CHAPTER ONE

1. Introduction

1.1 WHY STUDY THE MATING AND REPRODUCTIVE DECISIONS OF A PARASITOID WASP

Mating behaviours and reproductive decisions are of key importance to evolutionary fitness. However, it is often difficult to measure and compare the fitness of different individuals, especially in long-lived species, and for behaviours that are subject to multiple selective forces. A striking exception though is the field of sex-allocation research, which is a triumphant success of evolutionary theory (Charnov 1982). The reason for this success is that, following Fisher (1958), the fitness consequences of different sex ratios can be readily predicted and tested analytically. These predictions are very amenable to empirical testing and provide a platform to measure the precision of adaptation, which is crucial for understanding the nature of evolutionary change.

A valuable model organism in sex-allocation research has been the jewel wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae), a gregarious ectoparasitoid of large Diptera pupae (such as Calliphoridae and Sarcophagidae; Whiting 1967; examples of use in sex ratio research; Werren 1980; 1983; 1984; Parker & Orzack

1985; Orzack 1986; 1990; 1994; Orzack & Parker 1986; 1990; 1994; King & Skinner 1991b; 1991a; Orzack et al. 1991; King 1992; 1993; Orzack & Gladstone 1994; Hardy 1994; Molbo & Parker 1996; Flanagan et al. 1998; Reece et al. 2004; Shuker & West 2004; Shuker et al. 2004a; 2004b; 2005; 2006b; 2006c; 2007b). This small wasp's utility stems from its ease of laboratory culture and because it is a Hymenopteran parasitoid. Hymenopteran parasitoids offer a great opportunity to test facultative sex-ratio responses to environmental conditions because they are haplodiploid: sons develop from unfertilized eggs, and daughters from fertilized eggs (Cook 1993). Haplodiploidy, in principle, allows females to precisely determine the sex of each egg and thus the sex ratio of their broods, which are often heavily female biased (although the sex ratio may be a result of competing interests between a female and her mate; Shuker et al. 2006c). These biases can by explained, by Hamilton's (1967) theory of local mate competition, as a result of structured populations and an asymmetry in dispersal between the sexes (Hamilton 1979; Suzuki & Iwasa 1980; Werren 1980; 1983; Yamaguchi 1985; Frank 1985a; Herre 1985; Nunney & Luck 1988; Stubblefield & Seger 1990; Taylor & Crespi 1994; Frank 1998; Reece et al. 2004; Shuker et al. 2005). Conceptually, one can think of female biased broods reducing competition between sons whilst also providing more mates for them (in species where females mate primarily before dispersing), or as a way for females to maximize their number of grandchildren (Taylor 1981; Charnov 1982). An additional female bias is favoured in haplodiploids because inbreeding leads to mothers being relatively more related to their daughters than their sons (Frank 1985a; Herre 1985). The local mate competition model is the flag-ship of sex-ratio theory and has stimulated more than a generation of empirical studies and

spawned many theoretical developments (West et al. 2005). These theoretical developments do need to still be verified with empirical results, and not just with laboratory experiments but also with accurate descriptions of wild-type behaviour. Although *N. vitripennis* is a model organism for sex-ratio research, it has been little studied in the wild (Werren 1983; Molbo & Parker 1996), and several questions still arise. How informative are the results of controlled laboratory experiments in describing the sex ratio behaviour of wild *N. vitripennis*? Have laboratory experiments accurately captured the decisions wild females have to make? What cues do females respond to in the wild, have laboratory experiments provided the appropriate information and stimuli for the females to make these decisions? Essentially, are the assumptions the models make valid? For example, the size of male offspring is often considered of only marginal significance, is this truly the case?

In contrast to sex-allocation theory, sexual selection and sexual conflict are two areas still fuelled by a lack of consensus (Grafen 1990a; Basolo 1990; Kirkpatrick & Ryan 1991; Maynard-Smith 1991; Andersson 1994; Pomiankowski & Iwasa 1998; Kokko et al. 2002; 2003; 2006; Zeh & Zeh 2003; Tomkins et al. 2004; Arnqvist 2006; Lehmann et al. 2007; and for sexual conflict in particular; Holland & Rice 1998; Rice & Holland 1999; Gavrilets et al. 2001; Cameron et al. 2003; Chapman et al. 2003b; 2003a; Cordero & Eberhard 2003; Arnqvist & Rowe 2005; Rowe et al. 2005; Rowe & Day 2006). *Nasonia vitripennis* is an interesting study organism for mating behaviour because in contrast to the growing awareness of female insects being polyandrous, the females of *N. vitripennis* are still believed to be monoandrous (mate

only one male)**.** Female mating patterns are of central importance to understanding sexual selection and conflict, and largely shape local mate competition. However, despite intensive laboratory study, little is known about the wild mating behaviour of *N. vitripennis*. Are the females really monoandrous in the wild, and if so, why? Is their mating behaviour an adaptation to their ecology, or the outcome from a conflict between the sexes? If it is an ecological adaptation, will it change in response to the radically different ecology of laboratory culture? What role do males play?

1.2 SOME KNOWN BIOLOGY OF *NASONIA VITRIPENNIS*

1.2.1 General definition

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae) is a 2 to 3-mm-long, gregarious parasitoid wasp of dipteran pupae, with females laying clutches of eggs on a range of large Diptera pupae (such as Calliphoridae and Sarcophagidae; Whiting 1967). The species is ectoparasitic, with the eggs laid between the pupa and puparium wall, with adults emerging from the host puparium to mate. Like all Hymenopterans, *Nasonia* is haplodiploid, with females developing from fertilized (diploid) eggs, and males from unfertilized (haploid) eggs. This means that even virgin females can parasitise hosts and lay (an all male) brood.

The polygynous males are brachypterous (short-winged) and unable to fly, remaining at the site of adult emergence to compete with each other for access to emerging females. Typically males compete to guard exit holes in the hosts, whereby they

secure copulations with the virgin females as they exit the host (Van den Assem et al. 1980a). The extent of male dispersal is unknown but is arguably minimal because they are unable to fly. In contrast females are fully winged and presumably disperse away from the host after mating. Such a mating system typifies that assumed by LMC, and *N. vitripennis* has long been an outstanding model organism for the study of sex ratios. The sex ratio (proportion male) is often very low and this is probably in response to local mate competition (Hamilton 1967; Werren 1980; Orzack & Parker 1990; Molbo & Parker 1996; Flanagan et al. 1998; Reece et al. 2004; Shuker & West 2004; Shuker et al. 2004b; 2004a; 2005; 2006b; Grillenberger et al. 2008; Chapter 5).

Nasonia females are presumed, like most species of parasitoid wasps, to have low mating rates, with females typically only mating once before dispersal (Gordh & Debach 1978, found that 85% of species screened were monoandrous, N = 34; and Ridley 1993, found that 80% of species screened were monoandrous, N = 99). Laboratory studies have shown that virgin females are very willing to mate and appear to show little or no discrepancy over potential mates, however mated females are much, much, less willing to mate (Whiting 1967; Van den Assem & Jachmann 1999). The monoandry (single-mating) of wild females, along with the readiness of virgins to mate, can perhaps be explained by their ecology: in the wild, finding suitable hosts to parasitise is arguably the limiting constraint on female reproductive success, so any benefits of mating multiply or searching for 'better' mates would have to be quite substantial unless mates can be found in quick succession. However the sex ratio can be heavily female biased and thus males are rare, especially unrelated ones (Werren 1980; Molbo & Parker 1996). The best strategy for females

then will be to ensure they are mated as soon as possible and then to disperse in search of hosts to parasitise. However the results of an anecdotal study suggested that females of one *N. vitripennis* strain may have evolved an increased willingness to re-mate under the conditions of laboratory culture (Van den Assem & Jachmann 1999).

1.2.2 *Nasonia* Courtship and Mating

Nasonia vitripennis is sympatric with two congeners that are endemic to North America: *N. longicornis* in the west and *N. giraulti* in the east. Partial prezygotic reproductive isolation occurs in all three species as a result of behavioural differences in courtship (Van den Assem & Werren 1994; Bordenstein et al. 2000; Beukeboom & Van den Assem 2001; 2002) and mating system (Drapeau & Werren 1999; Leonard & Boake 2006).

Nasonia vitripennis males have a stereotyped courtship consisting of mounting the female in response to volatile compounds that signify a female's presence and performing multiple series of four to seven head-nods, with each series separated by an interval of 5-10 seconds (Van den Assem et al. 1980b; Beukeboom & Van den Assem 2001). Although courtship duration and the number of head-nod series varies: mean number of head nod series until males give up; 8.16 ± 0.23 (Beukeboom & Van den Assem 2001). During courtship, the male releases mandibular pheromones during the first head-nod of each series. The production and release of these pheromones is necessary to make the female receptive and is potentially costly to the male (Van den Assem et al. 1980b; for an example of costly pheromone production

see Johansson et al. 2005). Courtship is almost certain to induce receptivity in a virgin female, which she signals with the stereotyped lowering of her head and a retraction of her antennae towards her head, before the male backs up and establishes genital contact (Van den Assem et al. 1980b; Van den Assem & Jachmann 1999; Bordenstein et al. 2000).

Copulations are short, with a mean of approximately 14 seconds, and males are unable to force unreceptive females into copulating (Burton-Chellew et al. 2007a; Chaper 2). After copulating the male performs a stereotyped post-copulatory courtship performance that reduces future female receptivity but may have additional effects. When males are prevented from performing the post copulatory courtship, the female is more likely to mate with a subsequent courting male (Van den Assem & Jachmann 1999). Females appear to do less to facilitate courtship by changing their behaviour once mated (King et al. 2000). A singly mated female will normally receive sufficient sperm to fertilise several hundred eggs, but note that males that have recently mated with 50 or more females do produce smaller ejaculates (or fail to inseminate successfully: Barrass 1961). Larger males may also be able to transfer more sperm, in which case females that mate smaller males may become sperm limited sooner, with obvious consequences for their future sex allocation. Although doubly mated females are rare, they may occur at a frequency of approximately five percent (Burton-Chellew et al. 2007a; Grillenberger et al. 2008). According to Holmes (1974), about fifty percent (N = 39/79) of double mated females will produce mixed broods consisting of eggs inseminated by sperm from both males.

1.2.3 *Nasonia vitripennis* Sex Ratios and Offspring Size

As mentioned above it is known from both laboratory and field studies that the females adjust their sex ratios in response to the basic tenets of LMC (Werren 1983; Orzack et al. 1991; Molbo & Parker 1996; Shuker & West 2004; Grillenberger et al. 2008). *N. vitripennis* has also been extremely useful in testing the more complex LMC models, but so far these studies have been restricted to the laboratory (Werren 1980; Orzack 1986; Orzack & Parker 1990; Flanagan et al. 1998; Reece et al. 2004; Shuker & West 2004; Shuker et al. 2004a; 2006b; 2007b). There is therefore a need for empirical studies from the field. For example, although the mating system of *N. vitripennis* appears to conform to the assumptions of LMC models, one potential exception is that mating will often not be random within the whole patch, as assumed by most LMC models (Shuker et al. 2005; 2006b; 2007b). Laboratory experiments have shown that even when wasps emerge at very similar times, from hosts that are next to each other, they are more likely to mate with individuals that developed in their own host (Van den Assem et al. 1980a; 1980b; Shuker et al. 2005). It would be useful to know what the natural range of emergence times is for wild *N*asonia.

Nasonia vitripennis females will super-parasitise previously parasitized hosts, but only within a certain time frame, and females appear to be able to assess with relative ease whether a host has been previously parasitized (Werren 1984; King et al. 1995; Shuker et al. 2005; 2006b). The later laid offspring tend to develop faster so that the adult emergence of two different broods can be near synchronous (Werren 1983; Shuker et al. 2005). The benefit to the second female in this case is that her offspring get to mate with those of the first female. As the first female will probably have laid

a very female biased clutch, this allows the second female to, in a sense, parasitise this first clutch by laying just a few males to inseminate all the other females. However the cues females use to gather information on the extent of LMC, and how they interact with each other, are somewhat unknown but are at least being deciphered with the help of laboratory experiments. For example, a recent laboratory experiment showed that females lay less female biased sex ratios when co-foundress females are present, but that the primary cue is the eggs laid by those other females, and not the presence of the females themselves (Shuker & West 2004). Females are also less likely to oviposit on, and lay fewer numbers of eggs on, parasitized hosts that have had a greater numbers of eggs previously laid on them (Shuker et al. 2005). Whilst these results are intriguing, they still leave unresolved the issue of which cues are used in the wild and which are the most reliable or salient to the females. It should be noted that although there is in general a highly female biased sex ratio, there are also situations in nature when the sex ratio may be less female biased, and competition between males will be important (Werren 1983; Molbo & Parker 1996; Chapter 5). What determines male success under such competition has implications for LMC models, because the sex-allocation decisions of a mother may affect the competitive ability of her sons.

Plus, although females can presumably allocate sex adaptively, they can also invest in their offspring via egg size, and egg size correlates with adult size (Lalonde 2005). Size is probably important for adult females because it correlates with greater egg formation and maturation (Rivero & West 2002). More recent results suggest that female size may not be directly important, but appears to be because it correlates

with the degree of larval competition experienced, which has a strong bearing on adult fitness (Sykes et al. 2007). The effects of such larval competition may be asymmetric though, with females suffering greater costs, and individuals of both sexes suffering greater costs when sharing with more female biased broods (Sykes et al. 2007). Smaller females also have reduced longevity compared to larger females, but only when food is unavailable (Rivero & West 2002). The affect of size on males is unknown and the extent to which male *N. vitripennis* feed in the wild is unknown.

1.2.4 Culturing *Nasonia vitripennis*

I maintained wasp strains in mass culture, generally at 25°C, under 16 : 8 h, light : dark conditions. Under this regime, males start to emerge after 13 to 14 days and mate with females, which emerge soon after. I reared all wasps on *Calliphora vomitoria* hosts and maintained stock cultures in either two or four replicate transparent glass vials of 75 x 25 mm proportions. Typically, on the fourth day following adult emergence, I transferred approximately 40 females from just one of the culture vials to each of two or four new replicate vials of identical proportions and incubated them with around 40 fresh hosts (less than one month old at 4°C). I therefore avoided any inbreeding effects associated with small population size as population densities before re-culturing were typically in excess of 500 individuals. For my experiments I used approximately 20 parasitized *Calliphora* pupae (hosts) from each stock vial to collect virgin *N. vitripennisa* from each strain. I isolated individuals as pupae from the host puparia just prior to eclosion to ensure male and female virginity.

1.3 THESIS OUTLINE

In this thesis I investigate the reproductive and mating behaviour of *N. vitripennis*. In chapters 2, 3, and 4, I investigate mating behaviour by i) examining if female re-mating propensity has adapted in response to laboratory conditions (Chapter 2; Burton-Chellew et al. 2007a); ii) measuring the costs and benefits of mating in females (chap. 3); and iii) analyzing both the size-fitness relationship and the cost of mating in males (Chapter 4; Burton-Chellew et al. 2007b). Finally I describe a microsatelite analysis of a natural population, designed to examine the mating behaviour and reproductive decisions of females in the wild (Chapter 5; Burton-Chellew et al. 2008).

In the appendix I include other papers on which I worked, but where I was not first author. These include: (Appendix A) an investigation of the heritability of the re-mating propensity in *N. vitripennis* (Shuker et al. 2007a: I constructed the outbred experimental strain and advised on the experimental methodology); (Appendix B) a molecular analysis of the population structure of natural populations of *N. vitripennis* (Grillenberger et al. 2008; I genotyped the majority of the samples and reconstructed the foundress genotypes and that of their mate(s), thus measuring the degree of polyandry in the wild); (Appendix C) an experimental test of whether humans adjust their level of cooperation in response to population structure (West et al. 2006; I assisted with the experiment). In the remainder of this introductory chapter (Chapter 1), I provide a review of the puzzle of polyandry. I explain its importance to our

understanding of sexual conflict and sexual selection by exploring the theoretical history and empirical developments of the field. More specific reviews for the topics considered in each chapter, are provided in each chapter.

1.4 OPTIMAL MATING RATES AND THE EVOLUTION OF POLYANDRY: A REVIEW

1.4.1 The Role of Females in Mating

1.4.1.1 *Female choice as a cause of evolutionary change*

The examination of the female role in the evolution of mating behaviour extends back to its theoretical conception in Darwin's theory on Sexual Selection (1859). Darwin's elaboration of his theory (1871), purported that competition for mates was an additional force of selection that could favour traits even when they were detrimental to survival, providing that they increased mating success. The competition for mates was typically observed to be stronger in males and was the cause of sexual dimorphism. The competition could be direct, requiring costly armaments such as antlers and horns, or could be indirect and mediated by female choice. Female choice was therefore responsible for driving the evolution of conspicuous, and thus costly, male displays. Paradoxically, Darwin believed that sexual selection "*led to the improvement of the…species*", despite it favouring traits

that were not favoured by natural selection (quote taken from Arnqvist & Rowe 2005).

Darwin should be credited for acknowledging the role of females in mating, but even though he realised the potency of female choice, he did not articulate why females are often choosey, or a mechanism for the evolution of female preference. Indeed, it would be over a century before biologists asked what mating strategies females should employ (Ahnesjo et al. 1993). Victorian scientists, perhaps reflecting their society, readily accepted the idea of male-male competition fuelling the evolution of costly armaments, but readily rejected the idea of females dictating the outcome of mate competition, especially on the basis of an aesthetic sense (Andersson 1994). Despite some theoretical developments by Fisher (1915; 1930), that provided the first theoretical underpinning of the evolution of female preference (indicator models 1915; runaway selection 1930), female choice was largely ignored as unimportant, or only important in maintaining species integrity, up until the growth of behavioural ecology in the 1960's (see references in Andersson 1994; Arnqvist & Rowe 2005). Female choice is now a widely studied phenomenon and well supported as an agent of evolutionary change (see table 6.A in Andersson 1994), although the mechanisms involved are still hotly debated (Grafen 1990a; Kirkpatrick & Ryan 1991; Kirkpatrick & Barton 1997; Kokko et al. 2002; 2003; 2006; 2007; Cordero & Eberhard 2003; 2005; Lehmann et al. 2007).

1.4.1.2 *Why are females the choosier sex?*

Although Darwin (1871; 1859) highlighted the importance of female choice, and Fisher (1930; 1915) postulated how it's evolutionary origin and maintenance could be achieved, no one really explained why females are more choosey than males. In other words, no one showed why the competition for mates is greatest in males, until Bateman's experiments with fruit flies (1948) showed that the reproductive potential of females is more limited than males (because females invest relatively more per gamete). This was empirical evidence, albeit in one species (although for examples in other species see Jones et al. 2002; 2003), that males are favoured to seek multiple partners whereas females are favoured to be relatively chaste, or even monoandrous (mate only one male). Bateman's results were from a species with no parental care, and therefore the differences in the sexes derive purely from differential investment in gametes. Trivers (1972) then showed that the sex which invests more per offspring, be it via larger gametes, or parental care, is selected to be more careful with it's investment. Trivers reasoning was that the sex which invests more has more at stake, is taking more of a risk, and will suffer more from poor decisions. The sex with the more limited reproductive potential is under greater pressure to get each reproductive event 'correct'. In this case gender is irrelevant, for if males invest more in the offspring, then they should be more discriminating in how they allocate their resources. If males provide resources that help with raising offspring or improve female fecundity, then females may well end up competing for those male derived resources, in a form of sex role reversal (Clutton-Brock 1991; Gwynne 1991; Vincent et al. 1994; Andersson 1994; Eens & Pinxten 2000; Forsgren et al. 2004). Females tend to invest relatively more for various reasons, primarily because they,

by definition, have larger gametes, and because males are favoured to invest relatively less in parenting when their paternity is uncertain (Queller 1997). Mate choice can also be seen as a luxury that is only available to the sex that is in limited supply under the operational sex ratio (OSR; see below, Section 1.4.2.1). If one sex is in greater demand then it can afford to be selective without losing mating opportunities, a luxury unavailable to the opposite sex (Kvarnemo & Ahnesjo 2002; but see Johnstone et al. 1996; Kokko & Monaghan 2001). The number of available females is often limited because they have to take a 'time out' from mating during times of egg production, gestation, and parental care, and therefore there are relatively fewer females 'qualified to mate' over time (Ahnesjo et al. 2001; Kvarnemo & Ahnesjo 2002).

1.4.2 Optimal Mating Rates

The greater investment by one sex, and the corresponding limited reproductive potential, not only favours choosiness (but see Johnstone et al. 1996), but also a lower mating rate in general. This is because optimal mating rates are a trade off between the costs and benefits of mating, and this trade off typically differs for the sexes and especially so as the number of interactions increases. As first shown by Bateman (1948), male fitness increases linearly with access to mates whereas female fitness in contrast rapidly asymptotes with increased mating opportunities. This is because female reproduction is more generally limited by factors such as egg load and resources required for parental care (Trivers 1972; Parker 1979; Clutton-Brock & Parker 1992). Theoretically, females can obtain all the resources that mating

offers, such as sperm, from relatively few matings, and therefore additional matings offer females diminishing returns, whilst the ecological costs of each new mating (e.g. the loss of time and increased risk of predation and infection), remain relatively constant. This leads to a sexual conflict over the optimal mating rate, with selection favouring a lower mating rate for females than males, and the prediction that the variance in mating success will be larger for males (Arnqvist & Rowe 2005).

Closer examination of female mating behaviour in the wild revealed a different picture; females were actively soliciting additional copulations (Davies 1992; Griffith et al. 2002). When these observations were combined with the advent of paternity testing, it became clear that females of many phyla and species mate with many more partners than expected or required to fertilize a full complement of eggs (Ridley 1993; Birkhead & Moller 1995; Griffith et al. 2002). This has resulted in a greater examination of female mating tactics and a reappraisal of the female role in the evolution of mating behaviour.

1.4.2.1 *Optimal mating rates, mating systems, and operational sex ratios*

The operational sex ratio (OSR) is the average ratio of fertilizable females to sexually active males at any given time (Emlen 1976; Emlen & Oring 1977; Kvarnemo & Ahnesjo 1996; although see Clutton-Brock & Parker 1992; Parker & Simmons 1996; Ahnesjo et al. 2001) and is a main determinant of the opportunity for sexual selection (Andersson 1994; Kvarnemo & Ahnesjo 2002). If females have low mating rates then this will affect the OSR, reducing the number of females available

at any one time. The OSR predicts the degree of competition for mates and therefore correlates well with the strength of sexual selection, (Kvarnemo & Ahnesjo 2002) which feeds back into the evolution of the mating system, which can affect the primary sex ratio, which partially determines the OSR (see Figure 7.2.2 in Andersson 1994). Therefore female mating rates are both a result of, and a cause of, mating systems, the primary sex ratio and the OSR. For example, in *Nasonia vitripennis*, the females typically only mate before dispersal, which causes local mate competition (LMC; Hamilton 1967). Therefore the competition for mates is often between related males. Mothers are therefore selected to produce fewer sons, in order to minimise the cost of competition, and to provide more mates for their sons (Taylor 1981; Charnov 1982). If *N. vitripennis* females mated post dispersal, then this would affect selection on the primary sex ratio laid by foundresses.

1.4.3 Explanations for Polyandry

What is the adaptive significance of female promiscuity? The puzzle of female promiscuity, or polyandry (females having multiple mates) is more perplexing when one considers the empirical results documenting the often-substantial costs to females of mating. These costs are manifold and include an increased risk of predation, exhaustion of time and energy, risk of injury, parasites, and diseases, plus harmful effects of the male ejaculate (Daly 1978; Fowler & Partridge 1989; Arnqvist 1989; Rowe et al. 1994; Chapman et al. 1995; Gems & Riddle 1996; Telford & Webb 1998; Stockley & Seal 2001; Stutt & Siva-Jothy 2001; Blanckenhorn et al. 2002; Martin & Hosken 2003; Maklakov & Lubin 2004; Kuijper et al. 2006; Muller

et al. 2007). Although one should take care to differentiate between ecological costs and those costs arising from mate resistance, copulation, and insemination, as these costs are not the cause of sexual conflict, but rather the result (see below, Section 1.3.4 Sexual Conflict; and Watson et al. 1998; Holland & Rice 1999; Pitnick et al. 2001; Martin & Hosken 2003; Kuijper et al. 2006). Understanding why females are polyandrous is therefore crucial to the fields of sexual selection and sexual conflict. Is polyandry an adaptive strategy for females, or a suboptimal outcome of sexual conflict? Many explanations have been postulated and some are more consistent with sexual selection than others (Zeh & Zeh 2003). The hypothesised benefits polyandrous females obtain can be broadly classified as either direct (or material) benefits, that increase the lifetime reproductive success of the female, or 'indirect' (or 'genetic') benefits, whereby the female benefits from improved offspring quality.

1.4.3.1 *Direct benefits from polyandry*

Females may benefit directly from mating by gaining increased access to high quality resources that are under male control (Birkhead & Moller 1992). In insects, females can often use mating to gain nuptial gifts from males. These are food items (caught and offered to females, or created endogenously by males) that can increase female longevity and fecundity (Gwynne 1984; Vahed 1998; Arnqvist & Nilsson 2000; Sakaluk et al. 2004; but see Vahed 2007). It is debatable whether nuptial gifts represent mating effort, or parental investment (Simmons & Parker 1989), but the evidence appears to suggest that mating effort is the predominate explanation (Vahed 1998; for an example of females providing nuptial gifts see Arnqvist et al. 2003).

There is no reason to assume that this mating effort is benign, never mind beneficial, to females. Rather it may have evolved out of sexual conflict (see Section 1.4.4 Sexual Conflict), either as a sensory distraction (Vahed 2007), or in the case of voluminous ejaculates, as an attempt by males to influence the female endocrine system, which has subsequently been neutralized and metabolized by females (reviewed by Arnqvist & Rowe 2005). Females can also obtain other material benefits from ejaculates, such as defensive chemical compounds that they can use themselves or transfer to their offspring (Gonzalez et al. 1999; Iyengar et al. 2001), although this would appear to be a rather unusual situation that is unable to provide a generalized explanation for polyandry. Another direct benefit is the supply of sperm, which is obviously beneficial to virgins, but would only be beneficial to mated females if they do not receive ample sperm from one mating, or are unable to store sperm (but most insects can). Such a need for 'sperm replenishment' (Thornhill & Alcock 1983; Ridley 1988; Pitnick & Markow 1994; Choe & Crespi 1997) is somewhat problematic as an evolutionary explanation for polyandry, because it immediately poses the question of why males do not provide sufficient sperm with each mating? The answer may be because sperm allocation by males is a subtle trade-off, which sometimes has to optimise female quality and number of mates, whilst balancing the risks of sperm competition (Parker 1990a; 1990b; Simmons & Kvarnemo 1997; Galvani & Johnstone 1998; Wedell et al. 2002a). However, many females obtain enough sperm from one mating (Ridley 1988), and there are many species where females obtain nothing but an ejaculate from a male. In these cases, the females would appear to only be benefiting indirectly from the genes acquired for their offspring unless the ejaculate has a direct effect.

A meta-analysis by Arnqvist & Nilsson (2000) of the direct benefits of polyandry to female insects found that there is often a fecundity benefit (for both species with and without nuptial feeding) but that there is also a longevity cost for species with no nuptial-feeding. Arnqvist & Nilsson (2000) argue that this selects for an optimal mating rate in females, where the costs of mating are balanced by the benefits (although for evidence of a bimodal relationship, whereby intermediate rates are disfavoured, see Arnqvist et al. 2005). Arnqvist & Nilsson (2000) also argue that most examples of insect polyandry can be explained by direct effects i.e. with no recourse to genetic effects. However it was unclear if the increased fecundity and egg viability arising from the studies used by Arnqvist & Nilsson (2000) was because of stimulatory substances transferred in the male ejaculate (which could arise from multiple mating), or because of genetic benefits arising from polyandry (multiple mates). Experiments that disentangle the effects of multiple mating from multiple mates were required. Also, one questions the view that stimulation of egg production and oviposition in response to additional matings is a benefit that could favour polyandry, rather than an evolved response to polyandry. One has to ask could females not produce as many eggs or oviposit at the same rate when mated to one male? Surely if females produce more eggs when polyandrous, this is either, i) an evolved response to capitalize on some other, unknown, benefit of polyandry, such as genetic benefits (see below, Section 1.4.3.2 Indirect Benefits of Polyandry), or ii) the result of male manipulation of their reproduction (see below, Section 1.4.4.2 Post-Copulatory Sexual Conflict).

1.4.3.2 *Indirect benefits from polyandry*

Various models of indirect benefits often assume males vary in their genetic 'quality' and therefore explanations for polyandry are often inextricably entwined with explanations for female choice in general. Thus explanations for polyandry are often faced with the same difficulty as models of female choice, which is that they invoke directional selection upon the relevant male traits. Over time such selection will erode the necessary variation for females to select upon, in what is knows as the Lek Paradox (Kirkpatrick & Ryan 1991). Various models have investigated whether sufficient variation can be maintained, via selection for parasite resistance, or by deleterious, or biased, mutations for example (Hamilton & Zuk 1982; Pomiankowski et al. 1991; Rowe & Houle 1996; Kokko et al. 2002; 2006). The models of indirect benefits can be categorized into the following four classes (Simmons 2001a); i) 'Trading Up', whereby females mate sequentially with males of ever improving 'quality'; ii) 'Bet Hedging', whereby females produce broods of multiple paternity and benefit from a heterogeneous brood that is better adapted to an unpredictable environment or for parasite resistance; iii) Sperm Sorting, whereby females benefit from either 'cryptic' choice, selecting from diverse sperm, or by invoking sperm competition, and thus producing sons with superior sperm (if sperm ability is heritable); and iv) Incompatibility Avoidance, whereby females vary at the genetic or cytological level for their preferred mate partner, and polyandry increases the odds of finding a compatible partner. It has been shown that females discriminate against previous mates, even in invertebrates (Zeh et al. 1998; Bateman et al. 2001), and that females leave a molecular trail on previous mates to mark them out from future matings (Ivy et al. 2005). This shows that sometimes the costs of mating are relevant,

and that there must be some value from gaining extra partners, a value that is not available by re-mating the same male, i.e. a genetic value.

1.4.3.2.1 *Indirect benefits from polyandry: 'trading up'*

'Trading up' allows females to upgrade on their previous mates by accepting additional copulations from superior mates. Such a process is fully consistent with previous models of sexual selection and explains polyandry as an outcome of females accepting additional mates in a sequential manner when they offer an improvement on previous mates (Thornhill & Alcock 1983; Kempenaers et al. 1992; Graves et al. 1993; Hasselquist et al. 1996; Petrie & Kempenaers 1998; Jennions & Petrie 2000). When females come across mates sequentially, they have to balance the risk of being non-mated against mating with males of low quality, and this balance depends on the encounter rate with males; when males are rare, a virgin female may do better to accept any mating opportunity she can (Kokko & Ots 2006; Lehmann 2007). In this case, the risk of not being mated is a cost of mate choice that is analogous to standard mate choice models. Trading up is more likely in species with high last male sperm precedence, or when females can jettison previously stored sperm. If females agree on which males are desirable, then female polyandry should be predicted by the identity and order of their mates in experiments (as shown by Gabor & Halliday 1997; Bateman et al. 2001; and as falsified by Klemme et al. 2006).

1.4.3.2.2 *Indirect benefits from polyandry: 'bet hedging'*

'Bet hedging' refers to theoretical developments of life-history evolution that postulated an advantage of having a generalist strategy, or producing a brood comprising a mixture of specialist strategies, in unpredictable environments (see references in Yasui 1998). Therefore polyandrous females can produce heterogeneous broods as an adaptation to uncertainty. In uncertain times it pays to spread one's bets and polyandry can hypothetically result from two forms of uncertainty, i) an unpredictable environment, and ii) imperfect mate choice when searching for 'good genes' (Yasui 1998; Yasui 2001; Fox & Rauter 2003). In i) polyandrous females are favoured because they reduce the variance in fitness between generations, which is greatest in unpredictable environments. However a difficulty arises if the mate choice criteria is the same for monoandrous and polyandrous females, because the arithmetic mean fitness will be the same for both (Yasui 1998). In ii) if female identification of high quality males is imperfect, then it may pay for them to mate many males, to reduce sampling error and minimize the risks of fertilizing their eggs with 'poor' sperm (Watson 1991; Yasui 1998; Yasui 2001). Theoretical models suggest that heterogeneous broods can only be favoured under restrictive conditions, such as small population size, negative frequency-dependent selection on fitness related loci, or when there are negligible costs to multiple-mating (Yasui 1998; Yasui 2001). However full-sib competition can favour heterogeneous broods, as the diversity of offspring allows for a degree of niche partitioning and therefore reduces the costs of competition (Barton & Post 1986). This may be relevant to gregarious parasitoids, whereby larvae have to compete for access to shared resources in the host (Hardy 1992).

Genetic diversity among offspring can also be favoured to allow more efficient cooperation between half-sibs. Here the advantage stems from a division of labour, allowing greater specialisation and efficiency (Page et al. 1995). Greater diversity among offspring may also be useful in defence against parasites, especially at the colony level for eusocial insects (Sherman et al. 1988). Social insects produce large numbers of offspring, and in confined spaces that are prone to parasitization and infection, therefore they are more likely to benefit from heterogeneous offspring (Sherman et al. 1988; Liersch & Schmid-Hempel 1998; but see van Baalen & Beekman 2006), but polyandry has important implications for their social structure which may be more constrained by selection (Trivers & Hare 1976; Ratnieks 1988; Boomsma & Grafen 1990).

1.4.3.2.3 *Indirect benefits from polyandry: sperm competition*

The acquisition of multiple ejaculates may allow females to choose their mates cryptically, that is, it may allow females an additional opportunity to screen potential mates (Eberhard 1996). If females can selectively sort sperm, then this allows them to accept copulations without a commitment to use the acquired sperm. Therefore if males are rather coercive, females may be able to accept superfluous copulations from unwanted males and thus avoid the costs of mate rejection (see Section 1.4.3.3 Convenience Polyandry), whilst also avoiding using their sperm. Male sperm quality may correlate with quality for other male traits ('intrinsic' male quality), in which case females may use sperm competition to select better males (Madsen et al. 1992; Eberhard 1996; Olsson et al. 1997).

Or females may mate multiply with different males in order to invoke sperm competition, and thus produce sons fathered by males with superior sperm, although the evolutionary dynamics are debatable (Keller & Reeve 1995). Competitive sperm does seem to be a heritable trait though, as required (Radwan 1998; Bernasconi & Keller 2001; Konior et al. 2005). One problem for sperm competition is that there must be limited first or last male precedence, i.e. the order of mating must not be too important (Byrne & Roberts 2000). Another potential problem is that cytoplasmic organelles such as mitochondria, which may affect sperm quality, are probably inherited only from the maternal gamete. Therefore sons may not inherit their father's successful sperm, thus any response to selection on such organelles can only occur in the maternal line. Sexy sons are also at odds with any non-nuclear maternal genes, which bear the costs of male manipulation but don't reap any of the benefits from successful sons (Zeh 2004).

1.4.3.2.4 *Indirect benefits from polyandry: incompatibility avoidance*

One way to overcome the problem of female choice creating directional selection that erodes the variation of the male trait (the Lek Paradox, Kirkpatrick & Ryan 1991; See Section 1.3.3.2 Indirect Benefits of Polyandry) is to realise that one female's high quality male may not be so desirable for another female (Zeh & Zeh 1996; 1997; Jennions & Petrie 1997; Newcomer et al. 1999; Tregenza & Wedell 1998; Tregenza & Wedell 2000; Simmons 2001a). Such a scenario may stem from genetic or cytoplasmic incompatibilities, whereby the value of a male's genes depends on how they interact with the female's own genes. The genotypic basis of the immune system is one trait that conforms to such interactions, whereby females

are favoured to choose mates with different alleles that code for the Major Histo-Compatibility Complex (Wedekind et al. 1995; Penn & Potts 1999). Mate choice is always essentially a balance between the costs and benefits of inbreeding and outbreeding. Inbreeding increases the frequency of alleles identical by descent but suffers the cost of overdominance, and outbreeding provides a heterozygote advantage but causes the dissolution of co-adapted gene complexes (Pusey & Wolf 1996; Kokko & Ots 2006). Incompatibility avoidance is essentially the same as inbreeding avoidance and heterozygote advantage, but can be restricted to just parts of the genome, rather than across the board.

But why would incompatibility avoidance select for polyandrous females? If such incompatibilities are hard to detect, then females may mate a variety of mates to safeguard against having only incompatible sperm. Note that this situation is analogous to the one of 'bet hedging' to guard against imperfect mate choice (Yasui 2001; see Section 1.3.3.2.2 Indirect Benefits of Polyandry – 'Bet Hedging'). Except with incompatibility avoidance, if there is a mechanism by which more compatible sperm are more likely to fertilize eggs, or if maternal effects can favour more compatible zygotes, then polyandrous females will have a higher mean fitness (Tregenza & Wedell 2000). Essentially polyandrous females will be favoured because they are more effectively screening potential mates by processing more information about them and utilizing a post-copulatory veto to decouple insemination and fertilization success. They could do this by favouring the fertilization success of compatible sperm by screening them as they travel through the reproductive tract or by selectively investing more in zygotes formed with more compatible sperm. When

testing for a benefit from incompatibility avoidance, female fecundity and offspring viability should depend on their mates, but the effects of their mates should not be repeatable when they are mated to different females (Tregenza & Wedell 2000), as found in (Wilson et al. 1997; Tregenza & Wedell 1998; Clark et al. 1999).

1.4.3.3 *Convenience polyandry*

Of course a simple explanation for polyandry would be that it has no adaptive value for females and is just the result of male coercion (Halliday & Arnold 1987; Ridley 1990; Rowe et al. 1994). Males are certainly able to force copulations in many species, and do, as reported in garter snakes, *Thamnophis sirtalis (Shine et al. 2005)* and in chimpanzees, *Pan troglodytes schweinfurthii* (Muller et al. 2007), but there are also many species where females control copulation and yet are still polyandrous. Yet still, in these instances females may not be mating to obtain any benefits, but rather to reduce the costs of not mating. Just as some males may provide material benefits, such as nutritious spermatophores (reviewed by Vahed 1998), to provide benefits to negate the costs of mating, males could in theory choose to increase the costs of not mating. This is the theory behind convenience polyandry (Thornhill & Alcock 1983), whereby females accept superfluous copulations, mating above their optimal rate, in order to reduce the burden of excessively amorous males. Females may choose the lesser of two evils when the costs of male coercion exceed the threshold of a costly mating (Clutton-Brock & Parker 1995). Evidence is emerging to support the convenience hypothesis (in water striders, Rowe et al. 1994; Watson et al. 1998; in a seed-eating insect, Neacoryphus bicrucis, McLain & Pratt 1999b; in a

calopterygid damselfly, Cordero & Andres 2002; and in a marine turtle, Lee & Hays 2004), which may explain why some species fail to test for a benefit to multiple mating, as the experimental design may fail to control for the costs of harassment.

1.4.3.4 *Measuring the costs and benefits from polyandry*

Traditionally, studies have aimed to measure the costs and benefits of mating in polyandrous species (Tregenza & Wedell 1998; 2002; Evans & Magurran 2000; Konior et al. 2001; Worden & Parker 2001; Fedorka & Mousseau 2002; Kamimura 2003). Whilst valuable, this approach can sometimes be problematic because data from polyandrous species potentially confound the origin of polyandry with the maintenance of polyandry (Arnqvist 2006). The selection favouring the origin of polyandry may differ from that favouring its maintenance. This may in part be due to a change in the costs and benefits of mating as a result of the co-evolutionary dynamics of sexual conflict (i.e. different patterns of selection on males and females: Morrow & Arnqvist 2003; Arnqvist & Rowe 2005; Ronn et al. 2007). An alternative approach is to examine the origin and spread of polyandry, in response to a change in the selective environment, either in the laboratory or in the wild (Torres-Vila et al. 2002; Harano & Miyatake 2005). Another problem that can arise is when the experimental design allows females to select their own mating rate, and remating propensity is condition or size dependent in that species, then this can confound the estimates of the costs and benefits of mating (Torres-Vila et al. 2004).

Arnqvist and Nilsson (2000) argue that one reason there is no consensus on the beneficial nature of multiple-mating is that experimental studies obtain results dependant upon the relationship between the experimental mating rate and the optimal mating rate for that species. If the experimental rate is set too high or too low the study will conclude that multiple mating is not adaptive. To resolve this one needs to screen a range of mating rates for benefits and costs, or allow females to select their own mating rate, but this too has problems because it confounds the average effects of multiple mating with the benefits accrued by females who have the most to gain (Torres-Vila et al. 2004).

1.4.4 Sexual Conflict

Parker (Parker 1979) coined the term 'Sexual Conflict' following his earlier observations of mating behaviour in the dung fly, *Scatophaga stercorarial*. Parker observed that females often suffered heavy costs, including death (by 'drowning' in cowpats), from the excessive attention of courting males. Such observations were contradictory to the prevalent view of reproduction as a harmonious affair between cooperating individuals. Parker reconciled his observations with sexual selection by realising that selection could pull in opposing directions for the sexes. His term sexual conflict therefore refers to the conflict of (evolutionary) interests between the sexes. Although sexual reproduction is an act of cooperation, and between two actors that are interdependent, conflict still arises because they often have mutually exclusive goals; for even when cooperating, the evolutionary interests of individuals

are rarely perfectly aligned, as selection often favours different outcomes for different actors (Trivers 1985; Chapman 2006).

Parker outlined two main sources of conflict, or 'battlegrounds'(Parker 1984; Arnqvist & Nilsson 2000). The first occurs when willing males interact with unwilling females, and the second occurs following copulation. Willing males often encounter unwilling females because the optimal mating rates typically differ between the sexes, with females favouring a lower rate (Arnqvist & Rowe 2005; see Section 1.3.2 Optimal Mating Rates). This means that males will be favoured if they can 'overcome' female 'resistance'. However, the evolution of such a trait in males will greatly favour any females that can resist such males, resulting in Sexually Antagonistic Co-evolution (SAC) between the sexes and an ensuing arms race between male 'persistence' and female 'resistance' (Arnqvist & Rowe 1995; Chapman & Partridge 1996b; Chapman et al. 2003b; Hayashi et al. 2007; although for a challenge to this viewpoint, see Cordero & Eberhard 2003; Eberhard 2005). In an elusion to Fisher's Runaway Selection model (1930), Holland and Rice (1998) postulated that such selection pressures have combined to form a powerful engine of evolutionary change (the 'Chase-Away' model), responsible for much species divergence in mating behaviours and the presence of costly male traits (Rice et al. 2005; although for a comparative analysis challenging this, see Eberhard 2006). Such traits clearly were no longer believed to "[lead] *to the improvement of…the species*" as Darwin once thought (quote taken from Arnqvist & Rowe 2005)! Such conflict can be termed 'pre-copulatory' conflict.

In the second 'battleground', even if the sexes have equal optima for mating rates, there will be conflict, because males will be favoured if they can prevent their mates from remating with other males (Parker 1984; Arnqvist & Nilsson 2000). Such conflict can be termed 'post-copulatory' conflict. Although there is also conflict over parental investment (Trivers 1972), I do not go into detail in this review, primarily because such conflict does not drive as much evolutionary change as the conflict over mating does (Lessells 2006). This is perhaps because it is not very feasible to manipulate the parental investment of a partner.

1.4.4.1 *Pre-copulatory sexual conflict*

The conflict that occurs between an unwilling female and a willing male is perhaps the most important form of sexual conflict for generating evolution (Lessells 2006). Although the extent of post-copulatory conflict may be relatively obscured because it is often mediated internally, either way, 'courtship' and the union of male and female for mating is no longer assumed to be harmonious. The most successful males are not necessarily the most desirable, and the evolution of female preference necessitates the evolution of female resistance (Arnqvist & Rowe 2005). Evidence of pre-copulatory conflict can be found in the evolution of 'grasping' traits in males, or other such features that allow them to overcome female resistance, and the subsequent evolution of female traits to resist such adaptations. Such examples can be found in water striders (Arnqvist & Rowe 1995; Arnqvist & Rowe 2002a; Arnqvist & Rowe 2002b), and diving beetles (*Dytiscidae*) (Bergsten et al. 2001). In water striders, females with more modified genitalia are better able to repel unwanted males (Arnqvist & Rowe 1995). In diving beetles, females are

polymorphic for their surface structure, being either smooth or rough. Males from populations where the rough morph is more common have more developed adhesive structures. The female polymorphism suggests that there are non mating related costs to being rough, such as increased friction when diving, although the polymorphism may be maintained by non-random mating with respect to the conflicting traits (Hardling & Bergsten 2006). In dung flies, the females exert mate choice by vigorously shaking 'off' undesirable mates. In response, males of *Sepsis cynipsea* have evolved clasping forelegs that can cause injuries to female wings during these struggles (Muhlhauser & Blanckenhorn 2002). The males also evolved armored genitalia that injure females internally during copula, which may serve to increase success in sperm competition, and/or to induce harm in females so as to prevent them re-mating (Johnstone & Keller 2000; Blanckenhorn et al. 2002).

Males can also overcome female resistance in other ways. Some males have evolved traumatic insemination, whereby they 'puncture' the female and insert their sperm. This is costly for females but may increase a male's odds in sperm competition whilst also circumventing female resistance (Stutt & Siva-Jothy 2001). It may also serve to prevent females from remating (Johnstone & Keller 2000). Examples, with female counter-adaptations, can be found in bed bugs, where females have evolved an alternative reproductive tract (Siva-Jothy 2006; Reinhardt & Siva-Jothy 2007) and Malabar ricefish (*Horaichthys setnai*) (Arnqvist & Rowe 2005). Alternatively, males may sidestep female resistance by mating with females when they are so juvenile that they cannot yet resist (Markow 2000; Shine et al. 2003), as is common in Lepidoptera (Wiklund & Forsberg 1991; Zonneveld 1996). Often the costs to

females are side-affects of the intense male-male competition, such as in dung flies and garter snakes (Parker 1979; and Shine et al. 2005, respectively). Here, the males do not gain by harming the females, but the risk for persistent males of harming their mates is less than the risk for non-persistent males of not mating.

Males may also become better at 'seducing' females. Two striking examples are by providing dishonest, worthless, nuptial gifts (Sadowski et al. 1999; LeBas & Hockham 2005) and by infanticide, such as the striking example, (reviewed by Arnqvist & Rowe 2005) of infanticide in a spider, *Stegodyphus lineatus*. Males present females with worthless parcels to exploit the female sensory system, although these could be 'honest indicators' of male quality (Zahavi 1975; Grafen 1990a; Cumming 1994). By the time the female discovers the true value of her gift, the male has often already mated with her. With infanticide, males are deleting the benefits from previous matings, and thereby setting the clock back for the females to the position when they last found it profitable to mate. Although females are selected to avoid infanticide, once the act is done, they are again in need of sperm unless they have adequate stores and so may be adequately 'seduced', providing the environmental conditions are suitable. Males may also exploit female sensory preferences, shaped by natural selection, to lure females with modified body parts that hijack female responses to features of their environment, such as prey recognition (Basolo 1990; 1996; Endler & Basolo 1998; Arnqvist & Rowe 2005). Such manipulations are only likely when males tap into a sensory process that is under strong natural selection, such as prey recognition for example. Otherwise

females are predicted to evolve 'indifference' to male produced, manipulative, supranormal, stimuli (Rowe et al. 2005).

1.4.4.2 *Post-copulatory sexual conflict*

After mating, the interests of the sexes differ over the female's future mating history, and her offspring production. Males can reduce the odds of their mate from mating with another male through various means such as mate guarding (Sherman 1989), copulatory plugs (Devine 1975; Harcourt 1991; Matsumoto & Suzuki 1992; Langtimm & Dewsbury 1991; Barker 1994; Olsson et al. 1994; Dixson & Anderson 2002), or the transfer of ejaculatory substances that mediate a lack of receptivity to mating in females (Gillott 2003; Wedell 2005). However, whichever method males employ to prevent their mate from mating again with other males, they can rarely be completely sure of their future paternity, which is one reason they are less likely to provide parental care (Trivers 1972). It also means that there will be a conflict over the reproductive rate of the female. Animals are selected to reach an optimal trade-off for reproductive effort, a trade-off between immediate gains and future costs (Stearns 1992). Current reproduction provides immediate benefits but may have adverse effects on longevity, whereas future reproduction may be greater, but is less certain. Under strict monogamy, reproduction would be truly cooperative, and the evolutionary interests of both males and females would be aligned. In this case, the optimal reproductive rate would be the same for both sexes (Holland & Rice 1999). But without strict monogamy, the optimal rate of reproduction will differ, with males favouring a greater immediate reproductive effort, thereby increasing the use of their sperm, even if this comes at a cost to the female (Trivers 1972; Holland & Rice

1999; Arnqvist & Rowe 2005). The transfer of an ejaculate with sperm allows males the opportunity to mediate female reproductive effort and mating behaviour via interference with the female endocrine system (reviewed by Arnqvist & Rowe 2005). Male *Drosophila melanogsaster* have been shown to transfer accessory substances in their ejaculate that function to stimulate egg production and oviposition, whilst also inducing a refractory period in the female. These products also reduce the female lifespan (Chapman et al. 1995; Chapman et al. 2001; Wolfner 2002), however this will not be costly for the male, and may even be beneficial. This is because a truncation in female lifespan will only act to reduce her reproduction at a time when she is using the sperm from other males. Therefore female harm can evolve as a by-product of male manipulation (Civetta & Clark 2000; Morrow et al. 2003). Large, endogenous, nuptial gifts may have originated as attempts by males to influence females, and they may still (reviewed by Vahed 1998).

1.4.4.3 *Detecting sexual conflict*

It can be difficult to detect the evolutionary consequences of sexual conflict for many reasons. Generating predictions is difficult because different outcomes are possible (Rowe et al. 2005), one does not know 'where' in the evolutionary cycle a particular species may currently be (Arnqvist & Rowe 2005), mating behaviour is the outcome of many selective forces (Andersson 1994; Arnqvist & Rowe 2005; Rowe & Day 2006; Kokko et al. 2006), and finally, because the dynamics of SAC may obscure the process as one sex ameliorates the costs inflicted by the other (Arnqvist & Rowe 2002a; Rowe & Day 2006). Further difficulties arise because one does not know the optimal mating rates of females and thus it is unclear if they are mating at

maladaptive rates or not. Combined with females being able to respond by different ways, such as mating out of convenience, avoiding mating sites, evolving resistance structures, or simply by becoming indifferent to male signals, it can be very difficult to compare what females are doing with what they 'want' to be doing (Rowe et al. 2005). Although measuring optimal mating rates is difficult, it is important to consider them in experimental design when measuring the costs and benefits of polyandry. As one does not know the optimal mating rate, and as different experimental designs can give different results within the same study system, it is best to test females over a range of matings (Arnqvist & Nilsson 2000).

There are three useful methods of approach when investigating evolution by sexual conflict. The first is to conduct comparative analyses to reveal correlated evolution of male 'persistence' and female 'resistance' traits within clades, as used by (Bergsten et al. 2001; Arnqvist & Rowe 2002b), The second is to conduct manipulative experimental studies that infer the function of relevant traits from their absence (Rowe & Day 2006), and the third is to use population crosses to disassociate male adaptations and female counter-adaptations (Long et al. 2006). Long et al. (2006) cross-mated populations of *D. melanogaster* to show that females experienced greater costs of mating when they mated males from other populations. Females are predicted to evolve responses to male induced harm, and males are predicted to evolve a suite of manipulative traits. Long et al. (2006) therefore provide a compelling argument that the females lacked a defence to the offensive strategies of 'foreign' males. This result was consistent with the seminal paper of Holland and Rice (1999). Their classical study compared laboratory strains of *D.*

melanogaster that had been kept under enforced monogamy versus standard, polygamous, strains. When they performed reciprocal crosses between the strains, they showed that males lost their ability to harm under monogamy, and that females lost their protection against manipulative male traits under monogamy. They therefore showed that when the evolutionary interests of males and females are aligned, there is no conflict.

CHAPTER 2

2. Laboratory Evolution of Polyandry

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2.1 SUMMARY

The evolution and maintenance of multiple mating in females (polyandry) is an adaptive puzzle since females typically obtain all the resources they need from males in only one or a few matings. Females should therefore limit superfluous mating to avoid the well-documented costs of mating. Previous studies have tended to focus on the maintenance of polyandry, and have examined the wide range of costs and benefits to females of multiple mating in species with varying levels of polyandry. There is much less empirical work charting the evolution of female mating behaviour and the origins of the polyandry trait itself. I investigate a potential increase of female remating rate in the parasitoid wasp *Nasonia vitripennis*. I screened the mating and remating rates of females from 20 strains, 18 of which were collected from the same locality across 3 years and varied in the number of generations that they had spent in the laboratory. Females from strains that had spent longer in the laboratory were increasingly likely to be polyandrous, confirming a previous study

based on one laboratory strain. Experimental crosses between strains at opposite ends of the spectrum for time spent in the laboratory showed that heritable changes in the females were predominantly responsible for the change in mating behaviour, as males did not appear to influence the likelihood of a female remating.

2.2 INTRODUCTION

The evolution and maintenance of female multiple mating (polyandry) remains a puzzle in many taxa. Typically, females can obtain all the male-derived resources they need, especially sperm, from a limited number of matings (Arnqvist & Nilsson 2000). Yet it is now apparent that females of many species mate more than is required to fertilize a full complement of their eggs (Birkhead & Moller 1995; Fedorka & Mousseau 2002). Furthermore, multiple mating appears to be costly in a number of ways, including increased risk of predation, exhaustion of time and energy, risk of injury, parasites and diseases, and harmful effects of the male ejaculate (Daly 1978; Fowler & Partridge 1989; Chapman et al. 1995; Stockley & Seal 2001; Stutt & Siva-Jothy 2001; Blanckenhorn et al. 2002; Martin & Hosken 2003; Maklakov & Lubin 2004).

However, if additional matings can also bring benefits, then the optimal mating rate for a female may involve some degree of multiple mating (Arnqvist & Nilsson 2000). These benefits can include: (1) direct benefits such as sperm (Lopez-Arroyo et al. 1999; Drnevich et al. 2001), nourishing nuptial gifts (LaMunyon 1997; Vahed 1998; Arnqvist & Nilsson 2000; Torres-Vila et al. 2002; Wedell & Karlsson 2003) and access to necessary resources such as food or oviposition sites (Thornhill & Alcock 1983; Worden & Parker 2001), or (2) indirect genetic benefits which are realised through the production of genetically fitter offspring (Andersson 1994; Arnold & Duvall 1994; Jennions & Petrie 2000; Bernasconi & Keller 2001; Konior

et al. 2001). Alternatively, female mating rate may be a result of male attempts to coerce or force mating (Rowe et al. 1994; Shuker & Day 2001; 2002) leading to convenience polyandry as females act to reduce the costs of not mating as opposed to seeking gains from additional mates and copulations (Thornhill & Alcock 1983).

Studies have tended to concentrate on which of the above benefits can be identified in already polyandrous species (Tregenza & Wedell 1998; 2002; Evans & Magurran 2000; Konior et al. 2001; Worden & Parker 2001; Fedorka & Mousseau 2002; Kamimura 2003). An alternative approach is to examine the origin and spread of polyandry, in response to a change in the environment, either in the laboratory or in the wild (Torres-Vila et al. 2002; Harano & Miyatake 2005). Although the origin of polyandry is closely linked to its maintenance, the selection pressures can differ. This is due to a change in the costs and benefits of mating as a result of the coevolutionary dynamics of sexual conflict (Arnqvist & Rowe 2005). Here I investigate the significance of an anecdotal report by Van den Assem and Jachmann (1999) of a potential increase of female mating rate in one laboratory strain of the parasitoid wasp *Nasonia vitripennis*. Believed to mate only once in the wild, *N. vitripennis* females are very unlikely to remate when presented with additional males in the laboratory, despite the eagerness of virgins to mate under identical conditions (Holmes 1974; Van den Assem & Feuthdebruijn 1977; Grant et al. 1980). Microsatellite data from wild caught offspring indicate that only 2 of 49 foundresses had mated more than one male (Grillenberger et al. 2008). However, Van den Assem & Jachmann (1999) discovered that a laboratory-maintained strain (Leiden Lab II) of *N. vitripennis* had incidentally evolved an increased likelihood to remate, along with

a number of other behavioural differences, including an increased amount of courtship necessary for females to mate in the first place. Although their methods may have led to an inflated remating rate because males were prohibited from performing their post copulatory courtship, which may act to 'turn off' female receptivity. An evolved increase in mating rate has also been reported in laboratory maintained *Drosophila melanogaster* (Sgro & Partridge 2000). These observations suggested that mating behaviour had evolved over many generations of laboratory culture, and perhaps that laboratory culture could provide the opportunity to study the evolution of polyandry as it occurs.

My first aim was to test the generality of the Van den Assem & Jachmann (1999) findings by examining the relation between female multiple mating rate and time spent in laboratory culture, for a number of strains that had been maintained in the laboratory for variable lengths of time. If polyandry evolves repeatedly in the laboratory environment then I predicted a positive relation between the age of the strain and polyandry. My second aim was to test whether the change in mating rate was a result of changes in the behaviour of females themselves, or whether it involved interactions between males and females that could be both behavioural and physiological. I investigated this by examining mating behaviour of individuals from strains that had been in the laboratory for either a relatively short or a relatively long time paired with individuals from the opposite end of the spectrum.

2.3 METHODS

2.3.1 Study Organism

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae). For details on *Nasonia* biology please refer to Section 1.2.

2.3.2 Experimental Strains

I studied 18 strains that were collected from the same locality over consecutive summers from 2001 to 2003. Collections were made from bird nestboxes at Hoge Veluwe, the Netherlands, with no two strains originating from the same nestbox in the same year, so that each strain represents an independent replicate sample of the population. This is because each strain was derived from one or more mated female offspring that emerged from the parasitized pupae found in only one nest; the emerging wasps were likely to have been the offspring of only one or a few females. The wild-caught strains used were: B5; HV55; HV287; HV395 (collected 2001); HV202; HV236; HV307 (collected 2002); C51; C61; C62; C80; C130; C189; C194; C222/a; C223; C349; C378 (collected 2003). All strains were collected in June, July or August. Strains from the same year were not screened at the same time, providing a greater spread for the age of the strains. To compare these strains with long-established laboratory strains, I also used the strain Leiden Lab II, which is the same one used by Van den Assem & Jachmann (1999) to show evolved changes of mating behaviour in response to laboratory conditions. This strain was created from an individual *N. vitripennis* female caught in the *Calliphora* stocks of the Leiden University Physiology Department in 1971. I also used the red-eye mutant strain

STDR, which dates back to the 1950's (Whiting 1950; Whiting 1954; Saul & Kayhart 1956).

I pooled inexperienced males from the hosts before randomly assigning them to storage vials at a density of five males per glass vial (75 x 10 mm). I treated females the same way but stored them at a density of 10 per vial. I fed all individuals by using filter paper soaked with a honey solution at least once before any experiments and at least once every 48 h. I used individuals at an age of 1 to 3 days (post emergence) at the start of any experiment and to handle wasps I used an aspirator throughout. I removed all experimental subjects from the culture incubators and placed them at the observation locality (the same seat in the laboratory) at least 60 min prior to the observations to allow the wasps to acclimatize to laboratory conditions.

2.3.3 Experiment 2.1 Evolution of Polyandry

I measured polyandry as the proportion of females that remated when presented with a second male. I offered all females a preliminary mating with a virgin male from their own strain. This provided the mean mating score of virgins for each strain. Those females that copulated were then presented with another virgin male from their strain 18 to 24 h later. The proportion of females that copulated with the second male provided the mean remating, or polyandry, score for each strain. All trials took place in daylight between 1200 and 1900 hours.

I placed each virgin female into her own glass vial (50 X 10 mm). Males remained in their storage vials of five individuals until they were extracted individually for the mating trials. For each preliminary mating, I introduced a male to the female and observed them continuously for 3 min or until copulation terminated, after which I removed the male (males were always allowed to perform their post-copulatory courtship). This prevented any female from copulating more than once before the remating test. If a male was still courting or copulating at the end of the 3 min I continued the observation either until the male ceased courtship or a successful copulation was completed. Any female that copulated within the 3-min trial remained isolated in the observation vial and was returned to the culture incubator overnight, prior to her remating test the next day. I sampled 1366 female wasps in total (range of 36 to 106 per strain, with an arithmetic mean of 68.3).

To see whether a mated female remated the next day, I returned females in their observation vials to the laboratory as before and gave each a fresh virgin male. Females had approximately 21 h between trials (range 18 to 24 h). I observed the pairs continuously for 15 min or until a copulation terminated. If a male was in the act of courting or the pair were copulating at the end of the 15 min then I allowed them to finish and included them in the analysis. For most trials I recorded latency to courtship, courtship duration, number of courtship bouts, and copulation duration. I excluded from the analysis any females that were not courted by their male.

My measure of polyandry for each strain was a measure of how many mated females from each strain mated during this 15-min remating trial. This is different to the

measure of increased female receptivity reported in Van den Assem & Jachmann (1999), because their methods did not allow males to initiate and terminate post-copulatory courtship, which is an important determinant of female receptivity, limiting the likelihood that females remate (Van den Assem & Jachmann 1999). To ensure the copulations I scored involved sperm transfer, I checked in two ways, whether putatively mated females produced female offspring, indicating successful insemination (owing to haplodiploid sex determination). For the first check, I used 76 females that copulated in the preliminary mating trial, but then did not copulate in the remating screen. I gave each female three fresh hosts to parasitize. Seventy-two females oviposited, although 3 produced only diapause larvae, which cannot easily be sexed, and these were excluded from further analysis. Of the remaining 69 broods, all contained female offspring. For the second check, I took 50 virgin females (not used in the polyandry experiment) and paired each with a virgin male as above. Forty-six females mated, and of these 41 laid non diapause broods, all of which contained female offspring. Observed copulations are therefore clearly associated with at least some sperm transfer.

2.3.4 Experiment 2.2 Effect of Males

I carried out an experiment to determine whether variation in the rate of polyandry was due to variation in male or female behaviour. I compared the remating behaviour of females when given males from their own strain (results from above experiment) or from another strain. The mating trials were carried out as before, only in this case I gave females males from different strains (for both the first and second mating trial). I used a strain that had spent many generations in the laboratory and that has a

high score for polyandry (Lab II), with two relatively new strains, that both have low scores for polyandry (C223 and C349). Females from strains C223 and C349 were presented with males from Lab II, and vice versa, in a reciprocal behavioural test.

2.3.5 Statistical Analyses

I tested changes in female receptivity with respect to time spent in the laboratory by using the proportion of females mating as the response variable, and fitting models with two main effects: (1) female status (virgin or mated); and (2) time spent in laboratory culture. I used time spent in the laboratory instead of an estimate of the number of generations because the time spent was known with greater certainty but for the majority of laboratory cultures one can estimate a period of 2 weeks for one generation. This allowed me to explore whether female receptivity changed overall (including when first given a male as virgins) and whether it changed only with respect to being virgin or mated. A significant interaction would show that changes in receptivity varied between first and second matings. My main prediction was that receptivity in the second mating (polyandry) would increase with time spent in laboratory culture. Since some females contributed two data points (i.e. if they mated in the first trial), I used the proportion of females mating in a strain so that my degrees of freedom are based on the number of strains, rather than the number of females actually observed. I analysed the proportions of females mating as arcsine square-root-transformed data in general linear models (GLMs) weighted by the number of observations, using the JMP IN software, version 5.1 (SAS Institute Inc. Chicago, IL, U.S.A.). For the sake of clarity all figures show the proportions of females remating as untransformed. I measured time in laboratory culture in terms of

weeks and used log(weeks) for the analyses. To test for heritable change within strains from the same geographical population I also repeated the analyses after having excluded the non-Hoge Veluwe strains.

2.4 RESULTS

2.4.1 Experiment 2.1 Evolution of Polyandry

As expected, females were less willing to mate when they had mated previously, but this depended on the age of their strain, with strains that had spent more time in laboratory culture being more polyandrous (fig. 2.1). There was a significant effect of the female's mating status (virgin/mated; GLM: $F_{1, 39} = 252.30$, $R^2 = 0.81$, $P < 0.0001$), and the age of the strains ($F_{1, 39} = 6.04$, $R^2 = 0.02$, $P = 0.02$) and there was a significant interaction between these effects ($F_{1, 39}=16.23$, $R^2 = 0.05$, $P = 0.0003$). This significant interaction arose because the mean mating rate increases with time in the laboratory for mated females ($F_{1, 19} = 14.11$, $R^2 = 0.44$, $P = 0.001$,), but not for virgin females ($F_{1, 19} = 1.79$, $R^2 = 0.09$, $P = 0.2$).

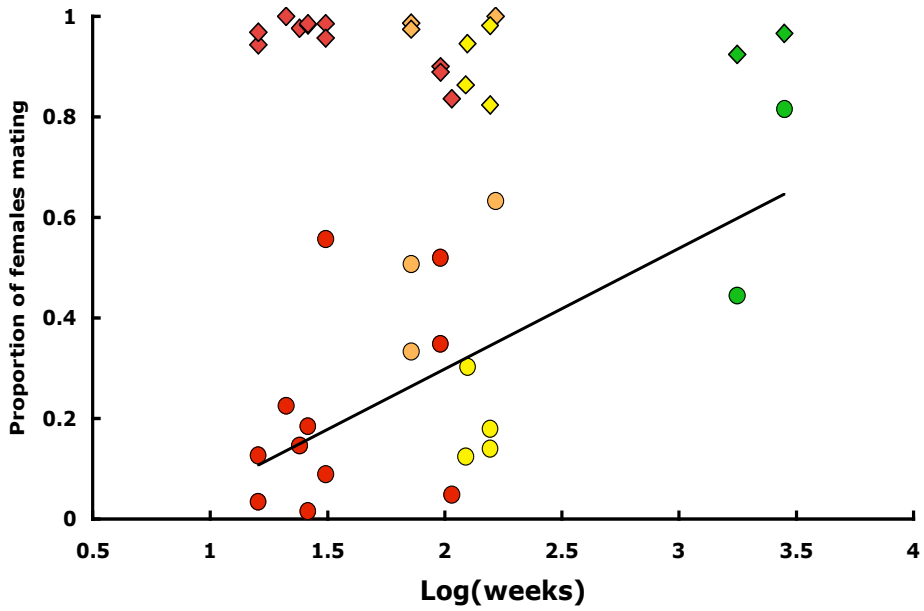


Figure 2.1. The relation between female receptivity to a courting male and her strain's time in the laboratory (Log(weeks)). Females were virgin (filled diamonds, non-significant) or once mated (filled circles; y = 0.04 + 0.26x) before each test. Red: collected 2003; orange: 2002; yellow: 2001; green: 1971 (Lab II) and 1950 (STDR).

I repeated the analysis excluding the two oldest strains, therefore analysing data only on the strains collected from Hoge Veluwe. In this case there was still a main effect of mating status (GLM: $F_{1, 35} = 251.45$, $R^2 = 0.87$, $P < 0.0001$); however, time spent in laboratory culture was no longer significant on its own ($F_{1, 35} = 0.53$, $R^2 = 0.00$, $P = 0.47$). There was a significant interaction again between the mating status of the female (virgin or previously mated) and the age of her strain ($F_{1,35} = 6.21$, $R^2 = 0.02$, $P = 0.02$,). When I analysed mated females only, I found no significant relationships although receptivity was marginally non significant ($F_{1, 17} = 3.53$, $R^2 = 0.18$, $P = 0.08$,).

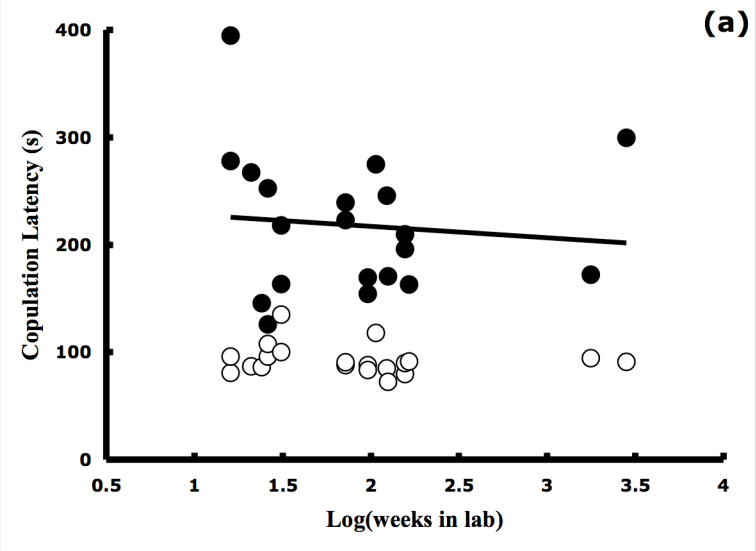
2.4.1.1 Experiment 2.1 Behavioural Changes

Mating behaviour varied in terms of both whether or not females were virgins and how long strains had been maintained in the laboratory (table 2.1, figs. 2.1 & 2.2).

Table 2.1. Comparisons of mating behaviour between virgin and mated females when presented with a virgin male

	Virgin female		Mated female	
	Mean ± SE	N	Mean ± SE	N
Courtship latency (s)	66.6 ± 1.3	1319	83.3 ± 2.2	524
Copulation latency (s)	92.6 ± 1.4	1260	156.5 ± 6.3	247
Courtship duration (s)[1]	23.6 ± 0.5	1211	62.6 ± 6.8	121
Copulation duration (s)	13.9 ± 0.1	1212	13.4 ± 0.2	106

[1]The courtship duration (s) for mated females includes only females that signalled receptivity to the first courtship bout for equal comparison with the virgin females.



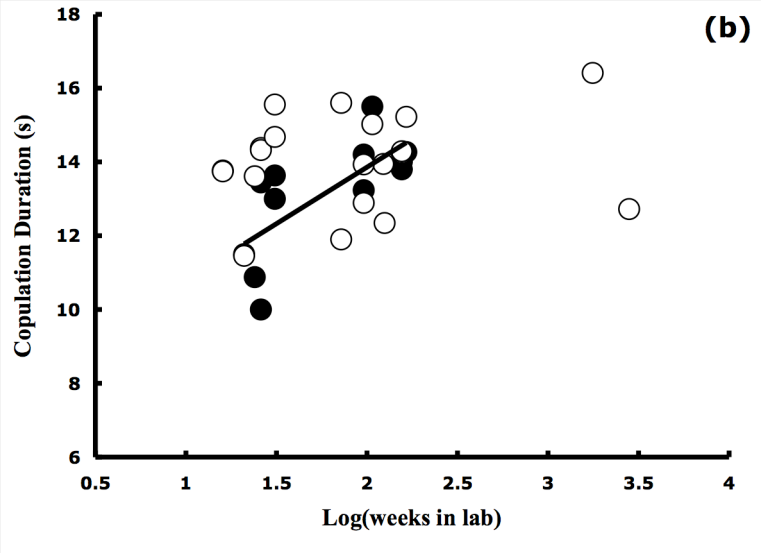


Figure 2.2. The relation between (a) copulation latency and (b) copulation duration and the strain's time in the laboratory (log(weeks)). Females were virgin (empty circles, non-significant) or once mated (filled circles; (a) y = 219.41 - 29.41x; (b) y = 1.84x + 10.18) before each test.

Virgin females copulated sooner than mated females (GLM: $F_{1, 36} = 60.98$, $R^2 = 0.65$, $P < 0.0001$,), and a positive interaction between mating status and the age of the strain showed that mated females remated sooner when their strains had spent more time in the laboratory ($F_{1, 36} = 4.58$, $R^2 = 0.05$, $P = 0.04$; fig. 2.2a). This was also true for mated females only ($F_{1, 16} = 5.88$, $R^2 = 0.28$, $P = 0.03$). The copulation duration did not differ between virgin and mated females ($F_{1, 31} = 0.04$, $P = 0.84$), but already mated females copulated for longer if their strain had spent longer in the laboratory ($F_{1, 11} = 5.97$, $R^2 = 0.37$, $P = 0.03$; Fig. 2.2b). Virgin females were also courted sooner than mated females ($F_{1, 33} = 5.64$, $R^2 = 0.15$, $P = 0.02$), regardless of how long they had been in the laboratory ($F_{1, 33} = 0.15$, $P = 0.70$), and they also required less courtship to become receptive ($F_{1, 33} = 11.35$, $R^2 = 0.22$, $P = 0.002$), again regardless of how long they had been in the laboratory ($F_{1, 33} = 1.24$, $P = 0.27$).

2.4.2 Experiment 2.2 Effect of Males

Female mating rates were consistent regardless of the strain used for her mates (table 2.2).

Table 2.2. The effect of males upon female remating

	Lab II males	C223 males	C349 males
Lab II females	0.47 (87)	0.48 (46)	0.38 (53)
C223 females	0.00 (52)	0.05 (43)	Not tested
C349 females	0.10 (30)	Not tested	0.10 (88)

Data are the proportion of polyandrous females for each mating combination (N).

Lab II females consistently showed a relatively high degree of polyandry whether they were with males from the same strain or one of the two newer strains (Fisher's exact test: $P = 0.77$). C223 females consistently showed a low degree of polyandry whether they were with males from the same strain or the older Lab II strain ($P = 0.20$). C349 females also consistently showed a low degree of polyandry whether they were with males from the same strain or the older Lab II strain ($P = 1.00$). This meant that male success depended upon the female genotype such that males courting mated Lab II females were more likely to mate than those paired with mated C223 or C349 females (Fisher's exact tests: all $P < 0.001$; table 2.2).

2.5 DISCUSSION

The longer *N. vitripennis* strains were kept in the laboratory environment, the more frequent polyandrous females became and they also remated sooner once paired with a male and for longer. This confirms the earlier finding of Van den Assem & Jachmann (1999) from one strain, Lab II, which had become more likely to signal receptivity when already mated, but there are subtle differences between the results. I found no changes among virgins and no effect of time in the laboratory on the length of courtship required, whereas Van den Assem & Jachmann (1999) found that virgin females had changed to require more courtship before signalling receptivity. My results confirm that the change in females is heritable, as females did not experience any males or male harassment prior to the preliminary mating, and experienced a male again only on the following day. This rules out female multiple mating as a plastic response to high male density and male harassment. I also showed that the change in female behaviour is independent of the male with which she interacts and that males have not evolved to be more or less likely to court a female. This suggests that a heritable change in female behaviour has taken place, rather than a change in the interaction between males and females. My results are comparable to those in a selection experiment with *D. melanogaster* (Mackay et al. 2005).

While there has been little focus on the evolution of polyandry compared to its maintenance, there has been a history of artificial selection studies exploring female receptivity more generally, particularly in *Drosophila*, and a rapid evolutionary

response to artificial selection and laboratory selection has been shown a number of times. For example *Drosophila ananassae* was artificially selected for both short and long remating intervals (Singh & Singh 2001): in just 10 generations selection produced a rapid divergence in remating times with high realized heritabilities ranging from 0.23 to 0.33 among replicate strains. Piñeiro et al. (1993) found that female receptivity in *D. melanogaster* responded to selection for both increased and reduced latency to copulation; however, the level of receptivity remained constant despite 42 subsequent generations of relaxed selection, suggesting that there was little selection pressure on receptivity in their laboratory conditions. Females from high receptivity lines had hybridized more frequently with *D. simulans* males, a result that mirrored the earlier finding that artificial selection for hybridization between these two species resulted in *D. melanogaster* females showing increased receptivity (Carracedo et al. 1991). Another study on *D. melanogaster* found an asymmetric response to selection on mating speed, in 29 generations, with females from the slow lines reducing their receptivity (Mackay et al. 2005). Estimates of realized heritability averaged 7%. The whole genome transcriptional response to selection was assayed and a large number of genes showed differential expression between the fast and slow replicate lines indicating substantial pleiotropy. Lastly, female receptivity also increased among laboratory populations of *D. melanogaster* in response to males with reduced courtship ability (McRobert et al. 1995); the stocks contained only males with a mutation (raised) that raises their wings and prevents them from performing their full courtship repertoire, so the females may have increased their receptivity to maintain their optimal mating rate (although selection at the level of the stock might have been the driving force in this unique

situation). An analogous situation may be driving the change in my laboratory cultures, where the high density of competing males might impair courtship (Van den Assem et al. 1980a). These examples are evidence that female mating behaviour can respond quickly to selection, although artificial selection is arguably stronger than any selection resulting from laboratory culture.

Although males had not evolved to be more or less likely to court a female, I did find that males initiated courtship later when the female was already mated: whether this is a result of male or female behaviour is unknown. Mated females may do less to facilitate courtship by changing their behaviour (King et al. 2000), or perhaps they can signal their mated status if mating induces a change in female cuticular hydrocarbons. In the wild it would be adaptive for males to discriminate against mated females, perhaps by sensing the presence of male-specific cuticular hydrocarbons or pheromone traces from the previous male. In response to females evolving polyandry, the males might have been expected to lose this preference for virgins, but the extent to which this discrimination occurs did not correlate with the time in the laboratory. My results are therefore again subtly different to those of Van den Assem & Jachmann (1999) who found a reduction in courtship intensity in laboratory-adapted males. This difference is probably because I have considered courtship only in terms of its initiation and termination rather than quantifying its components.

In summary, mated females from older strains were more likely to remate and remated sooner than mated females from younger strains. They also remated for longer which, along with remating sooner, is consistent with increased polyandry being the result of a heritable change in the females, as female *N. vitripennis* are in control of when copulation starts (they signal receptivity and need to open their abdominal orifice) and perhaps when it terminates.

Why does polyandry evolve under laboratory conditions in this species? The mass culture environment of the laboratory is one in which high densities of individuals (several hundred) emerge within a short period of time and where females are unable to disperse after an initial mating (at least until after 3 to 4 days some females are taken to found the next generation). During this time, females are subjected to high levels of repeated courtship, often with two or three males scrambling for access to one female. This is in contrast to the situation in the wild where females are free to disperse and forage for hosts as soon as they have mated. There are three straightforward explanations for the evolution of female multiple mating under this laboratory-induced change in mating system. First, polyandry has evolved because females have a much greater opportunity to sample and obtain indirect genetic benefits from the large number of potential mates now available to them (Jennions & Petrie 2000). Second, given the high levels of male harassment under laboratory conditions, multiple mating may have evolved as a way of limiting costly harassment, as envisaged by the convenience polyandry hypothesis (Thornhill & Alcock 1983). Third, the evolution of polyandry may be a pleiotropic effect of some

other laboratory-induced change, and so not under direct selection at all (Halliday & Arnold 1987; Grant et al. 2005).

Also, one should be cautious because although I showed that laboratory adapted females were more likely to be polyandrous under my experimental conditions, this does not necessitate that females are being polyandrous (or mating more often) under laboratory stock conditions. It is possible that the females now become receptive repeatedly in order to ensure that they become mated at all. In laboratory conditions the courtship attempts of males are often impaired by the high density of competing males, and therefore the scrambling males may only succeed to limit each other's mating success (Van den Assem et al. 1980a). Additionally, females are exposed to unnaturally high levels of male pheromones, a key component of male courtship (Van den Assem et al. 1980b; Van den Assem et al. 1981). It is possible that artificially high levels of pheromones induce female receptivity when they are not the focus of male attention, and thus their receptivity may not always lead to copulation (Van den Assem & Jachmann 1999). If females are fixed to only become receptive once, then this runs the risk of them not gaining any sperm. If a combination of these explanations is the true cause of female change, one wonders why the females do not evolve a change in their response to pheromones and courtship, rather than their willingness to remate? One could measure the degree of actual polyandry under laboratory conditions by sampling stock females and genotyping their progeny. Unfortunately this is not very feasible because the lack of genetic variation within strains will cause estimates of paternity and polyandry to be

underestimated due to the genetic similarity between mates. One could perhaps combine equal numbers of parasitised hosts from multiple strains into standard stock vials, but estimates will still be erroneously low if there is any assortative mating. One could break open the hosts to further mix the progeny, but then one is in danger of failing to replicate the standard stock environment.

To conclude, studying the evolution of polyandry as it happens provides three advantages to complement the alternative of examining the selection regime in natural populations. First, one can trace the behavioural changes as they happen in replicate populations. This means one can identify causal agents of the evolution of polyandry, rather than the possible agents of the maintenance of polyandry, including benefits that have arisen after polyandry itself evolved (see above). Second, one can explore the genetic basis of polyandry. For polyandry to evolve, the trait must show heritable variation, as seen in *N. vitripennis* (Shuker et al. 2007a). Although heritability was low for female polyandry and courtship duration, it was slightly higher for copulation duration. All three traits had high coefficients of additive genetic variance ($CV_A > 7.0$), suggesting that the additive effects are swamped by the nonadditive variation. Considerable dam effects were also found for all three traits, suggesting either dominance or maternal effects. Future experiments should investigate the number of loci involved and the mutability of the polyandry trait. Heritable variation in polyandry has also been shown in a number of species (Torres-Vila et al. 2002; Wedell et al. 2002b; Harano & Miyatake 2005), including the honeybee (*Apis mellifera*) (Kraus et al. 2005). Third, there is also a growing awareness of the link between mating behaviour, polyandry, and speciation (Parker

& Partridge 1998; Gavrilets 2000; Gavrilets et al. 2001; Gavrilets & Waxman 2002; Martin & Hosken 2004a). As mention in Section 1.2.2, *Nasonia vitripennis* is sympatric with two congeners that are endemic to North America: *N. longicornis* in the west and *N. giraulti* in the east. Partial prezygotic reproductive isolation occurs in all three species as a result of behavioural differences in courtship (Van den Assem & Werren 1994; Bordenstein et al. 2000; Beukeboom & Van den Assem 2001; Beukeboom & van den Assem 2002) and mating system (Drapeau & Werren 1999; Leonard & Boake 2006). This raises the possibility of testing how varying levels of polyandry in *N. vitripennis* influence the degree to which it is prezygotically reproductively isolated from *N. giraulti* and *N. longicornis*.

CHAPTER 3

3. The Female Costs and Benefits of Mating

3.1 SUMMARY

The optimal mating rate is a trade off between the costs and benefits of additional matings, and is typically lower for females than males, because their fitness is often limited by factors other than the number of mates obtainable. Despite the documented costs of mating, females are consistently found to mate multiply across a diverse array of taxa. In contrast to this trend, wild females of *Nasonia vitripennis* are generally monoandrous, although laboratory strains evolve an increased mating rate. I mated females 0, 1, or 2 times, with different mates, and measured the cost, in terms of reduced longevity, and the benefit, in terms of increased fecundity, of mating. I used four different laboratory strains of *N. vitripennis* that varied in the amount of time they had been cultured in the laboratory and hence their re-mating propensity. I found no significant cost of mating in any of the strains, but did find a substantial benefit of mating in one strain.

3.2 INTRODUCTION

Reproduction has traditionally been thought of as a harmonious act of cooperation in sexual species, but even when cooperating, the evolutionary interests of individuals are rarely perfectly aligned, as selection often favours different outcomes for different actors (Trivers 1985). Sexual reproduction is an act of cooperation between two actors that are interdependent, but which nearly always have mutually exclusive goals. This is because although mating is undoubtedly beneficial, it is not always equally beneficial for the sexes. Mating is also a costly business, and these costs have to be balanced against the benefits, and again these costs are not necessarily equal (Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Zeh & Zeh 2003; Martin et al. 2003; Arnqvist & Rowe 2005). The optimal mating rate is therefore a trade-off between the costs and benefits of additional matings (Arnqvist & Rowe 2005). This trade-off is typically different for the sexes, and especially so as the number of interactions increases. Male fitness increases almost linearly with increased mating opportunities, as their gametes are relatively cheap (compared to eggs) and the benefits large, unless paternal care is necessary (Bateman 1948). Therefore, because males generally invest relatively less in their offspring than females do, male fitness is usually limited by mating opportunities and number of mates (Bateman 1948; Trivers 1972). Female fitness in contrast, quickly asymptotes with increased mating opportunities as their fitness is more generally limited by factors such as egg load and resources required for parental care (Arnqvist & Nilsson 2000; Arnqvist et al. 2005). As females typically invest more per offspring, they are generally more discriminating when choosing mates, and this is the cornerstone of sexual selection

theory (Andersson 1994). Theoretically, females can obtain all the resources that mating offers, such as sperm, from relatively few matings, and therefore additional matings offer females diminishing returns, whilst the ecological costs of each new mating (e.g. the loss of time and increased risk of predation and infection), remain relatively constant (although search time can be abated by males occurring in close proximity). This leads to a sexual conflict over the optimal mating rate and the prediction that females should favour lower mating rates than males (Johnstone & Keller 2000; Arnqvist & Rowe 2005). However, since the advent of paternity testing it has become clear that females of many phyla and species mate with many more partners than expected or required to fertilize a full complement of eggs (Birkhead & Moller 1995; Fedorka & Mousseau 2002). This is especially perplexing as it contradicts not only theory but also empirical results documenting the often substantial costs to females of mating such as an increased risk of predation, exhaustion of time and energy, risk of injury, parasites, and diseases, plus harmful effects of the male ejaculate (Daly 1978; Fowler & Partridge 1989; Arnqvist 1989; Rowe et al. 1994; Chapman et al. 1995; Telford & Webb 1998; Stockley & Seal 2001; Stutt & Siva-Jothy 2001; Blanckenhorn et al. 2002; Martin & Hosken 2003; Maklakov & Lubin 2004). Although one should take care to differentiate between ecological costs and those costs arising from mate resistance, copulation, and insemination, as these costs are not the cause of sexual conflict, but rather the result (Watson et al. 1998; Holland & Rice 1999; Pitnick et al. 2001; Martin & Hosken 2003; Kuijper et al. 2006). Sexual conflict still occurs even if males and females have the same optimal mating rate, because males are favoured to prevent their mates from mating or reproducing with other males in the future.

Understanding why females are polyandrous (mate more than one male) is therefore crucial to the fields of sexual selection and sexual conflict. Traditionally studies have aimed to measure the costs and benefits of mating in polyandrous species (Tregenza & Wedell 1998; 2002; Evans & Magurran 2000; Konior et al. 2001; Worden & Parker 2001; Fedorka & Mousseau 2002; Kamimura 2003). Whilst valuable, this approach can sometimes be problematic because data from polyandrous species potentially confound the origin of polyandry with the maintenance of polyandry. The selection favouring the origin of polyandry may differ from that favouring its maintenance. This may in part be due to a change in the costs and benefits of mating as a result of the co-evolutionary dynamics of sexual conflict (i.e. different patterns of selection on males and females: Morrow & Arnqvist 2003; Arnqvist & Rowe 2005; Ronn et al. 2007). An alternative approach is to examine the origin and spread of polyandry, in response to a change in the selective environment, either in the laboratory or in the wild (Torres-Vila et al. 2002; Harano & Miyatake 2005; Burton-Chellew et al. 2007a; Chapter 2). Another problem that can arise is when the experimental design allows females to select their own mating rate, and remating propensity is condition or size dependent in that species, then this can confound the estimates of the costs and benefits of mating (Torres-Vila et al. 2004).

I studied the costs and benefits of mating in the parasitic wasp *Nasonia vitripennis*. In contrast to the result from field populations, females of *N. vitripennis* kept under laboratory conditions have been shown to evolve polyandry (Burton-Chellew et al. 2007a; Chapter 2). This change is a result of a change in female behaviour, as the

remating propensity of females was the same whether paired with males from wild or laboratory adapted males (Burton-Chellew et al. 2007a; Chapter 2). This suggests that the different 'ecology' that results from keeping wasps in the laboratory has altered the selection pressures that influence selection for polyandry. By investigating the costs and benefits of mating in this species, I hoped to discover which selection pressures are favouring the maintenance of monoandry in the wild and the spread of polyandry in the laboratory. The wild-type monoandry may not even be an adaptive female strategy, but rather, the result of male manipulation, with males having gained the upperhand in an ensuing sexual conflict. In this case polyandry may be beneficial and there could be evidence of other male effects on female fitness stemming from post-copulatory conflict. Such effects could be the induction of early oviposition at supraoptimal rates, and/or female harm, favoured because it reduces the female benefits of polyandry (Johnstone & Keller 2000) and/or female harm as a pleiotropic side affect of male attempts to influence female receptivity or oviposition (Civetta & Clark 2000; Morrow et al. 2003), or sperm competition (Crudgington & Siva-Jothy 2000; Morrow & Arnqvist 2003; Edvardsson & Tregenza 2005) or even of female mate choice (Pitnick & Garcia-Gonzalez 2002).

I tested for a cost of mating in terms of reduced female longevity. Such a cost, if found, would be indicative of sexual conflict (Holland & Rice 1999). I also used four different strains, two of which are adapted to the laboratory (30 months and have high remating rates), and two of which are relatively new to the laboratory environment (7 months), and presumably have low remating rates (although this has

not been specifically tested in these lines). If mating is harmful to all strains or only for laboratory strains, then this would give an insight into the level of conflict in the wild and in the laboratory. Specifically, if mating is only harmful for laboratory strains, then this suggests that males have adapted to females becoming polyandrous and importantly suggests that females are monoandrous in the wild for their own reasons. However, if mating is harmful for females from all strains, this suggests that there is conflict in the wild, which requires that polyandry is beneficial. Therefore the monoandry of wild females could be a result of male manipulation (if females were monoandrous anyway, there would be no advantage to males harming them as their interests would be aligned), or a latter response to males evolving harm. Or mating may only be costly for femnales from wild strains, in which case the laboratory adapted females may have evolved to subsume the costs of mating in response to increased exposure to males. The results of this experiment will not provide any definitive answers, but will allow one to speculate and to design future experiments accordingly.

I also tested for an effect on fecundity using the same strains. Admittedly a fecundity increase is not the only possible benefit of polyandry (Simmons 2005), but it is a direct benefit and thus relatively easy to measure, compared to the possible indirect benefits such as improved offspring quality (Tregenza & Wedell 1998; 2002; Wedell & Tregenza 1999). Plus, the general importance of increased fecundity as a direct fitness benefit of polyandry has been shown by a recent meta-analysis (Arnqvist & Nilsson 2000).

3.3 METHODS

3.3.1 Study Organism

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae). For details on *Nasonia* biology please refer to Section 1.2.

3.3.2 Experimental Strains

I used four strains (Hv2, Hv4, Hv236, Hv307), all of which were originally collected from the Hoge Veluwe, either by Prof. L. Beukeboom of the University of Groningen, Netherlands (Hv236 and Hv307; July 2002), or by myself in collaboration with E.M. Sykes (University of Edinburgh) and T. Koevoets (University of Groningen, Netherlands) (Hv2 and Hv4; August 2004).

3.3.3 Design of Experiment 3.1

I measured the longevity and fecundity of females from four different strains in response to being mated zero, one, or two times by males from their own strain. I initiated the experiment with 36 females per treatment (x3), per strain (x4), totalling 432 females. However the true sample sizes varied slightly due to accidental mortality, escape, and loss. I kept females in isolation from the moment I sexed them as pupae until I introduced them to a virgin male on their second day post eclosion.

Virgin females are very willing to mate and show no discernible mate choice when presented with a virgin male (Whiting 1967; Van den Assem & Jachmann 1999;

Burton-Chellew et al. 2007a; Chapter 2). In contrast, mated females are generally unwilling to re-mate (Van den Assem & Jachmann 1999; Burton-Chellew et al. 2007a; Chapter 2) and microsatellite pedigree-marker data indicate that polyandrous females are very rare in the wild (only 2 of 49 foundresses sampled were polyandrous; Grillenberger et al. 2008). However, the propensity of females to remate increases with the number of generations their strain has undergone lab culture (Van den Assem & Jachmann 1999; Burton-Chellew et al. 2007a; Chapter 2).

To avoid the potential problem of females selecting their own treatment level, I utilized a novel feature of *N. vitripennis* mating and courtship. After copulating, for around 14 seconds, *N. vitripennis* males return to the female's thorax/head region and perform what is currently termed a 'post-copulatory courtship', although the function of this remains unknown. It is unknown if it serves a common interest between the sexes or not. The manoeuvre may serve to communicate to the female that the copulation attempt is complete, or it may be an attempt by the male to influence the future mating and oviposition behaviour of the female (Eberhard 1996; Edvardsson & Arnqvist 2000). I prevented males from performing this post-copulatory courtship, which then allowed me to introduce a second male with increased chances of mating success. This secondary male then performs a standard courtship, and is able to induce receptivity and can copulate with the female. This effect is largely restricted to just two males; a repeat of the method with the second male, and the introduction of a third male, does not appear to work (conclusion from pilot study).

3.3.4 Measuring Longevity

I checked the females each morning for mortality. I did not provide the females with honey solution as a food source, but they could choose to feed upon the hosts I provided for oviposition.

3.3.5 Measuring Fecundity

I measured fecundity as the total number offspring emerging from parasitized hosts plus any diapause larvae that were found in the hosts. I provided females with up to 22 hosts to parasitise in their lifetime, in 3 separate batches of 6, 8, and 8 hosts. Females that died young therefore had access to fewer hosts. However any female living for 10 days or more had access to all 22 hosts. The overall experimental timeline was as follows; Day 2 - mated; Day 4 - provided with 6 fresh hosts; Day 7 - provided with 8 fresh hosts; Day 10 - provide with 8 fresh hosts.

3.3.6 Measuring Size

Size was determined after death by measuring the length of the right hind tibia using a Leica dissecting microscope (x100) and ocular micrometer. Tibia length is the most commonly used measure of body size in parasitoid wasps (Godfray 1994).

3.3.7 Statistical Analysis

I fitted general linear models, using stepwise deletion for longevity and fecundity using the JMP IN software, version 5.1 (SAS Institute Inc.). Initially, I fitted a full model to the data, including all explanatory variables and their interactions. I then removed terms from the full model by stepwise deletion until a minimum adequate

model remained, containing only significant interactions ($P > 0.01$) and their components, plus all significant main effects ($P > 0.05$) (Crawley 1993).

3.4 RESULTS OF EXPERIMENT 3.1

3.4.1 Longevity and the Cost of Mating

Mated females had the same longevity as virgins, although there was a negative trend ($F_{1,365} = 0.8$, $P = 0.37$; fig. 3.1).

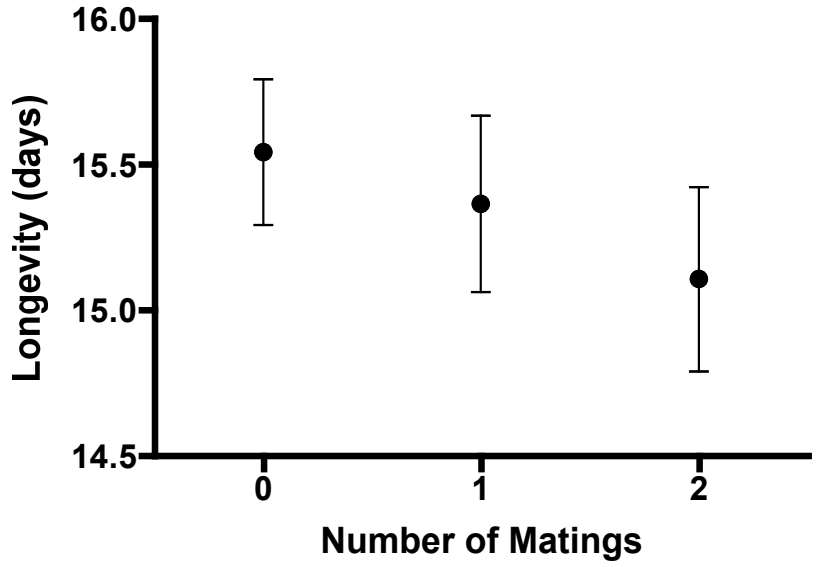


Figure 3.1. Female longevity with number of matings (by different males).

Strains varied in their longevity ($F_{3,366} = 3.8$, $P = 0.01$; table 3.1), which was positively correlated with female size ($F_{1,367} = 23.2$, $P < 0.0001$).

Table 3.1. A comparison of the mean size, longevity, and fecundity of the four strains.

Strain	Size (hind tibia length µm)	Longevity (days)[1]	Fecundity (emerged offspring)[2]
Hv2	666.7 ± 3.6	15.9 ± 0.3	89.1 ± 9.3
Hv4	668.5 ± 4.0	14.4 ± 0.6	80.0 ± 9.2
Hv236	659.4 ± 4.5	16.8 ± 0.3	104.1 ± 11.8
Hv307	662.2 ± 4.8	15.0 ± 0.6	79.7 ± 8.8

[1]To avoid the effects of mating, this is the longevity of virgins only.

[2]To avoid any effects of virginity or polyandry, this is the fecundity of once mated females only.

The number of times a female mated interacted with size in a way that suggests mating may be costly, but only for small females ($F_{1,365} = 3.2$, $P = 0.07$; fig. 3.2).

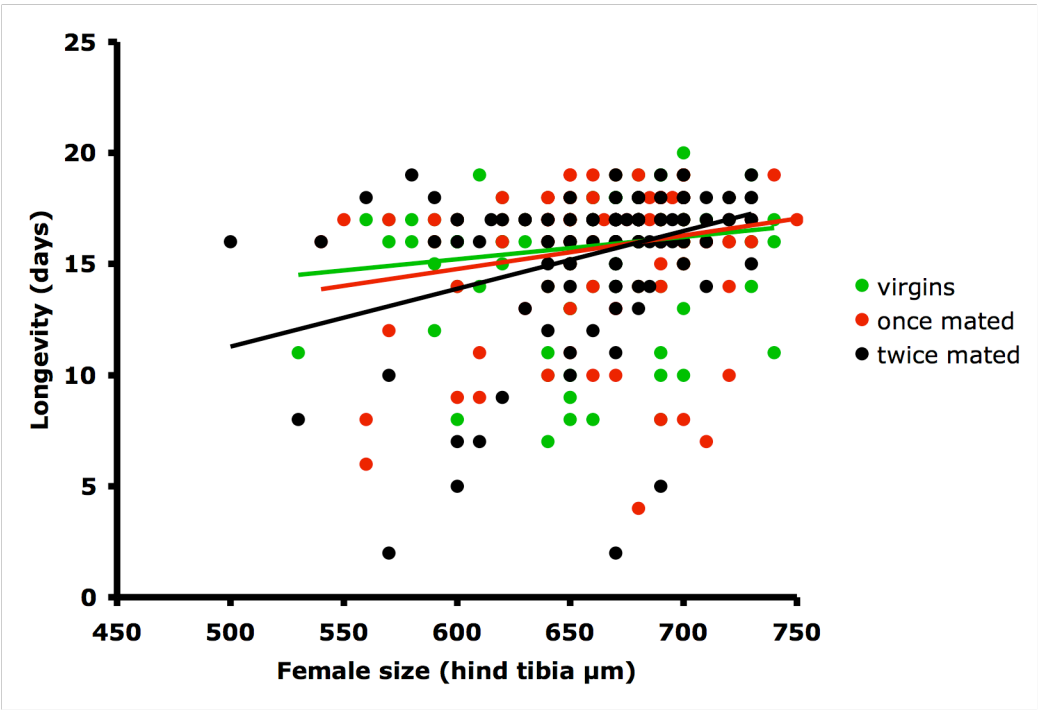


Figure 3.2. Female longevity by female size (hind tibia length, µm).

Analyzing the relationship between size and longevity within mating treatments, I found that size is increasingly significant with more matings, suggesting that the effect of size upon longevity is greater when paying the cost of mating (positive correlation between size and longevity; for virgins; $b = 0.011 \pm 0.005$, $F_{1,128} = 4.0$, $R^2 = 0.02$, $P = 0.05$; for once mated females; $b = 0.015 \pm 0.007$, $F_{1,117} = 5.3$, $R^2 = 0.04$, $P = 0.02$; for twice mated females; $b = 0.026 \pm 0.007$, $F_{1,118} = 15.9$, $R^2 = 0.12$, $P = 0.0001$; fig. 3.2).

Examining the strains separately, size was correlated with longevity for three of the four strains (not for Hv4), but number of matings still had no significant effect (fig. 3.3).

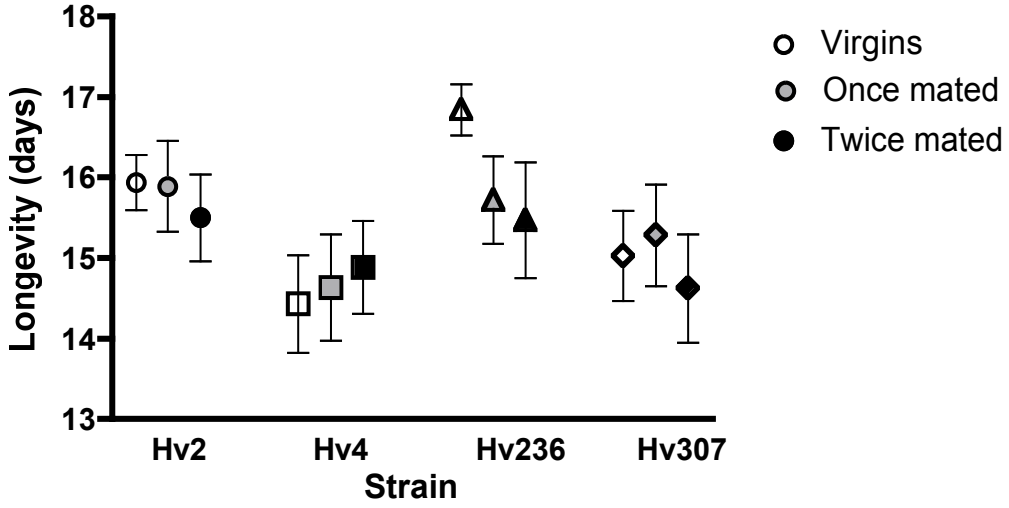


Figure 3.3. Female longevity by strain and the number of matings.

Again, the interaction between size and the number of matings was significant, in one strain (Hv2; $F_{1,75} = 4.4$, $P = 0.02$). When I investigated within mating treatments

for strain Hv2, size was only significantly correlated with longevity for double mated females (positive correlation between size and longevity; for virgins; $b = 0.011 \pm 0.011$, $F_{1,29} = 1.0$, $P = 0.32$; for once mated females; $b = 0.006 \pm 0.009$, $F_{1,22} = 0.4$, $P = 0.52$; for twice mated females; $b = 0.068 \pm 0.019$, $F_{1,24} = 12.8$, $R^2 = 0.35$, $P = 0.002$).

3.4.2 Fecundity and the Benefits of Mating

Females that mated twice had higher fecundity ($F_{1,361} = 3.4$, $P = 0.06$; fig. 3.4) but this effect depended on the strain ($F_{3,361} = 4.8$, $P = 0.003$; fig. 3.5).

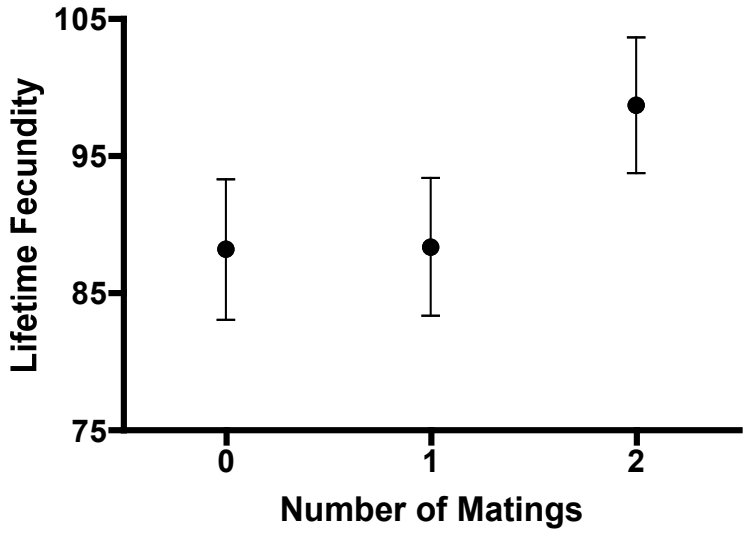


Figure 3.4. Lifetime female fecundity by the number of mates.

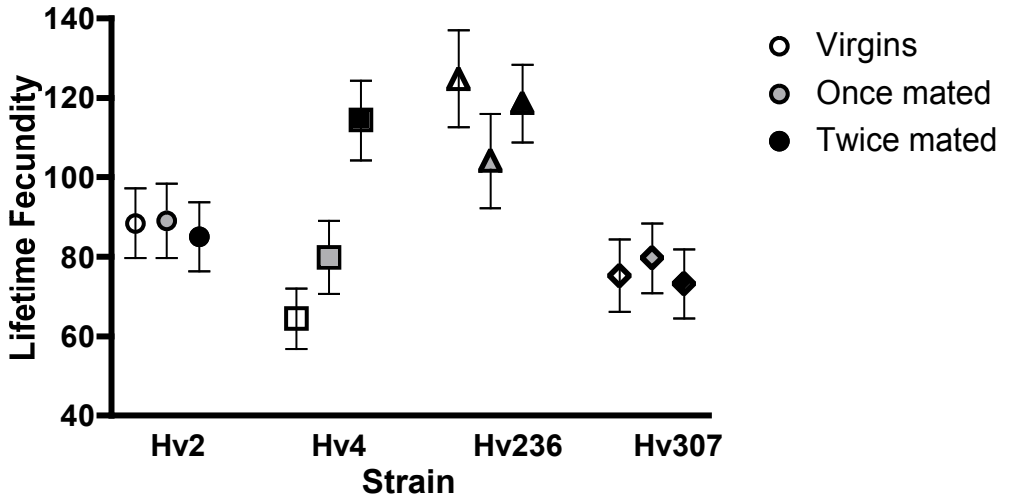


Figure 3.5. Lifetime female fecundity by strain and the number of matings.

Strains varied in their fecundity ($F_{3,361}$ = 15.0, P < 0.001; table 3.1), which was positively correlated with female size ($F_{1,361}$ = 71.1, P < 0.0001; fig. 3.6).

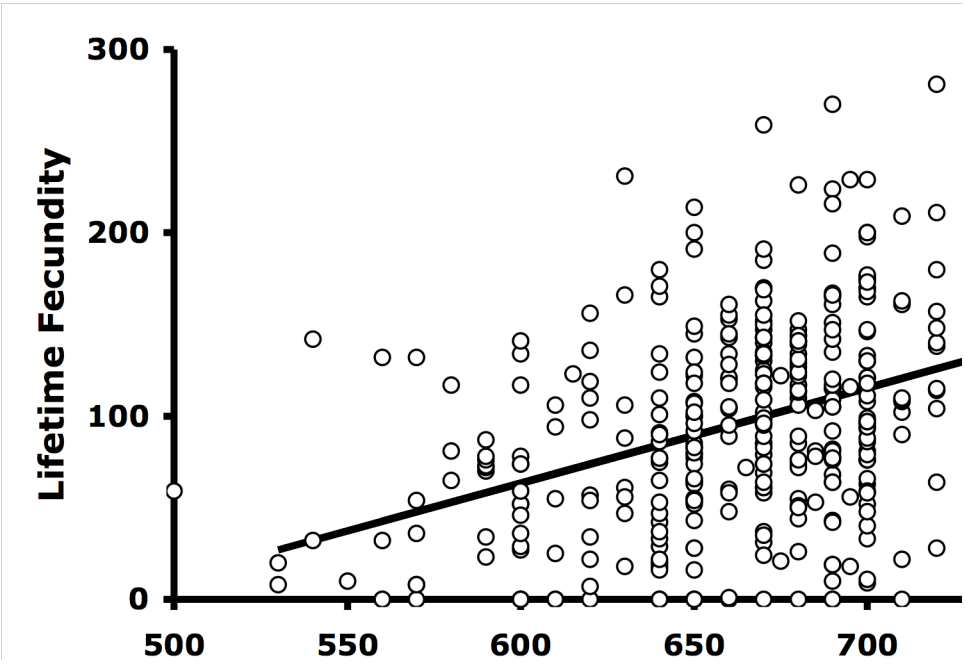


Figure 3.6. Lifetime female fecundity against female size.

Analyzing the strains separately reveals that within strain Hv4, females that mated twice had higher fecundity (Hv4: $F_{1,82} = 21.9$, $R^2 = 0.20$, $P < 0.0001$; virgin mean = 64.5 ± 7.5, once mated mean = 79.9 ± 9.2, twice mated mean = 114.3 ± 10.0; fig. 3.5). Females that mated twice did not have increased fecundity in the other three strains. Closer inspection of the oviposition behaviour of strain Hv4 reveals that the increased fecundity is only apparent in the first two batches of hosts offered (fig. 3.7).

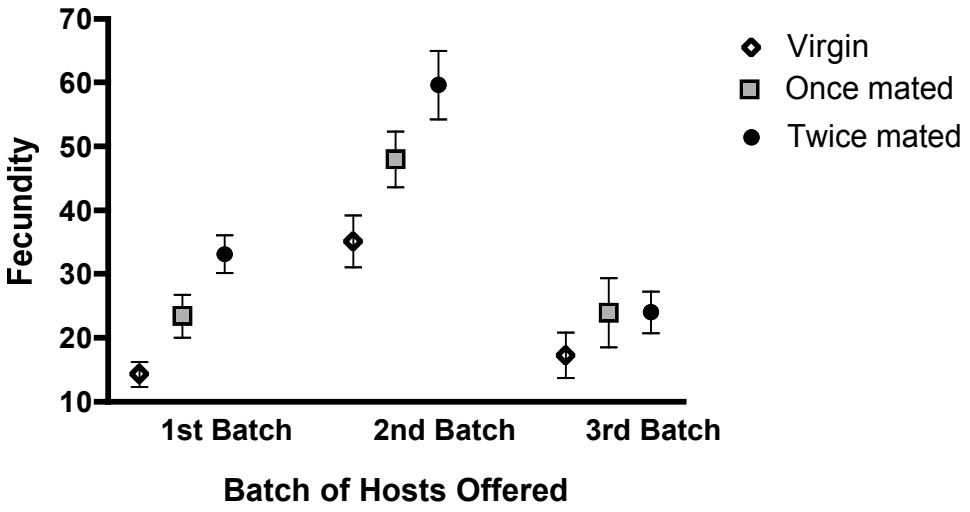


Figure 3.7. The fecundity of Hv4 females by number of mates and across batches of hosts (N.B. batch 1 contained 6 hosts, the others 8).

For the first batch of hosts offered, fecundity was positively correlated with both size ($F_{1,82} = 6.7$, $P = 0.01$) and the number of mates ($b = 10.6 \pm 1.9$, $F_{1,82} = 32.0$, $R^2 = 0.26$, $P < 0.0001$). For the second batch of hosts offered, fecundity was only correlated (positively) with the number of mates ($b = 12.3 \pm 3.2$, $F_{1,103} = 14.8$, $R^2 = 0.13$, $P =$

0.0002), whereas for the third batch, neither size nor the number of matings were correlated.

3.5 DISCUSSION

My experiment shows that mating, either one or two times, generally has very little effect, if any, upon female longevity and fecundity. However, it did significantly increase the fecundity of one strain (Hv4; fig. 3.7). There may be a cost to mating, in terms of reduced longevity, but this may only apply to small females, and maybe to only one strain (Hv2).

The lack of an effect of mating upon female longevity is in contrast to a significant and large negative effect of mating upon male longevity (Burton-Chellew et al. 2007b; Chapter 4), and this gender difference is repeated in the male-dimorphic dung beetle, *Onthophagus binodis* (Kotiaho & Simmons 2003). The discrepancy in the cost of mating may be because in Burton-Chellew et al. (2007b) the males were kept with four females for a 24 hour period, which is arguably more energetically demanding than just one or two copulations: it is unknown if merely one or two copulations are sufficient to induce a decrease in male longevity. Another difference between the sexes is that size was positively correlated with female longevity and fecundity in this study but in Burton-Chellew et al. (2007b; Chapter 4), size had no effect on male longevity, reproduction, or mating ability. This difference in fecundity is probably because size more directly affects egg formation and maturation than

spermatogenesis (Rivero & West 2002). The difference in longevity may only apply to mated females, as size was no more than marginally significant for virgins.

There are at least two possible reasons for my negative result in females. First, although I measured the longevity of females in response to zero, one, or two copulations, I potentially confounded longevity with reproductive effort. It would be useful to measure the longevity of females in the absence of hosts. Second, I limited the maximum number of matings to just two. A larger range of matings may have produced a greater, more detectable effect, but this is difficult to achieve (see Section 3.3 Methods, and for a discussion of experimental designs and their outcomes see Arnqvist & Nilsson 2000; Torres-Vila et al. 2004).

The direct benefits obtained by a female that mates compared to a virgin are clear: although haplodiploidy allows virgin females to lay sons, a mated female can lay both sons and daughters, and thus lay female biased sex ratios as is typically adaptive for this species (Hamilton 1967; Werren 1983; Stubblefield & Seger 1990; Shuker & West 2004; Shuker et al. 2005). There does not appear to be a fecundity benefit to mating in general or to additional mating, except for one strain. Mating may still affect longevity or fecundity under different conditions though. Various costs and benefits of mating and reproduction have been shown to be dependent upon nutritional condition in insects (Rivero-Lynch & Godfray 1997; Rivero & West 2005). For example, the cost of being small depends upon the availability of food in *N. vitripennis* (Rivero & West 2002), and the provisioning of food removes the cost of egg production in *Callosobruchus maculatus* (Tatar & Carey 1995), but allows for

females to mate more often, despite the costs, in *Drosophila melanogaster* (Chapman & Partridge 1996a). Female *N. vitripennis* are able to adjust their metabolism in response to factors such as nutrition and their size, which is correlated with lipid reserves, and thus they may also adjust in response to additional mating, but only when well fed (Rivero & West 2002). My females may have been unable to capitialize on any benefits of polyandry because despite being able to feed upon the hosts they were offered, they could not do so without impinging on their fitness. This is because fed upon hosts are a poor resource for their offspring (Rivero & West 2005). It would be useful to test for mating benefits under different feeding regimes. Polyandry may also be beneficial, but only to females with certain genotypes or in good condition. Unfortunately, the experimental method of assigning females randomly to each mating treatment, whilst valuable, can also bias against the detection of any benefits of polyandry in monoandrous species (Torres-Vila et al. 2004). This is because the pool of females assigned to the polyandry treatment may contain many females that are unable to respond or reap the benefits and thus the mean benefit is diluted.

Polyandry may also be beneficial indirectly, through increased offspring fitness, or by increased brood heterogeneity (Yasui 2001). Increased offspring quality has been shown in response to increased mate number in other insects and arachnids (Tregenza & Wedell 1998; Konior et al. 2001; Ivy & Sakaluk 2005), although the effects on sons in bulb mites appear to be the result of maternal effects (Kozielska et al. 2004). It would be useful if indirect fitness benefits such as improved offspring

quality, itself a very difficult parameter to measure, or the number of grandchildren, were measured in response to mating.

Why did strain Hv4 show increased fecundity when the other strains did not? This is unlikely to be a type I error (false positive) as I only tested four strains and the p-value is extremely small ($P < 0.0001$). Hv4 was also the only strain where size did not affect longevity. Increased fecundity in response to mating can be difficult to interpret because it can be explained by either cryptic female choice under sexual selection models (Eberhard 1996; Cordero & Eberhard 2003), or by male manipulation under models of sexual conflict (for examples of males stimulating females to increase oviposition rate see Chapman et al. 1995; Chapman 2001). Under polyandry an increased proportion of females will have mated an above average male (this truism is equally true for below average males), and if females can cryptically respond to mate quality by adjusting their fecundity, then the mean fecundity will be higher for polyandrous females. The alternative explanation under sexual conflict is that males in polyandrous species are favoured if they can increase their mates' immediate reproductive effort to above optimal levels, to counter against decreasing paternity assurance for future female reproductive events. Therefore, the early 'benefit' exhibited by both mated and double-mated Hv4 females (fig. 3.7) is suggestive of male manipulation (for an example of males stimulating earlier oviposition in females see Chapman et al. 2001). This male manipulation does not have to be ubiquitous (i.e. present in all strains), as an allelic polymorphism for a male induced cost of mating has been shown in *D. melangoaster* (Fiumera et al. 2006). One possible reason I detected no effects of mating in the other strains may

be because they have undergone correlated evolution between the sexes, with each sex shifting its biology in response to the adaptations of the other, and thus obscuring any underlying conflict and manipulation (Rowe & Arnqvist 2002; Rowe & Day 2006).

zzz Another problem with not controlling the mating rate between monandrous and polyandrous females is that if mating is costly, but polyandry is beneficial, then these costs and benefits may cancel each other out. Mating was not costly for my females, although there was nearly a significant interaction that suggested mating was costly, but only for small females. This could result in females being conditionally polyandrous, depending on their size, which has been shown elsewhere, such as in the polyandrous gift-giving butterfly *Pieris napi* (Bergstrom et al. 2002; Bergstrom & Wiklund 2002); and in the cellar spider, *Pholcus phalangioides* (Schafer & Uhl 2005). One could test to see if polyandry in laboratory-adapted females is more likely in larger females.

My ultimate aim was to understand why female *N. vitripennis* are monoandrous in the wild and polyandrous in the laboratory? Presumably if mating is costly then a higher mating rate will not evolve, but males can increase the optimal mating rate of females by either increasing the benefits of mating, or by increasing the costs of not mating (Clutton-Brock & Parker 1995). If an additional mating offers females a net cost then they are favoured to not mate, but if this cost is smaller than mate rejection, then they are favoured to copulate, out of convenience. Such 'convenience

polyandry' predicts that females evolve higher mating rates in order to reduce the negative consequences of persistent courtship and male harassment (Thornhill & Alcock 1983; Rowe 1992; Clutton-Brock & Parker 1995; Watson et al. 1998). Courtship is probably costly for male *N. vitripennis* (Burton-Chellew et al. 2007b; Chapter 4), but it is unknown if male courtship and attention is costly for females, as it is for female *D. melanogaster* (Friberg & Arnqvist 2003). If it is, then female *N. vitripennis* which mate may suffer less than those that persist to resist. The convenience hypothesis could be tested by measuring if i) courtship or male attention is costly to females, and ii) that higher mating rates actually reduce the amount or intensity of courtship endured. The acceptance of a male does not necessarily preclude subsequent attention from other males.

When *N. vitripennis* mating takes place under laboratory conditions there are many males competing for the females and competing males often interrupt and interfere with the attempts of courting males. Females may also become receptive under courtship from one male but be inseminated by a different male that profits opportunistically before the courting male has moved into position (Van den Assem et al. 1980a). Additionally, females are exposed to unnaturally high levels of male pheromones, a key component of male courtship (Van den Assem et al. 1980b; Van den Assem et al. 1981). It is possible that artificially high levels of pheromones induce female receptivity when they are not the focus of male attention, and thus their receptivity may not always lead to copulation (Van den Assem & Jachmann 1999). If females are fixed to only become receptive once, then this runs the risk of them not gaining any sperm. In either case, a change in female receptivity would not

be as a result of a change in the optimal mating rate (i.e. not because females are obtaining more benefits or avoiding the costs of male harassment), but instead a necessary change in order to still achieve the unaltered optimal mating rate under different conditions.

In summary, there is no good evidence that laboratory adapted females benefit more from mating than recently collected females, or that either set of females 'pay' more for mating. Therefore the evolution of polyandry in *N. vitripennis* remains an open question. The cost-benefit ratio of polyandry need not be equivalent for different sized females. It would be interesting to further explore the interaction between female size and mating upon longevity. It has been shown that female remating propensity can depend on female size, with smaller females less likely to remate (Bergstrom et al. 2002; Bergstrom & Wiklund 2002; Schafer & Uhl 2005). This may be because smaller females are unable to withstand the costs of multiple-mating. If mating truly is harmful in *N. vitripennis*, even if only for smaller females, then this is indicative of sexual conflict in the wild. Combined with the fecundity effect (in strain Hv4), which could be beneficial or the result of male manipulation, it may well be that wild males are collectively harming females and altering their oviposition behaviour. In this case, the wild monoandry may be because i) females have counter adapted their behaviour in response to male harm by only mating once, i.e. the benefits of polyandry are now outweighed by the increased costs of mating, or ii) because males have, along with harming and maybe altering oviposition, successfully manipulated females to be refractory to mating for longer. Quite how selection would maintain all these male traits is admittedly problematic, but it may

be that males vary for these abilities, both qualitatively and quantitatively, and that polyandry exists at low levels in the wild as either a female polymorphism, or as a result of male polymorphisms in the ability to manipulate females.

CHAPTER 4

4. The Cost of Mating and the Relationship between Body Size and Fitness in Males

This chapter was published in Evolutionary Ecology Research, as: Maxwell N. Burton-Chellew, Edward M. Sykes, Sophie Patterson, David M. Shuker, and Stuart A. West (2007). The cost of mating and the relationship between body size and fitness in males of the parasitoid wasp *Nasonia vitripennis*.*9: 921-934*.

4.1 SUMMARY

Question: Does male size affect fitness in gregarious parasitoids?

Hypothesis: Larger males achieve higher reproductive success by obtaining more matings when in a competitive scenario and by living longer. Although mating may be costly, larger males are better able to withstand these costs.

Methods: Three experiments: two assessed the effect of size on mating success, one with, and one without, the presence of a competitor; the third experiment explored the relationship between male size and longevity under alternative mating regimes.

Results: Mating success did not depend on male size even in the presence of an introduced competitor. Mating reduced male longevity, but it did so independently of size.

4.2 INTRODUCTION

The relationship between body size and fitness is predicted to influence a large number of reproductive behaviours (Stearns 1992). This relationship has attracted particular attention in work on parasitoid wasps, where theory predicts that it will influence behaviours such as host choice, host feeding, clutch size, superparasitism, and sex allocation (Godfray 1994). In solitary parasitoids, for example, where only one egg develops per host, it is commonly observed that female eggs are laid in relatively large hosts, and male eggs in relatively small hosts (West & Sheldon 2002). The explanation for this appears to be that larger wasps emerge from larger hosts, and that females gain a greater benefit from increasing body size than males (Charnov 1979; 1981). There is a considerable body of empirical knowledge detailing how female fitness varies with size in parasitoids, and recent studies have even begun to examine the underlying physiology (Kazmer & Luck 1995; Visser 1995; West et al. 1996; Ellers et al. 1998; Ellers & Jervis 2003; Rivero & West 2002; 2005). In contrast, there is a much poorer understanding of how body size influences fitness in males (Van den Assem et al. 1989; Heinz 1991; Kazmer & Luck 1995).

A number of recent studies have emphasised that the male size-fitness relationship can also influence sex allocation and male life history evolution in gregarious parasitoid species, where multiple wasps are able to develop in each host. In gregarious parasitoids, sex allocation is often dominated by local mate competition (LMC), where competition between brothers and sibmating favour the evolution of female biased sex ratios (Hamilton 1967; Taylor 1981; Frank 1985a; Herre 1985;

Godfray 1994; West et al. 2005). The male size-fitness relationship can influence sex allocation under conditions of LMC for at least two reasons. First, the mating opportunities and resources available for development can vary over time or between hosts in a patch (Abe et al. 2003a; 2003b; Shuker et al. 2005; Innocent et al. 2007). For example, eggs laid on previously parasitized hosts face greater competition for resources and tend to develop faster (Werren 1983), which can allow them access to more mates (Shuker et al. 2005), or place them in a position to kill competitors (Abe et al. 2003a; 2003b; 2005; Innocent et al. 2007). However, this also leads to smaller wasps, and so any possible advantages will depend upon how body size influences their ability to compete for mates, or their success in combat with competitors (Innocent et al. 2007; Reece et al. 2007). Furthermore, this same trade-off between size and development time will shape the evolution of development time in males. Second, if males and females experience asymmetric resource competition during larval development, then the evolutionary stable (ES) sex ratio (proportion males) is predicted to depend upon how competition for resources influences body size and hence fitness (Godfray 1986; Sykes et al. 2007). In particular, when males and females differentially affect the level of competition experienced by other members of the clutch, the ES sex ratio is biased towards the sex that causes the smaller competitive effect.

Here I use three experiments to investigate the fitness consequences of body size in male *Nasonia vitripennis*. First, I examined whether absolute male size influences mating success. I measured how variation in body size influenced the insemination ability of solitary males presented with ten females for a limited time. Whilst this

scenario is representative of field situations where there is a highly female biased sex ratio, there are also situations in nature when the sex ratio may be less biased, and competition between males will be important (Werren 1983; Molbo & Parker 1996; Chapter 5). Consequently, in my second experiment, I examined whether relative male size influences mating success in a competitive scenario, where two males compete for ten females. Third, the reproductive fitness of adult males will be determined not only by how many females can be inseminated in a given time, but also by other factors such as: the number of daughters that any females they mate produce; how long they can remain reproductively competent; their longevity; their ability, if any, to manipulate female behaviour; and ultimately the survival and reproductive capacity of their offspring. For my third experiment I estimated male fitness by measuring the lifetime mating success (LMS) of males provided with mating opportunities for the duration of their lives. My field data suggest that the emergence period of females on a patch can range from 1 to 19 days (mean 9.00 ±2.36, N = 9; Burton-Chellew *et al*. unpublished data). The mating success of a male will therefore be determined by his ability to inseminate females over time, whilst withstanding the costs of mating in terms of both courtship and insemination. I varied the mating regime in this experiment to examine whether there was a cost of mating, and if this cost differentially affected males of different sizes. For instance, smaller males may suffer a greater reduction in longevity as a result of mating, limiting lifetime mating success.

4.3 METHODS

4.3.1 Study Organism

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae). For details on *Nasonia* biology please refer to Section 1.2.

4.3.2 Experimental Strains

The three experiments utilised two strains: HV7 and STDR. HV7 (hereafter referred to as 'Wild-type') is a relatively out-bred lab-strain created by the mixing of seven previously inbred laboratory strains, all of which were originally collected from the Hoge Veluwe, by Prof. L. Beukeboom (University of Groningen, Netherlands). The red-eye mutant strain STDR (hereafter referred to as 'Red-Eye'), which dates back to the 1950's (Whiting 1954; Saul & Kayhart 1956), is commonly used in experiments because the red-eye phenotype, the result of a recessive allele, provides a useful marker for assigning parentage to progeny.

4.3.3 Experiment 4.1 Size and Mating Success

I tested males of varying size for their ability to court, mate, and inseminate up to 10 females in 15 minutes. In order to generate a large range in male size, I manipulated the foundress number on each host, thereby manipulating the intensity of larval competition. Every male developed in a host that had been presented for oviposition to one, two, or three virgin females as potential foundresses for three days. I used only one male from any given foundress group in the experiment. Female subjects all developed from a host that had been presented simultaneously to two mated females

as potential foundresses for three days. Female size therefore spanned a smaller range. I placed one virgin Wild-Type male in a glass 75 x 10 mm observation vial containing 10 virgin Wild-Type females. All females with a given male came from different mothers. All individuals were less than three days old when tested. After 15 minutes I removed the male and separated the females before giving them hosts to parasitize over a 48-hour period (two batches of three hosts for 24 hours each). I measured male mating and insemination success as the proportion of the ten females that produced daughters (diploid offspring) in any of their six hosts. Pilot trials showed that 15 minutes is the optimum time to differentiate male success (i.e. there is variance in male success). To terminate each trial I placed the test vial in a box of ice for 60 seconds, slowing down the wasps and allowing me to easily separate the males and females with a paintbrush. In total, I tested 99 males over three days. I randomly allocated males from hosts parasitized by one, two, or three females to each day. I determined male size after death by measuring the length of the right hind tibia using a Leica dissecting microscope (x100) and ocular micrometer. Tibia length is the most commonly used measure of body size in parasitoid wasps (Godfray 1994). In *N. vitripennis*, males have longer hind-tibias than females, even though they are smaller in other morphological traits (Whiting 1967).

4.3.4 Experiment 4.2 Size and Mating Success in Competition

I put two males into the same arena and tested for their competitive ability to inseminate up to 10 females in 15 minutes. The experimental protocol was the same as for the above experiment, except that two males, one Wild-Type and one Red-Eye, were placed simultaneously into a vial containing 10 Red-Eye females. The

focal Wild-Type males were generated as in the above experiment. Their competitor Red-Eye males were generated in the same manner except that they all developed in hosts that had been presented to two virgin females as potential foundresses. Therefore the range in Red-Eye male size was much smaller than that of Wild-Type males. Red-Eye females were generated in the same manner as the Wild-Type females for the above experiment. The insemination success of the focal Wild-Type males was measured as the proportion of the ten females that produced daughters (diploid offspring) with the wild-type eye colour phenotype. Females that produced daughters with the red-eye phenotype had mated the competing Red-Eye male. Given the generally low rate of multiple mating (Shuker et al. 2007a; Burton-Chellew et al. 2007a; Chapter 2), I considered it unlikely that any females would have mated both males within the 15 minutes. However some females (12 of 810) produced daughters exhibiting both the wild-type and red-eye phenotype. These females were scored as having mated both males. In total, 81 trials were performed over three days.

4.3.5 Experiment 4.3 Size, Lifetime Mating Success and the Cost of Mating

I measured the longevity and reproductive success of different sized males in response to varying levels of lifetime mating opportunities. Again, in order to generate a large range in male size, I manipulated the foundress number on each host, thereby manipulating the intensity of larval competition. Males emerged from vials containing either one female and four hosts (mean leg length = 723μm ± 3.2), or four females and one host (mean leg length = 664μm ± 5.5; these are significantly

different: $F_{1,102} = 84.58$, $P < 0.0001$). Again, I only used one male per foundress group. I created three mating treatments: (a) solitary unmated males (N = 28); (b) solitary males presented with four females for the first 24 hours of their life, and then kept alone (N = 36); and (c) solitary males kept with four females for their whole life, with the females replaced every 24 hours (N = 41). There was no female mortality within the 24 hours. Unfortunately, as the males lived longer than anticipated, it was not possible to continue to give the males four females each day and towards the end of the experiment I was often forced to provide them with only one female every 24 hours. Also it was not possible to give the males equal number of females. This added noise to my experiment but was random with respect to male size. All wasps were from the Wild-Type strain.

I measured longevity by recording the time of emergence and time of death, with checks being performed approximately every six hours. All males were provided each day with a circle of filter paper soaked in honey solution as a food and water source. Each female that had been presented to a male was then placed in a separate labelled vial with two hosts on which she could feed and oviposit. Male LMS was measured as the number of females that went on to produce daughters.

4.3.6 Statistical Analyses

In experiment one (Size and Mating Success), some females laid only diapause offspring (offspring that are in suspended development and can not be sexed easily as they are yet to develop adult morphologies), so I failed to determine if they had been inseminated or not. Therefore I analysed male success for experiment one as the

proportion of those females laying non diapause offspring that were thus known to have been inseminated. I also analysed the data assuming that all diapause offspring came from either, i) non-inseminated females, or ii) inseminated females. In all cases this did not affect the significance of the results and so I present only the actual known proportions. In experiment two (Size and Mating Success in Competition), I analysed male success both as a function of the focal male's size, and then again as a function of the ratio of his size to that of his competitor (relative size). For experiment three (Size, Lifetime Mating Success and the Cost of Mating), I fitted general linear models, using stepwise regression, for longevity and LMS using the JMP IN software, version 5.1 (SAS Institute Inc.).

I analysed the proportions of females inseminated in all three experiments using the GLMStat software, version 5.7.5 (http://www.glmstat.com). Proportion data usually have non-normally distributed error variance and unequal sample sizes. To avoid these problems whilst retaining maximum power, I analyzed the data with a general linear model analysis of deviance, assuming binomial errors, and a logit link function. The response variable was the number of females inseminated in a sample and the binomial denominator was the total number of females scored as either inseminated or not. This form of analysis weights each data point according to its sample size (total number of females scored as either inseminated or not) and so controls for the fact that different numbers of inseminations were counted from different samples, and that the error variance is greater with small samples. Initially, I fitted a full model to the data, including all explanatory variables and their interactions. I assessed all continuous explanatory variables for non-linearity by

fitting quadratic terms. I then removed terms from the full model by stepwise deletion (Crawley 1993). Whether the removal of a term caused a significant increase in deviance was assessed with a χ^2 test. I checked the appropriateness of my binomial error assumption by comparing the residual deviance with the residual degrees of freedom after fitting the explanatory variable. Large relative values of the residual deviance indicate over-dispersion, which may result in overestimation of significance levels. To account for this, I rescaled the deviance by the heterogeneity factor (HF), the ratio of the residual deviance to the degrees of freedom (McCullagh & Nelder 1983). After correcting for over-dispersion, I used an F-test to test the significance of a term (Crawley 1993). For the sake of consistency figures one and two show the number of females inseminated as proportion data and means are presented ± their standard errors (back-transformed from binomial estimates).

4.4 RESULTS

4.4.1 Experiment 4.1 Size and Mating Success

On average, the proportion of females inseminated was 0.53 ± 0.02, which in actual matings translates to 4.5 inseminations from 8.5 females. The maximum number of average inseminations was 8.5 and not 10, due to the fact that, on average 15% of the females in experiment one laid diapause offspring and so were not scored as inseminated or not. Actual known success varied from zero to eight females inseminated and all males were known to have failed to inseminate at least one

female. Although male mating success varied considerably, it did not depend on male size (fig. 1; $\chi^2_{(1)} = 0.72$, P = 0.40, N = 99). A quadratic term for male size was also not significant ($\chi^2_{(1)} = 0.70$, P = 0.40). Mean male size, which was randomly allocated, did not vary over the days of the experiment (Male size; $F_{1,95} = 1.93$, P = 0.15).

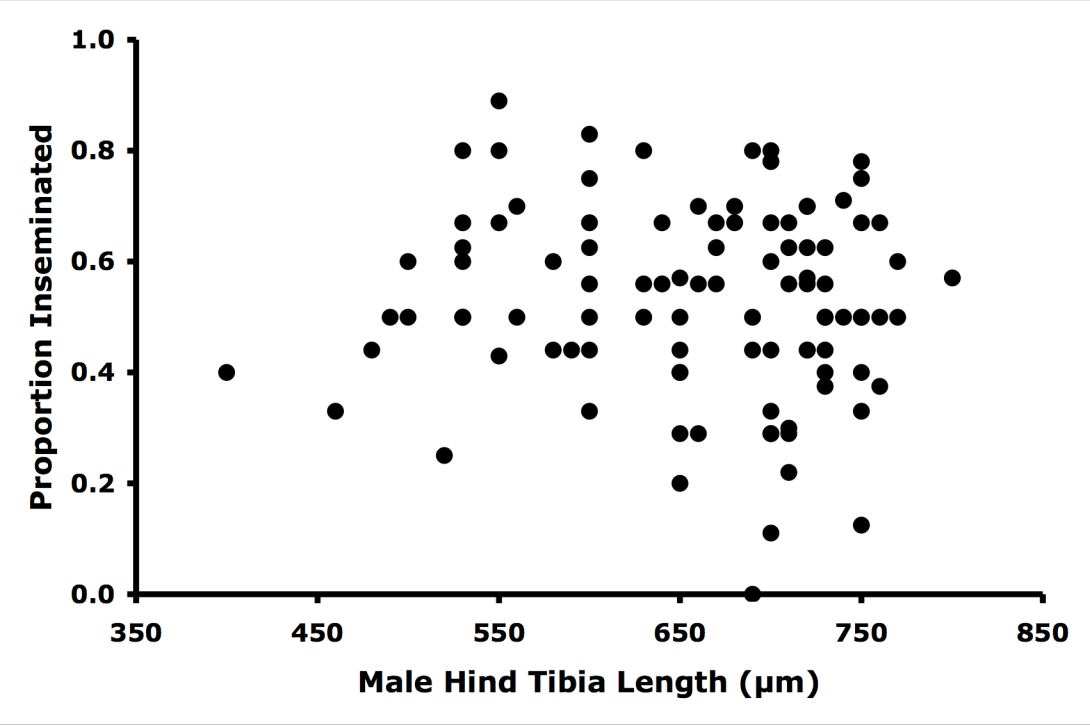
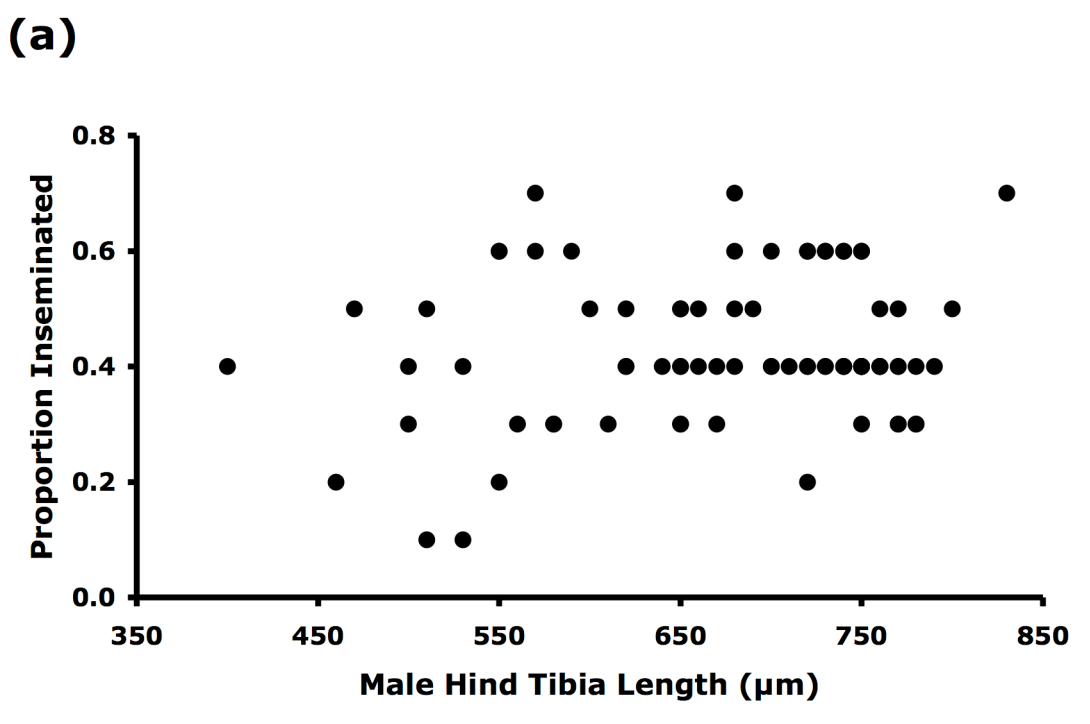


Figure 4.1. The insemination success of solitary males. Body size did not influence the proportion of females that a male could inseminate; when placed with 10 females for 15 minutes.

4.4.2 Experiment 4.2 Size and Mating Success in Competition

On average the two males combined to inseminate 9.1 ± 0.12 of the ten females, with the focal males averaging 4.3 ± 0.14 inseminations. The success of the focal male

was not related to his size ($\chi^2_{(1)}$ = 1.71, P = 0.19, N = 81; fig. 4.2a), or his size relative to that of the other male ($\chi^2_{(1)}$ = 0.80, P = 0.37; fig. 4.2b).



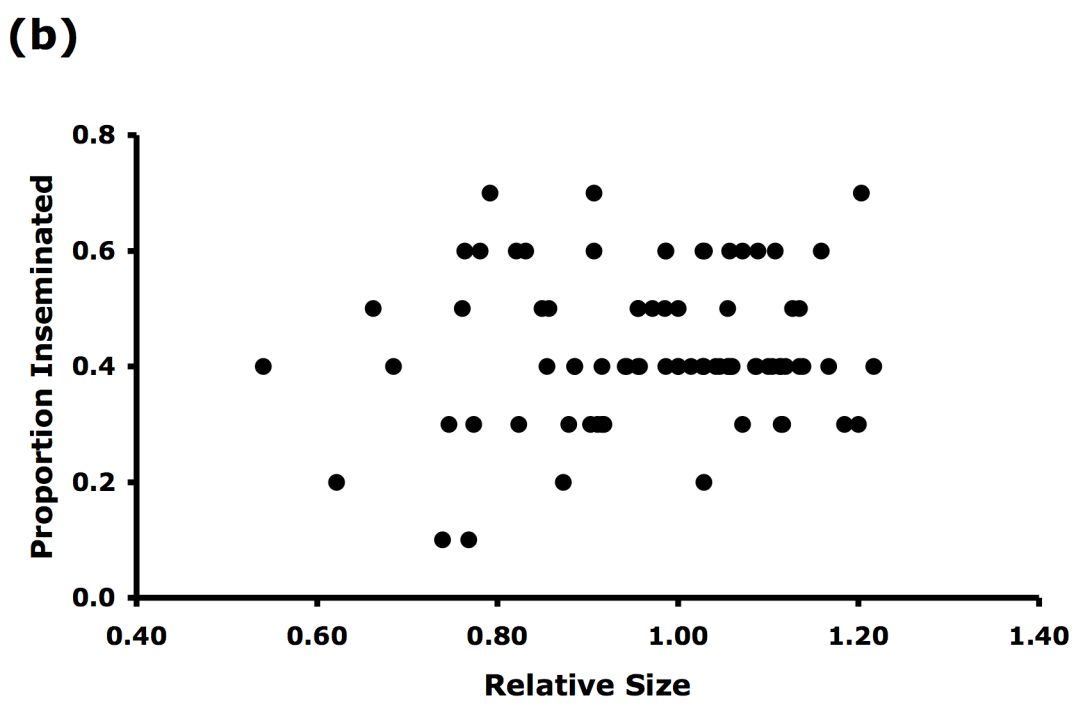


Figure 4.2 (a+b). The insemination success of males in competition. The proportion of females that a focal male inseminated, when placed with 10 females and one other male for 15 minutes, was not significantly influenced by either: (a) the size of the focal male; or (b) the relative size of the focal male to that of his competitor (ratio of focal male leg length to competitor male leg length).

Quadratic terms for focal male size or relative size were also non-significant (focal male size: $\chi^2_{(1)} = 0.47$, P = 0.21; relative size: $\chi^2_{(1)} = 0.41$, P = 0.52). When comparing the success of males in isolation with those in competition, the mean proportion of females inseminated was slightly higher for experiment one, with solitary males obtaining 0.53 ± 0.02, compared to only 0.43 ± 0.14 for males in competition ($\chi^2_{(1)} = 15.43$, P < 0.0001, N = 180).

4.4.3 Experiment 4.3 Size, Lifetime Mating Success and the Cost of Mating

Male longevity was not associated with male size ($F_{1,100} = 2.0$, P = 0.16; interaction term: $F_{3,98} = 1.23$, P = 0.30) but was significantly affected by the mating regime ($F_{2,101} = 20.41$, P < 0.001; fig. 4.3).

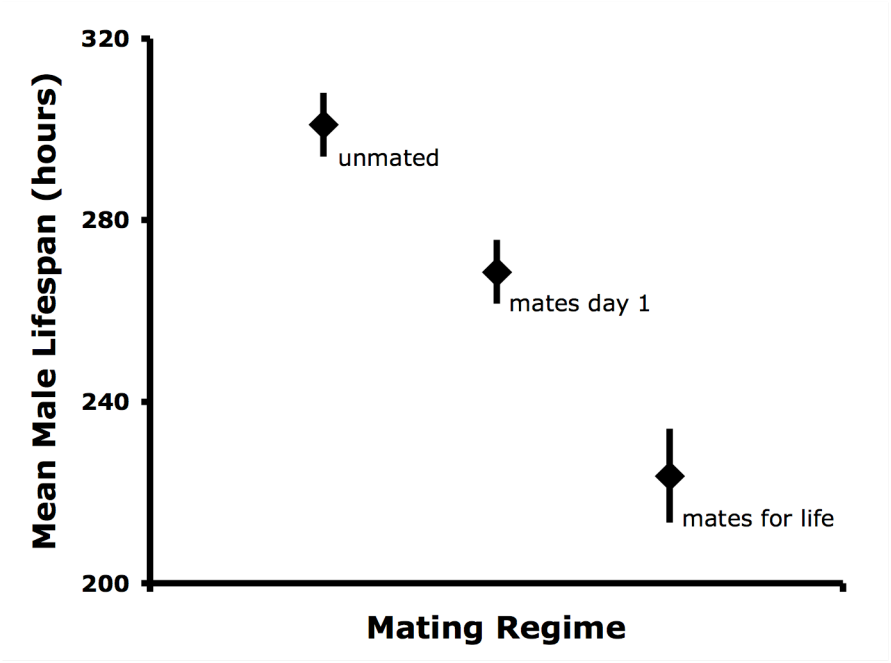


Figure 4.3. The effect of mating on longevity (mean ± SEM). Virgin males live significantly longer than mated males, which in turn live significantly longer than males that had mates for their whole lives.

Male size still had no effect on longevity when examined within each mating regime: unmated males ($F_{1,26} = 0.41$, $P = 0.53$), mated first day ($F_{1,34} = 3.40$, $P = 0.07$), and mates throughout life ($F_{1,39} = 1.33$, $P = 0.26$). Lifetime mating success, which was predicted to increase with longevity as a result of my experimental design, did not depend on male size ($F_{1,39} = 0.69$, $P = 0.41$; fig. 4.4). When I controlled for longevity, by fitting longevity, male size, and the corresponding interaction, longevity was a significant main effect as expected ($F_{1,39} = 18.45$, $P = 0.0001$), but male size was still non-significant ($F_{1,39} = 0.03$, $P = 0.86$), and the interaction was also non-significant ($F_{2,39} = 0.95$, $P = 0.37$).

Figure 4.4. The relationship between male size and total number of females inseminated (LMS). Male size had no effect on the total number of females inseminated, even when controlling for longevity or the number of females offered (treatment C only).

Although it is not an independent analysis, I also checked whether the same result held for the proportion of females inseminated and thus controlled for the number of females offered to a male. The proportion of females inseminated by a male across its lifetime was not significantly correlated with male size ($F_{1,39} = 1.22$, $P = 0.28$, HF = 1.82).

4.5 DISCUSSION

Male size had no significant affect on my measures of fitness in all three experiments. These experiments examined mating success when alone (fig. 4.1),

mating success when in competition (fig. 4.2), and lifetime mating success (LMS) when provided with daily access to mates (fig. 4.4). My final experiment also allowed me to examine whether there was a cost of mating to males in terms of reduced longevity. When males were allowed to mate greater numbers of females this led to reduced lifespan, but this cost did not depend upon the male's size (Fig. 4.3).

Experiments one and two were designed to match the scope of competition and number of potential mates that male wasps experience in the field, where extremely female biased sex ratios are common (Werren 1983; Molbo & Parker 1996; Chapter 5). Experiment one tested the speed at which males can court and copulate with multiple females. A possible limitation of my design was that the potential benefits of being large, such as increased energy reserves or sperm production, would only be relevant over longer periods than 15 minutes. However this limitation is addressed in experiment three. Experiment two tested the influence of male size in competition. Although the design was the same as experiment one but for the addition of another male, male size could be expected to be more important in such a scenario. This is because males could compete for access to females (Van den Assem et al. 1980a), or there could be female choice (Hughes & Hughes 1985; Hardy et al. 2005). However, again I found no effect of size. A possible limitation of this experiment is that a truly monopolizable resource might be required, such as an exit hole in the puparium that can be guarded. In addition, although the operational sex ratio was appropriate for field populations, it might have meant that the males did not have to interact or compete directly for females (e.g. the similarity in mating success of focal males

with or without a competitor). It would be useful if future experiments address these issues by looking at mate competition in more complex environments.

Experiment three measured male LMS, longevity, and the cost of mating. The key result was that although mating significantly reduced longevity, this was equally costly for males of all sizes. The costs of mating to females have been well documented in insects (Fowler & Partridge 1989; Chapman et al. 1995; McLain & Pratt 1999a; Blanckenhorn et al. 2002; Moore et al. 2003; Shuker et al. 2006a) but less attention has been given to the costs of mating for males (Cordts & Partridge 1996; Prowse & Partridge 1997; Cordero 2000; Kotiaho & Simmons 2003; Martin & Hosken 2004b; Sakaluk et al. 2004; Perez-Staples & Aluja 2006; Simmons & Kotiaho 2007). To an extent this is because they are less paradoxical: the costs to males are easily accounted for by the direct fitness benefits males accrue. In my study, the costs to males could stem from either the increased energy demands of extra copulations and inseminations, or the continual efforts of courting. The effort of courting may well explain my cost of mating. As my males were confined with females for either 24 hours or their whole lives, it is probable that they expended considerable energy in repeated courtship attempts, which necessitate the production and release of potentially costly pheromones (Van den Assem et al. 1980b; for an example of costly pheromone production see Johansson et al. 2005). Despite a longer latency to courtship when males are presented with mated females as opposed to virgins, they do still typically court frequently (Burton-Chellew et al. 2007a; Chapter 2); and courtship is known to be costly for males in other insects (Cordts & Partridge 1996). These repeated courtship attempts would most likely be

unsuccessful because of the low re-mating rate of female *N. vitripennis* (Burton-Chellew et al. 2007a; Chapter 2; only 2 of 49 females sampled in the wild were polyandrous; Grillenberger et al. 2008). Therefore the prolonged exposure to females would lead to an increase in courtship attempts, but not necessarily to an increase in copulations. Exposure to unreceptive females can actually be more costly as males often court more, and suffer more than males exposed to receptive females that they can mate (Cordts & Partridge 1996). Consequently, the cost of mating may be less in natural populations, where mated females will disperse, and so the continued presence of unreceptive females will be unlikely. The costs of copulation and superfluous courtship could be disentangled by either i) providing males with females that are replaced immediately after copulating, or ii) allowing males with ablated genitalia to court females.

But why are these costs not greater for smaller males? If the costs are a result of persistent unsuccessful courtship attempts, then it may be that larger males court more often or with more vigour. This greater courtship effort would not translate into increased copulations in my experiment and so my measure of fitness would fail to detect a size advantage, with larger males spending their greater energy reserves (if they have them) on superfluous courtship attempts. Alternatively, smaller males could limit mating costs by producing fewer sperm. However, smaller males may actually have paid a cost in terms of reduced sperm production with increasing age and mating experience since my experiment could only resolve differences in terms of the success or failure of insemination. Thus I did not consider male ejaculate quality quantitatively. How male *N. vitripennis* invest in sperm is not known in

detail. One could argue though that males would do better if they spread their resources across many ejaculates (i.e. individual ejaculates are cheap), because female *N. vitripennis* are more likely to be host-limited than egg-limited, and sperm competition will be a weak selective force in the wild because of the low female re-mating rate (Simmons 2001b; Shuker et al. 2007a; Burton-Chellew et al. 2007a; Chapter 2). How males invest in sperm production, and how this is related to body size, clearly merits further work, not least given the recent interest in the role of sperm depleted males in parasitoid wasp mating systems and its effect on sex allocation (Henter 2004; Damiens & Boivin 2006; Shuker et al. 2006c). Finally, my males were also fed daily and this may have allowed the smaller males to negate any of the costs of mating. Nutrition can play a major part in mediating the costs of mating in female *Drosophila melanogaster* (Chapman & Partridge 1996a), and the longevity costs of being small for female *N. vitripennis* only apply when food is not available (Rivero & West 2002). The extent to which male *N. vitripennis* feed in the wild is as yet unknown.

CHAPTER 5

5. Facultative Sex Ratio Adjustment in Natural Populations: Cues of Local Mate Competition and the Precision of Adaptation

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5.1 SUMMARY

Hamilton's theory of local mate competition (LMC), which predicts female biased sex ratios in structured populations, offers an excellent opportunity for examining the extent to which individuals adaptively adjust their behaviour in response to local conditions. Hamilton's original model has been extended in numerous directions to predict how different individuals on the same patch should lay different sex ratios in response to factors such as fecundity, time of oviposition and relatedness to either their mate or the other females on the patch. These models therefore assume that females use different sources of information about the environment when allocating sex, but have been little tested outside of the laboratory. I use microsatellite markers to describe the oviposition behaviour of individual females in natural populations of

the parasitoid wasp Nasonia vitripennis, and hence test the predictions of these various models. The offspring sex ratio produced by a female on a particular host was determined by the number of eggs laid on that host, relative to the number of eggs laid on that host by other females. In contrast, the offspring sex ratio was not directly influenced by other potentially important factors, such as the number of females laying eggs on that patch, relative fecundity at the patch level, or relatedness to either a mate or other females on the patch. These results emphasise that the degree to which one should expect real animals to fit the predictions of theory will be constrained by the availability and reliability of environmental cues.

5.2 INTRODUCTION

Sex ratio theory has provided excellent opportunities for examining the precision of adaptation (Charnov 1982; Herre 1987; Hardy 2002; West & Sheldon 2002; Boomsma et al. 2003; Shuker & West 2004). One of the most productive areas from this respect has been Hamilton's theory of local mate competition (LMC), which explains why female biased sex ratios are favoured in structured populations, where mating occurs before the females disperse (Hamilton 1967). Specifically, if N diploid females lay eggs on a patch, then the evolutionary stable (ES) sex ratio (r^*; proportion males) is given by $r^* = (N-1)/2N$ (Hamilton 1967). One way of conceptualising this is that a female bias is favoured as it reduces competition between sons (brothers), and increases the number of mates for sons (Taylor 1981). An additional bias is favoured in haplodiploid species because inbreeding makes females relatively more related to their daughters than their sons (Frank 1985a; Herre 1985). There is extensive empirical support for the basic predictions of LMC theory: females of numerous species have been shown to adjust their offspring sex ratios in response to the number of females laying eggs on a patch (N) (West et al. 2005).

Extensions of LMC theory have suggested that the pattern of sex ratio adjustment should vary depending upon how much information females are able to process about the environment. Hamilton's original prediction was based on a number of simplifying assumptions, such as females contributing the same number of offspring to each patch, and random mating within the patch (Hamilton 1967). These assumptions implicitly constrain what information females are thought to use. When

these assumptions are relaxed, offspring sex ratios are predicted to vary within the patch, between individuals, and over time and space (Werren 1980; Suzuki & Iwasa 1980; Yamaguchi 1985; Frank 1985a; Frank 1987; Stubblefield & Seger 1990; Taylor & Crespi 1994; Abe et al. 2003a; Reece et al. 2004; Shuker et al. 2005). For example, if one female produces a relatively smaller brood, then she should lay a less female biased, or even male biased, sex ratio (Werren 1980; Suzuki & Iwasa 1980; Yamaguchi 1985; Frank 1987; Stubblefield & Seger 1990). Table 5.1 summarises these models and identifies what variables are predicted to influence sex ratio. Whilst these models have been tested several times in the laboratory, there has been a conspicuous absence of field tests, examining what information females actually use when varying their sex ratio under LMC. This is largely because of the technical difficulties of recording oviposition behaviour in the field. However, the results of laboratory studies require the support of field tests, where the controlled environment of the laboratory does not apply.

Table 5.1. Models of sex allocation under Local Mate Competition, in terms of the information females are predicted to use and the variables associated with the models in my empirical study.

Model	Predicted information use	Empirical variables associated with the model
'Hamilton' (Hamilton 1967; Hamilton 1979)	Patch foundress number $s^* = (2N-1)(N-1)/N(4N-1)$	Patch foundress number
'Stubblefield & Seger model I (S&SI)' (Stubblefield & Seger 1990)	Knowledge of own fecundity, no knowledge of co-foundress fecundity ("imperfect knowledge")	Focal female fecundity (defined at the level of the host or patch)[1]
'Stubblefield & Seger model II (S&SII)' (Frank 1985a; Herre 1985; Stubblefield & Seger 1990)	Knowledge of own fecundity and co-foundress fecundity ("perfect knowledge")	Focal female and co-foundress fecundity (defined at the level of the host or patch) [1]
'Werren (host)[2] ' (Werren 1980; Suzuki & Iwasa 1980)	Relative clutch size (focal female relative to co-foundresses) on a given host	Relative clutch size of focal female on a host (as difference in clutch sizes between focal and co-foundress females)
'Werren (patch)[2] ' (Werren 1980; Suzuki & Iwasa 1980)	Relative clutch size (focal female relative to co-foundresses) across the patch	Relative clutch size of focal female on a patch (as difference in clutch sizes between focal and co-foundress females)
'Asymmetrical LMC' (Nunney & Luck 1988; Shuker et al. 2005)	Knowledge of own and co-foundress fecundities across both individual hosts and the patch as a whole	Focal female and co-foundress fecundities across hosts and patch
'Greeff' (Greeff 1996; Reece et al. 2004)	Relatedness to mating partner and foundress number	Relatedness to mating partner and foundress number
'Frank' (Frank 1985a; Taylor & Crespi 1994; Frank 1998; Shuker et al. 2004a)	Relatedness to co-foundresses and foundress number	Relatedness to co-foundresses and foundress number

1. Originally defined at the level of the patch, but if mating is increasingly non-random within a patch (Shuker et al. 2005), then each host effectively becomes patch.
2. The original Werren model is for sequential oviposition by two females, with the focal female being the second female. The predicted sex ratio is influenced by the primary sex ratio, the population inbreeding coefficient, as well as relative clutch size. I use it here in a general sense to consider sex allocation based on relative clutch size.

Here I address this problem by using microsatellite markers to trace the behaviour in the field of individual females of the parasitic wasp *Nasonia vitripennis*. *Nasonia vitripennis* is an ideal organism for such a study because it is known from both laboratory and field studies that the females adjust their sex ratios in response to the basic tenets of LMC (Werren 1983; Orzack et al. 1991; Molbo & Parker 1996; Shuker & West 2004; Grillenberger et al. 2008). *N. vitripennis* has also been extremely useful in testing the more complex LMC models, but so far these studies have been restricted to the laboratory (Werren 1980; Orzack 1986; Orzack & Parker 1990; Flanagan et al. 1998; Reece et al. 2004; Shuker & West 2004; Shuker et al. 2004a; 2006b; 2007b). Here I use the power of molecular techniques to test these extensions to LMC theory in the wild. Specifically, I test (1) to what extent females adjust their sex ratio in response to predicted environmental parameters (table 5.1), and (2) which models of LMC best approximate sex allocation in the wild. By genotyping more than 3500 offspring at four microsatellite loci, I was able to reconstruct the parental genotypes and hence determine the sex ratios produced by 49 females, in 350 broods across 18 natural patches. My results provide the first detailed analysis of individual sex allocation under LMC in the wild.

5.3 METHODS

5.3.1 Study Organism

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae). For details on *Nasonia* biology please refer to Section 1.2.

5.3.2 Sampling

I used two field sites, one in Hoge Veluwe (HV) National Park, the Netherlands, and one at a field site near Schlüchtern, Hessen, Germany (Schl). Full details of the sampling and subsequent genetic analysis of wasps are provided by Grillenberger *et al*. (2008)**.** That paper also describes the patterns of oviposition on the patches and the population genetics of the two study populations. Briefly, I collected *Nasonia vitripennis* broods in June 2004 from bird nestboxes ("patches"), either by searching for parasitized host puparia (HV) or by leaving unparasitized host puparia (*Calliphora vicina*) at nests as baits (patch size: 25 hosts, both HV and Schl). The HV samples consisted of nine nestbox samples and one baited sample, whilst the Schl samples were eight baited samples. All fly puparia were collected and incubated individually at room temperature.

Each day I brought out the incubated hosts into the daylight for at least 30 minutes before anesthetising any emerged individuals with CO_2 and storing them for molecular analysis. I checked for any un-emerged individuals by opening the fly puparia a month after the last emergence from that host. I recorded the origin of every individual in terms of field site, nest box, and host. The full details of the number of parasitised hosts and the individual broods are given in table S5.1.

Throughout I consider the number of emerged offspring to be the number of eggs laid by females (clutch size), thereby assuming negligible larval mortality. Whilst this has been shown to be the case under laboratory conditions (Werren 1984), I do not know the impact of larval mortality in the wild.

5.3.3 Molecular Genetic Analysis

I extracted whole genomic DNA from individual wasps by using either a standard high salt-chloroform protocol (Maniatis et al. 1982) or Chelex®100 (Bio-Rad California, USA). For genotyping I used four polymorphic, di-nucleotide repeat microsatellites (Nv-22, Nv-23, Nv-41, and Nv-46). Nv-22 and Nv-23 were originally developed by Pietsch et al. (2004) but the primers were redesigned for this study (table S5.2). I separated PCR products by fragment length using an AB 3730 DNA analyzer or ABI Prism 377 DNA sequencer (Applied Biosystems, California, USA), and analysed them using either GeneMapper v4.0® or GeneScan 3.1® (Applied Biosystems, California, USA).

I sexed all individuals by external morphology before DNA extraction, checking damaged individuals by their heterozygosity (e.g. heterozygotes have to be female). Parentage was assigned according to Mendelian rules of inheritance under haplodiploidy. The genotypes of the foundresses that oviposited on each host were reconstructed from the genotypic data of the offspring. Each patch was resolved with the minimum number of foundresses required to explain the offspring. For the analysis presented above, two patches were excluded. In the first case, a solitary foundress oviposited on one host in the nest, producing only sons. This female may

have therefore been a virgin and unable to produce daughters (a "constrained" female). I also excluded a nest box containing 16 parasitised hosts and up to 7 foundress females. In this case, assigning offspring to foundresses was difficult as some of the foundress females, and their respective mates, appeared to be very closely related. This meant that numerous offspring had multiple possible mothers. Inclusion of these two patches does not qualitatively alter the results presented. The following analysis therefore considers 16 patches, containing 324 clutches from 47 foundress females laid on 222 hosts. These clutches produced 3027 genotyped offspring that were assigned to a foundress.

I calculated the average relatedness between all foundresses on each patch, and between each foundress and her mate(s), following the principles of Queller and Goodnight (1989). I used the Relatedness 5.0.8 program (developed by Goodnight; 2001) to generate relatedness values on a scale from -1.0 to 1.0. I treated the HV and Schl samples as two distinct populations and the estimate of the population allele frequencies was bias-corrected for each foundress by excluding both herself and her mate. In the cases of a single foundress parasitizing a patch, I attributed a value of zero relatedness (i.e. the average relatedness of an individual to the population).

5.3.4 Statistical Analyses

I performed two analyses. First I tested explanatory variables at the host and patch level. For the second analysis I tested specific statistical models appropriate for different models of LMC. For the first analysis the explanatory variables were: patch foundress number; host foundress number; difference in fecundity of focal female

versus other foundresses on the host (or on the patch); patch size (as total number of hosts); numbers of parasitised hosts; proportion of parasitised hosts. I calculated the difference in fecundity between a focal female and the other females on the host (or patch) by subtracting the number of offspring produced by other foundress females from the number produced by the focal female. This allowed me to consider relative clutch size, a potentially important variable (Werren 1980), usually calculated as (focal female clutch size)/(non-focal female clutch size). However, this latter definition is undefined for females that oviposited by themselves, necessitating the use of difference in fecundity. When I specifically considered just those hosts with more than one foundress (i.e. superparasitism), the more usual relative clutch size of the focal female was used. For one patch, a recording error meant that I did not know the total number of hosts (parasitised plus unparasitized). Therefore the fixed effects "patch size" and "proportion of parasitised hosts" were tested on the subset of 15 patches with this information.

Sex ratios are best modelled within a generalised linear modelling framework assuming binomially distributed errors and with a logit link function (Wilson & Hardy 2002). Since females could contribute multiple clutches, for the first analysis I used a generalised linear mixed modelling approach (GLMM) including female identity as a random effect to take these multiple observations into account. GLMMs are still an area of active research and current tractable estimation methods do not generate true likelihoods but rather use approximations to complete the integration. I used restricted penalised quasi-likelihood (REPQL) as provided by the glme function in the Correlated Data library in S-Plus 7 (Pinheiro & Chao 2005). Other methods for

binomially distributed data (Laplacian and adaptive Gaussian Quadrature methods) force the dispersion parameter to be 1 (i.e. assume true binomial variance), but my data were slightly over-dispersed (dispersion parameter = 1.555). I tested the fixed effects using marginal t tests with approximate degrees of freedom (Pinheiro & Chao 2005). Given that several of the explanatory variables associated with different models of LMC are likely to be correlated with each other, I also tested variables alone in individual models.

For my second analysis, since GLMMs do not yield true likelihoods, I was unable to compare different models using techniques such as likelihood ratio tests or AIC (Akaike Information Criterion). In order to test how well different models of sex allocation predict wild sex ratios I therefore fitted specific models (table 1) to the sex ratio data using a maximum likelihood mixed effects framework. Model fit was examined by way of AIC. All statistics were performed in S-Plus 7 (Insightful Corporation, Seattle, WA, USA). Means are presented ± standard error (with asymmetric binomial standard errors for sex ratio).

5.4 RESULTS

5.4.1 Descriptive Statistics

The overall sex ratio across the 16 patches was extremely female biased (0.200 ± 0.007). The number of females laying eggs on patches ranged from 1 to 7, and on individual hosts from 1 to 4. The average clutch size per host per female was 9.34 ±

0.40 wasps (N = 324 clutches). For those hosts where only one female laid eggs it was 11.56 ± 0.64, and in those hosts where multiple females laid eggs it was only 7.74 ± 0.48. Sex ratio did not differ between populations ($t_{39} = 0.75$, P = 0.46) and so the analysis below considers both populations together. Sex ratios did vary significantly among females (among-female variance component = 1.225, 95% confidence intervals = 0.654 – 2.292). The average relatedness between foundresses on a patch varied from -0.46 to 0.28, with a mean of 0.09 ± 0.04 for HV and -0.05 ± 0.05 for Schl. The average relatedness of a foundress to her mate(s) suggested appreciable levels of sibmating: for HV the mean relatedness was 0.32 ± 0.04 (N = 27); and for Schl it was 0.22 ± 0.02 (N = 19), with values ranging from -0.43 to 0.82.

5.4.2 Sex ratios

Sex ratios varied with the relative clutch sizes that females produce on a host, with females producing more female biased sex ratios when they lay relatively more eggs on a host ($t_{270} = 5.00$, P < 0.0001; fig. 5.1).

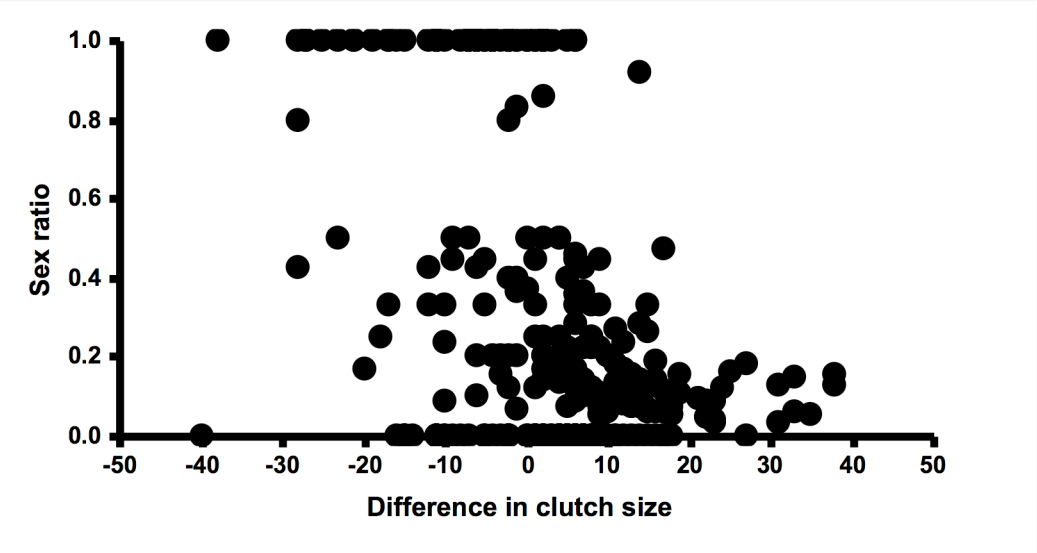

Figure 5.1. Sex ratios are negatively correlated with the difference in clutch size between females ovipositing on a host (including females ovipositing alone).

The quadratic term was not significant (t_{270} = 1.07, P = 0.28). When relative clutch size was fitted in the model, no other factors were significant (table 5.2).

Table 5.2. Analysis of sex ratio variation. Fixed effects are either fitted together in a model with the significance tested after the fitting of any other significant effects, or alternatively fitted alone in a model (apart from the relative fecundities which are fitted with their respective quadratic terms). t values are marginal t tests presented with approximate degrees of freedom. **Bold highlighting** indicates significance.

	Fitted together		**Fitted alone**	
Fixed effect	**t (d.f.)**	**P**	**t (d.f.)**	**P**
Patch foundress no	1.44 (270)	0.15	**2.74 (282)**	**0.007**
Host foundress no	1.11 (270)	0.27	**6.34 (282)**	**<0.0001**
Relative fecundity (patch)	0.40 (270)	0.69	1.64 (281)	0.10
Quadratic term	0.78 (270)	0.44	0.66 (281)	0.51
Relative fecundity (host)	**5.00 (270)**	**<0.0001**	**8.09 (281)**	**<0.0001**
Quadratic term	1.07 (270)	0.28	1.57 (281)	0.12
Focal female patch fecundity	1.02 (270)	0.31	**2.05 (282)**	**0.04**
No of hosts used by focal female	1.03 (270)	0.31	0.99 (282)	0.32
Total no of hosts used on patch	0.94 (270)	0.35	0.01 (282)	0.99
Patch relatedness	1.14 (270)	0.26	0.65 (282)	0.52
Mate relatedness	0.35 (37)	0.73	0.02 (37)	0.99

The relative number of offspring that a female produced on a host or a patch was negatively correlated with the number of females laying eggs on that host or patch (host foundress number and difference in fecundity on that host: r_{322} = -0.66; patch foundress number and difference in fecundity on that patch: r_{322} = -0.22; both P < 0.0001). When relative clutch size was not included in the model, the sex ratio was therefore positively correlated with both the number of females laying eggs on a host (t_{282} = 6.34, P < 0.0001; fig. 5.2) and the number of females laying eggs on a patch (t_{282} = 2.74, P = 0.007; fig. 5.2). There was also a weak negative correlation between sex ratio and the total number of offspring a female contributes to a patch when fitted alone (t_{282} = 2.05, P = 0.04).

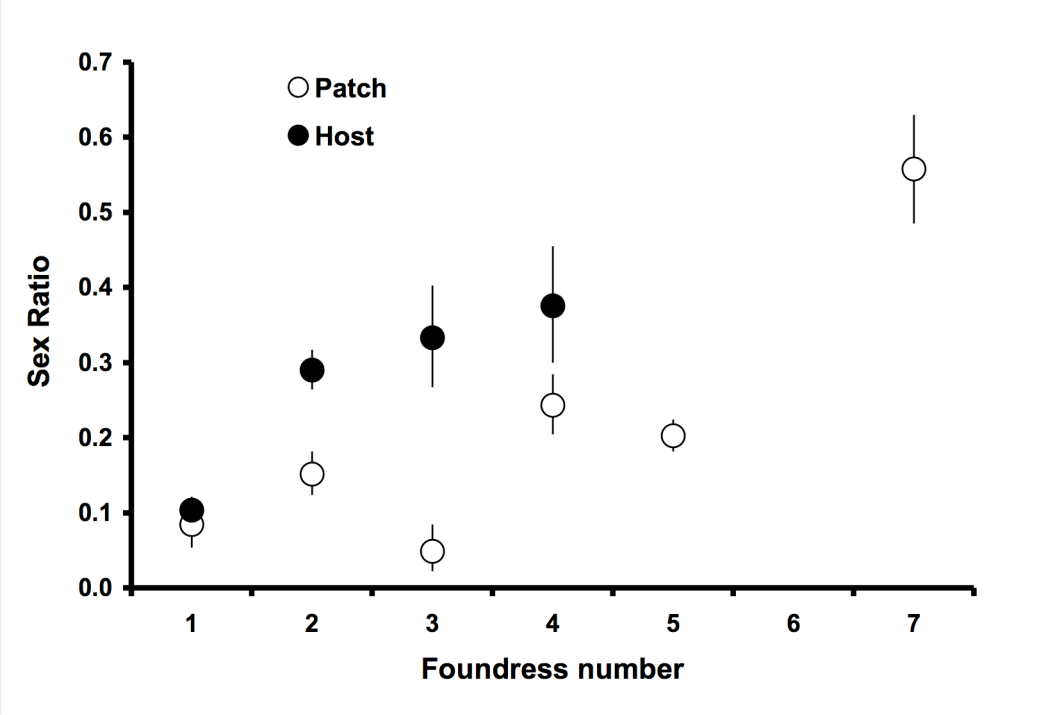

Figure 5.2. Sex ratios vary with the number of foundresses using the patch (open circles) or a particular host (filled circles). Error bars are 95% binomial confidence intervals.

The above data set considers all females and combines different patterns of patch and host use. It is also useful to consider some specific cases. In the simplest case, an individual female was the only foundress on a patch (N = 4). With no cues indicating reduced LMC, sex ratios were highly female biased (0.084 + 0.019, -0.016) and independent of clutch size (per host: $t_{12} = 0.59$, P = 0.57; per patch $t_2 = 0.12$, P = 0.92). Other females may have used a host individually, but shared the patch as a whole with other females (N = 27). Females did not shift their sex ratios on these hosts in response to the characteristics of the rest of the patch. Their sex ratios were not correlated with patch foundress number ($t_{23} = 1.24$, P = 0.23), clutch size on the host ($t_{91} = 1.05$, P = 0.30), total fecundity of the focal female on the patch ($t_{23} = 1.01$, P = 0.32), or with the difference in fecundity between the focal female and all the other foundresses across the patch ($t_{23} = 0.82$, P = 0.42). Finally, two or more females shared particular hosts (superparasitism, N = 35 foundresses). Sex ratios were highly significantly correlated with relative clutch size (defined here as {focal female clutch size}/{non-focal female clutch size}; see Section 5.2 Methods), with sex ratios declining with increasing relative clutch size as expected by theory (fig. 5.3).

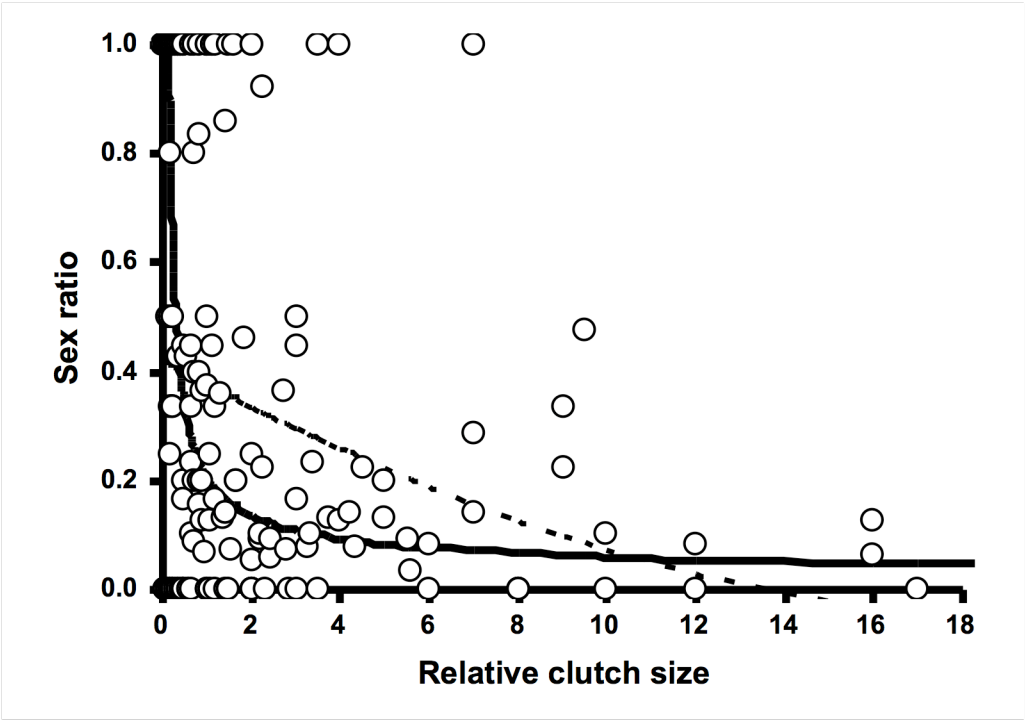

Figure 5.3. Sex ratios vary with relative clutch size when two or more females lay eggs on the same host (superparasitism). The dashed line is the relationship between sex ratio and relative clutch size (RCS) obtained from the analysis (sex ratio ~ $0.4211 - 0.0448*(RCS) + 0.0010*(RCS)^2$). The solid line is the prediction from Werren (Werren 1980) adjusted for haplodiploidy. For clarity, the largest relative clutch size has been omitted from the figure (RCS = 39.0, sex ratio = 0.154).

Both relative clutch size and its quadratic term were highly significant ($t_{151} = 4.47$, $P < 0.0001$, and $t_{151} = 3.81$, $P < 0.0001$). The theoretical prediction for sex allocation under superparasitism according to Werren (1980; adjusted for haplodiploidy) includes the sex ratio of eggs already present on a host and the inbreeding coefficient. Using the sex ratio produced by females when ovipositing on a patch alone and $F_{IT} = 0.197$ (Grillenberger et al. 2008)**,** the Werren model also predicts a highly significantly proportion of the variance in sex ratio ($t_{152} = 4.04$, $P < 0.0001$; fig. 5.3 and table 5.3).

Table 5.3. Testing models of sex allocation that assume different sources of information for estimating the level of LMC experienced by offspring: (a) all females; (b) only those females sharing hosts (superparasitism). Mixed effect models were fitted by maximum likelihood, with Female as a random effect. Model fit is described in terms of: AIC = Akaike Information Criterion; Log-lik = log-likelihood of the model; Residual = residual deviance of the model; % decrease = % decrease in residual deviance compared to the model with just the random effect. Models in bold represent the better fitting models. The model "Werren (host)" also represents the minimal model from my empirical analysis. For full details of the models see table 5.1.

Model	AIC	Log-lik	Residual	% decrease
(a) All females				
Random effect only	221.08	-107.54	0.3111	
Hamilton	217.54	-104.77	0.3100	0.35
S&S I (patch)	216.18	-104.09	0.3099	0.39
S&S II (patch)	211.46	-100.73	0.3094	0.55
S&S I (host)	180.08	-86.04	0.2901	6.75
S&S II (host)	**151.70**	**-70.85**	**0.2800**	**10.00**
Werren (host)	**149.96**	**-70.98**	**0.2804**	**9.87**
Werren (patch)	213.59	-102.80	0.3098	0.42
Asym LMC	**153.27**	**-69.63**	**0.2799**	**10.03**
Greeff	216.53	-103.27	0.3091	0.64
Frank	208.56	-99.28	0.3106	0.16
(b) Superparasitism				
Werren[(1)]	175.14	-83.57	0.3425	4.38[(2)]
Empirical model[(3)]	**171.52**	**-80.76**	**0.3405**	**4.94**

1. The specific version of the Werren (Werren 1980) model adjusted for haplodiploidy (Suzuki & Iwasa 1980; Greeff 2002) and parameterised using the single foundress sex ratio, relative clutch sizes, and inbreeding coefficient from this paper and Grillenberger *et al*. (2008)**.**
2. The residual deviance after fitting the random effect only is 0.3582.
3. Contains the variables Relative Clutch Size and (Relative Clutch Size^2).

5.4.3 Testing LMC Models

Models of sex allocation under LMC form a hierarchy, with more complicated models assuming that females use increasingly sophisticated information to estimate the level of LMC (table 5.1). Assuming that females process increasing amounts of information about the patch, by substituting in the appropriate variables for each

model, explains increasing amounts of the variation in sex ratios in the field (table 5.3). The best fitting models suggest that complete knowledge of the clutch sizes of the females on a given host, either in absolute terms or as the difference between them, is crucial for explaining the sex ratio. The best fitting model of all is the "Werren (host)" model. This also corresponds to the empirically derived minimal model from the above analysis, containing the difference in fecundity on a host. For the specific case of superparasitism, the empirically derived model above (relative clutch size and its quadratic term) fits the data marginally better than a fully-parameterised version of the Werren model (1980).

5.5 DISCUSSION

I used microsatellite markers to determine the sex ratio behaviour in the field of individual *N. vitripennis* females. I found that the only significant variable was the relative clutch size laid on a host: females produced a less female biased sex ratio when they laid relatively fewer eggs on a host (fig. 5.1). When this effect was included in the model, no other factors were significant (table 5.2). I also tested the extent to which different LMC models could explain variation in sex ratio. I found that whilst models based purely on the number of females laying eggs on a patch (Hamilton 1967), or the relatively fecundity on a patch (Stubblefield & Seger 1990), were statistically significant, they did not fit the data as well as models based on relative fecundity at the host level (Werren 1980; Suzuki & Iwasa 1980; Shuker et al. 2005; table 5.3).

My results suggest that females are adjusting their offspring sex ratio in response to variation in the extent of LMC, and that the primary cue on which they are basing their behaviour is the relative number of eggs that they are ovipositing on each host. In contrast, they do not appear to be using information about the total number of females on a patch, or the relative fecundity of different females on a patch. This result agrees with a recent laboratory experiment in which females were shown to lay less female biased sex ratios when co-foundress females were present, but that the primary cue was the eggs laid by those other females, and not the presence of the females themselves (Shuker & West 2004). I suggest that the explanation for these results is that females are responding to the cues that are the most reliable indicators of the extent of LMC that their offspring will experience under natural conditions. Females appear to be able to assess with relative ease whether a host has been previously parasitized (Werren 1984; King et al. 1995; Shuker et al. 2005; 2006b), and a higher proportion of previously parasitized hosts should correlate with less LMC. In contrast, females may not be able to directly assess the number of females that are laying eggs on that patch, especially if these females visit the patch sequentially.

Another potentially important factor is that mating will often not be random within the whole patch, as assumed by most LMC models (Shuker et al. 2005; 2006b; 2007b). Laboratory experiments have shown that even when wasps emerge at very similar times, from hosts that are next to each other, they are more likely to mate with individuals that developed in their own host (Van den Assem et al. 1980a;

1980b; Shuker et al. 2005). In nature, this effect will be increased because hosts can be spatially separated and emergence times can be very spread out, as they were for the HV population (emergence times for the Schl population were not recorded), where the mean duration of emergence from the first to the last individual in a patch was 9.00 ± 2.36 days. Sometimes the difference in emergence time between hosts from the same patch was as high as 18 days, which is considerably higher than the mean lifespan of approximately nine days for sexually-active males in the laboratory (Burton-Chellew et al. 2007b; Chapter 4). This means that the level of LMC actually experienced by wasps may differ from that expected by observers when considering the whole patch, and that wasps from different broods on the same patch may experience different levels of LMC (asymmetrical LMC: Shuker et al. 2005). Consequently, whether a host has been previously parasitised, and the relative number of eggs that a female lays on it, may be the most reliable indicator of the level of LMC that the brood laid on a host will actually experience. The importance of this in other species will depend upon natural history details: for example, emergence and mating may be staggered in many parasitoid wasps that attack clumps of hosts (Godfray 1994; West et al. 2005), whereas the relatively synchronous oviposition and emergence of fig wasps (Hamilton 1979; Frank 1985a; 1985b; Herre 1985; 1987) should lead to relatively random mating within the patch.

What information do females actually use to produce the observed negative correlation between offspring sex ratio and the relative clutch size that a female lays on a host (figs. 5.1 and 5.3)? Females may respond to their own fecundity, whether or not the host has been previously parasitized, or the number of previously laid eggs

on the host (Werren 1980; 1984; Orzack 1990). Support for the idea that females are responding to previous parasitism and the number of eggs laid previously is provided by the fact that there is: (1) no correlation between absolute clutch size and sex ratio when females lay eggs on a host alone (whether they share any of the other hosts on the patch or not; table 5.2); (2) a poorer fit to the data with a focal female's own fecundity when compared to a focal female's fecundity plus other foundress females' fecundity (table 5.2). In addition, previous experiments have shown that females are less likely to oviposit on, and lay fewer numbers of eggs on, parasitized hosts that have had a greater numbers of eggs previously laid on them (Shuker et al. 2005). Also my analyses of the field data will have underestimated the ability of individuals to assess the number of eggs previously laid on a host because, in superparasitized hosts, I do not know the order in which females laid eggs. Consequently, the first females to visit each host are also included in my analyses, despite the fact that they can have no knowledge of the number of eggs that will be laid later on the host. This limitation of a natural data set may also explain why I did not find support for the experimentally observed pattern that the sex ratio laid on a host is influenced by the extent to which other hosts on the patch have been previously parasitized (Shuker et al. 2005). Further complications include that females only respond to other hosts that are recently parasitized (Shuker et al. 2006b) and that, as discussed above, parasitization and emergence can be relatively spread out on natural patches. Females may also be sperm limited, and thus constrained to produce male-biased broods. One female, excluded from the analysis presented here, did produce only males, which could result from virginity or sperm-depletion. Whilst single mating in *N. vitripennis* usually provide sufficient sperm to fertilise several hundred eggs,

males that have recently mated with 50 or more females do produce smaller ejaculates (or fail to inseminate successfully: Barrass 1961). However, in my dataset only four from the 136 clutches laid singly on hosts had sex ratios in excess 0.4, none of which exceeded 0.5. Sperm limitation therefore seems unlikely to be common.

My analyses support the results from laboratory studies on *N. vitripennis* and other species that females do not adjust their sex ratio in response to their relatedness to their mate or the other females on the patch (Frank 1985a; 1998; 1994; 1996; Reece et al. 2004). Females are predicted to lay a more female biased sex ratio when mated to more closely related individuals, because then they will be relatively more related to their daughters than their sons (Frank 1985a; Herre 1985; Greeff 1996; Reece et al. 2004). Females are also predicted to lay a more female biased sex ratio when ovipositing with more closely related females, because this will increase the relatedness between the offspring developing on the patch, and hence increase the extent of LMC (Frank 1985a; 1986; Taylor & Crespi 1994). Whilst it could be argued that selection for an effect with relatedness to other females may be weak, because relatives rarely oviposit on the same patch, there is appreciable variation in relatedness to mates, as mating with both siblings and non-siblings is common. However, such sex ratio adjustment would require reliable cues for kin recognition, and theory suggests that sufficient variability in the cues is unlikely to be maintained (Reece et al. 2004). The reason for this is that more common alleles would be recognised more often, indicate a higher relatedness, and hence be under positive selection: less common alleles would thus be eliminated, along with the variability that is required for kin discrimination (Crozier 1986; Rousset & Roze 2007).

5.5.1 CONCLUSION

My results show that for species which are shown to fit simple models of LMC (11), techniques that allow the testing of more specific models in the wild can tell a great deal about what limits adaptive behaviour. My results also emphasise two general points about the extent to which one should expect data to fit theory. First, the ability of individuals to adjust their behaviour in response to environmental conditions depends upon the cues which they can use, and the reliability of those cues (West & Sheldon 2002; Boomsma et al. 2003; Shuker & West 2004). Here, I have found that cues concerning whether or not hosts are already parasitized are much more important than social cues, such as the presence of other females or the relatedness between individuals. Second, the pattern of social interactions in natural conditions can be much more complicated than that assumed by theory or laboratory experiments. More specifically, mating can be structured both temporarily and spatially within patches, leading to a higher likelihood of mating among individuals from the same host, in contrast to the usual assumption of random mating at the patch level (Shuker et al. 2005). Studies on sex ratio evolution have been extremely useful for illustrating such general points, because of the relative ease with which the key parameters can be measured and linked to their fitness consequences.

5.6 SUPPLEMENTARY INFORMATION

Table S5.1. A Summary of the field collection. Wasps were collected at two field sites, either from natural host puparia found in nest-boxes, or from baits, containing 25 laboratory host puparia, placed into nest-boxes. Not all the host puparia found or baited were parasitized. For various reasons not all offspring could be assigned to a foundress. The sex ratio is that of the assigned individuals within a patch (nestbox).

Nestbox (patch) and study site	**Parasitised hosts (total)**	**Number of foundresses**	**Total offspring (unassigned)**	**Sex ratio for analysis**
HV 8[1]	1 (15)	1	7 (0)	1.000
HV 13	27 (27)	5	607 (1)	0.211
HV 220	8 (unknown)[2]	5	171 (0)	0.199
HV 267[1]	16 (16)	7	476 (19)	0.222
HV 288[b]	11 (25)	1	141 (2)	0.086
HV 306	1 (6)	1	18 (0)	0.056
HV 323	6 (8)	2	203 (0)	0.094
HV 330	79 (82)	5	593 (3)	0.197
HV 344	4 (43)	1	79 (0)	0.063
HV 365	1 (35)	1	25 (0)	0.160
Schl 11[b]	15 (25)	4	204 (5)	0.317
Schl 13[b]	3 (25)	2	43 (6)	0.108
Schl 16[b]	4 (25)	2	24 (3)	0.333
Schl 20[b]	25 (25)	2	331 (11)	0.178
Schl 21[b]	9 (25)	7	186 (5)	0.558
Schl 22[b]	14 (25)	4	246 (1)	0.188
Schl 23[b]	1 (25)	2	8 (1)	0.125
Schl 28[b]	15 (25)	3	188 (2)	0.048
ALL HV	154 (262)	29	2320 (18)	0.186
ALL Schl	86 (200)	20[3]	1230 (33)	0.241
TOTAL	**240 (462)**	**49**	**3550 (59)**	**0.205**

HV = Sample from Hoge Veluwe (HV) National Park, the Netherlands.
Schl = Sample from Schlüchtern, Hessen, Germany.
[b] = samples collected from baits.
[1]These patches were ultimately not included in the analyses, because the foundress in HV 8 was believed to be a constrained or virgin female, and because assigning offspring in HV 267 was problematic due to the foundresses being closely related.
[2]The number is not known because of a recording error, but it is known to be nine or more, and thus nine is used when compiling the totals.
[3]The total number of foundresses for Germany does not equal the sum total because six foundresses parasitized puparia in two different nestboxes.

Table S5.2. Information regarding the four microsatellite primer sets used. Name (annealing temperature), location, sequence, size range of PCR products, and fluorescent dye used.

Primer	Chromosome*	Sequence 5'-3'	Size Range	Dye
Nv-22 (58°C)	I	F) GCT ATA ACA CTT TTC CGC TCT CA R) AAG ACC AGC TAG GGA AGA GGA TA	194-222	HEX
Nv-23 (58°C)	II	F) ATA CTC AAG CAA GCC ACA GCA TA R) GCG TAC CAA TCC ACA GAA AAT AG	235-257	FAM
Nv-41 (52°C)	V	F) GTC AGA CGT GGG CTT TGT C R) TTA TGC GCC ACA CAC ACC	326-358	NED
Nv-46 (58°C)	IV	F) TTA CGT CAA GGT ATA GCT GC R) GAA TAA GTG GCT GAA AGT TCC	235-267	FAM

*Chromosome designation according to Rütten *et al*. (2004).

CHAPTER 6

6. Discussion

6.1 SUMMARY

This thesis aimed to improve our understanding of the reproductive behaviour of a parasitoid wasp, *N. vitripennis*, and by doing so, to increase our understanding of how Natural Selection resolves the conflicting interests of cooperating individuals. Males and females are selected to cooperate by exchanging gametes, and they do. They may cooperate further by doing their utmost to facilitate the greatest reproductive success for their partner, but they do not, for they are selected to enhance their own reproductive success, even at the expense of their mate's success if necessary. Similarly, two foundresses that simultaneously parasitise a host may 'cooperate' by restricting their number of sons, and if they are related then they are favoured to show even greater restriction, but they will not, as Natural Selection is unable to continuously favour the ability to discern kin on the basis of genotypic similarity.

Proponents of the 'adaptionist programme' have sometimes been criticized for zealously applying adaptive explanations for all and any biological traits (Gould & Lewontin 1979). Such proponents, it is argued, fail to consider that many traits are merely 'by-products' arising from design constraints. If selection favours trait Y,

then trait X will tag along, in much the same way as the Spandrels of San Marco exist without serving any purpose (Gould & Lewontin 1979). Such a view may well have explained the extreme sex-ratios observed for *N. vitripennis*, and other similar organisms, as merely the result of some feature of the organism's internal anatomy, or a result of some environmental influence. In fact it was, by Whiting (1967), who imaginatively proposed a mechanism for the production of extreme sex ratios in *N. vitripennis* (Whiting suggested that the internal anatomy of females was responsible for a less than complete insemination rate of eggs, and thus the production of haploid offspring, i.e. sons). What a shame it would have been then if people had accepted such an explanation, an explanation that had it's own merit, but that belonged entirely to a 'proximate' viewpoint, rather than asking for a more 'ultimate' explanation! A shame because the ultimate, Darwinian, explanation, of Local Mate Competition (Hamilton 1967), has been instrumental in illuminating the dexterity of the hand of Natural Selection, with all of its intricacies and subtleties too. LMC research has helped to show how organisms interact with their environment and process information, and has even demonstrated when Natural Selection can be constrained. A greater appreciation of the dynamics of Natural Selection has also revealed reproduction to be a less than harmonious act and has helped to explain a variety of incongruous and perplexing behaviours and traits such as why males are under such pressure to evolve new and varied substances to join their sperm in their ejaculate - substances which are often harmful to females, if not at least manipulative.

This thesis has shown that we must not forget the role of females in sexual selection (Chapter 2) and that when males and females interact the path of evolution may not be predictable (Chapter 3). Sexual Selection and Sexual Conflict theory require that females mate adaptively and that the mutability of mating behaviour is key to speciation. Shuker et al. (2007a) have shown that there is additive variation for polyandry, and my results show that female mating behaviour can change rapidly (Burton-Chellew et al. 2007a). Adaptionist theory requires that these changes are beneficial, or at least not harmful. Polyandry may be beneficial for *N. vitripennis*, but if so, why are the females not polyandrous in the wild? Van den Assem and Feuthdebruijn (1977) postulated that females only mate once in the wild because insemination temporarily inactivates their ability to inseminate eggs and thus females that mate repeatedly will be at a disadvantage. This explanation is not satisfying though because, like the Whiting (1967) explanation for biased sex ratios, it relies on a proximate mechanism that is conceivably amenable to evolutionary change itself. More likely then, it is because of the drastically different selection pressures between the wild and the laboratory. If this is the case, then it is a strong reminder of the role of ecology in the evolution of mating systems.

It is also important that we continue to tackle problems with a diverse array of empirical approaches and most importantly that we combine theoretical approaches with both laboratory studies and field studies. For example, my results not only showed that laboratory adapted females are willing to remate, but also that females of wild-strains are un-willing to remate (Burton-Chellew et al. 2007a). When combined with my inference from microstatellite data of monoandrous females in the

wild (Grillenberger et al. 2008), this is compelling evidence that wild *N. vitripennis* are monoandrous. The high degree of inbreeding (Burton-Chellew et al. 2008; Grillenberger et al. 2008; Chapter 5) also strongly suggests that females are mating at the site of emergence. This is important data that confirms that *N. vitripennis* conforms to LMC in the wild (the more matings a female has, then arguably the less chance there is that they are all local), an often assumed fact of biology for parasitoids with female biased sex-ratios, but not a commonly tested and verified one (Orzack 2002).

The empirical approach can only be as good as its design. When testing for a general evolutionary response it is important to use many replicate strains or populations, as evolution is fuelled by the random process of mutation and is always contingent on prior events. Unfortunately, as far as experiment 3.1 is concerned, it is very difficult to do so with just four strains, or populations. When I used 20 strains, for experiment 2.1, a general evolutionary trend was detected for the trait in question.

Sometimes light can be shed upon evolutionary conflict from the results of apparently unconnected experiments. By engineering males of different sizes I was able to show that size does not affect mating success, either alone or in competition with other males, nor the lifetime reproductive competence of males continually exposed to females. This has implications for parent-offspring conflict, whereby offspring 'want' ('want' is shorthand for 'are favoured by selection if they can get…') more than their fair share of parental resources, but parents 'want' to partition their resources in an optimal manner (Trivers 1974). In parasitoids such as

N. vitripennis, mothers can invest in their offspring via egg size, and egg size correlates with adult size (Lalonde 2005). Mothers can also allocate more or less host resources to each offspring by adjusting brood size. Therefore if size is important to offspring, they will 'want' to develop in large eggs and in small broods. Although note, that for parasitoids, the optimal number of siblings is probably a balance between local resource competition and resource enhancement, as siblings may cooperate to overcome host defences and to metabolize host tissue (Godfray 1994; Lalonde 2005). However there are various asymmetries between sons and daughters in such cases. Daughters will 'want' to come from large eggs and small broods; for them size and enough males to ensure they get mated are important. Whereas sons will not 'care' about egg size, and will 'want' lots of sisters as potential mates. Therefore daughters will be in conflict with mothers, whereas sons will not. An added twist arises from haplodiploidy; fathers only improve their fitness through daughters, so will 'want' what is best for their daughters, whilst maximizing daughter number. Males may therefore have a conflicting influence with females over oviposition and sex allocation decisions, especially during superparistiztion, when females are selected to lay more male-biased sex ratios (Shuker et al. 2006c). The degree of polyandry will affect these asymmetries also. If females mate many males, then paternal genes will not be shared between daughters, further increasing sororial conflict over resources. Perhaps one reason *N. vitripennis* is monoandrous is to reduce sibling competition and to facilitate cooperative host-utilization (Boomsma & Ratnieks 1996).

The theoretical approach towards adaptation often considers traits in isolation but, as quite rightly pointed out by Gould and Lewontin (1979), organisms are holistic entities, and selection upon one trait may impinge upon selection upon another. This is because, at a fundamental level, organismal 'design' is the result of a series of trade-offs (Stearns 1992), and because various behaviour and traits feed back into the selection upon other traits. For example the mating system, operational sex ratio (OSR), the strength of sexual selection, and the favoured strategy for sex allocation all interact to affect each other (Emlen & Oring 1977; Andersson 1994).

For example, the optimal sex-ratio for a single foundress as predicted by Hamilton (1967) is 'zero', which is interpreted as the minimal number of males required to fertilize all her daughters. The costs of courtship, insemination, and the effect of male size on fitness, all interact to determine what 'zero' actually should be. This means that 'zero' may even vary for different females, and over an individual female's lifetime, depending on factors such as her size and nutritional state. This may explain why there is natural variation in the primary sex ratio of female *N. vitripennis* and quash some doubts about the optimality of Natural Selection (Orzack & Parker 1994; Orzack & Sober 1994; Parker & Orzack 1985). By varying the mating regime in experiment 4.3 I also showed a cost of mating in terms of reduced longevity, and that this cost was equivalent for males of all sizes. It would be interesting to break down the components of male reproduction such as courtship and ejaculation, to further clarify the costs involved. If ejaculates are costly then males may have to trade off mate number versus mate quality, and may direct their attention towards better females, e.g. younger or larger females (Rivero & West

2002). Plus, a quantitative analysis of sperm production could analyze how males strategically allocate their resources, a matter of increasing interest in parasitoids (Henter 2004; Boivin et al. 2005; Damiens & Boivin 2006; Shuker et al. 2006c).

As explained in Section 1.1, models of LMC are useful for testing more than just sex allocation theory as they allow more general questions of socio-biology to be investigated, such as kin recognition (Greeff 1996; Reece et al. 2004) inbreeding (Frank 1985b; Herre 1985; Werren 1987), and the relative strength of different levels of selection (Frank 1985a), and how animals process the relevant environmental information (Nunney & Luck 1988; Stubblefield & Seger 1990; Taylor & Crespi 1994; Greeff 1997; Boomsma et al. 2003; Shuker & West 2004). This is because the same theoretical principles underlie much of socio-biology, such as kin selection and sex allocation (Frank 1998).

We must take care though not to assume we know the underlying biology of the species we use to test such models. We must confirm that the mating systems conform to LMC (Orzack 2002) and appreciate the constraints limiting information processing if we wish to use optimality models (Krebs & Kacelnik 1991). However, if we can confirm LMC and gain realistic measures of reliable information available to individuals, we can use models of LMC to test adaptionist theory (West & Rivero 2000).

Although LMC models have been tested many times, there have been relatively few studies of the sex-ratio produced by individuals as opposed to by groups (Orzack

2002), and there have been few studies in the field. My study is an advance on previous studies by Werren (1980) and Molbo and Parker (1996) because I use microsatellites, which allow me to trace the conditional sex-ratios produced by individuals under different circumstances. This is important because individuals are predicted to be able to facultatively adjust to their environment (Stearns 1992). Not only that, but it allows one to test multiple models of LMC simultaneously, an advantage over laboratory studies, which although can elegantly manipulate the conditions, cannot test multiple hypothesis simultaneously. Describing field behaviour is essential also because it highlights which scenarios are more common, and thus which should be under stronger selection, and which models are likely to be of the most relevance.

In what ways is Natural Selection constrained, if at all? Well, despite the theoretical advantages to kin recognition for a species such as *N. vitripennis*, the empirical results strongly suggest that they lack such ability. Is this result a challenge to adaptionist theory then? Well no, because i) if females are arriving at different times to a patch, it would be unreasonable to expect them to assess each other's relatedness, and, ii) more importantly perhaps, evolutionary theory says that kin recognition is not evolutionary stable except for in very restrictive circumstances. This is because identifier alleles are under positive frequency dependent selection. The more common an allele, the more likely it is to be 'recognized', and thus the more likely it is to receive the benefits of kin recognition and thus increase in frequency (Crozier 1986). Such frequency dependant selection will rapidly erode the allelic polymorphism required for kin recognition, because once an allele is common,

it is no longer a reliable indicator of relatedness (Crozier 1986). Grafen (1990b) challenged this though by showing that if the kin recognition allele allowed for altruism, it could help individuals to avoid 'cheaters' and thus serve a dual purpose. This would allow kin recognition, especially if individuals could tailor the degree of their altruism, so as to be increasing altruistic when matched with rare alleles, thus providing a form of negative frequency-dependent selection to counter the affects of positive frequency dependence. Rousset and Roze (2007) offer a solution to this debate by showing that Grafen's (1990b) model only maintains kin recognition under conditions of low recombination rates combined with low rates of dispersal. Kin recognition is still possible though if extrinsic cues that correlate with kin are used as rules of thumb. For a good example of such extrinsic cues, see Ode et al. (1995), who show that individuals of *Bracon hebetor* can recognize brood mates. *N. vitripennis* could arguably develop cues of host origin, but this would only allow females to discriminate between males from different hosts, and not between brothers and unrelated male developing in the same host. The benefits would thus be small and the selection pressure would therefore be weak. Indeed laboratory experiments suggest that a facultative shift does not occur in response to true relatedness or proximate cues (Shuker et al. 2004a; Shuker et al. 2004b; Reece et al. 2004). Another reason *N. vitripennis* does not discriminate against kin in mate choice is perhaps because, as a haplodiploid, it is unlikely to suffer inbreeding depression (Werren 1993; Godfray 1994), whereas *Bracon hebetor* does (Ode et al. 1995). When using theory to predict animal behaviour it is important to remember that animal intelligence and strategies are embedded in the environment and constrained by the information available, and although theory can predict a myriad of adaptive

responses to different conditions, these conditions will not be equally common and selection will be weighted accordingly.

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