I examine whether the level of dispersal varies in response to the extent of local competition for resources, and the relatedness between competitors. I use the species *M. australica*, which readily produces two distinct female dispersing morphs, to show that the production of dispersing females increases with the competition for resources. I consider the parallels between the evolution of dispersal and of sex ratio. In Chapters 4 and 5, I examine male fighting in more detail and explore theory that predicts that when extreme conflict does evolve, the incidence of fighting varies with resource value, number of competitors, and the level of relatedness between males. I show that mating opportunities are

sufficiently valuable that male *Melittobia* will always engage in fighting irrespective of relatedness, that there is no opponent assessment prior to fighting, and that the intensity of fights increases with the number of competitors. This thesis highlights the importance of considering combinations of social traits and the interactions between them, to understand the evolution of social characters.

Declaration

The research described in this thesis is only possible through collaboration, details of which are provided below.

Chapter 2
Experiments 1 and 2 were carried out with an honours student, Jo Savage.

Chapter 4
Data for this experiment was collected by Sarah Reece and two honours project students, Andrew MacMath and Simon Donegan. Data analysis was principally carried out by Sarah Reece. I have written the chapter and produced the subsequent manuscript.

Chapter 5
Experiment 1 was carried out with an honours student Nita Rikkanen; and experiments 2 and 3 with a second honours student, Jenni Sanderson.

Unless otherwise stated, the remaining work and content of this thesis are my own.

Signed:

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Acknowledgements

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List of equations, tables and figures

Chapter 1 Introduction

1.1 *Social evolution: cooperation to conflict*

1.1.1 What are social behaviours?

A behaviour is termed social when it has consequences for both the actor (the individual carrying out the behaviour) and the recipient (Frank, 1998; West et al., 2007b). Clearly, this is often true for interactions, both cooperative and involving conflict. Social behaviours can be categorised depending upon their fitness costs and benefits (Hamilton, 1964): Selfishness benefits the actor, at a cost to the recipient; altruism benefits the recipient at a cost to the actor; mutualism benefits both actor and recipient; and spite has a cost for the actor and for the recipient (Table 1.1). Hamilton (1964) showed that a social behaviour will be favoured when rb>c, where b is the benefit to the recipient, c is the cost to the actor, and r is the relatedness of actor to recipient. Hamilton's (1964) rule underlies much of our understanding of social behaviour, and can be used to help explain behaviours ranging from sex ratio to dispersal and conflict; and exceptional examples where Hamilton's rule does not apply, such as when extreme conflict occurs despite high relatedness (West et al., 2001; 2002), can be particularly informative.

	Effect on recipient	
	+	**-**
+	mutualism	selfishness
-	altruism	spite

Effect on actor

Table 1.1: a classification of social behaviours.

1.1.2   Cooperation to conflict

Interactions between individuals can lie anywhere on a spectrum between peaceful cooperation and escalated conflict. For instance, relatively peaceful cooperation occurs between cooperatively breeding vertebrates, and in a number of bird species where helpers remain at the nest to raise the subsequent offspring of a related breeding pair (e.g. Clutton-Brock et al., 2000; Griffin et al., 2003; Komdeur et al., 1997). In cases where contests are more likely, such as when male red deer or fighting fish compete for matings, violent escalation is often prevented by the ritualised behaviour and assessment which mediates these contests (Clutton-Brock and Albon, 1979; Jakobsson et al., 1979). Some form of convention or social hierarchy may also limit conflict, as seen between queuing females of the paper wasp *Polistes dominulus* (Cant et al., 2006; Innocent and West, 2006). At the opposite extreme of this spectrum, wingless male fig wasps compete violently within fig fruit for access to locally emerging female mates (Hamilton, 1979; Murray, 1987; 1989).

Why do some interactions lead to cooperation when others end in conflict? The key to understanding the range of behaviours observed is to understand the balance of costs and benefits for individuals, and to consider the influence on these costs and

benefits of relatedness. For instance, high costs incurred from not cooperating, or benefits (direct or indirect) gained from cooperation, can favour cooperative interactions (West et al., 2007a). One way of looking at the importance of relatedness is that, if $r < 1$ then selection can favour the evolution of conflict between individuals, whilst if $r > 0$ then selection can favour cooperation; this sets up a spectrum, along which the balance of costs and benefits, and the specific level of relatedness can vary to produce different behaviours. In general, the most common strategy observed is one of conflict limitation, where mechanisms have evolved to minimise the occurrence of costly, violent conflict (Maynard-Smith and Price, 1973). In order to fully understand why the outcome of interactions varies so much, it is important to consider specific examples (and identify the costs and benefits); it is particularly useful to study the extremes of this spectrum, and the exceptions to the general rule of conflict limitation. In some instances, both extremes of cooperation and conflict are found within the same species, often associated with unusual natural history (Hamilton, 1979; Murray, 1987). And, as the factors that bring individuals together to cooperate will also bring them together to compete, both cooperation and extreme conflict can be favoured between relatives (West et al., 2001; 2002).

In this thesis, I consider the social evolution of the parasitoid wasp genus *Melittobia*, which exhibits behaviour at both spectrum extremes: these wasps display both potentially cooperative traits, such as sex ratio and dispersal (of females); and also extreme conflict, most strikingly in the form of fatal fighting (between males).

1.1.3   Sex allocation and dispersal as social behaviours

Within the field of social evolution as a whole, a large amount of work has considered two widespread social traits, sex allocation and dispersal. One reason for this is because the costs and benefits associated with social behaviours will vary, depending on the demographic context within which individuals interact; and the distribution of resources and individuals is a central component of this (Frank, 1998). Why are these two traits considered to be social? Both sex ratio and dispersal commonly occur in situations where a number of individuals interact, and often when

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these individuals are related. More specifically, with local mate competition (LMC; Hamilton, 1967b; see section 2.2), the optimal sex ratio will depend upon the level of competition between relatives. And in contrast, dispersal from a patch where related individuals must compete can be favoured in order to reduce the level of competition experienced by relatives (Hamilton and May, 1977; see section 3.1). Both sex ratio and dispersal have commonly been used as model traits for developing methods for social evolution theory (Frank, 1998; Taylor and Frank, 1996). In addition, sex ratio and dispersal are interesting traits to consider together because: (1) there are a number of similarities between the evolutionary processes leading to the evolution of dispersal and of sex ratio; (2) the evolution of these two traits is affected by the same factors, and they may influence each other. Therefore it is possible to consider sex ratio and dispersal independently, and also to try and understand the interaction between these traits within a wider social context.

1.1.4   Thesis aims & outline

In this thesis I consider aspects of the social evolution of the parasitoid wasp genus *Melittobia*. A number of aspects of the biology of *Melittobia* are unusual (details in chapters 2-5), and I use this system to ask questions about several of these social traits: sex ratio, female dispersal, and fatal fighting amongst males. Moreover, *Melittobia* are a good system for trying to understand different social traits at the same time, as this system allows us to ask questions about the relationship between social traits, as well as the traits themselves.

In chapter 2, I examine the pattern of sex allocation in *Melittobia,* and ask: What is the pattern of sex ratio observed in *Melittobia,* and is sex ratio adjusted with variation in LMC? Does fatal fighting between male offspring influence sex allocation? And are there additional/alternative influences? I describe the pattern of sex allocation in *Melittobia acasta*, which produces an extremely female-biased sex ratio, and shows a lack of sex ratio adjustment. I discuss the potential explanations for this unusual pattern of sex allocation.

In chapter 3, I ask: Is dispersal in this system influenced by competition between relatives? And is the pattern of dispersal associated with the pattern of sex allocation? Here, I consider the evolution of dispersal when relatives compete for resources. *Melittobia* species produce two female morphs, a dispersing and a non-dispersing form. I describe the patterns of morph production, and then examine whether variation in the level of dispersal is related to the pattern of sex ratio.

In chapters 4 and 5, I focus on the nature of extreme fighting behaviour observed between male *Melittobia*, and consider how this behaviour evolved. Here, I ask: What is the nature of fighting behaviour between male *Melittobia* in competition for mates? What influences the intensity of fighting between males? Is the relatedness between opponents important? And finally, is there evidence for kin discrimination in *Melittobia*? I test experimentally the importance of several biological variables predicted to be important in competition for mates. I also consider the relationship with sex ratio, and look for evidence of kin discrimination from the pattern of fighting behaviour with variation in relatedness (chapters 4 and 5).

In chapter 6, I summarise and discuss the key findings from experiments described in chapters 2-5, and make several general points highlighted by the work in this thesis.

Each chapter of my thesis has a specific introduction that describes the relevant literature. In the remainder of this introduction, I provide a brief overview of the relevant literature on the relevant aspects of sex allocation and dispersal.

1.2 Sex allocation

1.2.1 Fisher's theory of equal investment

Decisions about how to allocate resources to offspring, in particular, sex allocation - the division of resources between male and female offspring - have important implications for individual fitness. Sex allocation theory predicts that individual females should allocate resources to male and female offspring in a way that maximises the fitness returns gained from these offspring (Charnov, 1982). The optimal strategy for sex allocation in large panmictic populations - first considered by Darwin (1871), and first modelled mathematically by Düsing (1883, 1884; in Edwards, 1998; 2000) - was eloquently explained by Fisher (1930). Fisher (1930) argued that, as observed in many species, equal investment in male and female offspring is favoured by natural selection. He reasoned that the total reproductive success for males must equal that of females, because an individual must be the product of two parents, one male and one female. In a population where males were common and females rare, then on average the reproductive success would be higher for the rarer sex, females. Consequently, parents who produced a higher proportion of females would also produce a greater number of grandchildren and gain higher fitness; and selection would therefore favour parents that overproduce the rarer sex. As a result of this negative frequency dependant selection, the proportion of the rarer sex in the population would increase towards an equal (unbiased) sex ratio. Similarly, if males were rare, the reverse would apply. Overall, theory predicts that equal investment in male and female offspring will evolve as the evolutionary stable strategy (ESS; Maynard-Smith, 1982), reflecting equal investment of resources in males and females, not necessarily equal numbers of males and females (Fisher, 1930).

Fisher's (1930) theory of equal investment forms the basis of all sex allocation theory. It has been extended in numerous directions, by relaxing the implicit

assumptions, to explain cases of biased sex allocation (West, 2009). The field of sex allocation has a substantial theoretical underpinning, and makes specific predictions that have been widely tested empirically (Charnov, 1982; West et al., 2005; West, 2009). Much success in this area has followed from the fact that the important features of biology that influence sex allocation can be identified and modelled, and often sex ratio can be measured with relative ease in natural or laboratory conditions (West et al., 2000; 2005; West, 2009). Sex allocation theory is therefore able to explain variation in sex ratio and its fitness consequences across a wide range of taxa (Charnov, 1982; West et al., 2005; West, 2009). Consequently, sex allocation is one of the most productive and well-understood areas of evolutionary biology. This has made sex allocation a useful model trait for developing social evolutionary theory more generally (e.g. Taylor and Frank, 1996; Frank, 1998), and addressing more general biological questions (West et al., 2000; West, 2009). Within the field of sex allocation, most attention has focused upon cases where biased sex allocation occurs (West, 2009), and I discuss the relevant issues in more detail below.

1.2.2 Biased sex ratios & LMC: When more Mums = more sons…

One important reason for biased sex allocation is the influence of cooperative or competitive interactions between relatives (Hamilton, 1967a; Taylor, 1981; West, 2009). Local resource enhancement (LRE) occurs when producing one sex increases the fitness of relatives, and so selection favours sex allocation biased towards this sex (Trivers and Willard, 1973; Taylor, 1981). For instance in species where offspring of one sex remain at the natal site to help rear subsequent offspring, the sex ratio is biased towards the helping sex, as seen in the Seychelles Warbler (Komdeur et al., 1997); see also (Griffin and West, 2003; Griffin et al., 2005). In contrast, when the production of one sex increases competition for local resources, this can have a detrimental affect on the fitness of related competitors of the same sex; this is known as local resource competition (LRC), and favour biased sex allocation towards the less-competing sex (Clark, 1978; Bulmer and Taylor, 1980b; Taylor, 1981).

Hamilton's (1967) local mate competition theory (LMC) - the best-studied example of LRC - relaxes Fisher's assumption that mating occurs at random across a population and applies when a population is subdivided so that individuals compete for a discrete, ephemeral resource. Under conditions of LMC, related male offspring compete locally for mates, and these mates may be related females – in the extreme case of a single female foundress laying offspring on a patch, all competitors are brothers, and all mates, sisters. Hamilton's (1967) LMC theory makes two predictions with regards to sex ratio: first, that under LMC a female-biased offspring sex ratio is favoured; and second, that the extent of sex ratio bias will be proportional to the level of LMC, shifting in the direction of an equal sex ratio as foundress number increases (relatedness decreases). The ESS sex ratio (r, proportion males) for a female to produce under LMC with N foundresses on a patch is predicted by:

$$r = N-1/2N \qquad\qquad \text{(equation 1.1)}$$

Taylor (1981) showed that a female-biased sex ratio is favoured under conditions of LMC for two reasons. First, by producing a higher proportion of daughters, a foundress maximises the number of mates available to her sons. Second, by producing a lower number of sons, a foundress minimises competition between sons for mates (Taylor, 1981). When a single foundress produces offspring on a patch, equation 1 predicts that s = 0; this is interpreted to mean that a female is expected to produce the maximum number of daughters possible, and the minimum number of sons required to fertilise them (Hamilton, 1967a).

1.2.3   LMC & inbreeding in haplodiploids – Mummy's girls?

In haplodiploids, there is another, additional effect of LMC due to inbreeding (Frank, 1985; Herre, 1985). With haplodiploid sex determination a mother produces daughters from fertilised (diploid) eggs, and sons from unfertilised eggs (haploid) (Cook, 1993). If a female mates with a related male this mate will pass shared genes to female offspring, making a mother more related to her daughter under inbreeding. In contrast, male offspring will only receive gene copies from their mother, and so

inbreeding has no effect on the relatedness of a mother to her sons. Therefore, with inbreeding, a mother is relatively more related to her daughters than her sons. This favours an additional female-bias in offspring sex ratio beyond LMC predictions (Hamilton, 1972; 1979; Frank, 1985; 1986b; Herre, 1985; West, 2009). The ESS sex ratio (r, proportion male offspring) under LMC for haplodiploids is predicted by the following, modified, equation:

$$r = (N-1)(2N-1)/N(4N-1) \hspace{3cm} \text{(equation 1.2)}$$

The sex ratio pattern predicted by LMC theory for both diploids and haplodiploids, across a range of foundress numbers is shown in figure 1.1.
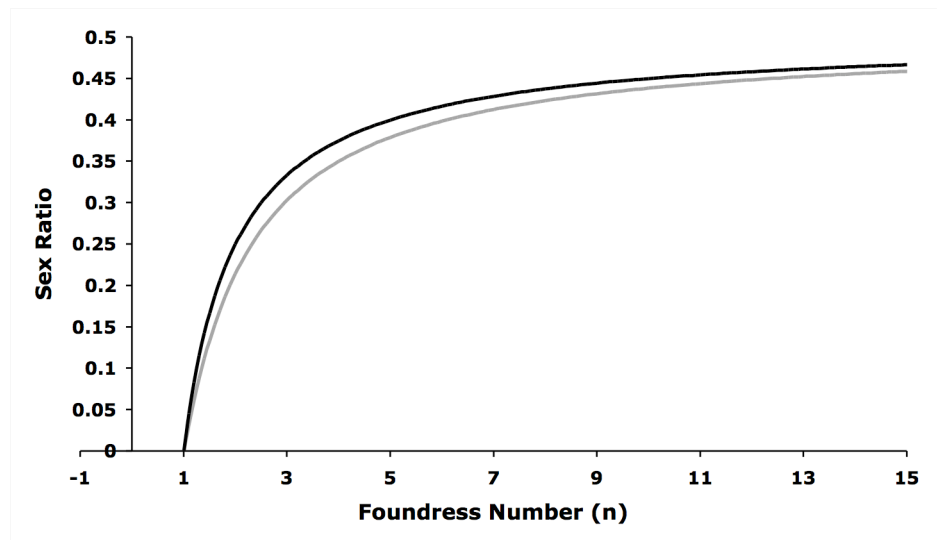
Figure 1.1: LMC curve illustrating the predictions for optimal sex ratio under LMC for diploid (black line) and haplodiploid (grey line) species.

1.2.4   Extensions of basic LMC Theory

Hamilton's original model of LMC has since been extended in many ways, to describe specific biological examples. For instance, LMC has been modelled for

cases where females lay different numbers of eggs on the same patch, with oviposition (egg-laying) either sequential (superparasitism; e.g. Werren, 1980) or simultaneous (Frank, 1985; 1986b). One specific extension of LMC models for sequential oviposition considers asymmetrical LMC (I test an example of this type of model for the sex ratio in *Melittobia*; chapter 2). Here, a male's mating success varies with the brood he's laid, and different males experience different (asymmetric) levels of LMC (e.g. Abe et al., 2003b; 2005; Shuker et al., 2005). For instance, early emerging males may have a mating advantage compared to later emerging males; this asymmetry will be more pronounced if early emergence confers a competitive advantage in male-male competition, such as an advantage in attack, or greater size (e.g. in *Melittobia*: Abe et al., 2003a; 2003b; 2005; Innocent et al., 2007). With asymmetry in the level of LMC, the fitness returns of laying males diminish with successive broods; this selects for superparasitising foundresses to lay more female-biased sex ratios than predicted by sequential LMC models – and in the extreme case, can favour little or no sex ratio adjustment (Abe et al., 2003a; 2003b; 2007; Innocent et al., 2007).

A second alternative scenario relevant to sex allocation in *Melittobia* occurs when multiple females oviposit on a patch, but the females are themselves related (see chapter 3). These models assume mating that female dispersal is limited, increasing the relatedness between co-founding females (and produce the same sex ratio for a given foundress number) (West, 2009). The general prediction here is that foundresses will lay a more female biased sex ratio than predicted by LMC (Bulmer, 1986; Frank, 1985; 1986b; 1998). A female-biased sex ratio is favoured because in this case multiple female foundresses on a patch is equivalent to a single foundress, since there is little variation in average relatedness as a consequence of increasing foundress number (Frank, 1985; 1986b). Thus, producing few sons minimises competition for mates between male relatives, and producing many daughters maximises the number of mates available to them (Frank, 1985; 1986b; Taylor, 1981).

1.2.5   Empirical tests of LMC

LMC theory is particularly well developed and has been thoroughly tested empirically, with wide-ranging support from a variety of taxa (Charnov, 1982; West et al., 2005; West, 2009). Precise theoretical predictions can be made, and these have been tested most comprehensively using the parasitoid wasp *Nasonia vitripennis*, and providing strong qualitative and quantitative support (Werren, 1980; 1983). First, the empirical data shows that under high levels of inbreeding the offspring sex ratio is extremely female biased (Werren, 1980; 1983). Second, the sex ratio in *N. vitripennis* is adjusted according to the number of foundresses, with a shift towards an equal (unbiased) sex ratio with increasing foundress number (Werren, 1980; 1983). Recent studies have also found a similar pattern of sex ratio variation with variation in foundress number in natural populations of *N. vitripennis* (Burton-Chellew et al., 2008; Grillenberger et al., 2008). More general tests of LMC in a range of taxa from flowering plants to malaria parasites qualitatively support the prediction that the average sex ratio should correspond to the intensity of LMC in populations (West et al., 2005; West, 2009). Support has also been found, in some cases, for facultative adjustment of sex ratio across species or populations with variation in the level of LMC. For instance, fig wasp species that experience greater variation in foundress number in natural populations (stronger selection for sex ratio adjustment), show greater facultative sex ratio adjustment; and species meet the predictions of LMC most closely for the foundress number(s) encountered most frequently (Herre, 1985; 1987; West and Herre, 1998b).

More generally, as sex allocation is a particularly well-understood area of evolutionary biology with strong empirical support, examples where sex allocation does not meet the predictions of LMC pose a problem, and need to be explained. The unusual pattern of sex ratio in *Melittobia* species is a striking exception: females produce an extremely female-biased sex ratio, which is adjusted little in response to variation in foundress number (Abe et al., 2003b; 2005; Cooperband et al., 2003; Innocent et al., 2007). The possible explanation for this unusual pattern is one of the central themes of this thesis (chapters 2, 3 and 6).

1.3 Dispersal

1.3.1 Dispersal as a social trait

Hamilton and May (Hamilton and May, 1977) first demonstrated theoretically that, when related individuals compete locally for a resource, dispersal can be considered a social trait. This is because dispersal will reduce competition between relatives, and increase the direct fitness of relatives (and the indirect fitness of the disperser), despite any direct cost to the disperser; it can therefore be considered a cooperative, potentially altruistic trait (Hamilton and May, 1977; West et al., 2007b). Extensions of this model (Comins et al., 1980; Comins, 1982) considered the effect of variation in foundress number, and therefore variation in relatedness on a patch, as average relatedness will decrease with increasing foundress number. In combination, this theoretical work predicts that selection for a higher level of dispersal will occur when a greater number of offspring are competing (for a resource), or when offspring are more highly related (as a result of being produced by a lower number of mothers), or both (Comins et al., 1980; Comins, 1982; Hamilton and May, 1977).

Hamilton and May's original model has subsequently been extended prolifically, in an attempt to consider more biologically realistic scenarios. The effect of inbreeding, population size, population structure, age structure, dispersal rate and distance, have all been considered (Bulmer and Taylor, 1980a; Comins et al., 1980; Comins, 1982; Frank, 1986a; 1998; Taylor, 1988; 1994; Crespi and Taylor, 1990; Taylor and Frank, 1996; Gandon, 1999; Gandon and Michalakis, 1999; Gandon and Rousset, 1999; Irwin and Taylor, 2000; Leturque and Rousset, 2002; 2003; 2004; Motro, 1982a; 1982b; 1991; Ronce et al., 2000; Rousset and Billiard, 2000; Rousset and Gandon, 2002; Wild and Taylor, 2004; Roze and Rousset, 2005; Wild et al., 2006).

1.3.2 Dispersal and sex ratio

A parallel can be drawn between the evolutionary processes leading to the evolution of dispersal, and of sex ratio, as both are influenced by competition between relatives (Bulmer and Taylor, 1980a; Frank, 1998; Leturque and Rousset, 2003; 2004; Motro, 1991; Taylor, 1994; Perrin and Mazalov, 2000; Rousset and Billiard, 2000; Wild and Taylor, 2004). Predictions can therefore be made for the combined effects of sex ratio and dispersal when individuals compete locally for resources (Hamilton, 1967a; Bulmer and Taylor, 1980a; Taylor, 1981; West, 2009). When one sex disperses less, relatives of this sex experience a greater level of competition, and so a sex ratio bias towards the more-dispersing sex is favoured (Hamilton, 1967a; Bulmer and Taylor, 1980a; Taylor, 1981). Similarly, when the sex ratio is biased towards one sex, a higher level of dispersal in that sex is favoured to reduce competition between relatives of the same sex (e.g. Taylor, 1981; Wild and Taylor, 2004). Overall, increasing foundress number predicts lower average relatedness between competing individuals, and therefore favours both less female biased sex ratios (Hamilton, 1967a; Taylor, 1981), and lower rates of dispersal (Hamilton and May, 1977; Comins et al., 1980; Comins, 1982).

Chapter 2   Lethal combat and sex ratio evolution in a parasitoid wasp

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2.1   Introduction

Hamilton (1967a) showed that when the offspring of one or a small number of females mate within the natal patch, prior to dispersal by females, a female biased sex ratio is favoured (Local Mate Competition, LMC). The explanation for this bias can be described equally well through an individual (inclusive fitness) or a hierarchical (group) selection approach (Colwell, 1981; Taylor, 1981; Harvey et al., 1985; Frank, 1986c; 1998; West et al., 2007b). The inclusive fitness approach suggests that the female bias is favoured in order to: (a) decrease competition between sons, and (b) increase the number of mates available to them (Taylor, 1981). In haplodiploids an additional bias is favoured because inbreeding increases the relative relatedness of mothers to their daughters (Hamilton, 1972; Frank, 1985; Herre, 1985). LMC theory predicts that in haplodiploids, the evolutionary stable (ES) sex ratio (*r*; proportion male) is given by the equation $r = (N-1)(2N-1)/N(4N-1)$, where *N* is the number of foundress females laying on the patch. There has been widespread support for this prediction, with female biased sex ratios being observed in a huge number of plant and animal species where LMC is likely to occur (Charnov, 1982). Furthermore, individuals of over sixty species, from a range of taxa, have been shown to adjust their sex ratio conditionally, in response to the number of females laying eggs on a patch (West et al., 2005).

In stark contrast, species of the parasitoid wasp genus *Melittobia* do not appear to shift sex ratio in accordance with LMC predictions (Abe et al., 2003b; 2005;

Cooperband et al., 2003). *Melittobia* species have a life history that meets the conditions where LMC is expected: competition for mates occurs between brothers, in order to gain mating opportunities with their sisters, and females disperse after mating (see methods section for a more detailed life history description). As expected from this, *Melittobia* species have extremely female biased average sex ratios, and were given as an example by Hamilton (1967), in his original LMC paper. However, recent studies on *M. australica* and *M. digitata* have shown that females fail to shift their offspring sex ratio with increasing foundress number as predicted by LMC theory (Abe et al., 2003b; Cooperband et al., 2003; see also Molumby, 1996). It has been suggested that a possible explanation for this relative lack of a shift in sex ratio is that males fight fatally, in order to gain mating opportunities (Abe et al., 2003a; 2003b; 2005). Theory has shown that if two females lay eggs on a patch sequentially, and males laid by the second female have a reduced mating success, then the second female can be selected to produce an extremely female biased sex ratio, in contrast to the predictions of classic LMC (Abe et al., 2003a; Shuker et al., 2005). The idea here is that the fitness gain from later laid males is reduced because they may be killed without any mating success. Support for this idea has been obtained from the observation that later emerging males tend to be killed by older, previously emerged males (Dahms, 1984; Gonzalez et al., 2004c; Abe et al., 2005).

However, another factor that can influence fights between males is body size. Larger males are more likely to win fights in *Melittobia* (Hartley and Matthews, 2003; Reece et al., 2007), as is the case in many animals, ranging from other insects (e.g. Murray, 1987; Greeff and Ferguson, 1999; Hartley and Matthews, 2003; Taylor and Jackson, 2003; Batchelor et al., 2005; Cook and Bean, 2006) to mammals (Clutton-Brock and Albon, 1979; Haley et al., 1994; McElligott et al., 2001; Preston et al., 2003; Lidgard et al., 2005). If this effect of size in *Melittobia* reduces or overrides the influence of emergence order, then fatal fighting will have a reduced influence on sex ratio evolution. In this situation we would predict an interaction between size and emergence order – for instance, emergence order may vary in importance as the asymmetry in the size of opponents changes. Furthermore, there may be an important distinction to be made between emergence order and age. Emerging early could be

advantageous if males gained an advantage because males are relatively defenceless before or during emergence, or if there is a period before cuticle sclerotisation occurs (Abe et al., 2005). Consequently, any advantage of emergence order may only be transient, and age itself may not be important once all males have emerged.

Here, I aim to test the potential importance of fatal fighting in restricting sex ratio shifts in *Melittobia*. In the methods section I provide a more detailed description of the natural history of wasps from this genus, emphasising similarities and differences relative to more familiar LMC model systems. The sex ratio behaviour of the species (*M. acasta*) I am working with has yet to be examined, so I first manipulated foundress number, to show that females of this species also fail to adjust their offspring sex ratios as predicted by LMC theory. I also manipulated the time that females were allowed to spend ovipositing, as this influences the number of eggs that a female can lay, which has been shown to influence offspring sex ratios in several parasitoid wasps (Godfray, 1994). I then carried out a series of experiments in which I examine the importance of both emergence order and age, and how these factors interact with size to influence male fighting success. I first control size and focus on emergence order, placing pairs of males who have emerged from their pupal cases with males who have not, to test the idea that emerged males have an advantage in combat because they kill other males either before, during or just after emergence. I then extend this scenario to include variation in size, to examine whether or not size advantage can overcome the potential gain from early emergence. Finally, I investigate the effect of emergence order beyond the time of emergence (age), to assess how the relationship with size (competitive ability) changes through the period of combat.

2.2   Methods

2.2.1   Natural history of the *Melittobia* genus

Melittobia acasta (Hymenoptera: Eulophid) is a gregarious ectoparasitoid wasp, with an exceptionally wide host range that includes Diptera and Lepidoptera, along with other Hymenoptera (Balfour Browne, 1922; Freeman, 1977; Van den Assem et al., 1980; Dahms, 1984; Gonzalez et al., 2004c). *Melittobia* species exhibit pronounced sexual dimorphism. Males are blind with reduced wings, and remain within the natal patch to compete for mating opportunities. Eclosing before females, males will fight to the death using their mandibles to attack and remove opponents' heads and limbs (e.g. Dahms, 1984; Gonzalez et al., 2004c). The male(s) remaining alive will then mate females within the natal host pupa as they emerge. In contrast, females have fully functioning eyes and wings, and will disperse after mating to find new patches of hosts. Females are typically able to lay large clutch sizes (~200 per host) and, since *M. acasta* is haplodiploid, females can adjust (offspring) sex ratio, producing daughters from fertilised and sons from unfertilised eggs. Virgin females have been observed to lay a small first clutch of males, and mate with one of these sons upon emergence, in order to lay a larger second clutch containing females (Balfour Browne, 1922; Dahms, 1984).

Available data suggest that the natural histories of species in the *Melittobia* genus are very similar (e.g. Van den Assem et al., 1980; Gonzalez et al., 2004a; 2004c). Female biased sex ratios have been reported from field studies, suggesting that a lack of sex ratio shift in the lab is representative of natural conditions (Van den Assem et al., 1980; Gonzalez et al., 2004a; Gonzalez et al., 2004c). Data suggests that foundress number is variable in natural populations, and is influenced by host size and density in a number of *Melittobia* species (*M. hawaiiensis*: Freeman and Ittyeipe, 1976; Freeman, 1977; *M. japonica*, *M. acasta*: Van den Assem et al., 1980; *M. femorata*: Molumby, 1996; Cooperband et al., 2003). Two female morphs are found, a long-winged dispersing and a short-winged non-dispersing morph, of varying

proportion between broods (*M. chalybii:* Schmieder, 1933; *M. australica*: Freeman and Ittyeipe, 1976; Freeman and Ittyeipe, 1982; Dahms, 1984; *M. digitata*: Consoli and Vinson, 2002a; Cooperband, 2003; *M. clavicornis*: Gonzalez et al., 2004a). The amount of dispersal relates to resource availability, which is known to vary across the host range, thus providing further (indirect) evidence of variation in foundress number.

Natural variation in male size has not yet been measured, however, all published data lies within the range I have found in the stock population (Balfour Browne, 1922; Hartley and Matthews, 2003; Gonzalez et al., 2004c). Furthermore, size is known to depend upon clutch size and host quality (Consoli and Vinson, 2002a). Consequently, as *Melittobia* species have an extremely wide host range (Balfour Browne, 1922; Freeman, 1977; Van den Assem et al., 1980; Dahms, 1984; Gonzalez et al., 2004c) male size is likely to vary in natural populations. The male emergence period can be relatively prolonged – 16 days at 25 C for *M. australica* (Abe et al., 2005). Furthermore, staggered emergence and overlapping generations of males are likely, given the possibility of multi-foundress scenarios, and the potential for non-dispersing female offspring to superparasitise large hosts (Schmieder, 1933; Freeman and Ittyeipe, 1976; Cooperband et al., 2003). Males are highly aggressive and will start fighting almost immediately upon emergence, with the majority of fights occurring before the first females start to emerge (Buckell, 1928; Van den Assem et al., 1980; Gonzalez et al., 2004c; Abe et al., 2005). Adult males will attack emerging pupal males, will almost always fight when encountering each other, and these violent encounters often lead to the death of at least one male (*M. acasta*: Balfour Browne, 1922; Dahms, 1984; *M. digitata*, *M. australica*: Abe et al., 2003b; 2005; Buckell, 1928; Gonzalez et al., 2004c).

2.2.2 General methods

For all experiments I used wasps from our UK stock population, established from wasps collected in the field in 2004 (by Mark Shaw, Royal Museum of Scotland, Edinburgh), and subsequently (mass) cultured in the lab on *Calliphora vomita* pupae.

All wasps were reared at 30 C, with a 16L: 8D photoperiod. Under these conditions generation time is 11-13 days for males, with females emerging 1-2 days later. For each generation I placed groups of approximately 100 females in 70 x 25 mm glass vials stoppered with cotton wool, containing ~ 20 host pupae for oviposition (0-200 offspring laid per host).

In order to produce the large numbers of males required for experimental work, I cultured virgin females taken from stock populations: thanks to the haplodiploid genetic system, virgins are limited to laying sons, and thus produce all-male clutches (Cook, 1993). I opened up hosts 10 days post oviposition and removed virgin female pupae, grouping and housing them with fresh hosts according to each experimental treatment (see below). After 6-8 days I was able to dissect male pupae from these hosts, which I isolated in gelatin capsules (volume =0.21ml, similar dimensions to *C. vomita* pupae) before emergence, to prevent fighting prior to the experiment.

2.2.3   Experiment 1: sex ratio adjustment and LMC

I first tested the predictions of LMC theory by manipulating the number of foundress females that are able to lay eggs (oviposit) on a single host, and thus the degree of local mate competition (LMC) experienced by offspring. Specifically, I set up 20 replicates within each of 5 levels of foundress number treatment: 1, 3, 10, 25 and 50 females (i.e. total sample size equals 100). I also manipulated the time females were allowed to oviposit upon hosts. Within each foundress treatment level, I allowed the females in 10 replicates to oviposit for 24 hours, and 10 replicates for 48 hours. Mated adult females were (randomly) chosen from stock populations approximately 24-48 hours after emergence, and randomly assigned to treatment levels. All replicates were placed in stoppered glass vials with a single *C. vomita* host. After either 24 or 48 hours I removed females and returned the host pupae to the vials, which I kept at 30 C until offspring emergence. I then opened each host pupa, and sexed and counted all (adult) offspring to calculate mean brood size and sex ratio.

2.2.4   Experiment 2: Size, age & fighting ability in males

I carried out three experiments to test how male fighting ability was influenced by male size, age, and their interaction. I have previously shown that relatively larger males have a higher probability of winning contests (Reece et al., 2007). Here I examine: (i) the importance of emergence order (i.e. emerged males versus unemerged males); (ii) the interaction between emergence order and size (a measure of fighting ability); and (iii) the effect of age beyond 24 hours and its relative importance compared to variation in size.

2.2.4.1   Experiment 2a: importance of emergence order

In this experiment I investigated the effect of emergence order upon the fighting success of males, using one-on-one arena experiments. I placed pairs of males from the same 'size treatment' (see experiment 2b) within gelatin capsules (volume=0.21ml). Each pair consisted of one adult male, emerged within the preceding 24 hour period; and one pupal male, due to emerge within the subsequent 24 hour period. In order to generate males emerging at 24-hour intervals I set up groups of virgin females every day for 18 days, so that their offspring emerged at 1 day intervals for 18 days.

I generated two size classes of male, large and small. To create males of different sizes I manipulated the number of hosts in a patch upon which groups of females were laying eggs, in order to vary the density of males developing within hosts. I manipulate host number rather than foundress number because virgin females are unlikely to oviposit if alone (Cooperband et al., 2003). I randomly assigned groups of 60 virgin females to treatments, and gave them either 1 or 40 hosts - for large and small treatments respectively. Males from the high male density treatment (high number of foundresses per host) had significantly larger body length ($F_{116,1}$=4.04, P<0.05) and head width ($F_{116,1}$=8.74, P=0.004). After 8 hours females were removed, and hosts incubated for 6-8 days. At this stage I opened hosts, dissected

male pupae and isolated them in gelatin capsules (see above). I then took a random sample of pupae from each size class, photographed them using a Leica dissecting microscope, and used Leica IM50 software to take measurements of head width and body length.

For the purpose of identification I marked adult males on their abdomen with coloured glass paint, before placing them into capsules with pupae. I checked each pair every 24 hours and recorded the date of pupal emergence, the identity of the winner and loser (where loser is defined as the first to die), and the longevity of both males. I discounted any replicates where there was no clear winner (i.e. neither male was dead) within 24 hours after the date of pupal emergence, as contestants were no longer considered to be emerging beyond this point.

2.2.4.2 Experiment 2b: importance of emergence order and size

In this experiment I wanted to examine the relative importance of emergence order and size upon contest outcome. I created different sized males as described in experiment 2a, but then incorporated the 2 size classes in a fully factored design to include the asymmetric parings (i.e. large versus small, small versus large). All pairs were composed of one adult male and one pupal male, giving the following fight combinations: large adult vs. large pupa; large adult vs. small pupa; small adult vs. small pupa; small pupa; small adult vs. large pupa. Once again I checked each pair every 24 hours and recorded the date of pupal emergence, the identity of the winner and loser (where loser is defined as the first to die), and the longevity of both males.

2.2.4.3 Experiment 2c: Size vs Age

I experimentally manipulated both the age and the size of male opponents within contests to examine the relative importance of these factors upon fighting success. The key difference between this and the previous experiment is that in this case I examined the effect of age beyond the 24-hour window around emergence.

I used size treatments as described above in 2a, and also created three age classes of male (young, middle-aged and old). To generate males of different age classes within size treatments, I staggered the hosting of groups of virgin females by one-day intervals. I set up groups of virgin females for each size treatment as described above, every 24 hours, for 18 days. I limited females to an 8-hour oviposition period in order to minimise overlap in the age of males from successive days. I was able to collect male pupae after 6-8 days, and then each day for an 18-day period, recording the date of emergence (and thus age) of every cohort. Experimental age classes were determined by preliminary work (unpublished data), based upon the longevities of a sample of 50 virgin males isolated in gelatin capsules and incubated at 30 C. I categorised males as 'Young' on the day they emerged (0-24 hours); 'Middle aged' 4 days after emergence (72-96 hours); and 'Old' 7 days after emergence (144-168 hours).

I placed two adult males into a gelatin capsule (volume=0.21ml), marked with gold or pink glass paint for identification. I randomly allocated males to pairs, using a fully factored design to set up all possible age-size combinations resulting from our 2 size classes and 3 age classes: large and small males considered 'young' 'middle-aged' or 'old', against both large and small males from all age categories. I also cross-factored paint colour marking (gold or pink) with all size and age treatment combinations. I checked all arenas every 24 hours, and recorded the identity and longevity of both the winning and losing male (as defined by order of death). I also set up controls from both size classes, placing individual males in capsules and recording longevity.

2.2.5 Statistical analysis

For maximum power when analysing proportion data, analyses should assume binomial errors and use a logit link function - as proportion data often have non-normally distributed error variance and unequal sample sizes (Crawley, 1993; Pickering et al., 2000). In this case, analyses using generalised linear models are

simplified using analyses of deviance, in which changes in deviance are compared to a Chi Squared distribution. However, proportion data can be overdispersed (residual deviance > residual degrees of freedom), and this can lead to overestimation of significance. If the dispersion parameter (heterogeneity factor; HF) is < 4, data can be scaled and F tests used to assess significance (Crawley, 2002). I carried out the analyses using R (R: Copyright 2005, The R Foundation for Statistical Computing, Version 2.1.0). I used binomial errors and Chi Squared distributions as, in all cases, HFs were < 1. Non-proportion data conformed to assumptions of GLM analyses using normal error distributions. I used the probability of the focal male winning the contest (P(winning)) as the GLM response variable, making it possible to fit size and age data as explanatory variables (Hardy and Field, 1998). I tested whether the offspring sex ratios produced by females showed less than binomial variation using the regression method developed by Green et al. (Green et al., 1982) and described in further detail by West & Herre (West and Herre, 1998b).

2.3 Results

2.3.1 Experiment 1: sex ratio adjustment and LMC

There was a significant increase in sex ratio with increasing foundress number (χ^2_4 = 13.60, P=0.0001; Figure 2.1). Treatments of 3, 10, 25 and 50 foundresses do not have significantly different sex ratios from one another but do have significantly less female biased sex ratios than the single foundress treatment (treatment contrasts: $P >$ 0.05 for all comparisons of 3, 10, 25, 50 foundresses and $P < 0.05$ for single foundress compared to multifoundress treatments). However, this effect represents a very slight shift in sex ratio of 3% (treatments mean ± se for single foundresses = 0.036 +0.008; -0.007 and multifoundresses = 0.069 +0.015; -0.012). The sex ratio was significantly lower when females were given 48hrs to oviposit compared to 24hrs (χ^2_1 = 14.90, P=0.009; mean difference = 3.8% ± 0.3; Figure 2.1). However, at 25 foundresses, the effect of time was not significant, resulting in a significant interaction (χ^2_4 = 10.62; P = 0.031) between foundress number and oviposition duration.

The sex ratios produced by females when ovipositing alone showed significantly less than binomial variation (termed precise sex ratios). I calculated the ratio of observed variance in the sex ratio versus that compared with that expected given a binomial (random) distribution. If the ratio of these numbers, termed the Green variance (GV), is significantly less than one, then this indicates that the sex ratios produced by females are precise and show less than binomial variation. I found that the variation in the offspring sex ratio was significantly less than binomial for both the 24 hour (GV=0.21; χ^2_7=1.49; P=0.02; n=9) and 48 hour (GV=0.07; χ^2_7=0.52; P=0.0006; n=9) oviposition periods.
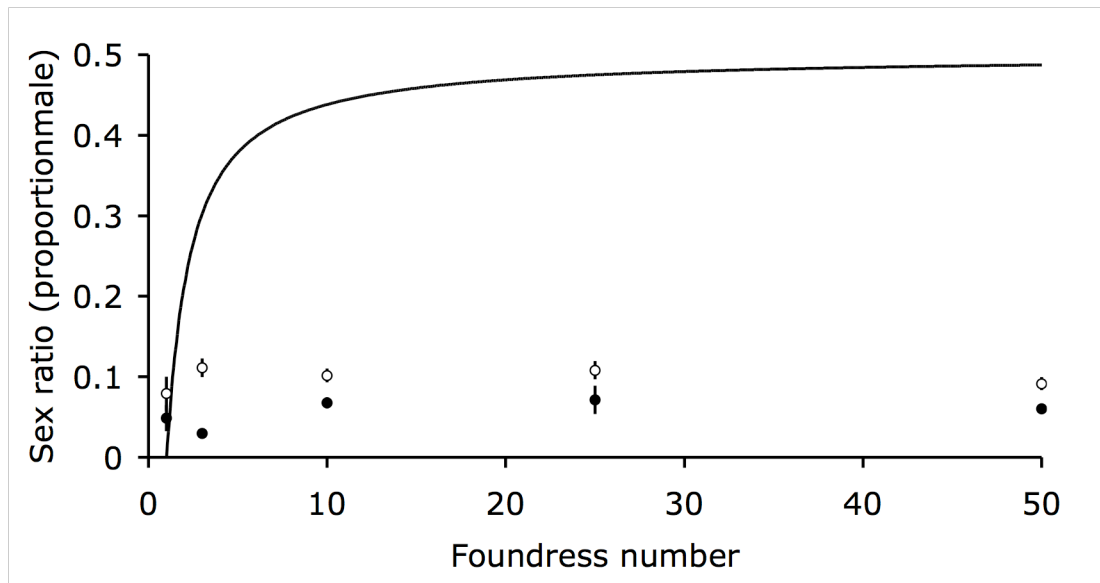
Figure 2.1: Mean brood sex ratios (proportion male) for each foundress number treatment when females are given either 24hrs (open symbols) or 48hrs (closed symbols) to oviposit. Note that in all cases of multi foundress treatments, observed sex ratios are lower than that predicted by LMC theory (solid line). The bars show asymmetric standard errors.

2.3.2 Experiment 2: Size, age & fighting ability in males

2.3.2.1 Experiment 2a: Importance of emergence order

Out of a total of 80 fights, 37 had a clear winner after 24 hours, therefore only these fights were considered in the analyis. Amongst these, emergence order had a highly significant effect on the likelihood of winning a fight, with 92% (34 of 37) of fights won by the male that was already emerged ($\chi^2_1 = 26.00$, $P < 0.001$; Figure 2.2a).

2.3.2.2 Experiment 2b: Importance of emergence order and size

Out of a total of 144 fights, 52 had a clear winner after 24 hours, therefore only these were considered in the analysis. Once again, emergence order had a significant effect on the likelihood of the emerged adult male winning when fighting a smaller or size-matched opponent (93-100% of fights won by the adult male in: Large adult vs. Small pupa; Large adult vs. Large pupa; and Small adult vs. Small pupa; Figure 2.2b). In the fourth group, where the size asymmetry favoured the pupal male, the effect of emergence order on the probability of winning was significantly less than expected. The probability of the emerged adult male winning in the Small adult vs. Large pupa treatment was significantly lower than all other treatments ($\chi^2_3 = 12.96$, $P = 0.005$; Figure 2.2b). This shows an interaction between size and emergence, where emerged males have a clear competitive advantage over emerging males, but only when fighting same-sized or smaller opponents.
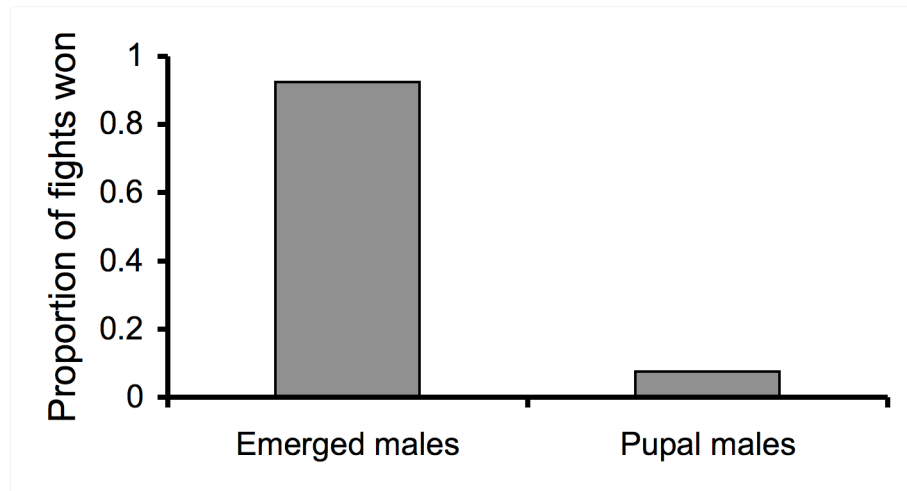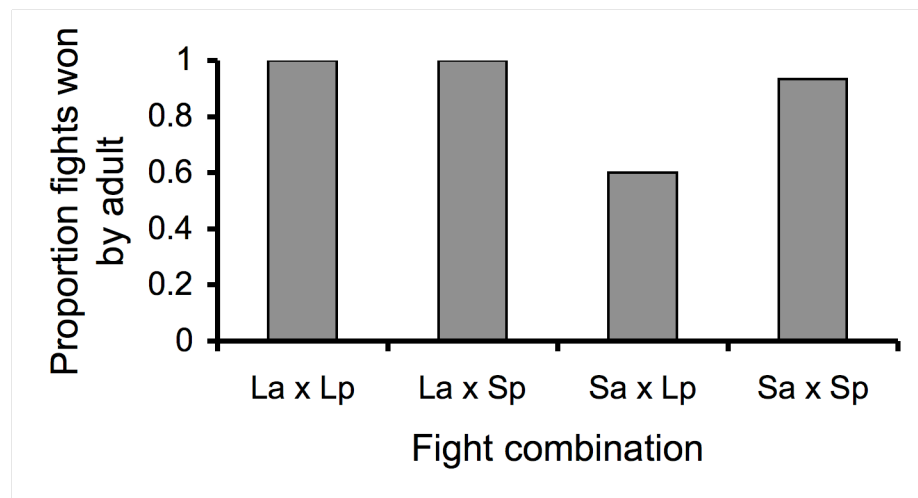
Figure 2.2: a) Proportion of fights won by emerged adult and emerging pupal males, when fighting a size-matched opponent. b) Proportion of fights won by the already emerged adult male in each of the four fight combinations, where La = Large adult, Lp = Large pupa, Sa = Small adult, and Sp = Small pupa.

2.3.2.3 Experiment 2c: Size vs Age Experiment

Experimental males had significantly shorter longevity than control males ($F_{1,367}$=379.80, P<0.0001), suggesting a cost of fighting. Identification colour had no effect upon either longevity ($F_{1,317}$=0.09, P>0.05) or the probability of winning (χ^2_1 =0.53, P>0.05); I therefore chose to use gold males as our focal males for further analyses.

When fitting the size and age of the focal male, irrespective of their opponent's characteristics, large males are significantly more likely to win contests ($\chi^2_{1,317}$= 23.88, P<0.0001) but there was no effect of the absolute age of the focal male. Large focal males won 27% more contests than small focal males.

When the relative difference in size and age between the focal male and his opponent are considered, relatively larger males have a significantly higher probability of winning contests ($\chi^2_{2,312}$=52.97, P<0.0001; Figure 2.3). Fight outcome was only influenced by relative age when the opponents were the same age: in this case the advantage of being large is reduced but there is still a disadvantage of being smaller ($\chi^2_{8,304}$=18.87, P=0.02).
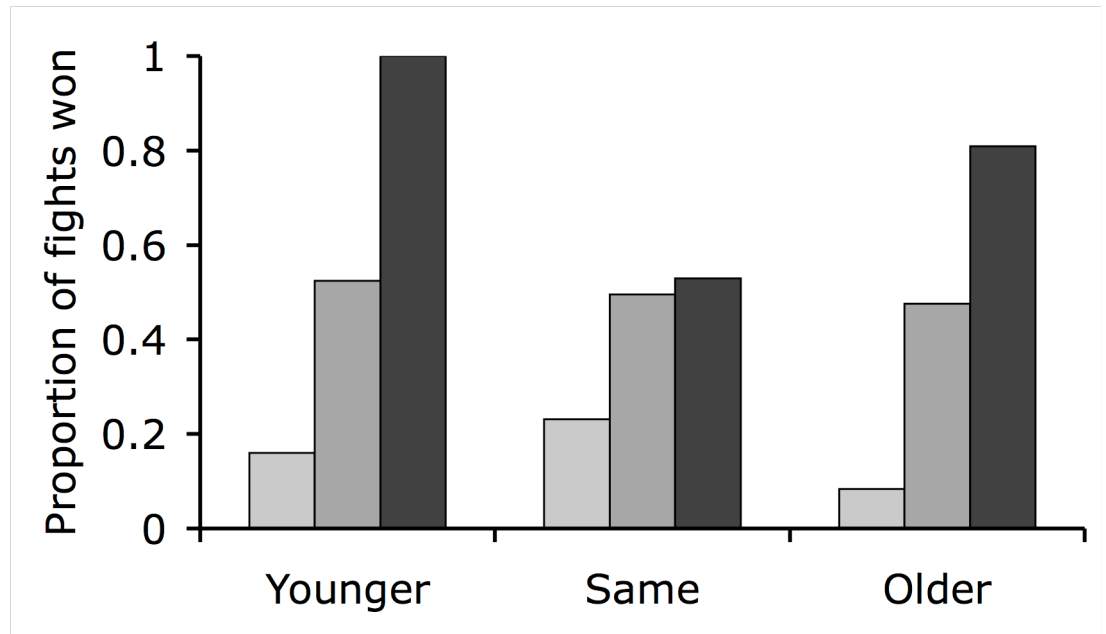
Figure 2.3: Proportion of fights won by focal males plotted against the age of focal males relative to their opponents. Shading represents the size of focal males relative to their opponents: smaller (unshaded bars); same size class (light grey); larger (dark grey).

2.4 Discussion

I have shown that average sex ratios in *M. acasta* are extremely female biased (9% male) and shift only marginally in response to foundress number (Figure 2.1). In addition, when ovipositing alone, females produce precise sex ratios with less than binomial variation, as is expected and observed in species with LMC (Green et al., 1982; Hardy, 1992; Hardy and Cook, 1995; Lopez-Vaamonde et al., 2005; Morgan and Cook, 1994; West and Herre, 1998b). Amongst fighting males, I have shown that asymmetry in emergence order of opponent's leads to a significant competitive advantage for early emerging adult males: these males are better able to attack emerging males during, or immediately after emergence (Figure 2.2a). Nonetheless, this advantage is mediated by the relative size of opponents – if the later emerging male is larger then this can override their opponent's advantage from early

emergence (Figure 2.2b). Furthermore, beyond a critical window of emergence (~24 hours), age is less important, and size is the major determinant of fighting success (Figure 2.3). Abe et al. (Abe et al., 2003a) suggest that fatal fighting can explain female-biased sex ratios if we assume that the early emerging male has a very large advantage compared to later emerging males, such that early emerging males have approximately 100% survival, and later males have approximately 5% survival relative to early emerging males. My experimental results suggest that the advantage to earlier laid males in fatal fights may not be sufficiently strong to explain the lack of sex ratio shift in *M. acasta* (Figure 2.1).

Our contest data suggest that the probability of winning a fight can be divided in to two parts: that resulting from emergence order, and that concerning the time beyond the emergence period. Emerged adult males are able to kill emerging males (Figures 2.2a & 2.2b), and behavioural observations suggest that this advantage may be due to attacking before emergence is complete. After this critical period any advantage of emergence order is reduced, and size becomes the key factor: large males have a considerable competitive advantage (Figure 2.3). If slower development allows larger body size, this raises a series of questions about how the success associated with earlier emergence is traded off against body size. More generally, the importance of size can be context dependant as, for instance, competitor density is known to influence both the frequency and severity of contests (Griffin and West, 2002; Murray, 1987; Murray and Gerrard, 1984; 1985; Reece et al., 2007; Reinhold, 2003; West et al., 2001), and opponent assessment is predicted to decrease contest frequency (Enquist and Leimar, 1983; Gammell and Hardy, 2003; Leimar and Enquist, 1984; Reinhold, 2003). However, in *M. acasta* there is no evidence of opponent assessment, suggesting that all males will fight in any contest regardless of opponent because of the high stakes involved (Reece et al., 2007). This is likely to be due to the large current value of the reward in comparison to its future value (Enquist and Leimar, 1983; 1987; 1990; Maynard-Smith and Price, 1973). For *Melittobia,* this explanation applies to both competitors in a fight, as all males are expected to emerge (and fight) before any of them get to mate (Balfour Browne, 1922; Dahms, 1984): this is not simply an explanation for early male advantage, but a potential

reason why any male should ever fight. Consequently, size is likely to be considered very important in determining male fitness.

Although I found a statistically significant shift in sex ratio with the number of females laying eggs on a patch, this shift is much smaller than predicted by LMC theory. For example, the predicted sex ratio for the 50 foundress treatment = 0.49, whereas the observed sex ratio is 0.08 (Hamilton, 1967a; 1972). In addition I observed that the 24-hour treatment had a significantly higher sex ratio than the 48-hour treatment, for all foundress numbers. A possible explanation for this may be the laying order of sons and daughters: this sex ratio pattern would occur if females chose to lay sons at the beginning of the oviposition period, and then daughters as time went on (Van den Assem et al., 1980). Since I have shown that earlier emerging *M. acasta* males have a greater chance of winning fights, laying sons first would maximise their chance of success. Moreover, the fact that I have observed significantly less than binomial variation in offspring sex ratios shows that females can choose with precision when to produce sons.

Does lethal male combat provide a reasonable explanation for the relative lack of sex ratio adjustment observed in *M. acasta* and other *Melittobia* species (Abe et al., 2003a; 2003b; 2005)? I have shown that earlier emerging males are able to kill later emerging males (Figure 2.2), as was also shown previously for *M. australica* (Abe et al., 2005). However, in order to completely explain the lack of conditional sex ratio, the advantage to early emerging males has to be very large (see above). Our results suggest two reasons why this may not be the case: (a) this age advantage only exists for a brief critical window around the time that the later emerging male is emerging (Figure 2.3), and (b) variation in male size can reduce this advantage (Figure 2.2b). Our data therefore suggests that whilst there is a possible role for lethal male combat in sex allocation (Abe et al., 2003a; 2005), it cannot fully explain the sex ratio pattern in *M. acasta*. Furthermore, the life history of this species suggests that brood emergence can take place over a relatively lengthy time, which is longer than male longevity (Abe et al., 2005; Buckell, 1928; Dahms, 1984; Gonzalez et al., 2004c). This means that even when females arrive sequentially on a patch, there can still be

considerable overlap in the timing of when their broods emerge, blurring the distinction between early and late emerging males (Abe et al., 2005; Gonzalez et al., 2004c; Van den Assem et al., 1980). In this case, the pattern of emergence and fighting may show some similarity to fig wasps, where conditional sex ratio adjustment has been documented numerous times (Frank, 1985; Herre, 1985; 1987; Herre et al., 1997; 2001; Kinoshita et al., 2002; Molbo et al., 2003; Moore et al., 2002; West and Herre, 1998a). In order to further assess the importance of male combat for sex ratio adjustment, it would be extremely useful to obtain data on natural populations to complement the available data from lab cultures (see methods for summary of natural history), examining variation in male size, synchrony of male emergence period and the temporal distribution of fighting, in conjunction with data on the simultaneous variation in host size/species/quality. For example, if it were found that competition for resources led later emerging males to be smaller, then this would select against producing later males, and hence could potentially increase the likelihood of lethal combat providing an explanation for the relative lack of sex ratio adjustment.

I conclude by discussing two alternative possibilities for the lack of sex ratio adjustment in *Melittobia* species (Abe et al., 2003a; Abe et al., 2003b; Abe et al., 2005; Cooperband et al., 2003). One possibility is that selection for sex ratio adjustment has been too weak. If females in a natural environment only ever encounter single foundress situations, then there will be weak selection for altering sex ratio behaviour in multifoundress situations (Herre, 1987; West and Herre, 1998b). However, multiple *Melittobia* females often appear to lay eggs on a single host in nature (Cooperband et al., 2003; Freeman, 1977; Freeman and Ittyeipe, 1976; Molumby, 1996). A second possibility is that co-founding females are usually highly related, in which case much smaller sex ratio shifts are predicted by LMC theory (Frank, 1985; 1986b; Shuker et al., 2004a). When females are related they are also related to each other's sons, therefore a more female biased sex ratio is favoured to reduce competition between males and provide more mates for them (Frank, 1986b). The natural history of *Melittobia* species, and the presence of a non-dispersing female morph suggest that co-foundresses will often be highly related (Consoli and

Vinson, 2002a; Cooperband et al., 2003; Dahms, 1984; Freeman and Ittyeipe, 1976; 1982; Gonzalez et al., 2004a; Schmieder, 1933; Van den Assem et al., 1980). Study of natural populations will be necessary to fully understand the frequency of multi-foundress scenarios, the relatedness between these foundress females, and the natural variation in the occurrence of the two female morphs. Furthermore, competition between related females could select for less female biased sex ratios, highlighting the need for specific theory that takes life history details into account (Bulmer, 1986; Courteau and Lessard, 2000; Frank, 1985; 1986b; Taylor and Crespi, 1994; West et al., 2002). This also raises the possibility that we would expect different patterns of sex ratio adjustment between dispersing and non-dispersing females (Taylor and Crespi, 1994).

Chapter 3 Competition between relatives and the evolution
of dispersal

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Evolutionary Biology*.

3.1 Introduction

Dispersal is a ubiquitous feature of natural populations, with important consequences
for individual fitness and population dynamics (Bowler and Benton, 2005; Roff and
Fairbairn, 1991; Ronce, 2007). By enabling the movement of individuals within their
environment, dispersal allows organisms to exploit new resources and habitats, but
doing so can be costly and involve high risk. For instance, the capacity for flight is
energetically costly, dispersal increases the mortality risk due to predation, and there
is a risk of not finding a suitable habitat. Across species, there is considerable
variation in patterns of dispersal, which can encompass movement across a range of
distances, the use of different dispersal mechanisms, and which can take place at
various life history stages (Bowler and Benton, 2005; Ronce, 2007). Moreover,
individuals vary in their ability to disperse, and likelihood of doing so – even within
the same species - which may be reflected in (adaptive) morphological differences
between them. For example in the cricket *Gryllus firmus*, long-winged, larger bodied
females disperse whereas smaller, short-winged females do not (Roff and Fairbairn,
1991).

A wealth of evolutionary theory has highlighted three factors that can favour the
evolution of dispersal: habitat quality, inbreeding, and competition between relatives.
Spatiotemporal variation in habitat quality can select for dispersal because it creates
uncertainty in resource availability – and in this instance, the direct benefits of
dispersal can outweigh the costs (Greenwood-Lee and Taylor, 2001; Leturque and
Rousset, 2002; Roff, 1986). If inbreeding depression is high, or inbreeding is

avoided, or both, then this can select for sex-specific dispersal to find unrelated mates (Gandon, 1999; Motro, 1991; Roze and Rousset, 2005). When non-dispersing individuals must compete for resources with relatives, Hamilton and May (1977) showed that dispersal can be favoured in order to reduce competition between relatives. In this case, dispersal is favoured because of its indirect fitness benefits to social partners, despite any direct costs incurred by the dispersing individual, and can be thought of as a cooperative, potentially altruistic trait (West et al., 2007b).

Here, I am concerned with how competition between relatives influences selection for dispersal. There have been numerous extensions of Hamilton and May's (1977) original model examining more detailed realistic scenarios to predict the consequences of factors such as dispersal rate, dispersal distance, population size, population dynamics, and age-structure (Bulmer and Taylor, 1980a; Comins, 1982; Comins et al., 1980; Crespi and Taylor, 1990; Frank, 1986a; 1998; Gandon, 1999; Gandon and Michalakis, 1999; Gandon and Rousset, 1999; Irwin and Taylor, 2000; Leturque and Rousset, 2002; 2003; 2004; Motro, 1982a; 1982b; 1991; Ronce et al., 2000; Rousset and Billiard, 2000; Rousset and Gandon, 2002; Roze and Rousset, 2005; Taylor, 1988; 1994; Taylor and Frank, 1996; Wild et al., 2006; Wild and Taylor, 2004). In contrast, there is a severe lack of experimental work directed at testing these predictions; instead, most empirical work has been focused on the direct costs and benefits of dispersal, rather than the social context of dispersal evolution (Crespi and Taylor, 1990; Roff and Fairbairn, 1991; 2007; Zera and Denno, 1997).

My main aim is to test empirically a number of assumptions and predictions arising from theoretical models of dispersal evolution, with a particular focus on how dispersal can be favoured due to competition between relatives. The parasitoid wasp *Melittobia australica* exhibits both sex-specific dispersal and a within-sex dispersal dimorphism: only female offspring disperse, and amongst females there is a pronounced dispersal dimorphism between dispersing (long-wing) and non-dispersing (short-wing) females (Consoli and Vinson, 2002a; Matthews et al., 2009). Dimorphic species generally provide excellent systems for studying the evolution of dispersal because the easily identified visible morphological differences correspond

to different patterns of resource investment into key life history traits (Roff and Fairbairn, 1991; Zera and Denno, 1997). Moreover, comparing dispersing and non-dispersing individuals within the same species controls for any difference due to phylogeny. I first examine the nature of the dispersal dimorphism in *M. australica* by comparing the morphology, dispersal behaviour, and life history traits of the two female morphs. This allows me to test whether morphological differences between females are indeed associated with differences in dispersal propensity and trade-offs between the life history traits of dispersing and non-dispersing individuals.

Second, I test theoretical predictions on how competition between relatives selects for dispersal by examining whether the production of non- and dispersing morphs is adjusted in response to local conditions. Theory predicts that selection for dispersal is increased when larger numbers of offspring are competing for a given resource, and when these offspring are related (i.e. produced by a lower number of mothers; Hamilton and May, 1977). I manipulated local resource competition and relatedness by varying the time female foundresses are given for oviposition (egg-laying), and the number simultaneously ovipositing on a host. Finally, I examine the correlation between the proportion of dispersing females, and the offspring sex ratio (proportion male offspring), to test several hypotheses suggested to explain the unusual lack of facultative sex ratio adjustment in response to local mate competition observed in *Melittobia* species (see discussion).

3.2 Methods

3.2.1 Natural history

Melittobia australica (Hymenoptera: Eulophidae) is a gregarious ectoparasitoid wasp and shares common natural history with other species in the *Melittobia* genus (Gonzalez et al., 2004a; Matthews et al., 2009; Van den Assem et al., 1980). *Melittobia* species are known to have an unusually wide host range, though most commonly parasitise other Hymenoptera (Balfour Browne, 1922; Cooperband and Vinson, 2000; Dahms, 1984; Freeman, 1977; Freeman and Parnell, 1973; Gonzalez

et al., 2004c; Matthews et al., 2009; Van den Assem et al., 1980). Pronounced sexual dimorphism is found across the genus. Males are blind and flightless, remaining on the natal patch to compete for local mating opportunities (Buckell, 1928; Dahms, 1984; Gonzalez et al., 2004c; Matthews et al., 2009). Mating competition is fierce: males eclose earlier than females and will fight to the death, employing their highly modified mandibles in attack to remove limbs and decapitate opponents (Abe et al., 2003b; 2005; Balfour Browne, 1922; Buckell, 1928; Dahms, 1984; see also Hamilton, 1979; Hartley and Matthews, 2003; Innocent et al., 2007; Reece et al., 2007). The males remaining alive at female eclosion will mate within the natal host. By contrast females have fully functioning eyes and wings, and can disperse. Females are able to lay large clutch sizes (200–1000+ depending on host) (e.g. Abe et al., 2005; Balfour Browne, 1922; Innocent et al., 2007; Matthews et al., 2009), and can adjust their offspring sex ratio through haplodiploid sex-determination – daughters are produced from fertilised eggs, sons from unfertilised eggs (Cook, 1993). Highly female-biased sex ratios have been reported for a number of species in the *Melittobia* genus, in the order of 85-95% female offspring for both natural populations and in the lab (Abe et al., 2003b; 2005; Cooperband et al., 2003; Gonzalez et al., 2004c; Innocent et al., 2007; Schmieder, 1938; Van den Assem et al., 1980). Moreover, a lack of sex ratio shift has been shown for laboratory populations of several species (Abe et al., 2003b; 2005; Cooperband et al., 2003; Innocent et al., 2007), despite evidence that foundress number varies and thus that there is variation in local mate competition (LMC) in natural populations (Cooperband et al., 2003; Dahms, 1984; Freeman and Ittyeipe, 1976; 1993; Matthews et al., 2009; Schmieder, 1933; Van den Assem et al., 1982). Previous studies have identified two distinct female morphs (Dahms, 1984; Freeman and Ittyeipe, 1976; 1982; Gonzalez and Matthews, 2008; Schmieder, 1933), and have suggested that morphological differences correlate with different patterns of dispersal and the associated life history strategies (Consoli and Vinson, 2002a; 2002b; 2004; Cooperband et al., 2003; Dahms, 1984; Freeman and Ittyeipe, 1976; 1982; Gonzalez et al., 2004a; Schmieder, 1933). However, data accurately describing the morphological differences between females are scarce, and the associated differences in life history strategies have not been formally tested (Consoli and Vinson, 2002a; Matthews et al., 2009).

3.2.2   General methods

The stock population of *M. australica* - from which I took all experimental wasps - was established from field collections made in Shiga, Japan (2000). I cultured all wasps on *Bombus terrestris* pupae (Koppert, The Netherlands) and reared them at 25°C with a 16:8 light:dark photoperiod. Development time is in the region of 14 days for males and non-dispersing females, and 14-21 days for dispersing females, under these conditions. To establish a new generation, I placed groups of approximately 50 adult females with an unparasitised, early-stage *B. terrestris* pupa in 25 x 70 mm glass vials, stoppered with cotton wool.

3.2.3   Experimental methods

3.2.3.1   General experimental methods

I carried out all experiments in two stages: the initial stage of experimental set up involved the manipulation of foundress females, and used a fully factorial design common to all experiments (Table 1); I then randomly allocated replicates to experiments to answer specific questions for the later stage (involving manipulation of offspring). A replicate consisted of the offspring generation produced by a single foundress female or group of females with a single host for oviposition. In this initial experimental design I manipulated the number of foundress females able to oviposit on a host, and simultaneously manipulated the length of time females were given for oviposition. This created variation in offspring relatedness across a range of clutch sizes, and thus generated variation in local mate competition (LMC) and local resource competition (LRC) between offspring. Specifically, I set up 40 replicates of each of 3 foundress treatment levels: groups of 1, 5, or 15 females (a total sample size of 120) and allowed females to oviposit on their host for 3- or 6-days. Overall this resulted in six treatment combinations: single females with 3- or 6-day oviposition, groups of 5 females with 3- or 6-day oviposition, and groups of 15 females with 3- or 6-day oviposition (Table 1). I used mated adult females for all six treatment combinations, which I chose at random from stock populations approximately 48 hours after emergence (to ensure mating occurred), and assigned

randomly to one of the six treatment levels. I placed all replicates in stoppered glass vials with early-stage *B. terrestris* pupae of known mass and age, and incubated them at 30°C until offspring emergence. I then randomly assigned each replicate to one of the following experiments in order to investigate 1) morphology; 2) life history and dispersal behaviour; 3) patterns of morph ratio and sex ratio. Specific methods for each of these investigations are detailed in the following sections. I ensured that all foundress by oviposition time treatment combinations were represented within each of the subsequent experiments. I did not include any replicates that failed to produce offspring, giving a total experimental sample size of 111 replicates.

		Foundress number		
		1	5	15
Oviposition time (days)				
	3	106	258	536
	6	206	640	791

Table 3.1: Summary of mean clutch sizes (± standard errors) for experimental treatment combinations (foundress x oviposition duration).

3.2.3.2 Morph characterisation

I first wanted to establish whether short-wing (SW), long-wing (LW) and intermediate (IM) female morphs existed in *M. australica,* and to characterise them. I sampled between 2 and 4 individuals of each morph class at random from each of 24 replicates, which spanned the full range of clutch size/foundress number combinations (see general methods, above). For each female sampled I a) scored the morph by eye (within 24 hours of emergence); b) photographed using an Olympus

SZX10 microscope (with DP20 camera) – with measurements of abdomen and wing length taken from these pictures; c) removed the hind-left tibia - later photographed and measured to control for body size (microscope as above; (Godfray, 1994); and d) dissected the abdomen on a glass slide and counted the number of fully developed eggs present (egg-load). I found distinct SW and LW groups, and so we tested for differences between them in key life-history traits - longevity and fecundity - along with differences in dispersal behaviour.

3.2.3.3   Longevity

In order to assess longevity, I randomly chose a single female of each morph (SW, LW) from each of 45 replicates, which were spread across initial treatment combinations. I isolated females in glass vials (10 x 75 mm) 24 hours after emergence (to allow for mating to occur, representative of natural conditions), and incubated them at 30°C. I gave females sugar solution every 3 days via small discs of filter paper, to allow more accurate discrimination of individual variation in longevity (Rivero and West, 2002). In addition, I repeated this treatment at 25 and 30 °C without sugar solution, to confirm the overall pattern of longevity. I checked all vials daily and recorded the date of death of each female, then I removed, photographed and measured the rear-left tibia in order to control for body size (as above). I recorded natal host mass for all replicates.

3.2.3.4   Fecundity

To determine fecundity, I chose 10 SW females and 10 LW females at random, each from a different replicate vial and I provided them each with an excess of host resources for oviposition. I placed each female in a stoppered glass vial with a single *B. terrestris* pupa of known age and mass, for eight days; eggs laid on this host were considered to be the 1st clutch. After eight days, I removed all female foundresses that remained alive, and provided each with a fresh host to lay their 2nd clutch; after the second eight-day period, I again moved females to new hosts (3rd clutch). This successfully provided a surplus of host resources, as only 39% of experimental

foundresses laid any eggs on their 3rd host, laying an average of only 10 eggs. I collected females after their 3rd period of oviposition, and removed and measured their rear-left tibias. I incubated all hosts at 30°C, and at offspring emergence I counted, sexed, removed, and (for females) scored morph of all individuals in each clutch. I found no significant correlation between host mass and total clutch size.

3.2.3.5   Dispersal behaviour

In order to estimate dispersal propensity I fitted 20 replicates - sampling the full range of treatment combinations - with a one-way dispersal hat, which enables individuals to leave the host vial, but prevents their return. This measure is used to indicate the likelihood of individuals to leave their natal patch. I collected individuals who 'dispersed' daily, counted and sexed them, and scored their morph. I removed individuals who remained on the natal host, counted and sexed them, and scored their morph every 4 days - in order to minimise disruption to natural dispersal behaviour.

3.2.3.6   Dispersal, sex ratio, and competition between relatives

In this experiment I investigated the production of offspring morph-ratio (proportion of long-wing females) and offspring sex ratio (proportion of males) simultaneously, varying both the number of foundresses and the extent of local competition. Females are predicted to alter the proportion of LW daughters in response to foundress number, which determines relatedness between competing offspring (Comins, 1982; Comins et al., 1980). This is analogous to the sex ratio literature where it has been shown that females adjust their sex ratio in response to foundress number (West et al., 2005) and do not use kin recognition or other direct cues of relatedness (Reece et al., 2004; Shuker et al., 2004a). I used 12-15 replicates from each initial foundress x time combination (81 replicates in total; treatments described above). These treatment combinations created variation in relatedness across a gradient of clutch sizes and therefore levels of local mate competition (LMC); this allowed me to distinguish between the effects of increasing clutch size alone, and any unrelated additional effects of high foundress number. All foundress females I used were of

LW morph, mimicking the likely pattern of LW females to disperse to new hosts in natural populations. I randomly assigned hosts across treatment levels and measured their mass. I collected foundresses after the given period of oviposition and removed, photographed and measured their rear-left tibia. I then incubated hosts were incubated at 30°C until offspring emergence. Once offspring began to emerge I inspected host's daily and removed, counted, sexed, and scored the morph of any emerging offspring. I calculated total clutch size, female offspring morph-ratio and offspring sex ratio for each replicate. I found no significant correlation between host mass and total clutch size (see table 1: summary of clutch sizes per treatment combination).

3.2.4   Statistical analysis

I carried out Principal Component Analysis (PCA) on morphological measurements, including tibia length, wing length, abdomen length and egg-load as y-variables. PCA combines these morphological variables to generate a series of linear variables (principal components) which best summarise the overall variation in the data set (Quinn and Keough, 2002). In order to test the validity of our morph groupings made by eye, I then performed Discriminant Function (DF) analysis upon the resulting principle component scores, with morph classification (as assigned by eye) as the x-variable. DF analysis defines significantly different groups within the dataset, and determines how accurately individuals are assigned to the original groups by comparison between DF and original scores (Quinn and Keough, 2002).

I used linear models to test for differences between morph groups in principal components 1 and 2 (PC1 & PC2), egg-load and body size. I also tested for differences in the life-history traits fecundity and longevity, and for order effects in the timing of egg laying in this way. I calculated mean ovigeny index (OI) for SW and LW females: OI is defined as the proportion of a females' lifetime egg complement present as mature eggs at emergence, and so I calculated the ratio of average initial egg-load (IEL) to average lifetime fecundity using data from both the morph description experiment (for egg-load), and from the life history experiment (for fecundity) (Godfray, 1994; 2004; Jervis et al., 2001; Rivero and West, 2002).

I used Generalised Linear Models (GLMs) to analyse proportion data, assuming a binomial error distribution and using a logit link function for maximum power. Model simplification was based upon analysis of deviance, where changes in deviance are compared to a chi-squared distribution. I calculated the heterogeneity factor (HF) to test for overdispersion of data (leading to possible overestimation of significance); in cases where HF <4, we scaled data and tested for significance using F-tests to correct for overdispersion (Crawley, 1993; 2002; 2007). I used GLMs to test for variation in sex ratio in response to variation in foundress number and oviposition duration; I included host mass and age in the maximal model as covariates. I transformed morph-ratio data using the arcsin-squareroot transformation, and used linear models to test for variation in response to foundress number, oviposition duration, and clutch size. Interactions are presented only where significant at the level of $P<0.01$ or above (Crawley, 1993; 2002; 2007).

I compared the dispersal behaviour of LW and SW females using linear mixed-effect models, where the probability of dispersal was used as the response variable; morph, clutch size, foundress number and oviposition time were then included as possible explanatory variables, and host was included as a random effect in the model. All multivariate analyses were carried out using the JMP statistics package (JMP version 5.0.1.2, Copyright © 1989-2003 SAS Institute Inc), linear mixed-effect models were run in Genstat (version 8.1, VSN International, UK), and I carried out all further analyses in R (R version 2.3.1, Copyright © 2006, The R Foundation for Statistical Computing).

3.3 Results

3.3.1 Morph description

I found two distinct female morphs, short-wing (SW) and long-wing (LW), and found that I could accurately identify them, both using morphological measurements and by eye. SW females had relatively shorter wings and an enlarged abdomen,

whilst LW females had longer wings than body length and a relatively reduced abdomen size, for a given body size (Figure 3.1). The majority of the variation in the morphological measurements I took (90%) was explained by principal components (PCs) 1 and 2 (which contributed equally; Table 3.2). Specifically, an increase in wing length for a given body size was associated with a decrease in both abdomen length and egg-load, described by the negative correlation between the contribution of wing length, and abdomen length and egg-load, to PC 1 (see Table 3.2). Overall, this suggests that there is a shape difference between the two morphs, and in addition I found a significant difference in this shape parameter (PC1) between the SW and LW groups (PC1: $F_{1,137}=153$, $P< 0.001$; Figure 3.2). For both morphs, increasing body size was associated with a proportional increase in the size of other morphological traits measured (positive/near-zero loading for PC2 for all traits; see Table 1), with no significant difference in body size (PC2 scores) between SW and LW females (PC2: $F_{1,137}=3.42$, $P=0.067$; Figure 3.2). I found the SW and LW morph groupings were significantly different (with individuals scored as IM falling in the SW group), illustrated by the clear difference in SW and LW group means from discriminant function (DF) analysis, based on individuals' scores for PC1 and 2. A number of intermediate (IM) individuals were identified when scored by eye, and approximately 12% of individuals (17 out of 139) were placed in the alternative group based upon their morphological measurements, from that which they were assigned to when scored by eye, by DF analysis; in nearly all cases these were IM individuals, which were the most likely to lie on the classification boundary between groups. I used these groups, SW (females scored SW or IM by eye) and LW (females scored LW by eye), to classify individuals for all further analyses.
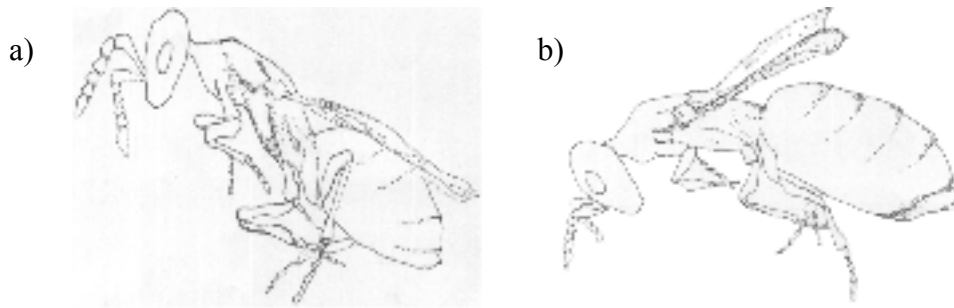
Figure 3.1: The two female morphs of *M. australica*: a) a long-wing (LW) female and b) a short-wing (SW) female (actual size approximately 1-2mm).

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	1.8439	1.7130	0.2793	0.1638
Percent	46.0977	42.8261	6.9813	4.0949
Cumulative Percent	**46.0977**	**88.9238**	95.9051	100.0000
Eigenvectors				
tibia length	0.07682	**0.70671**	-0.67456	0.19908
abdomen length	**0.65078**	**0.28006**	0.16501	-0.68617
wing length	**-0.28451**	**0.64529**	0.69077	0.15966
egg number	**0.69974**	**-0.07568**	0.20146	0.68121

Table 3.2: Results of principal component analysis (PCA), showing the amount of variation explained by principal components 1- 4 (eigenvalues), and the contribution ('loading') of each y variable to each principal component (PC1 – 4; eigenvectors).
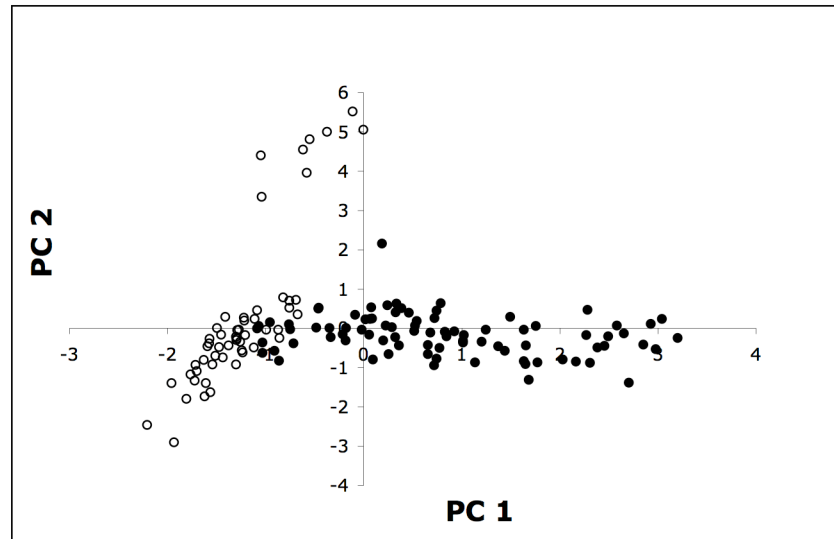
Figure 3.2: Score for principal component 1 (PC1) plotted against score for principal component 2 (PC2), taken from the principal component analysis based upon morphological measurements, for sampled individuals of SW (closed circles) and LW (open circles) morph.

3.3.2 Differences in life history and behaviour

I examined several key life history traits and dispersal behaviour to test whether morphologically different females adopt alternative strategies. SW and LW morphs differed in dispersal behaviour, but not overall body size or longevity. LW individuals showed a significantly higher propensity for dispersal from their natal patch than SW individuals (F_1=550.18, P<0.001; Figure 3.3a). Although SW females dispersed less than LW females, the dispersal of SW females - when compared across replicates of varying clutch size - increased with clutch size (F=85.81, P<0.001). In contrast, there was no significant difference in body size ($F_{1,137}$=3.1, P=0.08) or longevity (with sugar: $F_{1,104}$=0.092, P=0.7625; no sugar: F=2.32, P=0.13; Figure 3.3b) between the two morphological groups. The length of the hind-left tibia, our measurement of body size, was an average of 0.30mm (SE ±0.0009) for SW females, and 0.30mm (SE ±0.005) for LW females. The mean longevity was 6.7

days (SE ±0.2) for SW females, and 6.6 days (SE ±0.2) for LW females, at 30ºC (with sugar; Figure 3.3b).

Whilst SW and LW females did not differ in their overall fecundity, they did differ in when they produced eggs. SW females laid a mean total of 316 eggs (SE ±65), whilst LW females laid a mean total of 478 eggs (SE ±104) ($F_{1,20}$=1.12, P=0.30). In contrast, there was a difference between SW and LW females in both when they produced and when they laid eggs. Specifically: (a) SW females had a larger number of eggs developed at emergence than LW females - SW females carrying an average of 8.3 eggs (SE ±0.6), and LW females carrying an average of 0.7 (SE ±0.2) eggs at emergence ($F_{1,136}$=278, P< 0.001; Figure 3.3c); (b) SW females laid a higher proportion of eggs in the 1st clutch than LW females, and this pattern was reversed for the 2nd clutch (1st clutch, SW 10% more eggs: χ^2_1=232.7, P<0.001; 2nd clutch, LW 9 % more eggs: χ^2_1=183.3, P<0.001; Figure 3.3d). This pattern was also reflected in the ovigeny index (OI) of the two morphs. The OI measure shows where individuals lie on the continuum between synovigeny and proovigeny, and is equal to the proportion of a females lifetime eggs produced present at emergence, which varies from 0 to 1, where 0 represents no eggs mature at emergence (synovigeny) and 1 represents all eggs fully-developed at emergence (proovigeny) (Jervis and Ferns, 2004; 2003; Jervis et al., 2001). Whilst all females were relatively synovigenic (the majority of eggs are matured after emergence), the OI indicates that SW females were relatively more proovigenic than LW females (OI_{SW}=0.026, OI_{LW}=0.001).
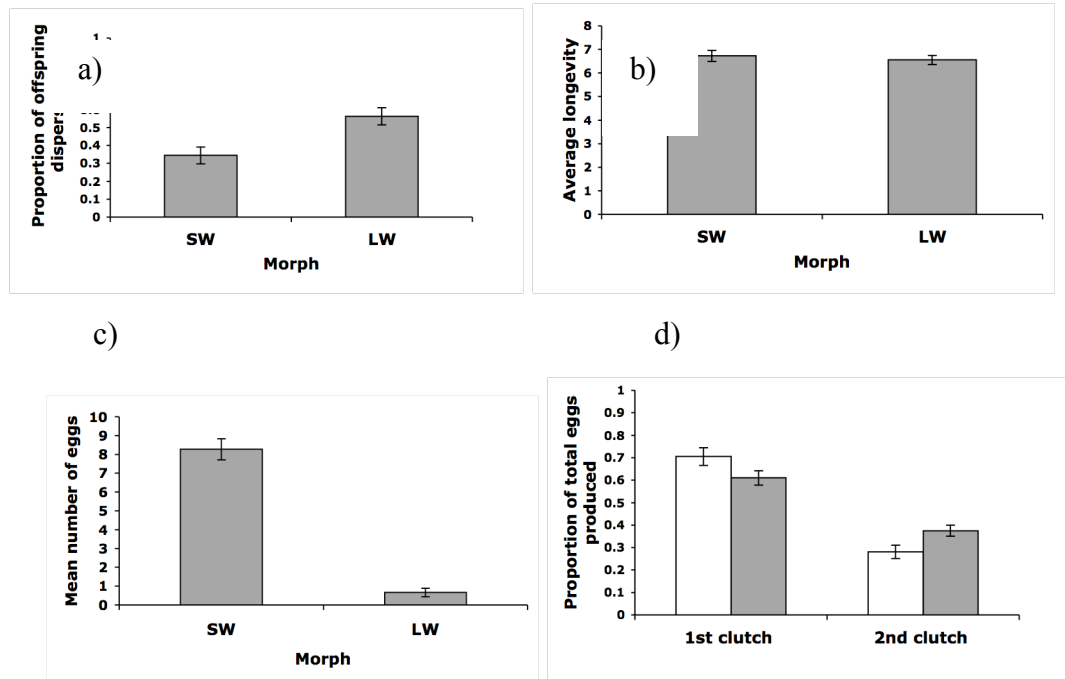
Figure 3.3: a) Mean proportion of female offspring within a brood that dispersed, from the total number of short-wing (SW) and the total number of long-wing (LW) females within a brood; b) mean longevity for short-wing (SW) and long-wing (LW) females; c) mean initial egg-load (IEL; number of eggs fully matured at emergence) for short-wing (SW) and long-wing (LW) morphs; d) proportion of total eggs laid in first and second clutches by SW females (white bars) and LW females (shaded bars). In all cases error bars indicate standard errors

3.3.3 Patterns of sex ratio and morph-ratio

The proportion of LW females varied with total clutch size and oviposition period, but not with the number of females laying eggs (foundress number). I found no significant variation in morph ratio with increasing foundress number ($F_{1,76}$=0.14, P=0.9; Figure 3.4). A higher proportion of long-wing females (larger morph ratio) were produced with both increasing clutch size ($F_{1,78}$= 58.9 , P<0.001; Figure 3.4) and also with longer oviposition period ($F_{1,78}$= 41.2, P<0.001; Figure 3.4). Considering the sex ratio, there was a significant increase in sex ratio with increasing

foundress number ($F_{2,78}$=3.9, P=0.02; Figure 3.5). However, this represents a very slight shift in sex ratio of 1.7% (from 2.4% to 4.1%), as the number of foundress females was increased from 1 to 15 - in comparison with the expected 46% shift under LMC theory (Hamilton, 1967a). There was no significant effect of the duration of oviposition upon offspring sex ratio ($F_{2,78}$=0.46, P=0.5; Figure 3.5) nor were there any significant interactions.
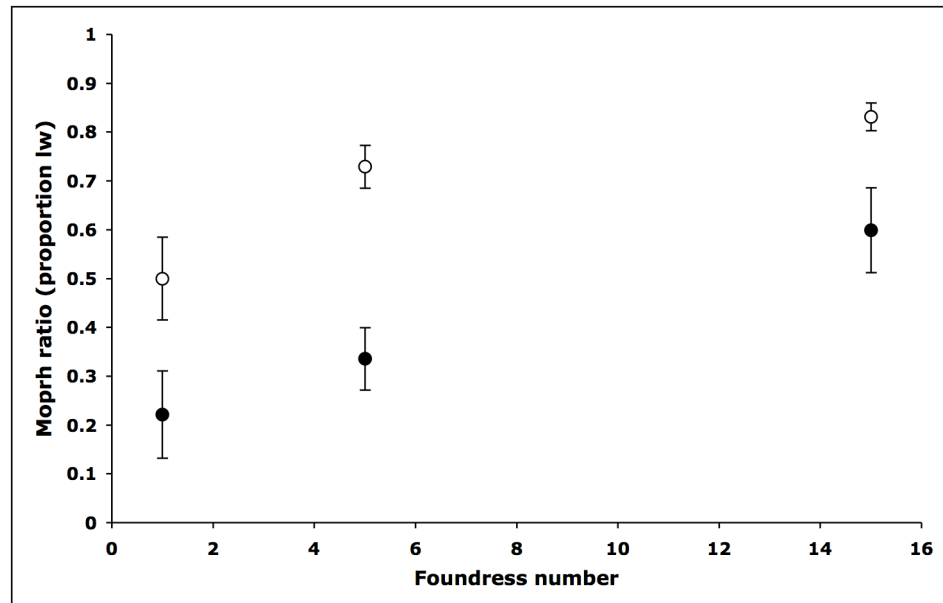
Figure 3.4: Variation in mean brood morph ratio (proportion long-wing females) across a range of foundress number treatments (1, 5 or 15 females); females were given an oviposition period of either 3 days (closed symbols) or 6 days (open symbols). Increasing foundress number corresponds to higher intensity of local mate competition (LMC). Error bars indicate standard errors.
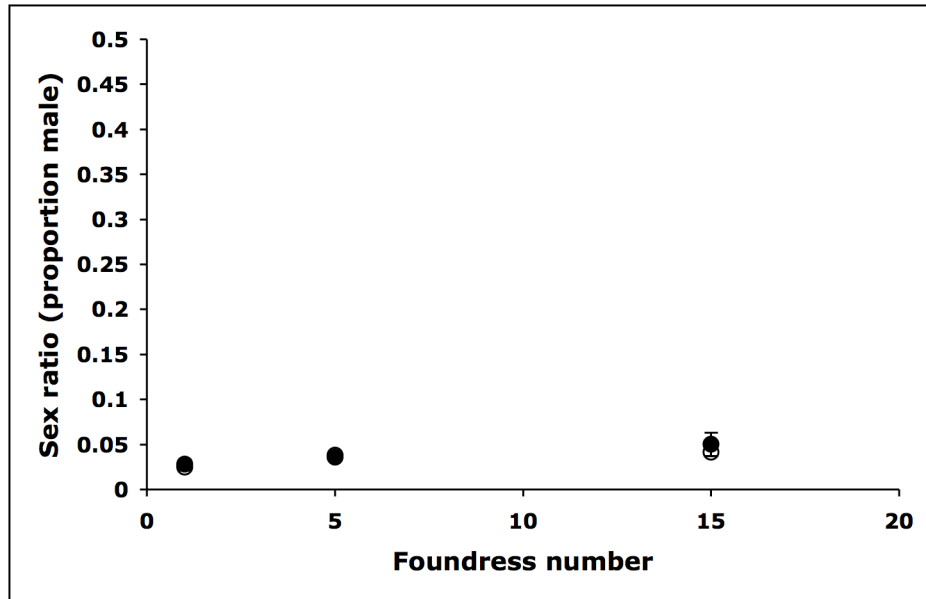
Figure 3.5: Mean brood sex ratios (proportion male offspring) across treatments of varying foundress number (1, 5 or 15 females), when females were given either 3 days (closed symbols) or 6 days (open symbols) for oviposition. Increasing foundress number corresponds to increasing intensity of local mate competition (LMC). The error bars indicate standard errors.

3.4 Discussion

I have shown that *M. australica* females have two morphologically distinct dispersal morphs – long-wing (LW) and short-wing (SW) (Figures 3.1 & 3.2). SW females had relatively shorter wings and larger abdomens, while LW females had reduced abdomen size and wings longer than body-length (Figure 3.1). Considering their life history strategies, SW females exhibit a lower propensity to disperse than LW females, emerge with a higher proportion of eggs fully developed, and lay a higher proportion of eggs in their first clutch (Figure 3.3). In contrast, the morphs did not differ in their body size, longevity, or overall fecundity (Figure 3.3). I then considered whether the ratio of the different female morphs was adjusted in response to local competition and relatedness, as predicted by theory. I found that a higher

proportion of dispersers was produced with both increasing clutch size and longer oviposition period, but that the proportion of dispersing offspring did not vary with the number of foundresses laying eggs on a patch - which determines the relatedness of competing females that do not disperse (Figure 3.4). My results suggest that resource competition, rather than relatedness, is the major determinant of variation in the dispersal rate in *Melittobia*.

3.4.1 Dispersal

How does the dispersal polymorphism in *Melittobia* compare with our understanding of dispersal polymorphism more generally? Across a range of polymorphic insect species, a trade-off between dispersal and other fitness-related life history traits has often been found, which most commonly manifests as increased fecundity coupled with decreased age of first reproduction for the non-dispersing morph (Roff, 1984; Roff and Fairbairn, 1991; Zera and Denno, 1997). In contrast, I found no difference in absolute fecundity between LW and SW females. However, SW females did have greater initial investment in egg production (higher ovigeny index), and laid a higher proportion of eggs earlier, suggesting that SW females have an earlier age of first reproduction – previous work suggests that SW *Melittobia* females start to lay eggs soon after locating hosts, whereas LW females must develop eggs before laying (Matthews et al., 2009). The majority of studies have found no difference in longevity between morphs, as I have shown for *M. australica* (Roff, 1984; Roff and Fairbairn, 1991). One possible explanation for the discrepancy between predicted life history trade-offs and experimental data is that - particularly in the case of parasitoids - differences in life history traits such as fecundity and longevity are unlikely to manifest fully under laboratory conditions (Godfray, 1994; Jervis and Ferns, 2004; 2003; Jervis et al., 2001). Nonetheless, physiological differences between morphs have been shown in other species, where nutrients were allocated differentially to different life history traits (Zera and Denno, 1997). Furthermore, a number of studies have shown that dispersal itself - and in particular the capacity for flight - is energetically costly, largely because the wing muscles of dispersing individuals are costly to develop and maintain, resulting in a compensatory decrease in metabolic

rate (Nespolo et al., 2008; Roff et al., 2003; Roff and Fairbairn, 2007; Roff and Gelinas, 2003).

Morphological differences between dispersal morphs have previously been shown to correspond to dispersal ability, usually higher in the winged morph (Roff and Fairbairn, 1991; Socha and Zemek, 2003). At the population level, a higher proportion of winged individuals has been found to correlate with both the increasing presence of wing muscles, and an increasing behavioural propensity of long-wing individuals to disperse (Roff and Fairbairn, 1991). I have shown that LW females have a higher propensity to disperse, and that dispersal propensity increases along with the proportion of dispersers – associated with increasing clutch size (Figures 3.3, 3.4). The process of morph determination is less well understood, with evidence that genetic, hormonal and environmental influences may be important (Roff, 1984; Roff and Gelinas, 2003; Zera and Denno, 1997). Previous work on *Melittobia* species suggests that morph determination is neither genetically controlled nor hormonally regulated (Consoli et al., 2004; Consoli and Vinson, 2002a; 2002b; 2004), and my experimental results provide indirect evidence that environmental conditions are important, as the proportion of dispersers was most strongly influenced by clutch size – a direct correlate of resource availability. Further work is needed to consider the potential for maternal or offspring control of morph determination.

Dispersal theory suggests competition between relatives can be important in the evolution of dispersal (Comins, 1982; Comins et al., 1980; Hamilton and May, 1977). Theory predicts that higher levels of dispersal will be favoured if fewer foundresses lay eggs on each patch, because this leads to a greater relatedness between competing non-dispersers (Comins, 1982; Comins et al., 1980). I find no support for this prediction (Figure 3.4), suggesting that variation in relatedness has little influence on selection for dispersal by females. A possible explanation could be that there is no selection on females to respond to variation in foundress number, if most females are solitary; in this case, we would expect a fixed rate of offspring dispersal (Herre, 1987). This is unlikely as data from natural populations of *Melittobia* suggest that foundress number does vary (Freeman, 1977; Freeman and

Ittyeipe, 1976; Gonzalez et al., 2004c). Alternatively, if females on natural patches are all highly related then variation in foundress number would not equate to substantial variation in offspring relatedness; in this case, we would also expect no variation in dispersal strategy (Frank, 1998).

Theory also predicts that the number of competitors can influence dispersal decisions, where an increase in the number of competitors for resources on a patch will favour an increased rate of dispersal (Consoli and Vinson, 2002a; Hamilton and May, 1977; Ronce et al., 2000). Here, I found support for this, with an increasing proportion of dispersing females produced with increasing clutch size (Figure 3.4). Increasing foundress number has two potential effects, lowering average relatedness, but also influencing the number of competitors; in this case, the effect of increased competition appears to be much more important. Previous studies on *Melittobia* species have also shown that SW females develop from the first eggs laid, and all later eggs develop into LW females (Abe et al., 2005; Consoli and Vinson, 2002b; 2004; Matthews et al., 2009); here I found indirect support for this pattern, as the proportion of LW females increased with greater length of oviposition period. Increasing competition for resources is expected to result in the production of more LW female offspring because the amount of resources available for oviposition decreases, and females must therefore disperse to find new hosts. Similarly, the pattern of producing SW females earlier in oviposition may be due to the low value of producing late-developing SW females: once the early-developing SW have laid eggs on the natal host, few resources remain for further oviposition by later SW.

3.4.2   Dispersal and sex allocation

The sex ratio behaviour of *Melittobia* poses a significant problem for sex allocation theory, as an exceptional case in a field that otherwise has extremely strong empirical support, and therefore needs to be explained (West, 2009; West et al., 2005). When offspring of one sex disperse less, related members of the non-dispersing sex experience a greater degree of competition, and so selection favours a sex ratio biased towards the dispersing sex (Bulmer and Taylor, 1980b; Hamilton, 1967a; Taylor, 1981). Hamilton (1967a) showed that, when mating occurs before only the

females disperse, a female biased sex ratio is favoured, that becomes less biased as more females lay eggs per patch. However, *Melittobia* females do not adjust their offspring sex ratios in response to the number of foundresses laying eggs per patch (Figure 5; Abe et al., 2003b; 2003a; 2005; Cooperband et al., 2003; Innocent et al., 2007). Several hypotheses have been proposed for the lack of sex ratio shift in these species: that foundress number does not vary in natural populations, high relatedness between foundress females, and fatal fighting between males (Abe et al., 2003a; 2007; Frank, 1998; Herre, 1987). Lack of variation in foundress number in natural populations would result in no selection for adjustment of sex ratio (Herre, 1987). Alternatively, if co-founding females are highly related a female biased sex ratio is predicted irrespective of foundress number, since there is little variation in relatedness between competing males in this case (Frank, 1998). Finally, fatal fighting between male *Melittobia* could select against the production of sons and favour a lack of sex ratio shift in response to LMC (Abe et al., 2003a; 2007; see also Shuker et al., 2005). While evidence supports the occurrence of multiple foundress scenarios (Cooperband et al., 2003; Freeman and Ittyeipe, 1976; 1993; Matthews et al., 2009; Schmieder, 1933; Van den Assem et al., 1982), empirical data give mixed support to the idea that selection due to male fighting can fully explain the sex ratio (Abe et al., 2003a; 2003b; 2005; 2007; Freeman and Ittyeipe, 1976; Innocent et al., 2007), and there is no conclusive explanation for this unusual pattern of sex allocation at present.

Given that the same selective forces influence both sex ratios and dispersal (Bulmer and Taylor, 1980a; Frank, 1998; Leturque and Rousset, 2003; 2004; Motro, 1991; Perrin and Mazalov, 2000; Rousset and Billiard, 2000; Taylor, 1994; Wild and Taylor, 2004), can the pattern of dispersal in *Melittobia* help us explain its unusual sex ratio behaviour? The various possible explanations of a lack of sex ratio adjustment in *Melittobia* wasps have different consequences for the evolution of dispersal. Theory predicts that just as an increasing number of (foundress) females laying eggs per patch selects for less female biased sex ratios (Hamilton, 1967a), it also selects for lower rates pf dispersal (Comins, 1982; Comins et al., 1980), which would translate into a higher proportion of the non-dispersing morph. The male

fighting hypothesis does predict variation in dispersal under LMC: in this case male fighting selects against sex ratio adjustment with variable foundress number, but does not select against variation in female dispersal rate (Abe et al., 2003a; 2007). In contrast, if the lack of sex ratio adjustment is due to foundress number (N) not normally varying (Herre, 1987), or high relatedness between foundresses (Abe et al., 2005; Frank, 1985; 1986b; 1998), then, as supported by our data, we would not expect the proportion of dispersing females to be varied with the number of foundress females. However, the lack of influence of foundress number on both sex ratio adjustment and variation in the proportion of dispersers should not be seen as definitive evidence against the male fighting hypothesis, because there are other possible reasons why the proportion of dispersers is not varied (see previous section). A key next step, which is already underway, is to obtain a greater amount of information about the population structure of natural populations of *Melittobia*.

Chapter 4 Lethal male-male combat in the parasitoid *Melittobia acasta:* are size and competitive environment important?

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4.1   Introduction

When individuals come into conflict, the outcome may lie anywhere on the spectrum between peaceful resolution and escalated violence. For example, ownership calls peacefully mediate territory defence by male pied wagtails, *Motacilla alba* (Davies and Houston, 1981), whereas male fig wasps will fight viciously in competition for mates (Hamilton, 1979). How can this variation in be explained? In particular, given that fighting can lead to injury and even death, why should it ever be favoured (Enquist and Leimar, 1990)? Theory predicts that the way in which conflict is resolved will depend upon the relative cost and benefit of fighting, with escalation to violence predicted in situations where the benefits of winning are relatively high (Enquist and Leimar, 1990; Maynard-Smith and Price, 1973).

Numerous factors are predicted to alter the costs and benefits of fighting, and hence determine the occurrence, frequency and severity of escalated contests (Anderson et al., 2003; Batchelor et al., 2005; Enquist and Leimar, 1990; Gammell and Hardy, 2003; Murray, 1987; Taylor and Elwood, 2003). A useful distinction can be made between those factors describing variation in an individual's environment, and those relating directly to variation in traits of the interacting individuals (such as size, experience, age and motivation). Important environmental variables to consider include the number and density of competitors, the availability and value of the contested resource, and the relatedness between competitors (Enquist and Leimar, 1987; Murray, 1987; Murray and Gerrard, 1985; West et al., 2001; 2002). Conflict is predicted to escalate when conditions are such that the value of a resource at the

present time is much greater than any potential future value of the resource (Enquist and Leimar, 1990). Wingless male fig wasps will fight violently when there are few females within the same fruit, as they represent a limited number of future mating opportunities; conversely, when females are numerous their relative value is lower so the level or intensity of fighting is reduced (West et al., 2001).

The cost:benefit ratio of fighting will also be influenced by an individual's relative fighting ability, since this will affect contest outcome. For example, large high quality individuals may be more likely to fight over a resource, particularly when in competition with a smaller individual (Maynard-Smith and Price, 1973). Assessment rituals may play a role in mediating the escalation of conflict (Enquist and Leimar, 1983; Enquist and Leimar, 1987; Gammell and Hardy, 2003; Leimar and Enquist, 1984; Taylor and Elwood, 2003). In some cases, individuals assess their own competitive ability, for example: orb weaving spiders, *Argyrodes antipodiana*, (Bridge et al., 2000; Taylor et al., 2001; Whitehouse, 1997) and fiddler crabs, *Uca mjoebergi*, (Morrell et al., 2005). In others, mutual assessment occurs, for example: the common toad, *Bufo bufo*, (Davies and Halliday, 1978); cichlid fish, *Nannacara anomala* (Jakobsson et al., 1979); and red deer, *Cervus elaphus*, (Clutton-Brock and Albon, 1979). In contrast, when opponents are more evenly matched in size or quality, there is a much higher chance that conflict will escalate to violence.

Here, I aim to test theoretical predictions about the nature of conflict and the factors influencing contest outcomes, using the parasitoid wasp *Melittobia acasta*. *M. acasta* males fight to the death, presumably due to the limited mating opportunities available within their natal host pupae, and as such provide a useful system for investigating conflict (Abe et al., 2003b; Abe et al., 2005; Griffin and West, 2002). Initially I consider environmental factors that influence the outcome of conflict between males. I manipulate the density of opponents, the number of available mates, and thus, the operational sex ratio of the competitive environment. I predict that the level of fighting will rise with: (a) increasing male density, because this will lead to more frequent encounters between males (Murray, 1987; also see discussion) and (b) decreasing female density, because this increases the value of any particular female

(Enquist and Leimar, 1990; West et al., 2001). I then focus upon traits related to individual fighting ability. Initially I search for evidence of opponent assessment mediating fighting behaviour by testing whether fights are more likely to occur between size-matched than asymmetric opponents. I then investigate how variation in individual fighting ability influences the outcome of contests by testing whether large males compete more successfully.

4.2 Methods

4.2.1 *Melittobia* life history and general methods

The Eulophid wasp genus *Melittobia* are gregarious Hymenopteran parasitoids with a world wide distribution (Gonzalez et al., 2004c). The only species of *Melittobia* found in Europe is *M. acasta*, and this species is also prevalent in parts of North and South America, Japan, India, Australia and New Zealand (Gonzalez et al., 2004c). *M. acasta* is a generalist ectoparasite, able to parasitise a wide variety of hosts, including hymenoptera, diptera, lepidoptera and coleopterans (Balfour Browne, 1922; Dahms, 1984; Gonzalez et al., 2004c). The stock population was collected in the field in 2004 by M. Shaw (Royal Museum of Scotland, Edinburgh) from solitary hymenopteran pupae, and subsequently cultured in the lab on *Calliphora vomita* pupae. The stock culture methods result in the clutches of several females developing in the same host, and several hundred wasps can successfully emerge from a single host. For each generation I housed approximately 60 females in glass vials, stoppered with cotton wool, and provided them with ~20 hosts to oviposit upon throughout their lifetime. I reared stocks at 30°C; at this temperature, egg to adult development time is ~13 days, with males emerging from pupae 1-2 days before females (Buckell, 1928; Dahms, 1984): eggs hatch 4-5 days post oviposition, larvae pupate after 7 days and males emerge as blind adults after 11-12 days (Balfour Browne, 1922; Buckell, 1928). Upon emergence, males attack each other, using their mandibles to chop off their opponents' genitals, head and appendages; the victors then mate with females as they emerge from their pupal case (Buckell, 1928; Dahms, 1984; Van den Assem et al., 1980). Females are normally long winged, have eyes, and after mating they disperse

to new hosts to lay their eggs, whereas males do not leave their natal patch. Females lay large clutches (up to ~250 eggs) in their lifetime (Balfour Browne, 1922), and as they are haplodiploid, can produce daughters by fertilising eggs, and sons by laying unfertilised eggs. In order to produce large numbers of experimental males, I opened up host pupae 10 days post oviposition, and dissected out virgin female pupae. As virgin females can lay only sons, I used them to produce large numbers of males.

4.2.2 Experiment 1: influence of competitive environment

In experiment 1 I manipulated the number of opponents and available mates, and thus, the operational sex ratio (OSR; proportion of adult males) of the competitive environment. For each competitive environment I measured whether males were engaged in combat or courtship behaviour. To produce males in large quantities, I kept virgin females in groups of ~100 and provided them with ~30 hosts. I opened hosts at 10 days post oviposition, and virgin male pupae were isolated and stored individually in gelatin capsules. I stored males at 30°C until emergence and randomly allocated each male to a contest arena. Within 24 hours of emergence, I added males and virgin females to their contest arenas, which consisted of gelatin capsules (with similar dimensions to *C. vomita* host pupae: volume = 0.21ml). I cross-factored the number of competitors (male number) with the number of mates (female number) in arenas. Male number had 3 levels: 1, 2, and 8 males, and female number had 5 levels: 0, 2, 8, 36 and 100 females. I chose these values to span the spectrum observed emerging from our stock-producing hosts and represent operational sex ratios (OSR) ranging from 0.01 to 1. I refrigerated both males and females at 5°C for 10 minutes in order to immobilise them long enough for the correct number to be counted and added to each arena. I set up 10 contest arenas per treatment combination for 2 and 8 males, and 5 per single male combination. I monitored each arena under a dissecting microscope, and recorded the number of males engaged in combat or courtship behaviour after 5 minutes, per arena. I classed behaviour as being either combat (interaction between two or more males) or courtship (interaction between a male and female including: mounting, abdomen exploration, antennal clasping and copulation). After this, I refrigerated each arena

for 5 minutes to immobilise the animals, and transferred them to a 1.5ml microfuge tube, which was stoppered with cotton wool and masking tape. I incubated the tubes at 30°C, and checked them at time intervals of 2, 4, 24, 48 and 72 hours, in order to record when the 1st male in each arena died. We used microfuge tubes because once mated, females will chew their way out of gelatin capsules in order to disperse from their 'natal' patch.

4.2.3 Experiment 2: importance of body size

In this experiment, I investigated how variation in individual body size influences contest outcome, and whether asymmetry in this trait can influence the probability and outcome of combat. I created different sized males by manipulating the level of larval competition experienced during development. I created large males by giving groups of 30 virgin females 40 fresh hosts in a stoppered glass tube. This created an environment with a low density of competitors, and resulted in low resource competition. Conversely, I created small males by giving groups of 30 virgin females only one fresh host. I opened all hosts 10 days post oviposition, isolated the male pupae, and stored them individually in gelatin capsules. I used a dissecting microscope to photograph each male pupae and Leica IM50 measurement software to measure the head width, body length and mandible length for each male. By measuring pupal traits I avoided measurement error introduced from attempting to photograph emerged males in a stationary and standard position. I initially measured the length of both mandibles, but as there was no significant asymmetry in the right and left mandible lengths ($T = 1.02$, $P = 0.318$, $DF = 27$), I only measured left mandible length for subsequent pupae. I stored measured males in individual gelatine capsules at 30°C until they emerged. Within 24hrs of emergence, I randomly allocated our measured, virgin males to $30 - 60$ replicates for each of 3 different contest categories: large vs. large; small vs. small; or small vs. large. I marked each male on his abdomen with either pink or gold paint, and colour was cross-factored with contest category. I set up contest arenas by adding the opponents to a gelatin capsule with approximately the same dimensions as *Calliphora* host pupae (volume: 0.21ml). I observed all arenas for 30 minutes after set up, and recorded the number of

fight. I recorded a fight when males contacted each other and began to fight. If contact between the opponents was made, but there was no escalation, I did not record a fight. I checked arenas every 24 hours, and the first male to die in each arena was recorded as the loser. I also set up arenas with single, marked males for each size treatment, and recorded longevity.

4.2.4 Statistical analysis

For maximum power when analysing proportion data, analyses should assume binomial errors and use a logit link function - as proportion data often have non-normally distributed error variance and unequal sample sizes (Crawley, 1993; Crawley, 2002; Hardy and Field, 1998). In this case, analyses using generalised linear models are simplified using analyses of deviance, in which changes in deviance are compared to a Chi Squared distribution. However, proportion data can be overdispersed (residual deviance > residual degrees of freedom), and this can lead to overestimation of significance. If the dispersion parameter (heterogeneity factor; HF) is < 4, the Chi Squared distribution should not be used but data can be scaled and F tests used to assess significance (Crawley, 2002). I carried out our analyses using R (R: Copyright 2005, The R Foundation for Statistical Computing, Version 2.1.0). I used binomial errors and Chi Squared distributions in cases where HFs were < 1 and quasi-binomial errors and F distributions when HFs were > 1 but < 4. For parametric data I used GLMs with normal errors where possible and Kruskal Wallis tests for non-parametric data.

4.3 Results

4.3.1 Experiment 1: influence of competitive environment

I was able to record combat data from all of the 2- and 8- male contest arenas that I set up. The proportion of males engaged in combat was significantly greater in arenas with more males ($F_{1,99}$ = 13.12; P <0.01; Figure 4.1a), and this decreased

with the density of females ($F_{4,99} = 7.96$; $P < 0.01$; Figure 4.1a), but there was no significant interaction between male and female density. These results are supported by further analysis showing that the proportion of males engaged in combat increases with operational sex ratio ($F_{1,99} = 14.78$; $P < 0.01$; Figure 4.1b).
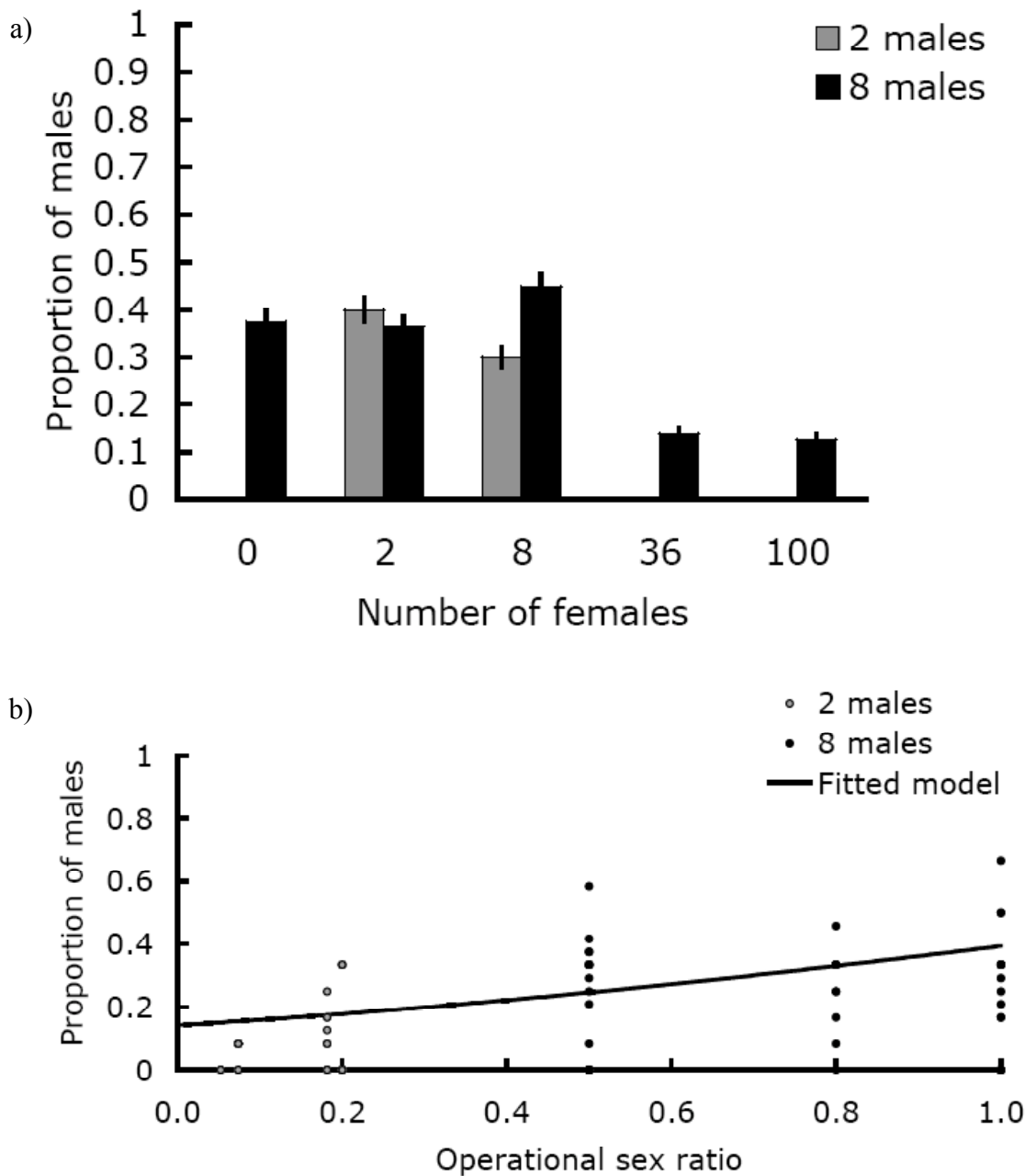
Figure 4.1: a) The mean ±SE proportion of males engaged in combat after 5 mins in arenas containing different numbers of opponents and mates; b) Proportion of males engaged in combat after 5 mins increases with the operational sex ratio (proportion male). We fitted the line from the logistic regression model, *proportion fighting* = $e^{(a+bx)}$ / $1+e^{(a+bx)}$ where *a* = -1.808, *b* = 1.375, and *x* = OSR.

I was able to record courtship data from all of the 1-, 2- and 8- male contest arenas set up. The proportion of males that were courting or mating was significantly greater in arenas with fewer males ($\chi^2 = 32.35$; *N = 125; DF = 2; P* < 0.0001; Figure 4.2a), and increased with female number ($\chi^2 = 152.15$; *N = 125; DF = 2; P* < 0.0001; Figure 4.2a). These results are supported by further analysis showing that the proportion of males courting decreased as the operational sex ratio increased ($\chi^2 = 165.89$; *N = 125; P* < 0.0001; Figure 4.2b).
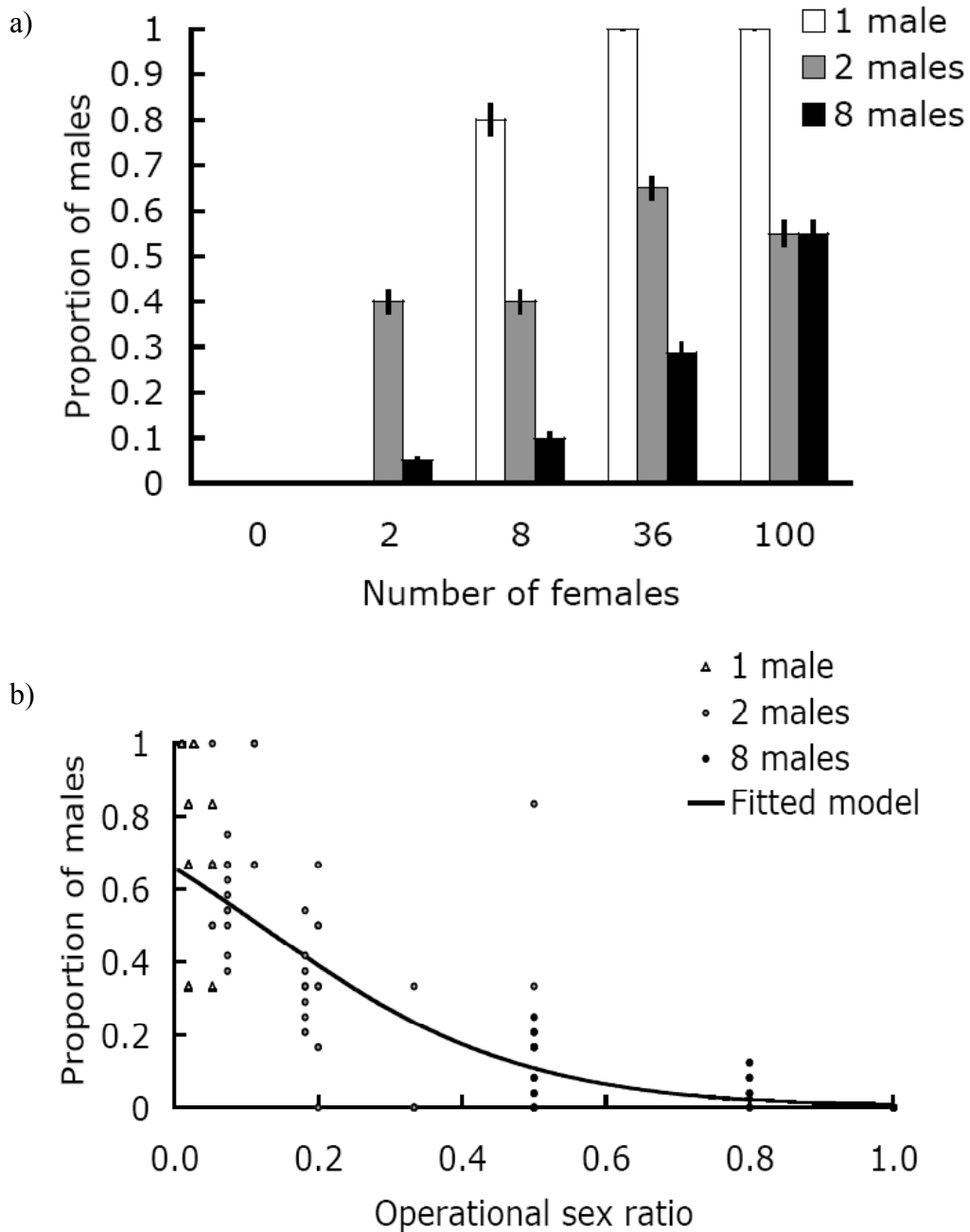
Figure 4.2: a) The mean ±SE proportion of males engaged in courting or mating after 5 mins in arenas containing different numbers of opponents and mates; b) Proportion of males engaged in courting or mating after 5 mins increases with the operational sex ratio (proportion male). We fitted the line from the logistic regression model, *proportion courting* = $e^{(a+bx)}$ / $1+e^{(a+bx)}$ where *a* = -0.656, *b* = -5.505, and *x* = OSR.

The median longevities for single males and those in 1-, 2- or 8- male arenas were 100, 72 and 24 hours respectively. The lifespan of the first male in each arena to die had a significant negative association with the number of males present ($\chi^2 = 377.98$, $DF = 3$; $P < 0.0001$).

4.3.2   Experiment 2: importance of body size

The larval competition treatments successfully created males that were significantly different in size (Table 1). Males from the low competition treatment had significantly larger body lengths ($F_{1,111} = 35.50$; $P < 0.0001$), head widths ($F_{1,111} = 44.18$; $P < 0.0001$), and mandible lengths ($F_{1,111} = 23.62$; $P < 0.0001$), than males in the high competition treatment.

	Large males		Small males	
	Mean	95% CI	Mean	95% CI
Body length	2.508	(2.433, 2.585)	2.217	(2.143, 2.290)
Head width	0.685	(0.663, 0.708)	0.588	(0.566, 0.610)
Mandible length	0.123	(0.117, 0.128)	0.106	(0.102, 0.111)

Table 4.1: Means and 95% confidence intervals of the sizes (mm) of males from the large and small treatments in experiment 2. Large males were significantly bigger for all three measured variables.

Of the 120 contest arenas that I set up, 114 (95%) had a clear winner. The number of fights observed in the first 30mins was not significantly different between large size

matched, small size matched and asymmetric contests ($\chi^2 = 1.95$; $DF = 2$; $P = 0.377$). This suggests that males do not assess their own competitive ability or that of their opponent. The colour of marks applied to males did not influence the probability of winning fights (57 pink and 57 gold males won fights; $P = 1.00$), so we chose gold males as our focal individuals. I used the probability of a gold male winning, P(winning), as our GLM response variable, thus allowing size data to be fitted as explanatory terms (Hardy and Field, 1998). When fitting size as the relative difference between the focal male and his opponent (i.e. the focal male is either smaller, the same size or larger), larger males are significantly more likely to win contests ($\chi^2 = 47.4$; $DF = 2$; $N = 114$; $P < 0.00001$).

I calculated the absolute differences in body length between each focal male and his opponent, and found that the probability of the larger male winning increased with the asymmetry in body size of opponents ($\chi^2_1 = 66.47$; $N = 114$; $P < 0.0001$; Figure 4.3). I also calculated the absolute differences in head width and mandible length, and found that all 3 size variables were significantly and positively correlated with each other (all $R^2 > 0.95$; $P < 0.0001$). Therefore, to establish the influence of head width and mandible length on contest outcome, we controlled for body size by using the residuals from the logistic regression analysis between body size and the probability of winning contests, as our response variable. We carried out separate analyses on these residuals for head width and mandible length to avoid problems associated with co-linearity. When controlling for body size, the probability of winning fights has a significant positive correlation with the magnitude of the difference in head width: the larger a male is compared to his opponent, the more likely he is to win ($F_{1,111} = 6.31$; $P = 0.013$; $R^2 = 0.05$; P(winning) = 0.009+2.22*difference). Also, when controlling for body size, males with larger mandibles were more likely to win fights, and this increased as the difference in mandible lengths of opponents increased ($F_{1,111} = 5.67$; $P = 0.019$; $R^2 = 0.05$; P(winning) = 0.0005+8.83*difference).
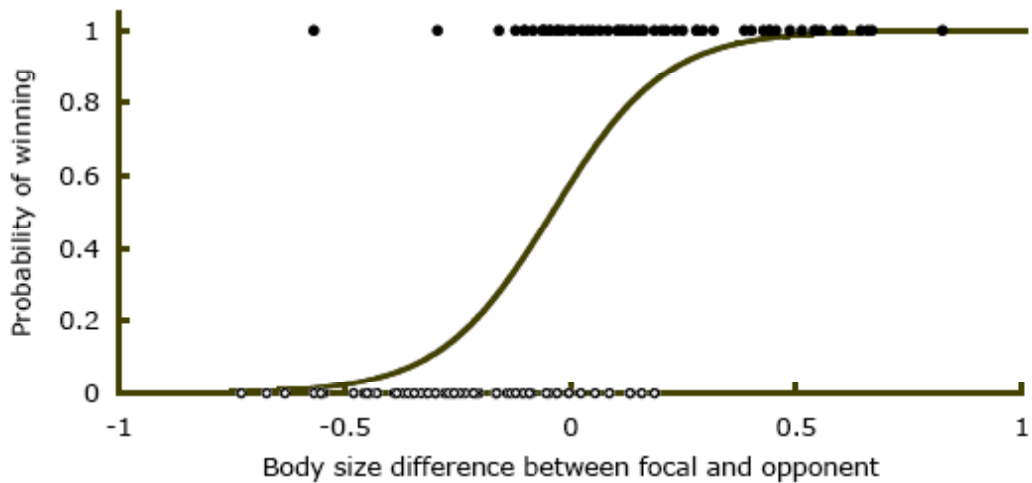
Figure 4.3: The probability of winning a fight is influenced by the magnitude of the difference (mm) in body size of opponents. Closed symbols represent fights in which the focal male won and open symbols represent the focal losing. We fitted the line from the logistic regression model, *P(winning)* = $e^{(a+bx)}$ / $1+e^{(a+bx)}$ where *a* = 0.318, *b* = 8.002, and *x* = size difference. In our experiment the mean difference in body size was ~0.3mm, range: 0.007 – 0.8mm.

4.4 Discussion

My results support theoretical predictions concerning how the competitive environment should influence the level of fighting. Specifically: (1) greater male density leads to an increased level of fighting behaviour (Figure 4.1), decreased level of courting behaviour (Figure 4.2), and a decrease in male longevity; (2) greater female density leads to an increase in the proportion of time males spent courting/mating, and a decrease in the time spent fighting (Figure 4.2). Within contests themselves, larger competitors were far more successful than smaller competitors (Figure 4.3). Furthermore, when controlling for body size, large head

width and mandible size increased competitive ability. Fighting was not more frequent when there was an asymmetry in fighting ability between opponents, which suggests that assessment does not mediate contest behaviour.

Variation in male density can have two effects, which influence fighting behaviour in opposing directions (Murray, 1987; Murray and Gerrard, 1985; Reinhold, 2003). First, increased male density will increase the encounter rate between individuals. This is predicted to lead to a greater number of fights, as has been observed in the fig wasp *Philotrypesis pilosa* (Murray, 1987). My measure of fighting corresponds to fight frequency, and I found a similar pattern (Figure 4.1). Second, male density alters the cost of fighting, and this is also predicted to have an effect on fight intensity (Enquist and Leimar, 1990; Murray, 1987; Murray and Gerrard, 1985; Reinhold, 2003). Specifically, increased density of competitors will decrease the benefit gained by fighting in any given fight, because: (a) fighting wastes time, as other males will be concurrently mating with females (Murray, 1987); (b) the probability of beating all competitors decreases with time spent fighting (Reinhold, 2003). Murray (1987) describes the interaction between these opposing factors, such that the greatest overall fighting cost is found at intermediate male densities; fights are more numerous than at low densities and fiercer than at high densities. Nonetheless, given the small number of males we used per treatment (1–8 males), I would still expect a positive correlation between density and cost of fighting over this range (see figure 2 of Murray 1987). High male densities could also lead to a reduction in the probability of initiating a fight (Reinhold, 2003), but my observations suggest that, in the case of *M. acasta*, fighting will always occur. Hence, these predictions are not met; however, Reinhold (2003) also suggests that, if kin recognition were present, the effect of male density may change.

Given the high cost of fighting (death upon losing), we might expect to find evidence of opponent assessment (Enquist and Leimar, 1983; Enquist and Leimar, 1987; Leimar and Enquist, 1984). In particular, given that large size significantly improves competitive success, we might expect small males to avoid fighting (Figure 4.3; (Petersen and Hardy, 1996)). However, behavioural observations suggest that *M.*

acasta males always fight, and that fighting between two males begins immediately upon encountering each other. There are two reasons why we might expect this to be the case. First, Enquist and Leimar (1990) showed that, if the value of the resource being contested is much larger than the value of potential future resources, all conflicts should be resolved by fighting rather than by retreating. In this case, it is not in the interests of weaker competitors to avoid a fight: even if the probability of winning is very small, as for *M. acasta*, there is a chance of winning, and this resource is extremely valuable. Put simply, males fight because they have little to lose and a lot to win (the 'desperado effect'; Grafen, 1987). Second, whilst models of fatal conflict assume that retreating is an option, it may be the case that *Melittobia* males are unable to run away and thus have to fight. As male *Melittobia* are blind, tactile cues may be the only way assess their opponents and close proximity may make fighting inevitable. The idea here is that the higher quality male must ensure he kills the lower quality opponent.

To conclude, this and previous work on *Melittobia* species has identified a number of factors that may influence fighting behaviour. I have focused upon the effects of male and female density, and body size on contest behaviour in *M. acasta*. Previous studies on *Melittobia australica* have shown that early emerging males will almost always kill pupal males upon eclosion, suggesting that early emergence confers a competitive advantage in lethal combat (Abe et al., 2003b; Innocent et al., 2007). Therefore, emergence order and body size have independent and striking effects on the probability of winning a fight, but their relative importance is unknown. It would be extremely useful to examine the interplay between fighting ability (e.g. body size) and ecological factors (e.g. emergence order) as, in most cases, competitive ability is the overriding factor (Batchelor et al., 2005; Petersen and Hardy, 1996). Furthermore, understanding the dynamics of fights between males could help explain the unusual lack of sex ratio adjustment that has been observed in *Melittobia* species, in response to the number of females laying eggs in a patch (Abe et al., 2003a; 2003b; 2005; Cooperband et al., 2003; Innocent et al., 2007). For example, if later born males are killed by previously emerged males, then the relative fitness of producing males late in a brood are reduced and so the sex ratio (proportion males) is

not expected to increase as more females lay eggs on a patch (Abe et al., 2003a; 2003b; 2005; Shuker et al., 2005).

Chapter 5 : Influence of relatedness and the environment on lethal combat in *Melittobia*

5.1 Introduction

When individuals compete for resources, these interactions span the entire spectrum of behaviour from cooperative resolution to escalated conflict (Maynard-Smith and Price, 1973). Violent interactions are rare and only predicted under conditions where the benefit of winning far outweighs the potential cost of conflict (Enquist and Leimar, 1990; Maynard-Smith and Price, 1973). Fatal fighting, where competing individuals risk death in violent contests, is expected only when competition occurs over a finite resource of extremely high value (Enquist and Leimar, 1987; 1990). Whilst competition over mates does not always lead to conflict almost all known examples of extreme conflict result from competition over mates or access to mating opportunities (Enquist and Leimar, 1987; 1990), such as in fig wasps, *Cardiocondyla* ants, and thrips (Anderson et al., 2003; Hamilton, 1979; Murray, 1987). As mating is so directly tied to an individual's fitness, this resource will be extremely valuable to all individuals, almost all of the time. Consequently, when a finite resource as valuable as mating opportunities is limited in time, space, or both, then extreme competition and lethal combat can evolve (Enquist and Leimar, 1990; Hamilton, 1979; Maynard-Smith and Price, 1973; Murray, 1987; Reinhold, 2003).

When extreme conflict does evolve, theory predicts that the occurrence and intensity of fights will vary with resource value (Enquist and Leimar, 1987; 1990), the number of competitors (Murray, 1987; 1989; Murray and Gerrard, 1984; 1985), and the level of relatedness between them (Reinhold, 2003). First, although mates are always a valuable resource, theory suggests that what matters for the evolution of extreme conflict is the ratio of the current resource value to its potential future value (Enquist and Leimar, 1990). When competitors have many future chances to mate, a single mating constitutes a small fraction of potential lifetime reproductive success, and so there is little value fighting over it (Enquist and Leimar, 1990; Hamilton, 1979). In contrast, if future mating opportunities are unlikely, or available mates diminish over

time, then a single mating represents a considerably larger proportion of lifetime reproduction, and so fatal fighting is more likely to be favoured (Cook et al., 1999; Enquist and Leimar, 1990; Hamilton, 1979; Murray, 1987). Second, variation in competitor density is predicted to have several, opposing effects upon the intensity or frequency of fatal fighting, or both. As the number of competitors increases, the encounter rate increases, resulting in a higher frequency of fights, but also lessening the value of winning each fight (Murray, 1987). Overall, these effects predict a peaked relationship between the number of competitors and the intensity of fighting, where fight intensity is highest at intermediate competitor density (Murray, 1987). Third, it has been argued more recently that if competitor number and relatedness are considered simultaneously, fight intensity is predicted to decrease with competitor number, rather than show a domed relationship (Reinhold, 2003). In addition, fight intensity is predicted to decrease when competitors are more closely related, if individuals are able to recognise kin, due to the indirect (fitness) benefits of harming non-relatives and not harming relatives (Hamilton, 1979; Reinhold, 2003). However, there have been few tests of these predictions, especially with experimental studies (Cook et al., 1997; Moore et al., 2008; Reinhold, 2003).

Here, I use the parasitoid wasp *Melittobia* to test all of these theoretical predictions, by manipulating resource value, competitor density, and level of relatedness independently. Male *Melittobia* have a limited opportunity within which to gain matings, as they are confined to their natal host, and are restricted to the female's eclosing from it (Hamilton, 1979; Matthews et al., 2009). As the entirety of their lifetime reproductive success is at stake when males compete, they engage in extremely violent lethal combat (see methods). First, I manipulate resource value - mating opportunities - by allowing some males to mate prior to fighting, but preventing others from doing so. This alters the ratio between the current value of the resource and its lifetime value, such that future reproductive opportunities are a greater component of lifetime reproduction for a virgin male than a mated male. I expect the resource (mates) to be of higher value to a virgin male than a mated male, meaning that virgin males will fight more often, more intensely, or both. Second, I manipulate the density of male competitors (spanning a natural range of density) by

placing males in groups of varied size, and measure fight intensity. I expect the frequency of fights to increase with group size, and fight intensity to be greatest at intermediate density (Murray, 1987), or decrease with increasing density (Reinhold, 2003; Figure 5.1). Third, I vary competitor density and relatedness simultaneously, by creating groups of different sizes within which males compete with either unrelated males, or a mixture of related and unrelated males. If individuals are able to recognise kin, then I predict lower overall fight intensity in more related groups (Reinhold, 2003). If, however, *Melittobia* do not recognise kin, I expect relatedness to have no effect on the frequency or intensity of fights, which would then simply be determined by competitor density (Reinhold, 2003). In all experiments I collect data to quantify fight intensity at a number of levels, considering the pattern of mortality; the incidence and severity of injuries; and measures of aggressive behaviour.

a)

b)
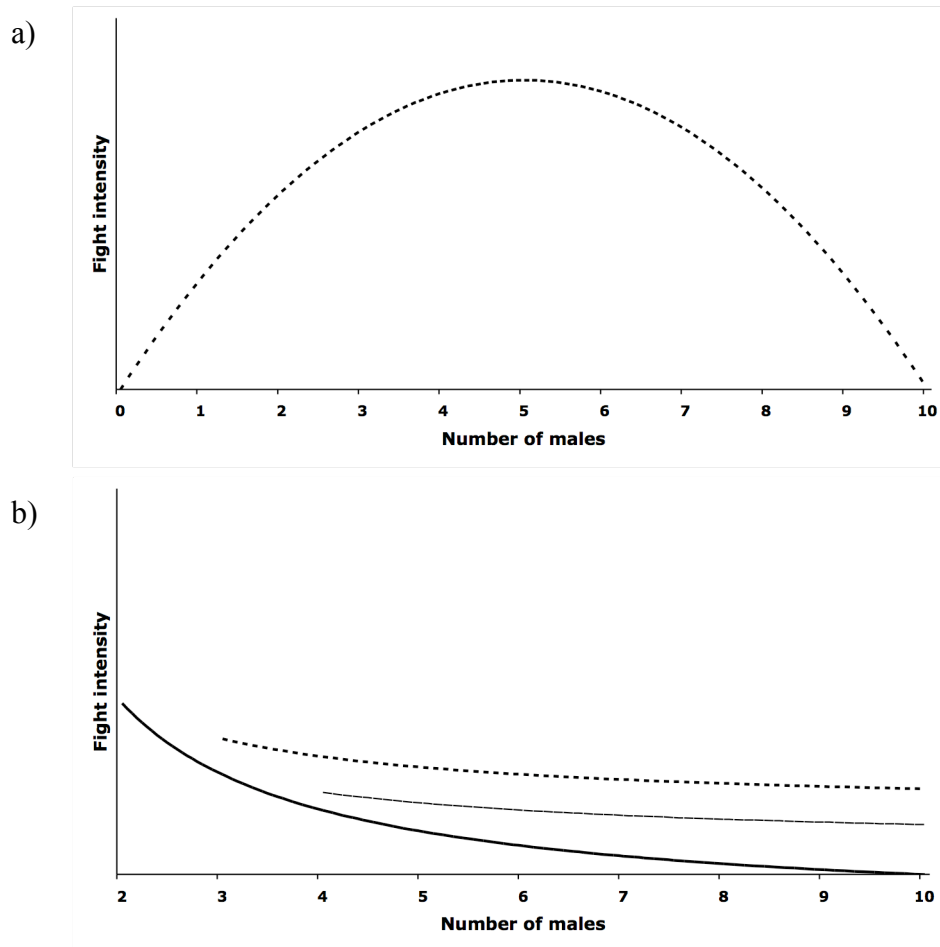
Figure 5.1: Theoretical predictions for the relationship between number of competitors and fight intensity; a) increasing encounter rate is counteracted by increased cost of fighting (Murray, 1987); b) fight intensity decreases with competitor number, for 1 foundress female (solid line), 2 foundresses (dotted line), and 3 foundresses (dashed line) (Reinhold, 2003). In both cases, the y-axis corresponds to increasing fight intensity.

5.2 Methods

5.2.1 Natural history and general methods

Melittobia acasta and *M. australica* (Hymenoptera: Eulophid) are gregarious ectoparasitoid wasps with natural history common to other *Melittobia* species

(Gonzalez et al., 2004a; 2004b; Matthews et al., 2009; Van den Assem et al., 1980); see Abe et al., 2003b; 2005; Innocent et al., 2007; Reece et al., 2007 for further details of natural history). *Melittobia* species parasitise a wide range of hosts, particularly other species of hymenoptera (Balfour Browne, 1922; Cooperband and Vinson, 2000; Dahms, 1984; Freeman, 1977; Freeman and Parnell, 1973; Gonzalez et al., 2004b; Matthews et al., 2009; Van den Assem et al., 1980). Sexual dimorphism between males and females is pronounced: males are blind, flightless and remain on the natal host to compete locally for mates, while females have fully-functioning eyes and wings, and may disperse after mating (Buckell, 1928; Dahms, 1984; Gonzalez et al., 2004b; Matthews et al., 2009). Male mandibles are highly modified weapons used in violent lethal combat prior to female eclosion: males sever competitor's limbs and decapitate opponents in fights to the death. Any male(s) remaining alive gain the opportunity to mate with females emerging from their host. Melittobia produce extremely female-biased offspring sex ratios (85-95% female; Abe et al., 2003b; 2005; Cooperband et al., 2003; Gonzalez et al., 2004b; Innocent et al., 2007) so the proportion of male offspring eclosing from a host is low; however, the precise number of males varies with both the number of females laying eggs on the host, and the host species. For example, the number of males per host in culture ranges from 0-1 (1 foundress, 24 hours oviposition) to 15-17 (50 foundresses, 48 hours oviposition) on *Calliphora vomitae* pupae, and from 0-7 (1 foundress, 72 hours) to as many as 80 (15 foundresses, 144 hours) males on *Bombus terrestris* pupae (Innocent et al., 2007; Innocent et al., in press). The degree of relatedness between male competitors is also influenced by the number of female foundresses, and by foundress relatedness (Innocent et al, in review). Given the potential for multiple foundresses and overlapping generations on a single host (Cooperband et al., 2003; Dahms, 1984; Freeman and Ittyeipe, 1976; 1982; 1993; Matthews et al., 2009; Schmieder, 1933; Van den Assem et al., 1982), male emergence may vary through time; as a result males differ in age and fighting ability e.g. (Abe et al., 2007; Innocent et al., 2007), and experience variation in the local or temporal availability of females.

Across *Melittobia* species, natural history, host range, sex ratio patterns and fighting behaviour are similar (e.g. Matthews et al., 2009), but patterns of development vary; I utilise the differences between *M. acasta* and *M. australica* in these experiments. *M. acasta* has more synchronous development in culture, enabling the production of large numbers of age-matched males (experiment 1), whereas a larger bank of iso-female lines is available for *M. australica* (experiments 2 & 3). I cultured *M. acasta* stock on *C. vomitae* pupae at 30ºC (see Innocent et al., 2007; Reece et al., 2007). *M. australica* lines were collected throughout Japan (by Jun Abe, 1999/2000; see (Abe et al., 2003b; Abe et al., 2005)). I reared all experimental *M. australica* lines on *B. terestris* pupae (Koppert, The Netherlands), incubating them at 30ºC with a 16:8 light:dark photoperiod.

To produce males, I collected virgin female pupae from appropriate stock culture: as sex determination in *Melittobia* is haplodiploid, virgins produce exclusively male offspring from unfertilised eggs (Cook, 1993). I placed groups of 60 virgin females with hosts for oviposition (as above) and incubated them at 30ºC. To minimise variation in age of male offspring, I gave virgin females hosts synchronously and limited oviposition to an 8-hour period. I collected male pupae from hosts approximately 8 days after oviposition, isolating each in a gelatin capsule of similar dimension to host pupae (volume = 0.21ml), preventing aggressive male-male interaction prior to the experiment. I checked males daily, grouped them by emergence date, and used males from the same 24-hour emergence period within experimental replicates. I collected data for measures of fight intensity based on behaviour, injury and mortality, using two types of fighting arena. I used holes punched in sheet metal (5mm diameter; 3mm thick) encased by glass cover slips as arenas for individual data collection (experiment 1), cleaned between replicates to avoid the influence of chemical signals from previous contests. To collect group-level data (experiments 2 & 3) I used gelatin capsules as arenas (as above).

5.2.2 Experiment 1: resource value

I tested Enquist and Leimar's (1990) prediction, that the extent of fatal fighting is related to the value of a contested resource. Specifically, I tested whether virgin

males compete more intensively over mating opportunities than previously mated males. I placed males from the mated treatment with 5 virgin females (from stock synchronised with male emergence) for 2 hours, at 30ºC. I placed the remaining virgin males at 30ºC for 2 hours, isolated in gelatin capsules. Subsequently I paired males in 3 combinations: mated male + mated male (MM), virgin male + virgin male (VV), and mated male + virgin male (MV). I painted each male's abdomen for identification; colour was assigned randomly across pairs and combinations. I observed each male separately for 5 minutes, recording the number of movements between sectors of the arena to estimate individual activity level. Next, I paired males in a new arena and observed for 30 minutes, recording: interaction start and end time; identity of the male initiating/retreating; and the occurrence of key aggressive behaviours, i) boxing, ii) locking of opponent, and iii) biting or lunging. I defined interactions as >5 seconds of physical contact between males, and considered them antagonistic if I observed aggressive behaviour. I scored the relative size of the two males by eye. After observation I incubated each pair into a gelatin capsule (as above) at 30ºC; I recorded the outcome of each contest at 24 hours (win/lose, or draw), the identity of male(s) remaining alive, and any visible injuries to either male.

5.2.3   Experiment 2: group size

I tested Murray's (Murray, 1987) prediction that fight intensity is influenced by the number of competitors, and greatest at intermediate male densities. I set up 6-12 replicates for each of five biologically realistic competitor densities: 2, 5, 10, 15 and 25 males. I placed groups of randomly assigned, age-matched males into capsule arenas (see above), and incubated them at 30ºC. I recorded the number of males dead at 2, 4, 6, 8 and 24 hours (Olympus SZX10 microscope), to estimate the time of the first death and proportion of males dead at 24 hours within arenas. I froze all arenas at 24 hours, scored visible injuries for each male within every replicate - according to a scale adapted from Murray (Murray, 1985; 1987; 1989; 1990; Murray and Gerrard, 1984; 1985) - and calculated mean injury per wasp (lifetime extent of injury, LEI), proportion of males injured and proportion with severe injury (score >7) for each arena.

5.2.4 Experiment 3: relatedness & group size

I tested Reinhold's (2003) prediction that the relatedness of competitors, in addition to their density, influences fight intensity within a group. I varied relatedness between males using two treatments: 'related' – all males came from the same line - or 'unrelated' – males came from 3 different lines (from 6 available lines); and competitor density using two group sizes, 3 or 6 males (see Table 5.1). For each of 3 lines contributing males to an unrelated replicate, I set up a separate, related replicate of the same group size (see Table 5.1). I simultaneously placed eight groups (1 of each combination; see Table 5.1) in gelatin capsules, which I mounted and observed under a microscope (as before) for 30 minutes. I recorded the number of fights and the number of males engaged in fighting at 1-minute intervals through this period, and any deaths throughout; non-aggressive interactions were not included. Following observation I incubated arenas at 30ºC, recording the number of males dead at 90, 180 minutes, and 24 hours in order to estimate the time of first death and calculate the proportion of males dead at 24 hours. I froze all arenas at 24 hours, scored injuries for each individual and calculated the LEI, proportion of males injured and the proportion with high injury per arena (as above).

	Related	**Unrelated**
3 males	aaa *or* bbb *or* ccc *etc*	abc *etc*
6 males	aaaaaa *or* bbbbbb *or* cccccc *etc*	aabbcc *etc*

Table 5.1: Treatment combinations for experiment 3, involving 2 group sizes (3 or 6 males) cross-factored with both a related (single line) and unrelated (3 lines: shown here as a, b, and c) treatment. Each letter represents an individual male.

5.2.5 Statistical methods

Where necessary, data were transformed to improve normality (square-root transformation on time of first death data, log transformations for behavioural measures, arcsine-square root transformation for proportion data). I used linear models to test for the effect of group size upon the time of 1st male death, the proportion of males dead at 24 hours, and the proportion of males injured in experiment 2; and all behavioural measures of fight intensity from experiment 1. I used Generalised Linear Models (GLMs) to analyse fight resolution and occurrence of injury data (experiment 1), assuming a binomial error distribution and using a logit link function for maximum power. Model simplification was based upon analysis of deviance, comparing changes in deviance between models to the chi-squared distribution. I tested for overdispersion of data by calculating the heterogeneity factor (HF); where HF<4 data was scaled and significance tested using the F-distribution to correct for overdispersion (Crawley, 1993; Crawley, 2002; Crawley, 2007). I included in the model resource value treatment, size difference between competitors, difference in activity level, and 2-way interactions with treatment as possible explanatory variables. Interactions are presented only where significant at the level of P<0.01 (Crawley, 1993; Crawley, 2002; Crawley, 2007). To examine the effect of both group size and relatedness between competitors on mortality, injury and behaviour in experiment 3, I used linear mixed effect models. I used the measures of fight intensity as response variables for each model, including group size and relatedness in the model as fixed effects, and fitting line as a random effect. All analyses were carried out in R (R version 2.3.1, Copyright © 2006, The R Foundation for Statistical Computing).

5.3   Results

5.3.1   Experiment 1: resource value

In contrast to Enquist and Leimar's (1990) predictions I found that variation in resource value – mating opportunities – as manipulated by whether males had previously mated with females, had no effect on fight intensity, as estimated by

mortality, injury, or behaviour. The likelihood of at least one male dieing within the first 24 hours was not influenced by resource value (male mating status: $F_{2, 55}=0.6$, $P=0.55$); size difference ($F_{1, 57}=0.47$, $P=0.49$); or individual activity level ($F_{1, 54}=0.26$, $P=0.61$). I scored all visible injuries but found no significant correlation with male mating status ($F_{2, 55}=1.95$, $P=0.15$), size difference ($F_{1, 55}=1.6$, $P=0.2$), or activity level ($F_{1, 55}=0.098$, $P=0.76$). I found no significant correlation between the mean number of fights per minute and male mating status ($F_{2, 55}=1.75$, $P=0.18$; Figure 5.2), size difference ($F_{2, 56}=3.38$, $P=0.07$), or difference in individual activity between males ($F_{1, 53}=0.01$, $P=0.91$). I also found the same qualitative pattern with two other measures of aggressive behaviour, the total number of fights and the proportion of the observation period individuals spent fighting ($P>0.25$ in all cases). There were no significant interactions.
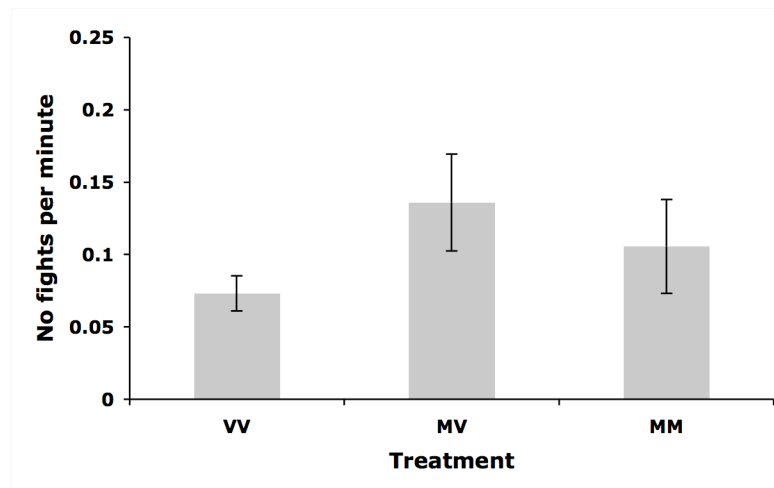
Figure 5.2: Mean number of fights per minute between male pairs within three treatment combinations: both virgins (VV), both mated (MM), and a mated versus a virgin male (MV). Error bars indicate standard errors.

5.3.2   Experiment 2: group size

In contrast to Murray's (Murray, 1987) prediction that fight intensity will be greatest at intermediate competitor density, I found that fight intensity increased

monotonically with group size. The proportion of males dieing within 24 hours was positively correlated with group size ($F_{1, 43}$=14.74, P =0.0004; Figure 5.3a), and the first male death was significantly earlier in larger groups ($F_{1, 43}$=48.32, P <0.0001). There was no significant quadratic relationship with group size in either case (P >0.1). We found no significant effect of increasing group size on the proportion of males injured within a group (linear: $F_{1, 43}$=1.17, P =0.29; quadratic: $F_{1, 42}$=0.26, P =0.61; Figure 5.3b). Similarly, there was no significant effect of group size on the proportion of males with high injury score, or mean injury per wasp (P >0.35); there were no quadratic relationships (P >0.35).
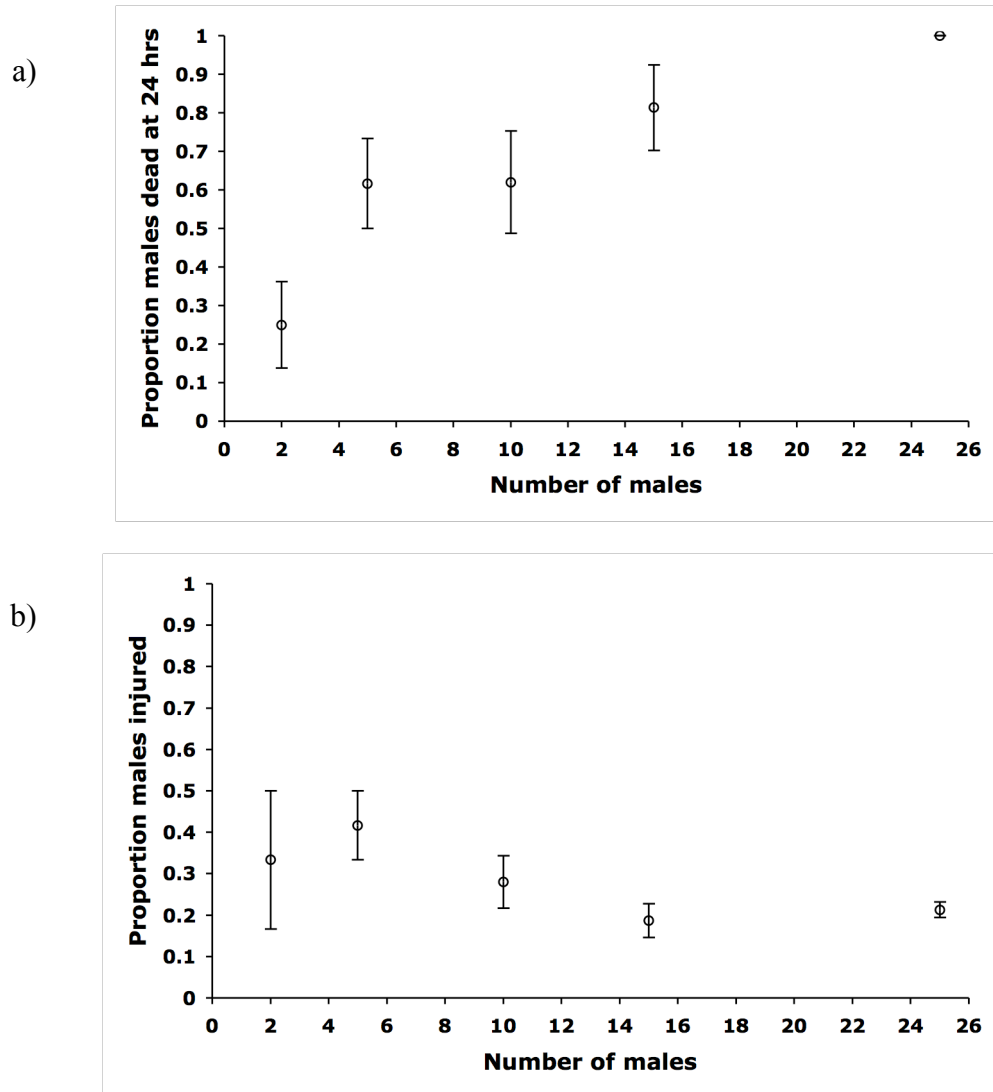
Figure 5.3: The influence of competitor density on mortality and injury measures within groups, for a range of group sizes; (a) the pattern of mortality, shown as the proportion of males within a group dead at 24 hours; and (b) the pattern of injury, shown as the proportion of males injured within 24 hours. Error bars show mean vales ± standard errors.

5.3.3   Experiment 3: relatedness & group size

I found no support for Reinhold's (2003) predictions. In contrast to Reinhold's (2003) prediction that lower fight intensity occurs when there is a higher level of

relatedness between competitors, I found that there was no effect of relatedness upon mortality, injury or aggressive behaviour. In addition, I found no support for Reinhold's (2003) prediction that increasing group size will lead to decreased fight intensity. The proportion of males dead at 24 hours increased significantly with increasing group size ($F_{1, 58}$=6.56, P =0.01; Figure 5.4a), but not with variation in male relatedness ($F_{1, 11}$=1.12, P =0.27; Figure 5.3a). Similarly, the time of first death was significantly earlier in larger groups ($F_{1, 58}$=12.23, P <0.0001), but was unaffected by relatedness ($F_{1, 11}$=2.39, P =0.13). The proportion of males injured did not vary significantly with increasing group size ($F_{1, 58}$=0.53, P =0.47) or relatedness within groups ($F_{1, 11}$=2.71, P =0.13; Figure 5.4b). I found a similar pattern for the proportion of males with severe injury and the LEI (P >0.1). The mean proportion of males fighting increased with group size ($F_{1, 58}$=11.34, P =0.001; Figure 5.4c), but did not vary with relatedness ($F_{1, 11}$=1.21, P =0.27). Similarly, the mean number of fights per minute increased with increasing group size ($F_{1, 58}$=38.9, P = 0.0001), but did not vary with relatedness ($F_{1, 11}$=1.87, P =0.18). There were no significant interactions.

a)
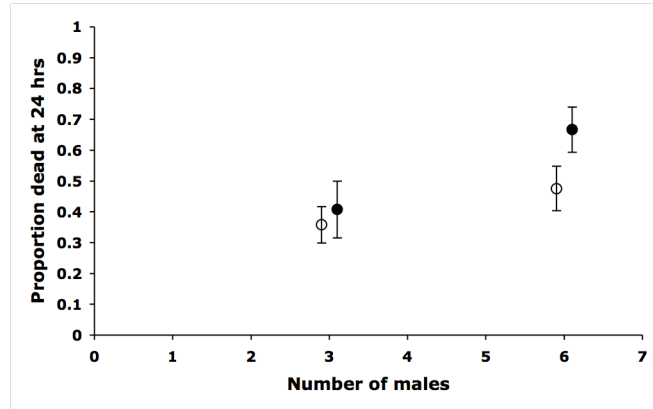
b)
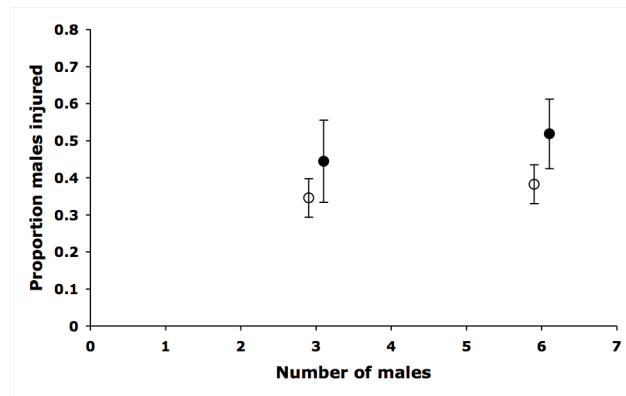
c)
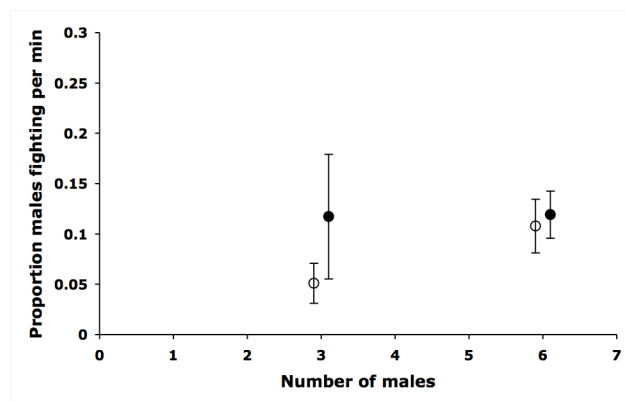
Figure 5.4: The influence of group size and relatedness on fight intensity (y-axis: 0 = low intensity, 1 = high intensity) in *M. australica* as measured by (a) mortality, shown as proportion of males dead at 24 hours; (b) proportion of males injured; and (c) proportion of males fighting per minute, for related (open circles) and unrelated (closed circles) groups of 3 or 6 male competitors. Error bars indicate mean value ± standard error.

5.4 Discussion

I used male *Melittobia* wasps to test theoretic predictions for how resource value, competitor density, and relatedness between rivals will influence the intensity of violent fighting (Figure 5.1). I found that: (1) males do not adjust their level of aggression in response to whether or not they had previously mated, and therefore the contested resource value (Figure 5.2); (2) the intensity of fighting increased monotonically with increasing competitor density (Figure 5.3); (3) there was no difference in the level of aggression between contests of either unrelated, or closely related, males (Figure 5.4). Overall my results suggest that male *Melittobia* exhibit a relatively fixed behavioural strategy, lack kin discrimination, and will always engage in potentially lethal combat.

Theory suggests that the more valuable a contested resource, the more likely competitors are to risk costly, escalated conflict to obtain it (Cook et al., 1999; Enquist and Leimar, 1987; 1990; Hamilton, 1979). Here, I find no evidence that the intensity of fighting between male *Melittobia* varies with resource value, or that it differs whether the contested resource has the same, or different value for the competitors (Figure 5.2). One possible explanation for this is that, given the short lifespan of males and limited opportunity to gain mates, any pre-existing fight advantage is lost if males stop to assess the merits of entering a fight, and it is therefore a better strategy to fight every time. Another possibility is that my manipulation does not alter resource value, because the value of past resources has no impact upon the ratio of current to future resource value (Dawkins and Carlisle, 1976). Here, the current resource always has far greater value than future resources, and so fighting is always favoured. How does fighting in *Melittobia* compare to other species where extreme contests are found? A common feature of species where males engage in lethal combat is that females are aggregated both spatially and temporally (for a short time), and that these females are the only mates available to males (Enquist and Leimar, 1990; Hamilton, 1979). For instance, wingless male fig wasps engage in lethal combat within fig fruit for access to locally emerging females, and wingless male *Cardiocondyla* ants will kill rivals within the nest during

competition for mates (Anderson et al., 2003; Bean and Cook, 2001; Cook and Bean, 2006; Cook et al., 1997; 1999; Hamilton, 1979; Murray, 1987; 1989; 1990; Murray and Gerrard, 1984; 1985).

The intensity of fighting is predicted to show either a domed, or decreasing relationship with competitor density (Murray, 1987; Reinhold, 2003). As competitor density increases this leads to a higher number of interactions between males, and hence the possibility for more violent conflicts; but this can be negated at high density if this leads to scramble competition and hence the benefit of fighting decreasing (Murray, 1987). I found that a greater number of competing males led to a monotonic increase in fight intensity (Figure 5.3). If encounter rate does increase with the number of competitors, as is likely to be the case, (Murray, 1987; Reece et al., 2007), then these results suggest that males do not modify their fighting behaviour in response to increasing costs of fighting with, and defending resources against, an increasing number of opponents (Murray, 1987; 1989; Murray and Gerrard, 1984). Furthermore, this confirms previous observations that conflict-limitation through opponent assessment does not occur in *Melittobia* (Reece et al., 2007).

Theory predicts that, if individuals are able to discriminate relatives from non-relatives (kin discrimination), then competition should be less aggressive between relatives (Hamilton, 1979; Reinhold, 2003). Another way of looking at this is that individuals should be more violent to non-relatives, if it reduces the level of competition experienced by relatives who are also competing with these non-relatives – because fighting may be costly, this can represent a spiteful or indirectly altruistic behaviour (Gardner et al., 2007; Gardner and West, 2004). I allowed male *Melittobia* to interact with both relatives and non-relatives, and found no evidence that they adjust their fighting behaviour in response to relatedness (Figure 5.4; see also Abe et al., 2003b). This suggests that *Melittobia* are unable to discriminate kin, which is consistent with data from other non-social insects, where kin discrimination is rarely found (Fellowes, 1998; Reece et al., 2004; Shuker et al., 2004a). A lack of kin discrimination can be expected from a theoretical perspective, because kin

discrimination based on genetics will often not be evolutionarily stable. The reason for this is that it would favour common alleles, which would be recognised more often, and hence kin discrimination would eliminate the genetic diversity that it requires in order to operate (Crozier, 1986; Rousset and Roze, 2007). More generally, the observed lack of kin discrimination supports the lack of an effect of mean relatedness on the level of fighting in fig wasps, because the local competition within fig fruits means that any kin selected benefit of reduced conflict with closer relatives is negated by increased competition with other relatives (West et al., 2002). Put simply, there is no benefit in being less aggressive with a brother, if any benefit they obtain comes at the cost to another brother.

Chapter 6 Discussion

Each chapter of this thesis has its own specific discussion of experimental results. Here, I summarise these findings in a broader context, suggest future directions, and highlight the implications of my work for social evolution more generally.

6.1 Summary of results

6.1.1 Sex ratio in Melittobia

In chapter 2, I present a series of experiments that examine the pattern of sex allocation in *Melittobia*. Specifically, I describe the extremely female biased offspring sex ratio in M. acasta, and attempt to understand the unusual lack of sex ratio adjustment in response to variation in local mate competition (LMC; Hamilton, 1967a) observed across Melittobia species. I show that females produce a sex ratio of 85-95% female offspring, and that this sex ratio is precisely controlled. And, while LMC theory predicts that the sex ratio will become increasingly less biased with increasing foundress number, I found that the sex ratio in *Melittobia* shifts only marginally relative to predictions. I examine a possible explanation for this pattern by testing a model of asymmetrical LMC, where earlier emerging males have a competitive advantage in competition for mates, which selects against the production of a less female-biased sex ratio (Abe et al., 2003b). In *Melittobia* mating competition takes the form of fatal fighting. I show that early emerging males do have a competitive advantage that could enhance the asymmetry in mate competition, as early emerging males are able to attack later emerging males at the vulnerable point of emergence. The pattern of sex ratio also suggests that male eggs are laid first, which would maximise emergence advantage.

The magnitude of the advantage required by early males to explain lack of sex ratio adjustment is large, and I show two reasons why this might not be realistic. First, male size is closely tied to fighting ability, and the probability of winning fights; any emergence advantage is reduced by the advantage of body size of opponents (chapter

4 and 5). Second, the advantage of early emergence lasts only as long as the period of emergence; after this point, all males are equally prepared for combat, which is less than half the duration of their lifespan. However, there are two alternative explanations for the unusual sex ratio pattern in *Melittobia*. If foundress number varied little in natural populations, then selection for sex ratio adjustment would be weak; here, little sex ratio adjustment would be predicted (Herre, 1987). Data suggests, that foundress number does vary in natural populations; and in addition, variation in female morph can lead to overlapping generations on a single host (Freeman, 1977; Freeman and Ittyeipe, 1976; Gonzalez et al., 2004c; Matthews et al., 2009). Similarly, if foundress females were related, and therefore were also related to each other's male offspring, then a female biased sex ratio would be predicted (Frank, 1998). To fully assess these alternative explanations, more data is needed from natural populations of *Melittobia*. If, for instance, resource competition leads to earlier emerging males also being larger, then the advantages of early emergence and fighting ability would be combined, and fatal fighting between males may provide a more substantial explanation for the lack of sex ratio shift. And, even if foundress females are often related, this could lead to increased competition between relatives cancelling the advantage of laying a female biased sex ratio. Finally, the pattern of sex ratio could be affected by the pattern of dispersal (chapter 3); an interesting extension to these experiments would be to understand more about the variation in female morph production and pattern of dispersal in natural populations.

Within the field of sex allocation, patterns of sex ratio that cannot be explained by the predictions of theory are rare. Sex allocation is one of the most theoretically well-understood areas of evolutionary biology, and has a large amount of empirical support from a wide range of taxa (West et al 2005; West, 2009). In some cases, the predictions of sex allocation theory, and in particular Hamilton's local mate competition theory (LMC), are met quantitatively as well as qualitatively (Werren, 1980, 1983). Considered in this context, the extremely female biased sex ratio, and lack of sex ratio adjustment in *Melittobia* species is particularly striking. Attempting to explain this pattern has prompted more recent extensions of LMC theory tailored to the biology of this system (Abe et al 2003). So far, empirical tests of theoretical

predictions have not provided a conclusive explanation for this unusual example of sex allocation. This may be because we need more information on the details of biology in natural populations, and may be because theoretical models are missing some of the important details of the biology of more unusual systems. In either case it is important, and interesting, to explain exceptional cases such as *Melittobia*, promoting further empirical testing of existing sex allocation theory, and in turn driving further development of theory.

6.1.2 Dispersal in Melittobia

The experiments I present in chapter 3 describe the dispersing and non-dispersing female morphs of *Melittobia australica*, and test theoretical predictions that the level of dispersal will vary with the extent of competition for resources and the level of relatedness between potential competitors. The two female morphs differ in morphology, pattern of egg production, and dispersal behaviour. I show that the production of dispersing females, and thus the level of dispersal, increases with increasing competition for resources. If related individuals compete for resources, dispersal can be favoured to decrease the level of competition faced by relatives, and can therefore be considered a cooperative trait (Hamilton and May, 1977). Dispersal theory predicts that higher levels of dispersal will be favoured when fewer foundresses lay eggs on a patch, because the relatedness between non-dispersing competitors will be higher (Comins, 1982; Comins et al., 1980). In contrast to these predictions, I found that the level of relatedness has no affect upon the pattern of dispersal in *Melittobia*. While this is unlikely to be because there is little variation in foundress number in natural populations (Herre, 1987); it is possible that high relatedness between co-founding females would favour lower levels of dispersal, due to greater relatedness (Frank, 1998). Variation in relatedness created by changes to foundress number has implications both for the evolution of dispersal and sex allocation, and this may help discriminate between possible explanations for why the sex ratio in *Melittobia* shifts little in response to LMC (chapter 2; Hamilton, 1967). When dispersal and sex ratio are considered simultaneously, theory predicts that if one sex disperses less, a sex ratio biased towards the more-dispersing sex will be favoured, as this sex faces less competition locally (Bulmer and Taylor, 1980a;

Hamilton, 1967a; Taylor, 1981). In *Melittobia*, fighting between males would favour variation in female dispersal with changes in foundress number. In contrast, both weak selection for sex ratio adjustment and high relatedness between co-founding females, would predict no variation in dispersal, along with no sex ratio adjustment; this is consistent with my findings. There are a number of reasons for variation in the production of dispersal morphs in *Melittobia*; testing these explanations and their influence on sex ratios requires more data from natural populations.

6.1.3 Extreme conflict in Melittobia

In chapters 4 and 5, I examine the nature of mating competition – in the form of fatal fighting - between male *Melittobia*. I show that greater male density results in a greater intensity of fighting, and shorter male lifespan, while greater female density may reduce the frequency of fights. Fighting ability is closely connected to body size in *Melittobia*, with large males having a high probability of winning fights. I found no variation in the frequency of fights whether opponents were evenly matched or there was asymmetry in fighting ability; although conflict limitation is common in most species (Maynard-Smith and Price, 1973), this result suggests that no opponent assessment occurs between male *Melittobia*. When extreme conflict does evolve, theory predicts that the occurrence and intensity of fights will vary with resource value, the number of competitors, and the level of relatedness between them (Enquist and Leimar, 1987; 1990; Murray, 1987; Reinhold, 2003). I found no influence of resource value on levels of fight intensity; this is consistent with the theoretical prediction that when the current value of a resource far outweighs any potential future value, and is closely associated with lifetime reproductive success, fighting will always be favoured (Enquist and Leimar, 1990). In such cases, all that matters is the current resource value – here, the availability of female mates – and the future resource value, which for male *Melittobia* is low, given their short lifespan and limited number of female mates available. I show that male *Melittobia* adopt a relatively fixed behavioural strategy, and do not modify fighting behaviour in response to variation in the likely costs of fighting.

Theory predicts that, if males are able to discriminate kin, then competition between relatives should be less aggressive, as males are predicted to be more violent towards non-relatives, in order to reduce the competition faced by relatives competing with the same pool of opponents (Gardner and West, 2004; Reinhold, 2003). Here, I found no variation in fight intensity with the level of relatedness between opponents, which suggests that *Melittobia* are unable to discriminate kin – consistent with studies of other non-social insects (Fellowes, 1998; Reece et al., 2004; Shuker et al., 2004b). Moreover, this is an example of where the local scale of competition means that any kin selected benefit of reduced conflict with closer relatives, is negated by increased competition with other relatives (West et al., 2001; 2002).

6.2 Conclusions

6.2.1 From cooperation to conflict

Peaceful cooperation between individuals, and violent contests when conflict escalates, could not appear to be more different. In fact, all interactions stem from a conflict of interest between the individuals involved, where each will act to maximise their own fitness. But, a conflict of interest can be resolved in many ways, which manifests as this spectrum of interactions ranging from cooperation to extreme conflict. Behaviour at both extremes of the spectrum is less common: it is unlikely to be good for an individual to help others with no benefit to themselves, and is rarely worthwhile incurring the extreme costs of escalated conflict. Understanding the costs and benefits of a behaviour to different individuals, and considering the relatedness between individuals that interact, can help explain why we see certain behaviours in particular scenarios. And, the most informative examples are often those that are unusual, and lie at the extremes of the spectrum of potential behaviour. With a natural history that is unusual in a number of respects, *Melittobia* is a good system for trying to explain the full range of interactions between individuals, from cooperation through to extreme competition. While LMC theory predicts that individuals should produce less female-biased sex ratios when many females lay eggs on a patch, a social evolution approach predicts that when these females are

related, all will benefit by cooperating and laying a higher proportion of daughters. Cooperation of this kind may partly explain the unusual pattern of sex allocation in *Melittobia*. Conversely, when competition is local, individuals will not benefit from favouring related competitors, if this advantage is counteracted by an increase in competition between these relatives. This may explain why male *Melittobia* will engage in violent, escalated conflict with brothers.

6.2.2 "Social behaviour must be analysed in its full ecological and demographic context" (Frank, 1998)

Social evolution considers behaviours where individuals interact, and which have consequences for the fitness of all individuals involved. A number of social traits have been theoretically modelled extensively, including those where the relevant biological variables are easy to identify, such as sex ratio and dispersal. In such cases, these biological variables can also be measured empirically with relative ease. Social behaviours rarely occur in isolation, and are predicted to interact in all manner of ways. While it is possible to try and understand traits by considering them in isolation, this does not always lead to a close match between theory and observation; moreover, although theory is able to explain many of the patterns observed, this may not capture the full picture of social behaviours in a system. Therefore, social evolutionary theory also considers combinations of social traits, and the interactions between them – for instance, sex ratio and dispersal. While the predictions of these models may be harder to test empirically, systems such as *Melittobia* allow some questions to be asked about how different social traits inter-relate, and provide some interesting links between the different areas of social evolution.

References

Abe J, Kamimura Y, Ito H, Matsuda H, Shimada M, 2003a. Local mate competition with lethal male combat: effects of competitive asymmetry and information availability on a sex ratio game. Journal Of Evolutionary Biology 16:607-613.

Abe J, Kamimura Y, Kondo N, Shimada M, 2003b. Extremely female-biased sex ratio and lethal male-male combat in a parasitoid wasp, Melittobia australica (Eulophidae). Behavioral Ecology 14:34-39.

Abe J, Kamimura Y, Shimada M, 2005. Individual sex ratios and offspring emergence patterns in a parasitoid wasp, Melittobia australica (Eulophidae), with superparasitism and lethal combat among sons. Behavioral Ecology And Sociobiology 57:366-373.

Abe J, Kamimura Y, Shimada M, 2007. Sex ratio schedules in a dynamic game: the effect of competitive asymmetry by male emergence order. Behavioral Ecology 18:1106-1115.

Anderson C, Cremer S, Heinze J, 2003. Live and let die: why fighter males of the ant Cardiocondyla kill each other but tolerate their winged rivals. Behavioral Ecology 14:54-62.

Balfour Browne F, 1922. On the life history of *Melittobia acasta*, Walker; a chalcid parasite of bees and wasps. Parasitology 14:349-370.

Batchelor TP, Hardy ICW, Barrera JF, Perez-Lachaud G, 2005. Insect gladiators II: Competitive interactions within and between bethylid parasitoid species of the coffee berry borer, Hypothenemus hampei (Coleoptera: Scolytidae). Biological Control 33:194-202.

Bean D, Cook JM, 2001. Male mating tactics and lethal combat in the nonpollinating fig wasp Sycoscapter australis. Animal Behaviour 62:535-542.

Bowler DE, Benton TG, 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80:205-225.

Bridge AP, Elwood RW, Dick JTA, 2000. Imperfect assessment and limited information preclude optimal strategies in male-male fights in the orb-weaving spider Metellina mengei. Proceedings Of The Royal Society Of London Series B-Biological Sciences 267:273-279.

Buckell ER, 1928. Notes on the life history and habits of *Melittobia chalybii* Ashmead (Chalcidoidea: Elachertidae). Pan-Pacific Entomolgy 5:14-22.

Bulmer MG, 1986. Sex-Ratio Theory In Geographically Structured Populations. Heredity 56:69-73.

Bulmer MG, Taylor PD, 1980a. Dispersal And The Sex-Ratio. Nature 284:448-450.

Bulmer MG, Taylor PD, 1980b. Sex-Ratio Under The Haystack Model. Journal Of Theoretical Biology 86:83-89.

Burton-Chellew MN, Koevoets T, Grillenberger BK, Sykes EM, Underwood SL, Bijlsma K, Gadau J, van de Zande L, Beukeboom LW, West SA, Shuker DM, 2008. Facultative sex ratio adjustment in natural populations of wasps: Cues

of local mate competition and the precision of adaptation. American Naturalist 172:393-404.

Cant MA, Llop JB, Field J, 2006. Individual variation in social aggression and the probability of inheritance: Theory and a field test. American Naturalist 167:837-852.

Charnov EL, 1982. The theory of sex allocation. Princeton (NJ): Princeton University Press.

Clark AB, 1978. Sex-Ratio And Local Resource Competition In A Prosimian Primate. Science 201:163-165.

Clutton-Brock TH, Albon SD, 1979. Roaring Of Red Deer And The Evolution Of Honest Advertisement. Behaviour 69:145-&.

Clutton-Brock TH, Brotherton PNM, O'Riain MJ, Griffin AS, Gaynor D, Sharpe L, Kansky R, Manser MB, McIlrath GM, 2000. Individual contributions to babysitting in a cooperative mongoose, Suricata suricatta. Proceedings Of The Royal Society Of London Series B-Biological Sciences 267:301-305.

Comins HN, 1982. Evolutionarily Stable Strategies For Localized Dispersal In 2 Dimensions. Journal Of Theoretical Biology 94:579-606.

Comins HN, Hamilton WD, May RM, 1980. Evolutionarily Stable Dispersal Strategies. Journal Of Theoretical Biology 82:205-230.

Consoli FL, Tian HS, Vinson SB, Coates CJ, 2004. Differential gene expression during wing morph differentiation of the ectoparasitoid Melittobia digitata (Hym., Eulophidae). Comparative Biochemistry And Physiology A-Molecular & Integrative Physiology 138:229-239.

Consoli FL, Vinson SB, 2002a. Clutch size, development and wing morph differentiation of Melittobia digitata. Entomologia Experimentalis Et Applicata 102:135-143.

Consoli FL, Vinson SB, 2002b. Larval development and feeding behaviour of the wing dimorphics of *Melittobia digitata* Dahms (Hymenoptera: Eulophidae). Journal of Hymenoptera Research 11:188-196.

Consoli FL, Vinson SB, 2004. Wing morph development and reproduction of the ectoparasitoid Melittobia digitata: nutritional and hormonal effects. Entomologia Experimentalis Et Applicata 112:47-55.

Cook JM, 1993. Sex Determination In The Hymenoptera - A Review Of Models And Evidence. Heredity 71:421-435.

Cook JM, Bean D, 2006. Cryptic male dimorphism and fighting in a fig wasp. Animal Behaviour 71:1095-1101.

Cook JM, Bean D, Power S, 1999. Fatal fighting in fig wasps - GBH in time and space. Trends In Ecology & Evolution 14:257-259.

Cook JM, Compton SG, Herre EA, West SA, 1997. Alternative mating tactics and extreme male dimorphism in fig wasps. Proceedings Of The Royal Society Of London Series B-Biological Sciences 264:747-754.

Cooperband MF, Matthews RW, Vinson SB, 2003. Factors affecting the reproductive biology of Melittobia digitata and failure to meet the sex ratio predictions of Hamilton's local mate competition theory. Entomologia Experimentalis Et Applicata 109:1-12.

Cooperband MF, Vinson SB, 2000. Host-acceptance requirements of Melittobia digitata (Hymenoptera: Eulophidae), a parasitoid of mud dauber wasps. Biological Control 17:23-28.

Courteau J, Lessard S, 2000. Optimal sex ratios in structured populations. Journal Of Theoretical Biology 207:159-175.

Crawley M, 1993. GLIM for ecologists: Oxford: Blackwell Scientific.

Crawley M, 2002. Statistical computing. Chichester: Wiley (UK).

Crawley M, 2007. The R Book. Chichester: Wiley (UK).

Crespi BJ, Taylor PD, 1990. Dispersal Rates Under Variable Patch Density. American Naturalist 135:48-62.

Crozier RH, 1986. Genetic Clonal Recognition Abilities In Marine-Invertebrates Must Be Maintained By Selection For Something Else. Evolution 40:1100-1101.

Dahms EC, 1984. A review of the biology of species in the genus *Melittobia* (Hymenoptera: Eulophidae) with interpretations and additions using observations on *Melittobia australica*. Mem Queensl Mus 21:337-360.

Darwin C, 1871. The descent of man and selection in relation to sex. London: John Murray.

Davies NB, Halliday TR, 1978. Deep Croaks And Fighting Assessment In Toads Bufo-Bufo. Nature 274:683-685.

Davies NB, Houston AI, 1981. Owners And Satellites - The Economics Of Territory Defense In The Pied Wagtail, Motacilla-Alba. Journal Of Animal Ecology 50:157-180.

Dawkins R, Carlisle TR, 1976. Parental investment, mate desertion and a fallacy. Nature 262:131-133.

Edwards AWF, 1998. Natural selection and the sex ratio: Fisher's sources. American Naturalist 151:564-569.

Edwards AWF, 2000. Carl Dusing (1884) on The Regulation of the Sex-Ratio. Theoretical Population Biology 58:255-257.

Enquist M, Leimar O, 1983. Evolution Of Fighting Behavior - Decision Rules And Assessment Of Relative Strength. Journal Of Theoretical Biology 102:387-410.

Enquist M, Leimar O, 1987. Evolution Of Fighting Behavior - The Effect Of Variation In Resource Value. Journal Of Theoretical Biology 127:187-205.

Enquist M, Leimar O, 1990. The Evolution Of Fatal Fighting. Animal Behaviour 39:1-9.

Fellowes MDE, 1998. Do non-social insects get the (kin) recognition they deserve? Ecological Entomology 23:223-227.

Fisher RA, 1930. The genetical theory of natural selection. Oxford UK: Clarendon.

Frank SA, 1985. Hierarchical Selection Theory And Sex-Ratios.2. On Applying The Theory, And A Test With Fig Wasps. Evolution 39:949-964.

Frank SA, 1986a. Dispersal Polymorphisms In Subdivided Populations. Journal Of Theoretical Biology 122:303-309.

Frank SA, 1986b. The Genetic Value Of Sons And Daughters. Heredity 56:351-354.

Frank SA, 1986c. Hierarchical Selection Theory And Sex-Ratios.1. General-Solutions For Structured Populations. Theoretical Population Biology 29:312-342.

Frank SA, 1998. Foundations of social evolution. Princeton, N.J.: Princeton University Press.

Freeman BE, 1977. Aspects Of Regulation Of Size Of Jamaican Population Of Sceliphron-Assimile Dahlbom (Hymenoptera-Sphecidae). Journal Of Animal Ecology 46:231-247.

Freeman BE, Ittyeipe K, 1976. Field Studies On Cumulative Response Of Melittobia Sp (Hawaiiensis Complex) (Eulophidae) To Varying Host Densities. Journal Of Animal Ecology 45:415-423.

Freeman BE, Ittyeipe K, 1982. Morph Determination In Melittobia, A Eulophid Wasp. Ecological Entomology 7:355-363.

Freeman BE, Ittyeipe K, 1993. The Natural Dynamics Of The Eulophid Parasitoid Melittobia-Australica. Ecological Entomology 18:129-140.

Freeman E, Parnell JR, 1973. Mortality Of Sceliphron-Assimile-Dahlbom (Sphecidae) Caused By Eulophid Melittobia-Chalybii-Ashmead. Journal Of Animal Ecology 42:779-784.

Gammell MP, Hardy ICW, 2003. Contest duration: sizing up the opposition? Trends In Ecology & Evolution 18:491-493.

Gandon S, 1999. Kin competition, the cost of inbreeding and the evolution of dispersal. Journal Of Theoretical Biology 200:345-364.

Gandon S, Michalakis Y, 1999. Evolutionarily stable dispersal rate in a metapopulation with extinctions and kin competition. Journal Of Theoretical Biology 199:275-290.

Gandon S, Rousset F, 1999. Evolution of stepping-stone dispersal rates. Proceedings Of The Royal Society Of London Series B-Biological Sciences 266:2507-2513.

Gardner A, Hardy ICW, Taylor PD, West SA, 2007. Spiteful soldiers and sex ratio conflict in polyembryonic parasitoid wasps. American Naturalist 169:519-533.

Gardner A, West SA, 2004. Spite and the scale of competition. Journal Of Evolutionary Biology 17:1195-1203.

Godfray H, 1994. Parasitoids: behavioural and evolutionary ecology. Princeton (NJ): Princeton University Press.

Gonzalez JM, Abe J, Matthews RW, 2004a. Offspring production and development in the parasitoid wasp Melittobia clavicornis (Cameron) (Hymenoptera: Eulophidae) from Japan. Entomological Science 7:15-19.

Gonzalez JM, Genaro JA, Matthews RW, 2004b. Species of Melittobia (Hymenoptera: Eulophidae) established in Bahamas, Costa Rica, Cuba, Hispaniola, Puerto Rico, and Trinidad. Florida Entomologist 87:619-620.

Gonzalez JM, Matthews RW, 2008. Female and male polymorphism in two species of Melittobia parasitoid wasps (Hymenoptera: Eulophidae). Florida Entomologist 91:162-169.

Gonzalez JM, Teran JB, Matthews RW, 2004c. Review of the biology of Melittobia acasta (Walker) (Hymenoptera: Eulophidae) and additions on development and sex ratio of the species. Caribbean Journal Of Science 40:52-61.

Grafen A, 1987. The Logic Of Divisively Asymmetric Contests - Respect For Ownership And The Desperado Effect. Animal Behaviour 35:462-467.

Greeff JM, Ferguson JWH, 1999. Mating ecology of the nonpollinating fig wasps of Ficus ingens. Animal Behaviour 57:215-222.

Green RF, Gordh G, Hawkins BA, 1982. Precise Sex-Ratios In Highly Inbred Parasitic Wasps. American Naturalist 120:653-665.

Greenwood-Lee JM, Taylor PD, 2001. The evolution of dispersal in spatially varying environments. Evolutionary Ecology Research 3:649-665.

Griffin AS, Pemberton JM, Brotherton PNM, McIlrath G, Gaynor D, Kansky R, O'Riain J, Clutton-Brock TH, 2003. A genetic analysis of breeding success in the cooperative meerkat (Suricata suricatta). Behavioral Ecology 14:472-480.

Griffin AS, Sheldon BC, West SA, 2005. Cooperative breeders adjust offspring sex ratios to produce helpful helpers. American Naturalist 166:628-632.

Griffin AS, West SA, 2002. Kin selection: fact and fiction. Trends In Ecology & Evolution 17:15-21.

Griffin AS, West SA, 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science 302:634-636.

Grillenberger BK, Koevoets T, Burton-Chellew MN, Sykes EM, Shuker DM, De Zande LV, Bijlsma R, Gadau J, Beukeboom LW, 2008. Genetic structure of natural Nasonia vitripennis populations: validating assumptions of sex-ratio theory. Molecular Ecology 17:2854-2864.

Haley MP, Deutsch CJ, Leboeuf BJ, 1994. Size, Dominance And Copulatory Success In Male Northern Elephant Seals, Mirounga-Angustirostris. Animal Behaviour 48:1249-1260.

Hamilton WD, 1964. Genetical Evolution Of Social Behaviour I. Journal Of Theoretical Biology 7:1-&.

Hamilton WD, 1967a. Extraordinary Sex Ratios. Science 156:477-&.

Hamilton WD, 1967b. Natural Selection Of Unusual Sex Ratios. Heredity 22:163-&.

Hamilton WD, 1972. Altruism and related phenomena, mainly in social insects. Annual Review Of Ecology, Evolution, And Systematics 3:193-232.

Hamilton WD, 1979. Wingless and fighting males in fig wasps and other insects. In: Sexual selection and reproductive competition in insects (Blum MS, Blum NA, eds). New York: New York: Academic Press; 167-220.

Hamilton WD, May RM, 1977. Dispersal In Stable Habitats. Nature 269:578-581.

Hardy ICW, 1992. Nonbinomial Sex Allocation And Brood Sex-Ratio Variances In The Parasitoid Hymenoptera. Oikos 65:143-158.

Hardy ICW, Cook JM, 1995. Brood Sex-Ratio Variance, Developmental Mortality And Virginity In A Gregarious Parasitoid Wasp. Oecologia 103:162-169.

Hardy ICW, Field SA, 1998. Logistic analysis of animal contests. Animal Behaviour 56:787-792.

Hartley CS, Matthews RW, 2003. The effect of body size on male-male combat in the parasitoid wasp *Melittobia digitata* (Hymenoptera: Eulophidae). Journal of Hymenoptera Research 12:272-77.

Harvey PH, Partridge L, Nunney L, 1985. Evolutionary Biology - Group Selection And The Sex-Ratio. Nature 313:10-11.

Herre EA, 1985. Sex-Ratio Adjustment In Fig Wasps. Science 228:896-898.

Herre EA, 1987. Optimality, Plasticity And Selective Regime In Fig Wasp Sex-Ratios. Nature 329:627-629.

Herre EA, Machado CA, West SA, 2001. Selective regime and fig wasp sex ratios: towards sorting rigor from pseudo-rigor in tests of adaptation. In: Adaptationism and optimality (Orzack S, Sober E, eds). Cambridge: Cambridge University Press; 191-218.

Herre EA, West SA, Cook JM, Compton SG, Kjellberg F, 1997. Fig wasp mating systems: pollinators and parasites, sex ratio adjustment and male

polymorphism, population structure and its consequences. In: The evolution of maing systems in insects and arachnids (Choe J, Crespi BJ, eds). Princeton (NJ): Princeton University Press; 226-239.

Innocent TM, Savage J, West SA, Reece SE, 2007. Lethal combat and sex ratio evolution in a parasitoid wasp. Behavioral Ecology 18:709-715.

Innocent TM, West SA, 2006. Social evolution: Cooperation by conflict. Current Biology 16:R365-R367.

Irwin AJ, Taylor PD, 2000. Evolution of dispersal in a stepping-stone population with overlapping generations. Theoretical Population Biology 58:321-328.

Jakobsson S, Radesater T, Jarvi T, 1979. Fighting Behavior Of Nannacara-Anomala (Pisces, Cichlidae) Males. Zeitschrift Fur Tierpsychologie-Journal Of Comparative Ethology 49:210-220.

Jervis MA, Ferns PN, 2004. The timing of egg maturation in insects: ovigeny index and initial egg load as measures of fitness and of resource allocation. Oikos 107:449-460.

Jervis MA, Ferns PN, Heimpel GE, 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. Functional Ecology 17:375-383.

Jervis MA, Heimpel GE, Ferns PN, Harvey JA, Kidd NAC, 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. Journal Of Animal Ecology 70:442-458.

Kinoshita M, Kasuya E, Yahara T, 2002. Effects of time-dependent competition for oviposition sites on clutch sizes and offspring sex ratios in a fig wasp. Oikos 96:31-35.

Komdeur J, Daan S, Tinbergen J, Mateman C, 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. Nature 385:522-525.

Leimar O, Enquist M, 1984. Effects Of Asymmetries In Owner Intruder Conflicts. Journal Of Theoretical Biology 111:475-491.

Leturque H, Rousset F, 2002. Dispersal, kin competition, and the ideal free distribution in a spatially heterogeneous population. Theoretical Population Biology 62:169-180.

Leturque H, Rousset F, 2003. Joint evolution of sex ratio and dispersal: conditions for higher dispersal rates from good habitats. Evolutionary Ecology 17:67-84.

Leturque H, Rousset F, 2004. Intersexual competition as an explanation for sex-ratio and dispersal biases in polygynous species. Evolution 58:2398-2408.

Lidgard DC, Boness DJ, Bowen WD, McMillan JI, 2005. State-dependent male mating tactics in the grey seal: the importance of body size. Behavioral Ecology 16:541-549.

Lopez-Vaamonde C, Godfray HCJ, West SA, Hansson C, Cook JM, 2005. The evolution of host use and unusual reproductive strategies in Achrysocharoides parasitoid wasps. Journal Of Evolutionary Biology 18:1029-1041.

Matthews RW, Gonzalez JM, Matthews JR, Deyrup LD, 2009. Biology of the parasitoid *Melittobia* (Hymenoptera: Eulophidae). Annual Review Of Entomology 54:251-266.

Maynard-Smith J, 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press.

Maynard-Smith J, Price GR, 1973. Logic Of Animal Conflict. Nature 246:15-18.

McElligott AG, Gammell MP, Harty HC, Paini DR, Murphy DT, Walsh JT, Hayden TJ, 2001. Sexual size dimorphism in fallow deer (Dama dama): do larger, heavier males gain greater mating success? Behavioral Ecology And Sociobiology 49:266-272.

Molbo D, Machado CA, Sevenster JG, Keller L, Herre EA, 2003. Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. Proceedings Of The National Academy Of Sciences Of The United States Of America 100:5867-5872.

Molumby A, 1996. The evolutionary ecology of a gregariously nesting wasp, *Trypoxylon politum*. Chicago (IL): University of Chicago.

Moore JC, Compton SG, Hatcher MJ, Dunn AM, 2002. Quantitative tests of sex ratio models in a pollinating fig wasp. Animal Behaviour 64:23-32.

Moore JC, Obbard DJ, Reuter C, West SA, Cook JM, 2008. Fighting strategies in two species of fig wasp. Animal Behaviour 76:315-322.

Morgan DJW, Cook JM, 1994. Extremely Precise Sex-Ratios In Small Clutches Of A Bethylid Wasp. Oikos 71:423-430.

Morrell LJ, Backwell PRY, Metcalfe NB, 2005. Fighting in fiddler crabs Uca mjoebergi: what determines duration? Animal Behaviour 70:653-662.

Motro U, 1982a. Optimal Rates Of Dispersal.1. Haploid Populations. Theoretical Population Biology 21:394-411.

Motro U, 1982b. Optimal Rates Of Dispersal.2. Diploid Populations. Theoretical Population Biology 21:412-429.

Motro U, 1991. Avoiding Inbreeding And Sibling Competition - The Evolution Of Sexual Dimorphism For Dispersal. American Naturalist 137:108-115.

Murray MG, 1985. Figs (Ficus Spp) And Fig Wasps (Chalcidoidea, Agaonidae) - Hypotheses For An Ancient Symbiosis. Biological Journal Of The Linnean Society 26:69-81.

Murray MG, 1987. The Closed Environment Of The Fig Receptacle And Its Influence On Male Conflict In The Old-World Fig Wasp, Philotrypesis-Pilosa. Animal Behaviour 35:488-506.

Murray MG, 1989. Environmental Constraints On Fighting In Flightless Male Fig Wasps. Animal Behaviour 38:186-193.

Murray MG, 1990. Comparative Morphology And Mate Competition Of Flightless Male Fig Wasps. Animal Behaviour 39:434-443.

Murray MG, Gerrard R, 1984. Conflict In The Neighborhood - Models Where Close Relatives Are In Direct Competition. Journal Of Theoretical Biology 111:237-246.

Murray MG, Gerrard R, 1985. Putting The Challenge Into Resource Exploitation - A Model Of Contest Competition. Journal Of Theoretical Biology 115:367-389.

Nespolo RF, Roff DA, Fairbairn DJ, 2008. Energetic trade-off between maintenance costs and flight capacity in the sand cricket (Gryllus firmus). Functional Ecology 22:624-631.

Perrin N, Mazalov V, 2000. Local competition, inbreeding, and the evolution of sex-biased dispersal. American Naturalist 155:116-127.

Petersen G, Hardy ICW, 1996. The importance of being larger: Parasitoid intruder-owner contests and their implications for clutch size. Animal Behaviour 51:1363-1373.

Pickering J, Read AF, Guerrero S, West SA, 2000. Sex ratio and virulence in two species of lizard malaria parasites. Evolutionary Ecology Research 2:171-184.

Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K, 2003. Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. Proceedings Of The Royal Society Of London Series B-Biological Sciences 270:633-640.

Quinn GP, Keough MJ, 2002. Experimental Design and Data Analysis for Biologists. Cambridge: Cambridge University Press.

Reece SE, Innocent TM, West SA, 2007. Lethal male-male combat in the parasitoid Melittobia acasta: are size and competitive environment important? Animal Behaviour 74:1163-1169.

Reece SE, Shuker DM, Pen I, Duncan AB, Choudhary A, Batchelor CM, West SA, 2004. Kin discrimination and sex ratios in a parasitoid wasp. Journal Of Evolutionary Biology 17:208-216.

Reinhold K, 2003. Influence of male relatedness on lethal combat in fig wasps: a theoretical analysis. Proceedings Of The Royal Society Of London Series B-Biological Sciences 270:1171-1175.

Rivero A, West SA, 2002. The physiological costs of being small in a parasitic wasp. Evolutionary Ecology Research 4:407-420.

Roff DA, 1984. The Cost Of Being Able To Fly - A Study Of Wing Polymorphism In 2 Species Of Crickets. Oecologia 63:30-37.

Roff DA, 1986. The Evolution Of Wing Dimorphism In Insects. Evolution 40:1009-1020.

Roff DA, Crnokrak P, Fairbairn DJ, 2003. The evolution of trade-offs: geographic variation in call duration and flight ability in the sand cricket, Gryllus firmus. Journal Of Evolutionary Biology 16:744-753.

Roff DA, Fairbairn DJ, 1991. Wing Dimorphisms And The Evolution Of Migratory Polymorphisms Among The Insecta. American Zoologist 31:243-251.

Roff DA, Fairbairn DJ, 2007. Laboratory evolution of the migratory polymorphism in the sand cricket: Combining physiology with quantitative genetics. Physiological And Biochemical Zoology 80:358-369.

Roff DA, Gelinas MB, 2003. Phenotypic plasticity and the evolution of trade-offs: the quantitative genetics of resource allocation in the wing dimorphic cricket, Gryllus firmus. Journal Of Evolutionary Biology 16:55-63.

Ronce O, 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annual Review Of Ecology Evolution And Systematics 38:231-253.

Ronce O, Gandon S, Rousset F, 2000. Kin selection and natal dispersal in an age-structured population. Theoretical Population Biology 58:143-159.

Rousset F, Billiard S, 2000. A theoretical basis for measures of kin selection in subdivided populations: finite populations and localized dispersal. Journal Of Evolutionary Biology 13:814-825.

Rousset F, Gandon S, 2002. Evolution of the distribution of dispersal distance under distance-dependent cost of dispersal. Journal Of Evolutionary Biology 15:515-523.

Rousset F, Roze D, 2007. Constraints on the origin and maintenance of genetic kin recognition. Evolution 61:2320-2330.

Roze D, Rousset F, 2005. Inbreeding depression and the evolution of dispersal rates: A multilocus model. American Naturalist 166:708-721.

Schmieder RG, 1933. The polymorphic forms of Melittobia chalybii Ashmead and the determining factors involved in their production (Hymenoptera chalcidoidea, Eulophidae). Biological Bulletin 65:338-354.

Schmieder RG, 1938. The sex ratio in Melittobia chalybii ashmead, gametogenesis and cleavage in females and in haploid males (hymenoptera chalcidoidea). Biological Bulletin 74:256-266.

Shuker DM, Pen I, Duncan AB, Reece SE, West SA, 2005. Sex ratios under asymmetrical local mate competition: Theory and a test with parasitoid wasps. American Naturalist 166:301-316.

Shuker DM, Reece SE, Taylor JAL, West SA, 2004a. Wasp sex ratios when females on a patch are related. Animal Behaviour 68:331-336.

Shuker DM, Reece SE, Whitehorn PR, West SA, 2004b. Sib-mating does not lead to facultative sex ratio adjustment in the parasitoid wasp, Nasonia vitripennis. Evolutionary Ecology Research 6:473-480.

Socha R, Zemek R, 2003. Wing morph-related differences in the walking pattern and dispersal in a flightless bug, Pyrrhocoris apterus (Heteroptera). Oikos 100:35-42.

Taylor PD, 1981. Intra-Sex And Inter-Sex Sibling Interactions As Sex-Ratio Determinants. Nature 291:64-66.

Taylor PD, 1988. An Inclusive Fitness Model For Dispersal Of Offspring. Journal Of Theoretical Biology 130:363-378.

Taylor PD, 1994. Sex-Ratio In A Stepping-Stone Population With Sex-Specific Dispersal. Theoretical Population Biology 45:203-218.

Taylor PD, Crespi BJ, 1994. Evolutionarily Stable Strategy Sex-Ratios When Correlates Of Relatedness Can Be Assessed. American Naturalist 143:297-316.

Taylor PD, Frank SA, 1996. How to make a kin selection model. Journal Of Theoretical Biology 180:27-37.

Taylor PW, Elwood RW, 2003. The mismeasure of animal contests. Animal Behaviour 65:1195-1202.

Taylor PW, Hasson O, Clark DL, 2001. Initiation and resolution of jumping spider contests: roles for size, proximity, and early detection of rivals. Behavioral Ecology And Sociobiology 50:403-413.

Taylor PW, Jackson RR, 2003. Interacting effects of size and prior injury in jumping spider conflicts. Animal Behaviour 65:787-794.

Trivers RL, Willard DE, 1973. Natural-Selection Of Parental Ability To Vary Sex-Ratio Of Offspring. Science 179:90-92.

Van den Assem J, Denbosch H, Prooy E, 1982. Melittobia Courtship Behavior - A Comparative-Study Of The Evolution Of A Display. Netherlands Journal Of Zoology 32:427-&.

Van den Assem J, Gijswijt MJ, Nubel BK, 1980. Observation of courtship and mating strategies in a few species of parasitic wasps (Chalcidoidea). Netherlands Journal Of Zoology 30:208-221.

Werren JH, 1980. Sex-Ratio Adaptations To Local Mate Competition In A Parasitic Wasp. Science 208:1157-1159.

Werren JH, 1983. Sex-Ratio Evolution Under Local Mate Competition In A Parasitic Wasp. Evolution 37:116-124.

West SA, 2009. Sex Allocation. Princeton, N.J.: Princeton University Press.

West SA, Griffin AS, Gardner A, 2007a. Evolutionary explanations for cooperation. Current Biology 17:R661-R672.

West SA, Griffin AS, Gardner A, 2007b. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. Journal Of Evolutionary Biology 20:415-432.

West SA, Herre EA, 1998a. Partial local mate competition and the sex ratio: A study on non-pollinating fig wasps. Journal Of Evolutionary Biology 11:531-548.

West SA, Herre EA, 1998b. Stabilizing selection and variance in fig wasp sex ratios. Evolution 52:475-485.

West SA, Herre EA, Sheldon BC, 2000. The benefits of allocating sex. Science 290:288-290.

West SA, Murray MG, Machado CA, Griffin AS, Herre EA, 2001. Testing Hamilton's rule with competition between relatives. Nature 409:510-513.

West SA, Pen I, Griffin AS, 2002. Conflict and cooperation - Cooperation and competition between relatives. Science 296:72-75.

West SA, Shuker DM, Sheldon BC, 2005. Sex-ratio adjustment when relatives interact: A test of constraints on adaptation. Evolution 59:1211-1228.

Whitehouse MEA, 1997. Experience influences male-male contests in the spider Argyrodes antipodiana (Theridiidae:Araneae). Animal Behaviour 53:913-923.

Wild G, Greenwood-Lee J, Taylor PD, 2006. Sex allocation and dispersal in a heterogeneous two-patch environment. Theoretical Population Biology 70:225-235.

Wild G, Taylor PD, 2004. Kin selection models for the co-evolution of the sex ratio and sex-specific dispersal. Evolutionary Ecology Research 6:481-502.

Zera AJ, Denno RF, 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review Of Entomology 42:207-230.

Appendices