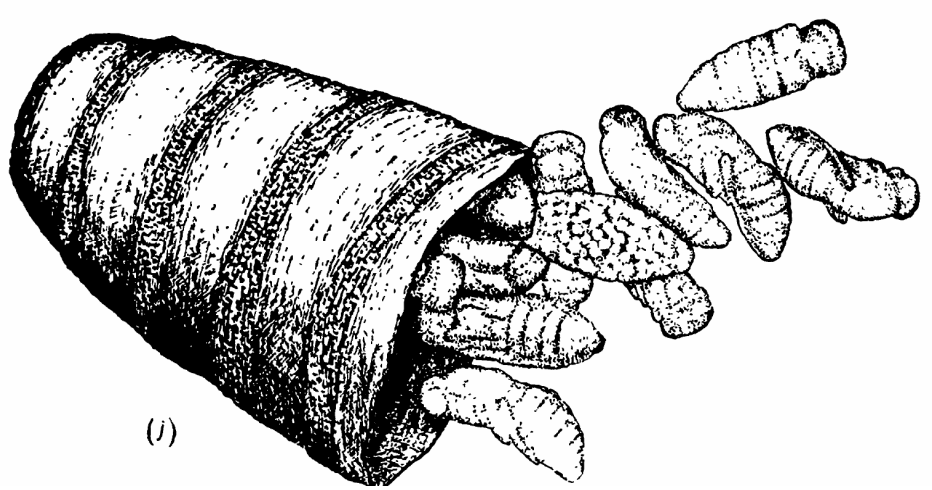
Life-history evolution in the parasitoid wasp *Nasonia vitripennis*



by

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Declaration

I declare that this thesis is entirely my own work, except for the collaborations mentioned below:

Chapter 3: The first two of the three experiments were created and carried out by Maxwell Burton. I had sole responsibility for my experiment and we wrote the resulting manuscript together.

Chapter 4: The mathematical modelling and associated figures (4-5 and 4-6) are the work of Ido Pen.

Chapter 5: The inbreeding experiment was set up by Kathleen Reynolds. I then gathered and analysed the data.

The wasp drawings in Figure 2-1 were created by Ana Rivero.

Acknowledgments

This is the part where I try and thank everyone that has helped me to finish my thesis. Unfortunately, I know that I can't do them justice. Without their help, in all its myriad of forms, I would not have even come close to finishing. First and ultimately, my supervisor Stu West was always able to put me back on track. His ability to simultaneously see the bigger picture and focus on the key points in my waffle was priceless. Secondly, Dave Shuker whether offering me a fresh idea or a nudge in the right direction was always persevering, perhaps in vain, to teach me how to think and I am extremely grateful. However, I wouldn't even have been in the position to need their help if it weren't for the vital funding that NERC so generously gave me.

During the four years that I was involved with The Westgroup and everyone at IEB, I had the chance to work and play with a fantastic bunch of people, in particular: Tabi Innocent, the gift-seeker and motivator extraordinaire; Stu Killick, the Panto Gimp and both Aleta Graham and Gav Ballantyne who kept me sane in the lab when, by all rights, I should have gone completely mad. At this point, I should also give a mention, though they'll never know it, to J.K. Rowling and Stephen Fry, for even if some people didn't appreciate their genius, it certainly kept me going! I was also lucky enough to have the joys of running across the hail-strewn, crater-pitted snow fields of Jack Kane with Sporting ICAPB (the world's greatest football team to field evolutionary biologists) and diving some of the best (and worst!) sites in Britain and Oman with EUSAC and Project NEMO. When my funding stopped in my fourth year it was actually a blessing in disguise as it meant that I was able to meet and work with everyone from the hideously-named, but wonderfully-fun Scottish Initiative for Biotechnology Education. There I was able to indulge in one of my favourite past-times, the act of communicating science to others, completely forgetting the overshadowing PhD five mornings per week – until of course Lara Crossland and the others sent me to the library.

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"Science aims at simple explanations of complex things"; Stearns 1992

Abstract

Reproductive success is heavily influenced by life-history traits; a series of energy investment trade-offs that organisms must optimise according to their environmental conditions. These include considerations such as how many offspring and when to reproduce? The consequences of multiple trade-offs can be extremely complex, making research difficult. However, there are notable exceptions. Simple clutch size theory enabled great strides in assessing trade-offs in resource allocation, though it quickly becomes more complicated when considering investment in current versus future reproduction. Arguably, even greater success has come from consideration of investment in a particular sex. Sex allocation theory provides simple models that can be empirically tested, and has provided some of the strongest evidence for natural selection and evolution. Much of this work has focused on certain parasitoids due to their extraordinary sex ratios and the finite resources available to offspring in a host. Whilst clutch size and sex allocation theory have provided many answers, there are still questions regarding the impact of other life-history traits. In this thesis I have used the gregarious parasitoid wasp *Nasonia vitripennis* in laboratory experiments to assess some of these traits. I have focused on the impact of larval competition, inbreeding, host condition and host feeding on longevity, fecundity, sex allocation and mating success. By manipulating host quality through host-feeding, I was able to vary the level of resources available to offspring. Simultaneously, by manipulating the mated-status and number of females ovipositing on a host, I was able to vary the number and sex ratio of offspring competing for resources. My research has provided an insight into how larval competition and host-feeding impact on optimal clutch size and sex allocation. Furthermore, I have attempted to assess the extent to which body size, which is commonly associated with reproductive success, can be used to predict fitness. The appendix includes work using molecular data to understand the mating behaviour and population structure of *N. vitripennis* in the wild, enabling models based on assumptions of laboratory-based behaviour to be applied to wild populations.

Chapter 1. General Introduction

To maximise fitness, individuals must trade off energy between the competing concerns of growth, maintenance and reproduction. For reproduction this often means balancing offspring quantity with offspring quality (Stearns 1992; Roff 2002). An individual must determine how many offspring to produce, and how many of each sex, encompassing the fields of clutch size and sex allocation theory respectively. Both sets of theory have produced a wealth of theoretical and empirical research (Werren 1984; Strand 1986; Antolin & Strand 1992; Godfray 1994; Nagelkerke 1994; Mackauer 1996; Mayhew & Glaizot 2001; Elzinga *et al.* 2005; Pexton & Mayhew 2005). In particular, sex allocation theory has been remarkably successful as it produces simple, mathematical predictions that can be tested empirically (Suzuki & Iwasa 1980; Charnov 1982; Hardy 1994; Hardy 2002; West & Herre 2002; West & Sheldon 2002; Reece *et al.* 2004; Shuker *et al.* 2005).

Lack (1947) revealed that although increasing clutch size results in more offspring, it also increases competition for resources, reducing the quality of each individual in the clutch. Lack's hypothesis (1947) shows how parents can be selected to produce an optimum clutch size that maximises the product of quality versus quantity. Subsequent work investigated why parents might deviate from the "Lack clutch" so as to retain energy for survival and future reproduction (Visser & Lessells 2001) or because of a limited resource such as time or egg number (models; Rosenheim 1999b; Rosenheim *et al.* 2000). Life-history theory predicts how, as far as adaptive plasticity allows, parents should behave under particular environmental conditions (Stearns 1992). As such, the Lack clutch has come to mean the number of offspring that will generate the greatest parental fitness from total generations, rather than the clutch that fledges the most offspring (Charnov & Skinner 1984; Mock & Parker 1997). However, clutch size alone will not maximise fitness, as individuals may be selected to invest more in one sex than the other.

Most sexual species are thought to invest equally in sons and daughters (Clutton-Brock & Iason 1986) but it was not immediately obvious why. Fisher (1930) showed that equal investment can be a result of frequency dependent selection. If offspring of each sex have

equal cost and as long as certain ecological assumptions are met (e.g. large population, mating occurs at random, linear fitness return from investment in each sex and equal maternal relatedness to each gender of offspring; Suzuki & Iwasa 1980; Godfray 1994; Hardy 1994), then an excess of females will increase the reproductive success of males as, on average, they will obtain more than one mate. This will give males a higher fitness in comparison to females. Consequently, individuals producing a relative excess of male progeny will be favoured and natural selection will drive the sex ratio back to 50:50 in a frequency-dependent manner.

However, Fisher's conditions are not always met and selection has generated more extraordinary sex ratios across a range of taxa (Madsen & Shine 1992; Komdeur *et al.* 1997; Creel *et al.* 1998; Kruuk *et al.* 1999; Aviles *et al.* 2000; Jordal *et al.* 2002; West & Sheldon 2002) that can be both greatly and precisely adjusted (West & Sheldon 2002; Reece *et al.* 2004; Shuker *et al.* 2004a; Shuker *et al*. 2004b; Shuker & West 2004; Shuker *et al.* 2005). West and Sheldon (2002) propose that the precision of sex allocation will be greatest in organisms for whom the fitness benefits associated with facultatively adjusting the sex ratio are high and the costs low, such as in parasitoids, where individuals are frequently able to predict their offspring's environment.

1.1. The use of parasitoids and *Nasonia vitripennis*

Godfray (1994) classifies parasitoids as insects whose larvae develop by feeding on and killing a single host, also usually an insect. The host, which may be an egg, adult or developmental stage in between, is a finite resource that must be shared between the developing offspring. In solitary species the offspring consist of a single larva, or at least following aggressive competition, a single adult, whilst in gregarious species there may be hundreds of larvae. These larvae can either develop within the body of the host (endoparasitoid) or on its surface (ectoparasitoid), where they gain sufficient nutrients to develop into adults. Having reached the adult stage, females mate and seek out new hosts, which they paralyse either permanently (idiobiont) or temporarily (koinobiont) whilst they lay their eggs. In many cases, eggs are laid using a highly modified ovipositor that

can drill through plant or wood material as well as the body of a host (for a full review see Godfray 1994).

The use of parasitoids has been extremely successful in research both on clutch size and sex allocation. Clutch size studies originated in birds, but interest in invertebrate clutch size (reviews in Godfray 1987; Godfray & Parker 1991) and parasitoid clutch size in particular has been a fast-growing area of research (Werren 1984; Waage & Greathead 1986; King 1993; Mayhew & Glaizot 2001; Jervis & Ferns 2004; Elzinga *et al.* 2005; Pexton & Mayhew 2005; Thorne *et al.* 2006). Modelling can predict clutch size behaviour when individuals are resource limited (Rosenheim 1999a; Rosenheim 1999b; Rosenheim *et al.* 2000; Mayhew & Glaizot 2001; Jervis and Ferns 2004) and in a variety of environmental conditions (Rosenheim 1999b; Rosenheim *et al.* 2000). However, the field of research best served by parasitoids is undoubtedly sex allocation (Charnov 1982; Waage & Godfray 1985; Waage & Greathead 1986; King 1987; Godfray 1994; West *et al.* 2002).

The majority of parasitoids are Hymenoptera (Godfray 1994) and, therefore, haplodiploid (diploid females, haploid males) enabling precise sex allocation as females either fertilise, or leave unfertilised, each egg before ovipositing. In solitary wasps, sex allocation has focused on the importance of host quality (Heinz 1991; Charnov 1992) and how different resource requirements can increase selection for production of a particular sex (Werren 1983; Heinz 1991; Hemerik & Harvey 1999; West *et al.* 2002). In both solitary and gregarious parasitoids, females often produce extremely low proportions of males (low sex ratio) as a means of reducing the number of related male competitors and increasing the potential number of mates (Hamilton 1967). Therefore, as relatedness between competing males increases, so does the advantage of a female-biased sex ratio, or vice-versa (Werren 1980; Werren 1983; Werren 1984a; King & Skinner 1991; King 1992; Godfray 1994; Molbo & Parker 1996; Shuker *et al.* 2005). Further work on the impact of local mate competition (Hardy 1994; West & Sheldon 2002; Reece, Shuker *et al.* 2004; Shuker *et al.* 2004b; Shuker *et al.* 2005) and superparasitism (Suzuki & Iwasa 1980; Shuker & West 2004) is considered later in the chapter.

In the course of this work, I have used the gregarious parasitoid wasp *Nasonia vitripennis*, a small, (approximately 2mm long) iridescent wasp found throughout the world (Whiting 1967). Commonly known as the Jewel wasp, it is an idiobiont, ectoparasitiod. *N. vitripennis* parasitises true-flies (dipterans) at the pupal stage, especially blowfly pupae (Calliphoridae, Sarcophagidae; Werren 1984a). When the host is in its final larval instar, it produces an outer layer that hardens and becomes a stiff outer shell (puparium) separated by an air space from the pupa within (Whiting 1967). Upon encountering a host, a female is able to assess whether it has already been parasitised and whether it is suitable for oviposition (Whiting 1967; Werren 1984b). If the host is suitable, the female then drills through the wall of the puparium with her ovipositor and stings the pupa, paralysing it permanently (Whiting 1967). Females are able to feed on the host's haemolymph and replenish lipid stores accumulated during the larval stage, but spent on egg production and adult maintenance. Females are able to continue egg production throughout their lives, provided they have sufficient nutrient resources (Jervis & Kidd 1986; Rivero & West 2002; Rivero & West 2005). Host-feeding reduces the nutrients in the host, but does not preclude oviposition on the host (Whiting 1967).

The proteinaceous venom used by *N. vitripennis*, which has recently been chemically analysed (Rivers *et al.* 2006), is stored in its active form in a reservoir in the wasp (Ratcliffe & King 1967; Ratcliffe & King 1969 from Rivers *et al.* 2006) and is mostly only effective on host species such as *Sarcophaga bullata* (Rivers *et al.* 1993). The venom inhibits the host's immune response (Rivers *et al.* 2002) and stops it from developing (Rivers & Denlinger 1995a). The longer the adult can be suppressed for before death, the greater the yield of offspring (Rivers & Denlinger 1995b). The venom not only inhibits the immune system and arrests development, in preferred hosts it also causes lipid levels to migrate from the host's haemolymph to its fat body (Rivers & Denlinger 1995b; Rivers *et al.* 1998); the preferential feeding site for parasitoid larvae.

The female oviposits clutches of sticky eggs (typically between 20 – 50 depending on the host; I have found up to 50 individuals in *Calliphora vomitoria* pupae) to the surface of the host pupa underneath the wall of the puparium. The eggs are actually wider than the

ovipositor, or hole, through which they are passed but flex back into shape once attached. The eggs hatch into larvae that fix upon a site away from the oviposition area and begin to feed. They remain there as they develop through four instars until they are fully-grown and pupate themselves. Development time is inversely correlated with temperature, but normally takes 14 days at 25°C (approximately two days as an egg, nine as a larva and three as a pupa). Adults emerge from their pupal case (eclose) and chew an exit hole through the side of the fly puparium. Males are smaller than females and they develop and eclose first, whereupon they wait at the exit hole for females. As the females emerge, the males begin a stereotypical courtship ritual that virgins almost always accept (Burton-Chellew *et al.* 2007). Mating occurs and whilst males remain at the host (they are flightless due to vestigial wings and can manage only limited dispersal), the winged-females disperse in search of new hosts. Females are able to store sperm internally in spermatheca, enabling them to fertilise selectively each egg that they lay. For a more thorough review of development see Whiting (1967) and Godfray (1994).

N. vitripennis is perfectly suited for studying aspects of clutch size and sex allocation theory. Females of the species can create eggs throughout their life (synovigenic), so can be both egg and time-limited, factors that are considered to be of the utmost importance in many models of clutch size behaviour (Godfray 1994). Further, because females mate before dispersal and males have very limited dispersal, *N. vitripennis* can suffer from local mate competition, driving selection for a female-biased sex ratio (Hamilton 1967). However, because more than one female can parasitise a host (superparasitism) levels of local mate competition can vary greatly, selecting high levels of sex allocation precision. I further investigate the issues of clutch size and sex allocation theory in the remainder of the chapter.

1.2. Thesis plan

During the course of this thesis I investigate how clutch size and sex allocation combine to influence offspring fitness in a variety of larval environments. By manipulating host quality through host-feeding, I vary the level of resources available to offspring. Simultaneously, by manipulating the mated-status and number of females ovipositing on a host, I vary the number and sex ratio of offspring competing for resources. Using the data from these techniques, I assess how resource competition inside the host affects larval development and the implications that this has for life-history decisions. In Chapter 2, I manipulate larval conditions and assess whether outcomes of offspring fitness (longevity, fecundity and sex allocation) are correlated with female size. In contrast, Chapter 3 focuses on the correlation between fitness and male size, in terms of longevity and mating success. Differences between male and female competitive ability and nutritional requirements can lead to asymmetric larval competition (described further in 1.3.5), a phenomenon that I test for in Chapter 4. In Chapter 5, I investigate whether inbreeding can also reduce offspring fitness by reducing female longevity or fecundity.

In the appendix, I include other papers on which I worked that either did not involve experimental work, or where I was not the first author. These include: (Appendix A) a commentary on the evolution of sexual reproduction published in Current Biology (Sykes & West 2005) – I was first author; (Appendix B) a study on male influence over sex allocation in *N. vitripennis* (Shuker *et al.* 2006) in which I carried out experiment three; (Appendix C) a microsatellite analysis of mating behaviour in natural populations (Burton-Chellew *et al.* submitted) – I worked in both the field and laboratory, assisted with molecular analysis and commented on the manuscript; (Appendix D) a molecular analysis of the population structure of natural populations (Grillenberger *et al.* 2007) – I worked in the laboratory and assisted with molecular analysis; (Appendix E) an experimental test of whether humans adjust their level of cooperation in response to population structure (West *et al.* 2006) – I helped carry out the experimental data collection for this paper.

In the remainder of this introductory chapter (Chapter 1), I provide a review of clutch size and sex allocation theory in parasitoids. Life-history theory focuses on how an individual should allocate energy in order to maximise fitness (Stearns 1992; Roff 2002a). Energy can be devoted to growth, maintenance or reproduction and the optimal allocation of resources can differ for each individual (Roff 2002a; Stenseth *et al.* 2002). Key areas of investment are clutch size and sex allocation, for which an individual must tailor a response based on their own resources and the environment. More specific reviews for the topics considered in each chapter, are provided in each chapter.

1.3. Clutch Size Theory

Lack's clutch size hypothesis (1947), originating from work on birds, was that overproduction of eggs would simply lead to less food for each offspring, resulting in the death of the weakest. This would not only be a waste of energy in egg production, but also wasted energy going to feeding offspring that never fledged. Further, it could reduce the food, and thus fitness, available for all the other members of the clutch. Instead of laying as many eggs as possible, females should be selected to lay the maximum number they could successfully raise to fledging size. However, a Lack clutch only maximised success from a single reproductive event. In order to survive and reproduce in the future, models showed that frequently clutch size should be reduced owing to the energetic costs of rearing chicks (Williams 1966). However, when clutch size theory was applied to parasitoids it was realised that rather than rearing costs, females are more likely to be constrained by time (or number of hosts) and eggs (Charnov & Skinner 1984; Parker & Courtney 1984; Charnov & Skinner 1985; Waage & Godfray 1985; Wilson & Lessells 1994).

1.3.1. Host quality

The quality of a host is extremely significant, not only because it affects suitability for oviposition, but also because it impacts on the size and quality of the clutch that it can support (Godfray 1994). Gregarious species lay larger clutches on larger hosts (Mayhew & Glaizot 2001), and offspring body size increases with host size both within and across

species (Opp & Luck 1986; Le Masurier 1987; Hardy *et al.* 1992; Ode *et al.* 1996; Mayhew 1998; Mayhew & Hardy 1998). The amount and quality of food available to larvae, have a tremendous impact on fitness directly (Godfray 1994; Jervis & Copland 1996; Rivero & West 2005) and through adult body size, which is frequently correlated with fitness (Godfray 1994; Jervis *et al.* 2003; but for exceptions see King 1989; West *et al.* 1996; Rivero & West 2002; Chapters 2 and 3). Maternal longevity and, particularly in the case of synovigenic individuals, fecundity (Rosenheim 1996), can be linked with resources either sequestered from larval reserves (Rivero & Casas 2001; Rivero & West 2002) or host-feeding (Jervis & Kidd 1986; Heimpel & Collier 1996).

Nasonia vitripennis parasitises a variety of dipteran pupae, ranging from *Musca domestica* (host weight 14 – 27 mg) to *Sarcophaga bullata* (120-132 mg) and *Peckia abnormis* (144-161 mg), with approximate clutch sizes of 6, 50 and 46 individuals respectively (Rivers & Denlinger 1995). Large *N. vitripennis* females have larger eggs (O'Neill & Skinner 1990; Lalonde 2005), larger numbers of eggs and greater lipid and protein reserves, than smaller females (Rivero & West 2002). Lipid availability is the main factor mediating the relationship between size and fitness (Rivero & West 2002). Body size is also positively correlated with longevity when adults are unable to feed (Rivero & West 2002; Chapter 2) giving them more time to find new hosts; larval reserves quickly become exhausted even when sugar-fed (Rivero & West 2002).

1.3.2. Time, host and egg-limited

For many parasitoid species, finding and preparing hosts proves a great cost (Godfray 1994). Preparation can take the form of drilling through plant-tissue or wood to reach a protected host on which to oviposit. Even in the case of parasitoids that must simply find their host, there can be a strong cost of effort involved with host-location. Parasitoids use a number of cues to locate hosts, ranging from the colour change caused by host damage to plants (*Pimpla turionella*; Fischer *et al.* 2004) to learned associations of chemical cues (*Aphytis melinus;* Morgan & Hare 1998), host movement (*Exorista meller*; Stireman 2002) in fruit (Biosteres longicaudatus; Lawrence 1981) and chemicals from larval frass (*Tiphia vernalis, T. pygidalis*; Rogers & Potter 2002). As a result, theory adapted from

optimal foraging theory (marginal value theorem) predicts that the optimal clutch size is strongly influenced by the time taken to locate a host and prepare it for oviposition (Godfray 1994; Sevenster *et al.* 1998; Rosenheim 1999b).

When hosts are scarce, or require preparation time, theory predicts that females should lay large clutches due to the high cost of finding new hosts (Godfray 1994; Sevenster *et al.* 1998; Rosenheim 1999b). Janssen (1989), mirroring low host-encounter rates from observations in the field, found that as the solitary species *Asobara tabida* and *Leptopilina heterotoma*, are increasingly time limited, they accept hosts of lower quality and become more likely to superparasitise. Previous experience of host quality can also affect future time allocation; increasing time between hosts leads to more time spent on each patch and a greater likelihood of superparasitism (*L. heterotoma*, Visser *et al.* 1992; *Trichogramma thalense*, Keasar *et al.* 2001). Increasing inter-patch distance also increases patch-residence time and number of ovipositions in *Diadegma semiclausus* (Wang & Keller 2003). Bezemer and Mills (2003) found that a gregarious parasitoid (*Mastrus ridibundus*) reduces clutch size as host frequency increases in the laboratory, but not in the field, where brood sizes are unchanged, but consistently higher. These findings suggest that time-allocation can play an important, and complex role in clutch size and host use, but that laboratory conditions may frequently be testing adaptation to extreme environments not found in the wild.

The number, frequency and quality of hosts are not the only possible limiting factors. A variety of models focuses on the effects of egg number and egg maturation rates (Briggs *et al.* 1995; Getz & Mills 1996; Shea *et al.* 1996; Murdoch *et al.* 1997; Rosenheim 1999b; Rosenheim *et al.* 2000). Whilst the first egg of a clutch gains a large increment in fitness for the parent, if resources are limited then each subsequent egg gains a successively smaller return. At the extreme, therefore, females can be selected to maximise fitness per egg by laying just one per host (Godfray 1994). The simplest models indicating this response focused on pro-ovigenic females that produced a constant clutch size throughout their life (Parker & Courtney 1984; Waage & Godfray 1985). These models predict smaller clutch-sizes in longer-lived species and in species that emerge carrying fewer eggs. Clutch size should also decrease as maternal condition

declines, but increase if there is an increased likelihood of death (Godfray 1994; Elzinga *et al.* 2005). Both models and studies of egg-limitation have found that host-encounter rate can exceed egg-maturation rate, leading to daily episodes of temporary egg-limitation (Heimpel *et al.* 1998; Heimpel & Rosenheim 1998; Casas *et al.* 2000; Rosenheim *et al.* 2000).

However, whilst there has been considerable debate in the literature over the relevance of time-limited and egg-limited models (see Sevenster *et al.* 1998; Rosenheim 1999b; Ellers *et al.* 2000; Rosenheim *et al.* 2000; West & Rivero 2000), many models now include both parameters, with pro-ovigenic parasitoids being time or egg-limited and synovigenic species being both time and egg-limited (Heimpel *et al.* 1998). West and Rivero (2000) proposed that, at least in solitary species, parasitoids lie on a scale between extreme egg limitation and extreme time limitation. Purely egg-limited females should lay an equal sex ratio, whilst time limited females should produce a sex ratio that reflects the number of hosts most suited to production of a particular sex.

1.3.3. Host-feeding

Approximately 98% of parasitoids are synovigenic (Jervis *et al.* 2001), potentially reducing the effects of egg-limitation (but see Rosenheim *et al.* 2000 which predicts more scenarios for egg-limitation due to temporary egg-limitation). In order to continue producing eggs, synovigenic females can use hosts for host-feeding (Godfray 1994). *N. vitripennis* females are able to both feed and oviposit on the same host which results in more eggs but reduces nutrients for offspring (Heimpel & Collier 1996; Rivero & Casas 2001). Rivero and West (2005) found that females emerging from intensely host-fed hosts were 5% smaller than females emerging from standard hosts. Field estimates of size-fitness relationships in other species suggest that this can decrease fitness by up to 30% (West *et al.* 1996). The 5% body size reduction correlated with 10ug fewer lipid reserves at emergence and a decrease in subsequent egg production (Rivero & West 2005). Host-feeding, therefore, offers another trade-off between current and future reproduction.

1.3.4. Maternal condition and ovigeny index (OI)

Egg-limitation, egg maturation rate, host-finding efficiency and longevity are influenced by female condition (Iwasa *et al.* 1984; Charnov & Skinner 1988; Heinz 1991; Visser 1994; Kazmer & Luck 1995; Rivero & West 2002; Bezemer *et al.* 2005; Rivero & West 2005). Female condition is largely influenced by the quality of host in which a female developed, her age and recent feeding and oviposition behaviour (Godfray 1994; Rivero & West 2002; Rivero & West 2005; Sykes *et al.* 2007; Chapter 2). These separate factors can make it hard to predict the effects of maternal condition across species. However, the ovigeny index (proportion of potential lifetime eggs already being carried by a female upon emergence) has been proposed as a means of grading maternal condition within and between species (Jervis *et al.* 2001; Jervis *et al.* 2003).

The ovigeny index (OI) ranges from zero (no eggs mature at eclosion) to one (entire lifetime egg supply mature at eclosion). Body size and OI are negatively correlated both between (Ellers & Jervis 2003; Jervis *et al.* 2003; Ellers & Jervis 2004; Jervis & Ferns 2004) and within species (Jervis *et al.* 2001; Ellers & Jervis 2003; Ellers & Jervis 2004; Jervis & Ferns 2004; Bezemer *et al.* 2005). OI is also negatively correlated with lifespan both between and across species (Jervis & Ferns 2004; Thorne *et al.* 2006). The perceived reason for these correlations is that by virtue of being larger and having greater resources, females with a low OI can allocate similar resources to egg production as smaller females but still have extra resources for somatic maintenance and longevity (Ellers & van Alphen 1997).

A lower OI means that a smaller proportion of lifetime eggs are available at any one time, but offers greater reproductive plasticity. In contrast, with a high OI, small, or short-lived females can maximise their reproductive success at the first opportunity but are less adapted to varying environments. OI is the degree to which lifetime egg production is concentrated into the early phase of adult life (Jervis & Ferns 2004). For this reason, strict pro-ovigeny (OI = 1) is theoretically linked to (i) uniform host distribution (Ellers, *et al.* 2000; Ellers & Jervis 2003; Ellers & Jervis 2004) and (ii) a very small body size relative to the cost of finding host patches (Ellers & Jervis 2004). In contrast, synovigenic

females with low OI are linked to stochastic host distribution, but have a high reliance on external food sources for egg production. *N. vitripennis* has a very low OI (using data from Rivero and West 2002).

Females with a low OI must host-feed to gain nutrients for further egg production, and host-feeding may be obligate in some species; Mondy *et al.* (2006) found that *Eupelmus vuilletti* emerge with only 30% of the required sterols for oogenesis during their lifetime and must gain the remaining 70% from host-feeding. The costs and benefits of host-feeding are, therefore, dynamic as they are dependent on the female's current condition, number of mature eggs and propensity to reproduce in the future. Burger *et al.* (2004) tested this in a model with the whitefly parasitoid *Encarsia formosa*, which showed that host-feeding is adaptive when hosts are frequent, or life-expectancy is high, but maladaptive when host-frequency is low or life-expectancy short. However, in this model, the parasitoid is able to gain egg-maturing nutrients from other sources. Casas *et al.* (2005) tested a model of lipid and carbohydrate use for the synovigenic parasitoid *E. vuilletti*. The model indicates that lipids from host-feeding are used for egg maturation, whilst carbohydrate and protein stores were allocated to maintenance. However, it did not model the frequency, or occasions on which females should host-feed.

1.3.5. Superparasitism

Superparasitism is the act of adding eggs to a host already parasitised by a conspecific (Godfray 1994). Ovipositing females were originally thought to be acting in error when superparasitising hosts, because the act will tend to push the brood size above the host's carrying threshold and later offspring lag in development, so are more likely to be out-competed. However, the behaviour can be favoured (van Alphen & Visser 1990). In solitary parasitoids, where a single offspring develops from each host, superparasitisim is favoured if: (i) the female is not searching alone; (ii) unparasitised hosts are rare and particularly; (iii) when parasitoids have large supplies of unlaid eggs (Iwasa *et al.* 1984; egg-limited model, van der Hoeven & Hemerik 1990; time limited model, Visser *et al.* 1992). However, a parasitoid searching alone in a patch is unlikely to gain fitness by parasitizing a host it has already attacked, as otherwise a female would have laid two

eggs initially (Godfray 1994). Pexton and Mayhew (2005) recently predicted and found that solitary parasitoids increase their clutch size if there is a high likelihood of superparasitism by a conspecific. They argue that self-superparasitism is preferable to superparasitism by another individual as it reduces further superparasitism and guarantees at least some fitness return. In gregarious parasitoids, such as *N. vitripennis*, superparasitism is predicted when unparasitised hosts are rare and members of the second clutch will still have sufficient resources to develop (Strand & Godfray 1989). The second clutches are both predicted and found to be smaller than the first due to the increased competition for resources (Werren 1980; Werren 1984b; Shuker *et al.* 2005).

1.4. Sex Allocation Theory

The act of superparasitism also has implications for sex allocation, which is the other area of study in which parasitoids have been particularly useful (Hamilton 1967; Werren 1984; Charnov 1992; Godfray 1994; West & Sheldon 2002; Shuker & West 2004). Superparasitism results in a more male-biased sex ratio (Walker 1967) as predicted by local mate competition theory (Suzuki & Iwasa 1980; Werren 1980), but first I shall discuss the impacts of relatedness, local mate competition and host quality.

1.4.1. Relatedness

Fisher's theory of equal investment (1930) includes, amongst others, the assumption of equal maternal relatedness to each gender of offspring. Almost all parasitoids are insects and the majority of these are Hymenopterans and therefore, haplodiploids (Godfray 1994). Haplodiploidy, as recognised by Hamilton in his kin selection theory (1964), results in the curious effect of unequal relatedness between relatives. Sister-sister relatedness (0.5) and brother-to-sister relatedness (0.5) are higher than sister-to-brother relatedness (0.25). Furthermore, when sisters breed with their brothers, as frequently happens in gregarious parasitoids, females have higher relatedness to their daughters (0.75) than to their sons (0.5) and therefore the higher relatedness favours overproduction of females. However, this is not considered to be the over-riding factor for extreme sex ratios in parasitoids, for many of them are diploids (approximately 13,000 species;

Godfray 1994), though inbreeding still plays a part. Instead, Hamilton proposed that the ultimate cause was related to how increasing the proportion of female offspring could reduce the number of brothers competing for mates, at the same time as increasing the number of potential mates (Local mate competition theory; Hamilton 1967).

1.4.2. Local mate competition (LMC)

When offspring mate prior to dispersal there is competition among siblings for resources, namely mates. Local mate competition theory (LMC; Hamilton 1967) predicts females will be selected to reduce competition between related males, and increase the number of available mates, by producing a reduced sex ratio. If offspring have the opportunity to mate with non-siblings, as occurs when multiple females lay on a patch or superparasitise, then local mate competition is reduced and females are selected to produce a higher sex ratio (Werren 1980; Werren 1983).

However, in order for females to optimise their sex allocation, they must have the means of assessing when local mate competition will be reduced. Females of some species respond to the number of females contributing offspring to a patch, both in the laboratory and the field (Werren 1983; Frank 1985; Herre 1985; Molbo & Parker 1996; Herre *et al.* 1997; Flanagan *et al.* 1998). *N. vitripennis* is one such species, (Werren 1980) although rather than focus on the presence of other females (Shuker & West 2004), ovipositing females pay more attention to estimates of egg numbers in a host, yet they seem unable to detect the sex of the eggs themselves (Werren 1984b). Theory predicts that if females can detect the relatedness of other females ovipositing on a patch then they should also adjust the sex ratio accordingly, though there is no evidence of this in *N. vitripennis* (Reece *et al.* 2004; Shuker *et al.* 2004b).

Local mate competition can, however, vary over time as mating structures across a patch enable even limited dispersal to reduce competition (Hardy 1994; Shuker *et al.* 2005). Virgin *N. vitripennis* females are not prone to dispersal but in the absence of mates, virgin males have been known to walk to other hosts, parasitised ones in preference to unparasitised ones (Hardy 1994). Shuker *et al.* (2005) investigated the effect of

asymmetric local mate competition caused by asynchronous emergence. *N. vitripennis* females lay higher sex ratios on an unparasitised host if a parasitised host exists in the patch. Assuming that the time lag is not too great, the males from the focal host will suffer reduced competition as they will be able to mate with offspring from both hosts. Similarly, if laying on an unparasitised host and then a parasitised one, females will increase the sex ratio for the same reason.

1.4.3. Host quality and asymmetric larval competition (ALC)

The fitness of parasitoid larvae normally declines as clutch size increases (Godfray 1994; Rivero & West 2002; Sykes *et al.* 2007). However, it is quite likely that the decline in fitness is not identical for offspring of each sex (Heinz 1991; Godfray 1994; Kazmer & Luck 1995; Rivero & West 2005), leading to overproduction of the sex that leads to lower competition (Bulmer & Taylor 1980; Taylor 1981). There are two forms of asymmetric competition: density dependent competition and composition dependent competition (Godfray 1986). Density dependent competition occurs when increasing the number of competing individuals causes greater fitness costs in one sex than in the other. In contrast, composition dependent competition happens when, at a given clutch size, altering the sex ratio causes a greater fitness cost to a particular sex. As selection favours overproduction of the less damaging sex (Bulmer & Taylor 1980; Taylor 1981) this could alter optimal clutch size and sex allocation. These asymmetries have been observed in species subject to LMC such as parasitoid wasps, but their quantitative effects on clutch size and sex allocation are still being researched. Pickering (1980) discovered that female *Pachysomoides stupidus* are favoured because they outcompete males. In *Bracon hebetor*, increasing offspring sex ratio has a marginal increase on female offspring body size (Ode *et al.* 1996). Van Baaren *et al.* (1999) found that in *Anaphes victus*, superparasitising females must balance the benefits of reduced LMC, against the costs of reduced fitness to second-clutch males that are out-competed by first-clutch females.

Rivero and West (2005) found that male *N. vitripennis* are less affected by larval competition than females are. They found that female offspring size decreases as a function of the proportion of males in a clutch (2005; but see Sykes *et al.* 2007 where

fitness was reduced by the proportion of females not males). This effect on size could be very important for females as fecundity correlates greatly with body size in parasitoids (Visser 1994; Kazmer & Luck 1995; Rivero & West 2002; Jervis *et al.* 2003; Bezemer *et al.* 2005; but see Sykes *et al.* submitted, Chapter 2). Male success seems less impeded by reduced body size (Godfray 1994). This suggests that females will suffer to a greater extent than males from developing in poor quality hosts (Charnov *et al.* 1981; Werren 1984a) and could impact on both clutch size and sex allocation, although asymmetric larval competition may have its greatest influence on sex ratio evolution in species without local mate competition (Sykes *et al.* 2007; Chapter 4).

1.5 Discussion

This review has shown how clutch size and sex allocation theory have developed through work with parasitoids, whilst highlighting the areas specific to parasitoid ecology. I have tried to establish the importance of the interconnecting factors of maternal condition, host quality and mating structure for both sets of theory. However, it is also evident that there are still areas of research that require investigation, such as the relative importance of larval reserves and body size and how they impact optimal investment in egg production and sex allocation. Further, there is a significant gap in the understanding of the impact of host nutrient levels on both male and female fitness. In the ensuing chapters I attempt to investigate these areas further by assessing the relationship between larval competition and fitness, both between and within male and female offspring.

Chapter 2. Relating body size to female fitness

This chapter is being submitted as:

Does body size predict female fitness in the parasitoid wasp *Nasonia vitripennis*?

Ed Sykes, Stuart West, David Shuker

2.1. Abstract

Body size is frequently used as an indicator of female fitness, particularly in parasitoid wasps. This is largely due to both perceived and tested correlations between body size and resource levels, most notably those affecting longevity and fecundity. Here I assess the efficacy of body size as a predictor of female fitness. My findings suggest that whilst body size is a strong predictor of starvation resistance, it is less strongly linked with fecundity or longevity in the presence of food. Instead, larval competition proves more apt in predicting fecundity - I suggest this is due to competition for a resource that can only be amassed during larval development.

2.2 Introduction

Parasitoid wasps have long been considered excellent models for testing a variety of theories on the evolution of life-history strategies (Godfray 1994). When faced with a suitable host, a female wasp must decide what percentage of her resources to allocate to current versus future reproduction. Should she feed and/or oviposit on the host and if she is to oviposit, how many eggs should she lay (clutch size) and what proportion should be male (sex ratio)? A female's response may be influenced not only by her condition but also the host's, and her social environment, such as the presence of other ovipositing females (Hamilton 1967), the size of the other females (Flanagan *et al.* 1998), eggs laid by other females (Werren 1980; Shuker *et al.* 2005), and competitive interactions within or between the sexes (Godfray 1986; Abe *et al.* 2003a; Sykes *et al.* 2007). For many of these decisions, theories exist that can closely predict a female's reproductive strategy. This accuracy is due to the relatively simple link between reproductive behaviour and fitness - the fitness consequences of choosing to feed or lay on a host, for example, are immediate and relatively clear.

A major determinant of fitness in parasitoids and other insects is body size (Godfray 1994). A wasp's body size is influenced by the amount of resources available during development, which is determined by the host's size and quality, along with the number and sex of other wasps also developing within that host (Godfray 1994; Rivero & West 2002; Rivero & West 2005; Sykes *et al.* 2007). The quantity and quality of these resources may affect not just overall body size but also factors such as lipid reserves, which can be used to prolong life in the absence of food (Rivero & Casas 1999a; Rivero & Casas 1999b; Rivero & West 2002). If, upon emergence, the level of lipid reserves or other resources can vary independently of body size, then body size would be less strongly correlated with fitness. For example, in the absence of food, if lipid reserves are the primary determinant of longevity, then fitness may be more strongly linked to lipid reserves than body size (Rivero & West 2002). Complications such as this could help explain why the relationship between size and fitness can vary greatly with the environment being tested (e.g. whether a food source or hosts are provided; Visser 1994;

West *et al.* 1996; Ellers *et al.* 2001), and the fitness measure used (e.g. egg production or longevity, Ellers 1998; Rivero & Casas 1999).

Here, I manipulated the different factors that can influence resource availability for developing larvae and tested how they interact in the parasitoid wasp *Nasonia vitripennis*. I varied: (i) the previous levels of adult feeding on a host so as to vary the amount of resources available (i.e. host quality); (ii) the number of females laying eggs on a host in order to vary the number of larvae competing for those resources. I then determined the consequences of this for the body size, lifespan and reproductive output of the female offspring that emerged from the hosts. My primary aim was to determine whether the influence of host quality (level of previous host feeding) and social environment (number of competitors) could be solely explained by their consequences on body size, or whether they elicited more complicated interactions. For example, is there an effect of host feeding in addition to any consequences for wasp size? If this were the case, one might expect to see variation in reproductive strategies. For instance, individuals with lower lipid stores and thus reduced longevity may invest in early reproduction (Rivero & West 2002). This work represents part of a long-term study examining the precision of reproductive behaviour, especially sex allocation, in this species (Flanagan *et al.* 1998; Reece *et al.* 2004; Shuker *et al.* 2004a; Shuker *et al.* 2004b; Shuker & West 2004; Shuker, *et al.* 2005; Shuker *et al.* 2006a; Shuker *et al.* 2006b; Sykes *et al.* 2007).

2.3. Methods

2.3.1. Study organism

Nasonia vitripennis is a small (females are approximately 2mm long), gregarious, parasitoid wasp that parasitizes large fly pupae (Whiting 1967). Females seek out a host puparium, paralyse the fly pupa within, and oviposit on the pupa's surface. Females can lay in excess of 60 eggs per host, depending on host species and host size. Upon emergence the males wait outside the host puparium for the females, who develop more slowly. They then mate and the females disperse to find new hosts. The flightless males

remain on the natal patch and die. Females are able to develop eggs throughout their lives by feeding on hosts (synovigeny). Feeding does not preclude oviposition on a host but it does reduce the available nutrients for the prospective larvae (Rivero & West 2005). Feeding results in a trade-off between current and future reproduction concerning how nutrients from a host are utilised (Rivero & West 2005).

N. vitripennis has been used extensively as a model organism for studying local mate competition (LMC) and its relationship with sex allocation behaviour. Females produce highly female-biased sex ratios when ovipositing alone (proportion of males = 0.1 - 0.2), through to highly male-biased sex ratios (> 0.9) if laying eggs on previously parasitised hosts (superparasitising; Whiting 1967; Werren 1980; Werren 1983; Werren 1984a; Werren 1984b; Skinner 1985; Orzack & Parker 1986; King & Skinner 1991; King 1992; King & Lee 1994; King *et al.* 1995; King 1996; West *et al.* 1999; West & Sheldon 2002; Reece *et al.* 2004; Shuker & West 2004; Shuker *et al.* 2005; Shuker *et al.* 2006a). In the laboratory, once a host is parasitized there is a window of approximately 48 hours during which further females tend to parasitise the host. Superparasitism rarely occurs after this period because by then the first brood will have developed sufficiently to start utilising the host's resources, leaving too little food for later larvae (Werren 1984; Shuker *et al.* 2005).

2.3.2. Laboratory stock

The focal wasps were from the laboratory strain HV7, originally collected from bird boxes in Hoge Veluwe National Park in the Netherlands by Prof. L.W. Beukeboom in the summer of 2002. I also used a red-eye mutant strain (STDR) to generate experimental hosts that had previously been fed upon. For each strain, stocks were maintained by placing approximately 50 mated female wasps in each of 6 glass vials (25 x 75 mm) plugged with a sponge bung. I provided these females with about 50 hosts (*Calliphora vomitoria* fly pupae) on which to feed and lay their eggs. The vials were kept in an incubator (25 °C, 16H light: 8H dark) and after approximately 14 days the new offspring emerged and mated for 3-5 days. I then again randomly selected approximately 50 mated-females per vial to repeat the whole process.

2.3.3 Experimental design

In order to test how larval conditions affect offspring fitness and how well fitness is predicted by body size, I created a range of competitive larval environments by manipulating the number (1-3) of females ovipositing on the host and the quality of the host itself (standard or fed upon). This influenced both the number of offspring competing for resources in each host and the amount of nutrients available (Fig. 2-1).

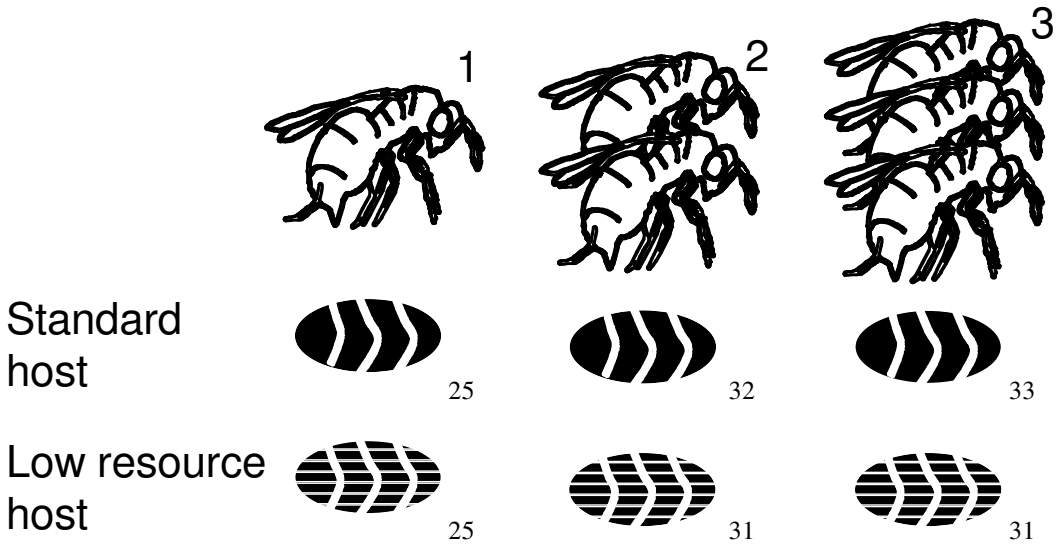


Figure 2-1. Experimental design. Between one and three foundresses were allowed to oviposit on a single host. Each host was either standard or had previously been fed-upon by an STDR (red-eye mutant) female for 2 hours. Emerging focal females were allowed 24 hours in which to mate with males from their brood. I then collected 11 females at random from each brood. 10 of these were placed in labelled, individual glass vials to measure their longevity. They were not supplied with food but were observed regularly until death. The 11th female from each brood was placed in a separate vial and given 6 fresh hosts to parasitise every 48 hours until death. The brood size and sex ratios from these combined hosts were then counted upon emergence of the offspring. Numbers (25 – 33) show sample sizes for each treatment.

A large sample of mated females was collected from the stock cultures of both HV7 and STDR following their emergence and subsequent mating for 24 hours. Each female was individually placed in a 10 x 75 mm vial stopped with cotton wool. I gave each HV7 female a host for 24 hours and then a disc of honey solution-soaked filter paper for a further 24 hours; this period gave the wasps time to feed and mature their eggs.

Simultaneously, the STDR females were each given a host for 2 hours upon which to host-feed (any hosts found to contain red-eye offspring at emergence were rejected; Rivero & West 2002; unpubl. data). I then allocated the HV7 females to one of 6 treatments (approximate sample size of 40 per treatment) varying foundress number between 1 and 3 and providing them with either a standard or previously fed-upon host (Fig. 2-1). Each host was available for a period of 6 hours, after which the ovipositing females were removed and the hosts were placed in the incubator at 25 ºC (16H light: 8H dark conditions) until the offspring emerged. I excluded treatment broods containing diapause individuals, red-eye individuals or just males, as here I am concerned with directly assessing the effects of specific larval environments on female fitness. This meant that my sample sizes varied from 25 to 33 broods per treatment. Diapause occurred more frequently (ranging from 0 to 8 samples) as foundress number increased and host quality decreased. The loss of samples was less than had been anticipated which is why high foundress sample sizes were slightly greater.

I used four measures to assess the consequences of larval competition on female offspring fitness: starvation resistance (individuals were denied access to food following eclosion then monitored to record their lifespan; (Hoffmann & Parsons 1989), reproductive lifespan, fecundity and sex ratio of offspring produced. I wished to assess both starvation resistance and reproductive lifespan because females in the presence of oviposition resources (hosts) are also capable of host-feeding and this may reduce the costs of reproduction, influencing the role of larval competition on adult fitness. I allowed the emerging female wasps from the treatment broods to mate for 24 hours post-emergence and then I randomly selected 11 females from each brood. Out of the 11 females taken from each host, I placed ten into separate 10 x 75 mm vials and recorded their starvation resistance, scanning them every 3 hours between 5 am and 11 pm. I used the mean lifespan of the 10 females from each brood to avoid pseudo-replication. The 11th female from each brood was used to assess the effects of my treatments on fecundity and reproductive lifespan. Each female was placed in a separate 10 x 75 mm vial, stopped with cotton wool, and provided with 6 fresh hosts every 48 hours until death. The parasitised hosts were then placed in fresh vials so that the offspring could develop, emerge and be sexed and counted. I recorded the brood sizes and sex ratios for the $1^{st,}$ 4^{th},

7^{th} and 10^{th} 48-hour oviposition periods (the maximum number of oviposition periods was 13) to explore how female fecundity changed over the lifetime. Hosts were dissected to count diapause and non-developed larvae. These larvae were used for clutch size data but not sex ratio work. I assume that there is negligible larval mortality and that the number of emerging adult offspring is a good proxy for the number of eggs laid. In total, the 177 experimental females produced 49,803 offspring across the four oviposition periods. Once the 11 experimental females had been taken from each treatment brood, the remaining offspring were left to die, then counted and sexed. I added these data to the eleven collected females to estimate the degree of larval competition experienced by the focal females in each brood. I also measured the right-hind tibia of up to 5 females from each brood as an estimate of body size (Godfray 1994; Rivero & West 2002). To do this I dissected each leg and used a Leica dissecting microscope fitted with an eyepiece micrometer at x100 magnification. Body size and fitness components were therefore measured using different subsets of female offspring from each brood.

2.3.4. Analysis

I used a general linear modelling approach. First I tested that my treatments had created variation in brood size and offspring body size, and then I explored the relationships between female body size and components of fitness (starvation resistance, reproductive lifespan, fecundity, sex ratio of offspring). For each fitness component I first assessed how much variation could be explained using all relevant variables, including female body size. I fitted full models containing all the main effects and their interactions, and then manually simplified the models to the minimum adequate model through stepwise deletion of non-significant terms ($p > 0.05$ for main effects, $p > 0.01$ for second order interactions; (Crawley 2002) using the statistical package JMP IN 5.1 (SAS Institute Inc.). When appropriate I then assessed how much variation could be explained by female body size alone in comparison to the amount of variation explained by the minimum adequate model.

Sex ratio data were analysed using arc-sine square root transformed data, since the data were too over-dispersed for analysis with binomial error structures (see Wilson & Hardy

2002). Female reproductive effort could theoretically change over the course of her life. To establish the factors that influenced the brood size or sex ratio produced by a female at any given point during her lifetime, I performed a generalised linear mixed-effects model (GLMM) using the statistical program GENSTAT (Lawes Agricultural Trust 2007). I introduced wasp identification number as a random effect because I was testing repeated measures. In each case I ran the full model then used stepwise simplification, by removing terms with $p > 0.05$ for main effects, $p > 0.01$ for second order interactions, until I had reached the minimal model.

2.4. Results

2.4.1. Effect of treatment on brood size, body size and sex ratios

By varying the number of foundresses that oviposited on each host I successfully manipulated the size of broods and the resulting body size of the females emerging from them - an increase in foundress number correlated with an increase in brood size (Table 2-1; $F_{1, 174} = 54.77$, $P < 0.0001$) and a decrease in body size (Table 2-1; $F_{1, 167} = 69.85$, $P < 0.0001$). Using hosts that had previously been fed-upon reduced brood size (Table 2-1; $F_{1, 174} = 4.45$, $P = 0.036$) and increased body size (Table 2-1; $F_{1,167} = 11.77$, $P = 0.0008$). There was no interaction between foundress number and host quality on brood size ($F_{1, 173} = 2.48$, $P = 0.117$) or body size ($F_{1, 166} = 3.72$, $P = 0.055$). The proportion of males in the treatment broods correlated positively with

Foundresses		Brood size			Sex ratio			Leg-length		
		Mean	SE	N	Mean	SE	N	Mean	SE	N
Std	1	31.36	1.46	25	0.11	0.01	25	658.00	7.58	25
	2	35.28	1.69	32	0.23	0.02	32	639.84	5.94	31
	3	43.46	2.99	33	0.32	0.03	33	611.13	9.16	31
Poor	1	25.00	1.20	25	0.15	0.03	25	692.87	5.10	25
	2	30.26	2.11	31	0.23	0.02	31	662.65	8.34	27
	3	43.58	1.61	31	0.30	0.02	31	618.01	5.97	31

Table 2-1. Varying the number of foundresses and quality of hosts used (standard or previously fed-upon for 2 hours) caused variation in both the size and sex ratio of the brood and the body size of the females developing in the brood (measured as length of left-hind tibia).

foundress number (Table 2-1; $F_{1, 175} = 85.93$, $P < 0.0001$). In contrast host quality had no effect on the broods' sex ratio ($F_{1, 172} = 0.29$, $P = 0.588$), nor was there any interaction between foundress number and host quality ($F_{1, 171} = 1.58$, $P = 0.210$).

The variation in brood size also correlated with variation in body size, as measured by leg-length. I reduced the full model (containing brood size in which female developed and host quality) to the minimum adequate model. Body size was negatively correlated with the size of the brood in which the female developed (Fig 2-2; $F_{1, 167} = 348.590$, $P < 0.0001$) and the females developing in standard quality hosts were smaller than those developing in poor quality hosts ($F_{1, 167} = 5.987$, $P = 0.016$). There was no significant interaction between host quality and brood size on leg-length ($F_{1, 166} = 1.724$,$P = 0.191$).

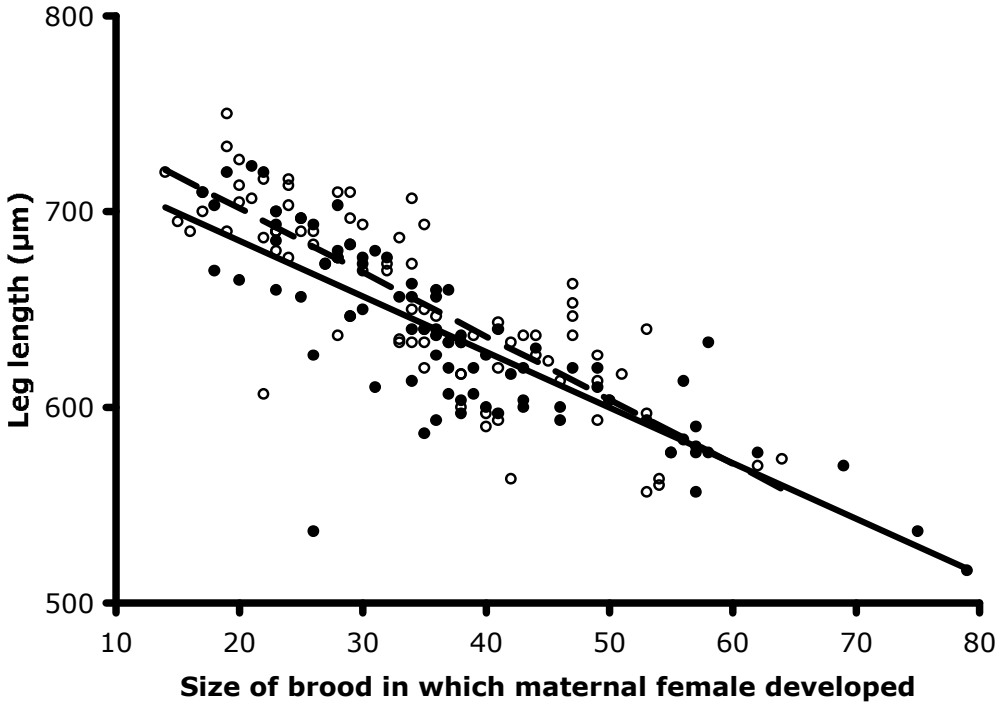


Figure 2-2. The relationship between larval competition faced by females and their body size as measured by hind tibia length. Females either developed in standard hosts (filled circles; solid line) or in hosts that had previously been fed-upon (open circles; dashed line). Body size negatively correlated with brood size in females from poor (Rsq = 0.686; -3.27*brood size + 767.19) and standard hosts (Rsq = 0.673; -2.84*brood size + 741.93).

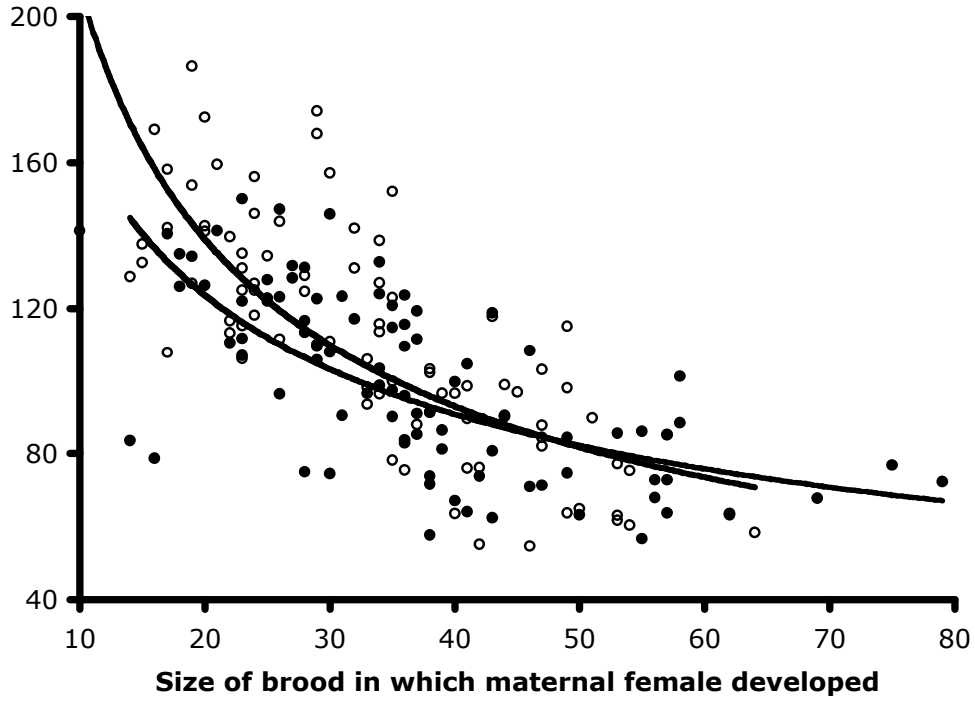
2.4.2 Effect of treatment and body size on female starvation resistance

I first determined the minimum adequate model for female longevity when deprived of food as an adult. The full model contained body size (female leg length), the size and sex ratio of the brood in which the female developed and host quality. Starvation resistance was positively correlated with body size (Fig. 2-3b; $F_{1, 162} = 32.497$, $P < 0.0001$) and had a negative quadratic correlation with the size of the brood in which the female developed. The effect of each additional wasp reduced as brood size increased (Fig. 2-3a; $F_{1, 162} = 4.871$, $P = 0.029$). There was also a significant interaction between the quality of the host and the proportion of males in the brood in which the female developed; females from broods containing higher proportions of males in standard quality hosts had higher starvation resistance than those in poor quality hosts (Fig. 2-3c; $F_{1, 162} = 12.664$, $P = 0.0005$). In contrast, starvation resistance showed no significant correlation with host quality ($F_{1, 162} = 0.876$, $P = 0.351$) or brood sex ratio as main effects ($F_{1, 162} = 0.029$, $P = 0.866$) but they were kept in the minimum model due to their significant interaction. No other interaction from the full model was significant ($P > 0.01$ in all other cases). Overall the minimum adequate model explained 68.2% of the variation.

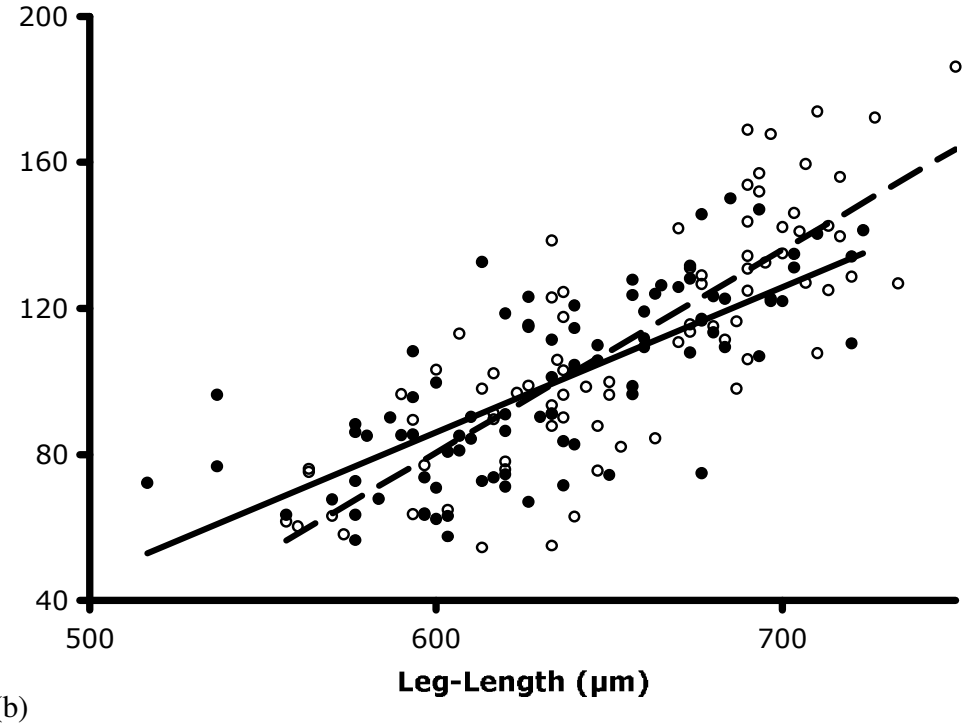
Due to the high correlation between body size and the size of the brood in which the female developed, I also assessed the full models without each of these factors present. When body size was excluded, the minimum adequate model consisted of host quality, size of brood and their interaction. This model explained 55.8% of the variation. In contrast, when size of brood was excluded, the minimum model consisted of body size, host quality, sex ratio of brood and an interaction between these last two. This model explained 63.8% of the variation. When the effect of body size was analysed on its own, without any other explanatory variables, it explained 61% of the variation ($F_{1, 167} = 260.98$, $P < 0.0001$).

2.4.3. Effect of treatment and body size on female reproductive lifespan

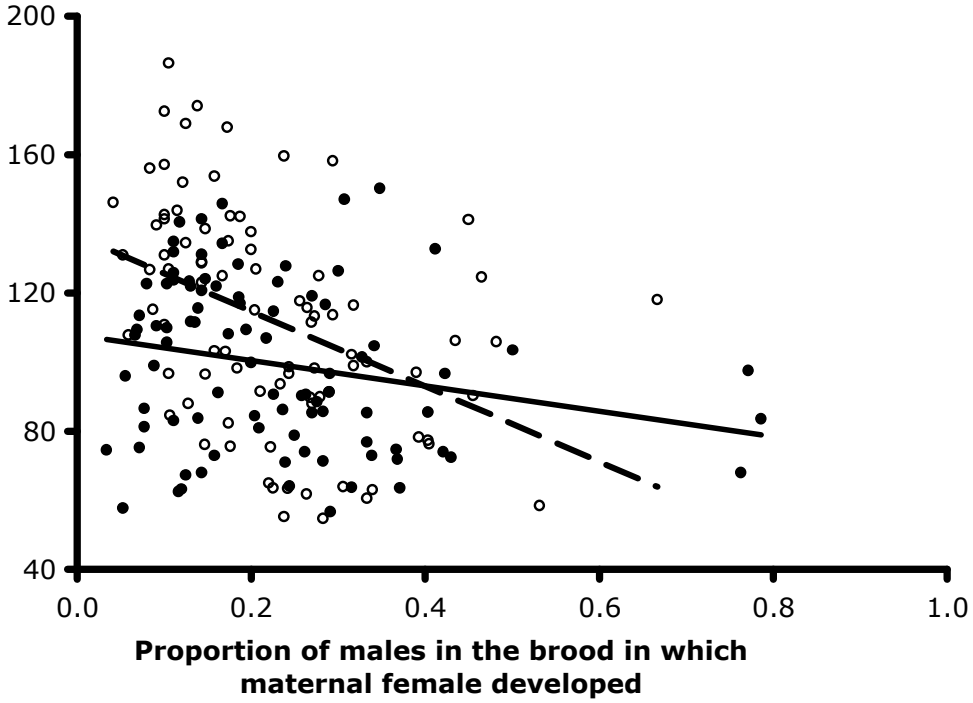
I first determined the minimum adequate model for the longevity of females with access to hosts. Longevity was slightly greater in females that had developed in poor quality hosts ($F_{1, 167} = 3.957$, $P = 0.048$). Host quality explained 2.3% of the variation. No other


200
160
120
80
40
10
20
30
40
50
60
70
80
Size of brood in which maternal female developed

(a)


200
160
120
80
40
500
600
700
Leg-Length (µm)

(b)



c)

Figure 2-3. This shows the relationship between female longevity in the absence of food and (a) her body size (b) the size of the brood in which she developed and (c) the proportion of males in that brood. The data include mothers from standard hosts (filled circles; solid line) and hosts that had previously been fed-upon (empty circles; dashed line). In Fig. 2-3(a) longevity is positively correlated with the size of brood in which the female developed (Poor hosts: Rsq = 0.609; -2.03*brood size + 180.021; Standard hosts: Rsq = 0.415; -1.17* brood size + 142.985). In Fig. 2-3(b) longevity is positively correlated with body size (Poor hosts: Rsq = 0.654; 0.55* body size -251.64; Standard hosts: Rsq = 0.545; 0.40* body size – 152.95). In Fig. 2-3(c) longevity is influenced by the interaction between host quality and the proportion of males in the brood in which the maternal female developed (Poor hosts: Rsq = 0.199; -110.39*transformed sex ratio +162.95; Standard hosts: Rsq = 0.043; -33.43*transformed sex ratio + 114.803).

effect (including body size ($F_{1, 165} = 0.386$, $P = 0.535$), initial brood size ($F_{1, 160} = 1.12$, $P = 0.292$) and proportion of males in the brood ($F_{1, 166} = 2.486$, $P = 0.117$)) or interaction was significant ($P > 0.01$ in all cases). I assessed whether the proportion of females that oviposited varied with treatment over time. Whilst the proportion decreased at successive oviposition periods ($F_{1, 22} = 100.335$, $P < 0.0001$), there was no effect of treatment ($F_{1, 21} = 1.213$, $P = 0.283$).

2.4.4. Effect of treatment and body size on total female fecundity

Total female fecundity was estimated by summing the number of offspring produced over the four oviposition periods (every 48 hours females were given 6 fresh hosts; I counted offspring from the 1st, 4th, 7th and 10th periods). I reduced the full model (containing host quality, size and sex ratio of brood in which maternal female developed and maternal size) to the minimum adequate model. Total fecundity was negatively correlated with the size of the brood in which the maternal female developed (Fig. 2-4; $F_{1, 175} = 16.061$, $P < 0.0001$) and explained 8.4% of the variation. No other main effect (sex ratio, $F_{1, 174} = 0.071$, $P = 0.790$; host quality, $F_{1, 172} = 0.093$, $P = 0.761$; leg-length, $F_{1, 170} = 0.096$, $P = 0.757$) or interaction was significant ($P > 0.01$ in all cases).

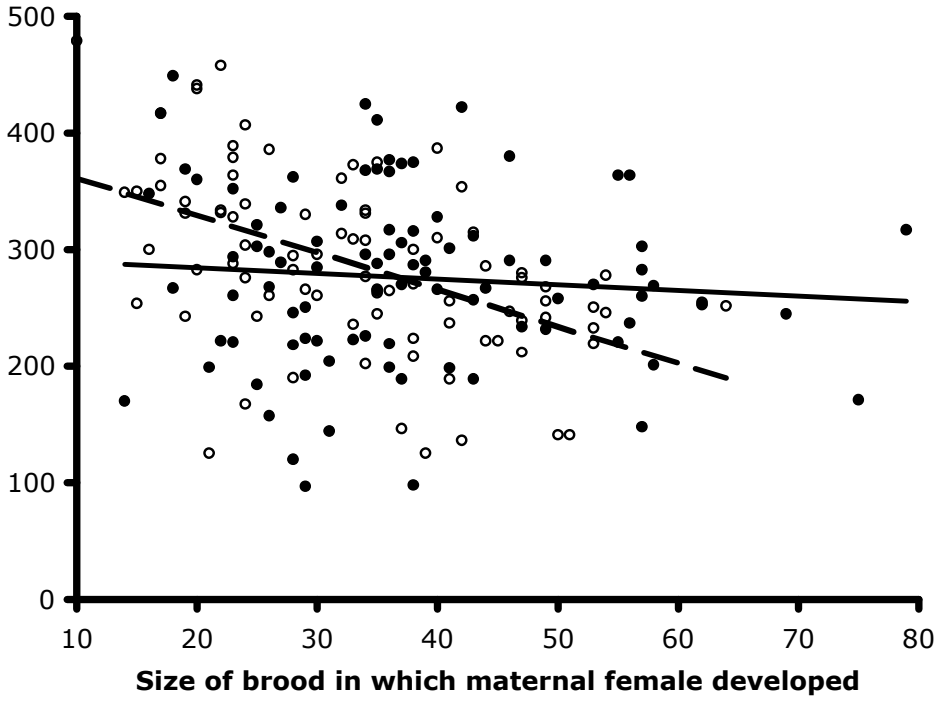


Figure 2-4. This shows the relationship between the number of brood mates that a female competed with during larval development and my sample of her lifetime fecundity. Lifetime fecundity was established by totalling the number of offspring produced at the 1st, 4th, 7th and 10th ovipositioning opportunities. The data include mothers from standard hosts (filled circles; solid line) and hosts that had previously been fed-upon (empty circles; dashed line). Female fecundity was negatively correlated with the size of the

brood in which she developed (Poor hosts: Rsq = 0.257; -3.17*brood size +392.85; Standard hosts: Rsq = 0.007; -0.49*brood size + 294.32).

2.4.5. Effect of treatment and body size on female fecundity over time

The size of any given brood produced by a female was negatively correlated with oviposition period in a quadratic fashion with the decline in size increasing between each successive oviposition period (Fig. 2-5; $F_{1, 623} = 149.875$, $P < 0.0001$). I wanted to determine whether the effect of treatment and body size changed over the course of a female's lifetime as a result. I established the minimum adequate model and discovered that the negative correlation between brood size and oviposition period explained most of the variation ($F_{1, 530} = 911.894$, $P < 0.0001$). The size of brood produced was negatively correlated with the number of brood-mates that a female

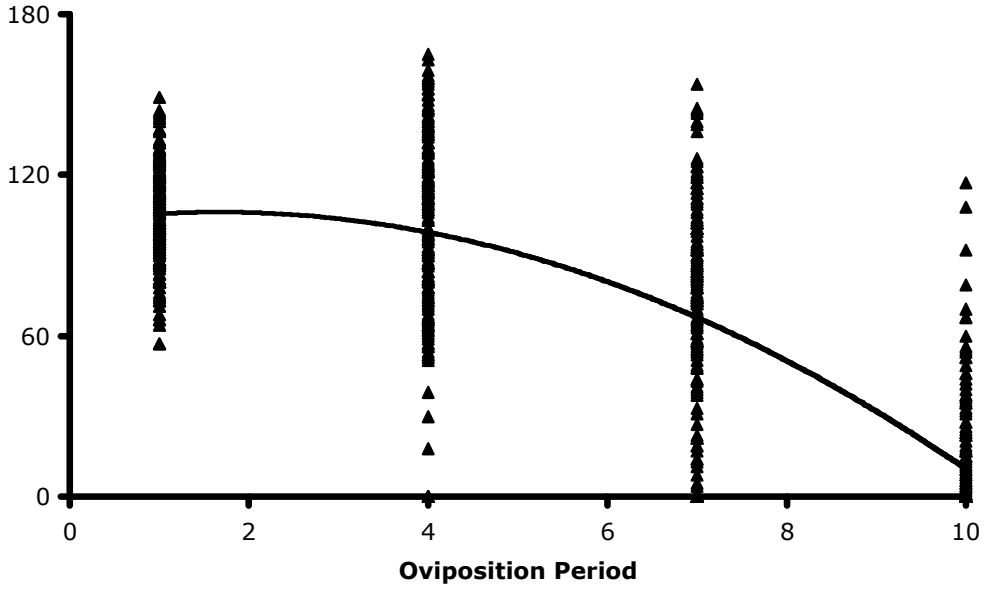


Figure 2-5. This shows the quadratic relationship between the size of brood produced by a female and the oviposition period at which she produced the brood. The data include brood sizes produced from females that developed in both poor and standard quality hosts. The size of the brood produced was negatively correlated with the oviposition period (Rsq = 0.545; -10.63*oviposition period + 128.24).

developed with ($F_{1, 173} = 16.009$, $P = 0.001$) and was also affected by a significant interaction between the number of brood-mates that a female developed with and the

quality of host that she was developing in; the negative correlation between brood-mates and brood size produced was far steeper for females that developed in poor quality hosts, compared to those that developed in standard hosts (Fig. 2-6; $F_{1, 173} = 10.056$, P = 0.0018). In contrast, brood size showed no significant correlation with female body size ($F_{1, 163} = 0.011$, P = 0.918) or host quality ($F_{1, 173} = 0.959$, P = 0.329) as main effects but host quality was kept in the minimum model due to its significant interaction with brood mates. No other interaction from the full model was significant. Overall, the minimum adequate model explained 9.20% of the variation.

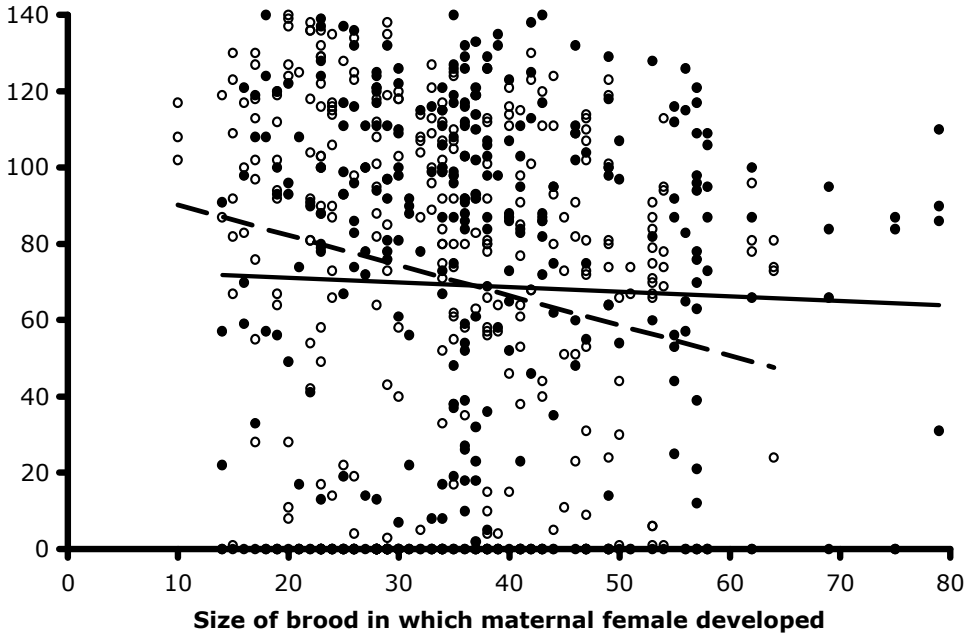


Figure 2-6. This shows the relationship between the size of a brood produced by a female and the size of the brood in which she developed. The data include mothers from standard hosts (filled circles; solid line) and hosts that had previously been fed-upon (empty circles; dashed line). The size of brood produced was negatively correlated with the size of the brood in which the maternal female had developed (Poor hosts: Rsq = 0.048; -0.79*brood size + 105.23; Standard hosts: Rsq = 0.002; -0.13*brood size + 85.28).

2.4.6. Effect of treatment and body size on sex ratio of offspring at a given time

I reduced the full model (containing quality of host in which the female developed, oviposition period, size of the brood in which she developed, her body size and all the available interactions) to the minimum adequate model. The sex ratio of the offspring produced by a female was negatively correlated with size of the brood produced ($F_{1, 376}$ = 189.832, P < 0.0001) but positively correlated with the oviposition period ($F_{1, 376}$ = 185.525, P < 0.0001). Offspring sex ratio was also positively correlated with the size of the brood in which the female developed ($F_{1, 175}$ = 12.065, P = 0.0006). There was also a significant interaction between oviposition period and larval competition ($F_{1, 376}$ = 13.192, P = 0.0003); as the oviposition period increased, females from small initial broods produced much higher proportions of males than did females from larger initial broods. Body size had no significant effect ($F_{1, 165}$ = 0.227, P = 0.634) nor did any of the other interactions (P > 0.01 in all cases). Overall, the minimum adequate model explained 32.43% of the variation.

2.5. Discussion

My results show that whilst female body size provides a good indication of longevity in the absence of food, larval competition gives a more accurate representation of fecundity. Starvation resistance was positively correlated with body size and, to a lesser extent, negatively correlated with the size of the brood in which the female developed. However, body size had no significant effect on any of my other measurements of fitness (reproductive lifespan, fecundity, offspring sex ratio). In contrast, increasing larval competition faced by a developing female not only reduced her starvation resistance but also her fecundity and the proportion of female offspring that she produced. Females developing in poor quality hosts had lower starvation resistance, in broods of high sex ratio, but a greater reproductive lifespan. This effect could have had implications for the methodology of the experiment as females developing in poor quality hosts may have delayed their reproductive effort. However, I found no effect of treatment on the

proportion of females that laid at any given oviposition period. Poor quality hosts did result in reduced female fecundity as larval competition increased.

Fitness is generally measured in terms of the number of successful offspring produced and for parasitoids the main limits to fitness are either being host or egg limited (Rosenheim 1996; Sevenster *et al.* 1998; Rosenheim 1999b; Ellers *et al.* 2000). A female is host (or time) limited if the number of hosts she can locate and oviposit on limits her reproductive success. In contrast, a female is egg limited if the number of eggs she is carrying limits her reproduction. The measures of fitness that I use in my experiments, longevity in the absence of hosts, and fecundity, correspond to host and egg limitation respectively. In nature, individuals will often be in an intermediate position along the continuum between extreme host or egg limitation (Hunter & Godfray 1995; Ellers *et al.* 1998; West *et al.* 1999; West & Rivero 2000). *Nasonia vitripennis* females emerge without any mature eggs, but with reserves of lipids and glycogen that they can use for developing eggs and sustenance whilst searching for fresh hosts (Rivero & West 2002).

Consequently, my findings suggest that body size can be used to predict the fitness cost of being host limited (longevity), whilst larval competition can predict the likelihood of being egg-limited (fecundity). The results support the conclusion that body size predicts longevity when females are not feeding, because larger individuals have greater lipid reserves (Rivero & West 2002). Lipid reserves can sustain females when carbohydrates are in short supply and larger females, carrying more reserves, have a larger window in which to find fresh hosts. In contrast to Rivero and West (2002; 2005) however, body size was a less useful predicter of fecundity. In the 2002 study they found that larger females carry more eggs, however, to dissociate host-feeding and egg-laying their experimental females were fed on a diet only of water or honey-solution before being dissected to count their egg load; they were not given access to a host. In the 2005 study they were assessing the costs associated with laying eggs in a host that had already been used for host-feeding. Therefore they tested the size of brood produced by females offered hosts of varying quality, as opposed to the size of brood produced by females developing in hosts of varying quality.

My finding that larval competition and host quality, but not body size, affect fecundity suggests that a key component other than lipids and independent of body size, is amassed during the larval stage. As female fecundity declined over time this also suggests that the component cannot be harvested through host-feeding as an adult. Added to this, the fact that being raised in poor quality hosts increased the cost of larval competition at high densities, and reduced the number of female offspring produced, suggests a further cost of host-feeding (see (Rivero & West 2005).

Body size is frequently used as an indicator of female longevity and fecundity (Charnov & Skinner 1984; Godfray 1994; Rivero & West 2002). My findings highlight that whilst body size may be a strong predictor of starvation resistance, environmental conditions may reduce its efficacy in predicting fecundity. In this instance, larval competition and host-feeding highlight the importance of another, as yet unknown resource. This study adds further detail to the ongoing work examining the importance of resource allocation in the precision of reproductive behaviour (Jervis & Kidd 1995; Flanagan *et al.* 1998; Rivero & Casas 1999a; Rivero & Casas 1999b; Reece *et al.* 2004; Shuker & West 2004; Shuker *et al.* 2006; Sykes *et al.* 2007).

Chapter 3. Relating body size to male fitness

This chapter is available in advance on-line and is in press for Evolutionary Ecology Research as:

The cost of mating and the relation between body size and fitness in males of the parasitoid wasp *Nasonia vitripennis*

Maxwell N. Burton-Chellew*, Edward M. Sykes, Sophie Patterson, David M. Shuker, and Stuart A. West.

3.1 Abstract

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.

3.2 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 and 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; Rivero & West 2002; Ellers & Jervis 2003; 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 1985; 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). 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. 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 is a cost of mating to males, and if this cost differentially affects males of different sizes. For instance, smaller males may suffer a greater reduction in longevity as a result of mating, limiting lifetime mating success.

3.3 Methods

3.3.1 Study organism

Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae) is a 2-3 mm long, gregarious parasitoid wasp of dipteran pupae, including numerous species of *Calliphoridae* and *Sarcophagidae* (Whiting 1967). Like all Hymenopterans, *Nasonia* is haplodiploid, with females developing from fertilized (diploid) eggs, and males from unfertilized (haploid) eggs. The sex ratio is often very female biased as a response to local mate competition (Hamilton 1967; Werren 1980; 1983; Orzack 1986; Orzack & Parker 1990; Orzack, Parker *et al.* 1991; Flanagan *et al.* 1998; Reece *et al.* 2004; Shuker *et al.* 2004a; 2005; 2006a; 2007b). Females typically mate once before dispersing to find new oviposition sites (Burton-Chellew *et al.* 2007; Shuker *et al.* 2007a). The polygynous males are brachypterous and unable to fly, remaining at the site of adult emergence to compete with each other for access to emerging females. 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).

N. vitripennis males exhibit a stereotyped courtship performance consisting of mounting the female in response to volatile compounds that signify a female's presence and performing multiple series of 4-7 head-nods, with each series separated by a 5-10 second interval (Van den Assem *et al.* 1980b; Beukeboom & Van den Assem 2001). During courtship, the male releases mandibular pheromones during the first head nod of each series. 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.* 1980; Van den Assem & Jachmann 1999; Bordenstein *et al.* 2000). Copulations are short, with a mean of approximately 14 seconds (Burton-Chellew *et al.* 2007), although

courtship duration varies with number of head-nod series: mean number of head nod series until male gives up 8.16 ±0.23 (Beukeboom & Van den Assem 2001). Males are unable to force unreceptive females into copulating. After copulating the male performs a stereotyped post-copulatory courtship performance that serves to reduce future female receptivity; 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).

3.3.2 Experimental strains and maintenance

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. Wasp strains were maintained in mass culture, generally at 25°C, under 16h:8h, light : dark conditions. Under this regime, males start to emerge after 13-14 days and mate with females who emerge soon after. All wasps were reared on *Calliphora vomitoria* hosts. Stock cultures were maintained in replicate transparent glass vials of 75 x 25 mm proportions. Typically, on the fourth day following adult emergence, approximately 40 females were transferred to each of several new replicate vials of identical proportions and incubated with around 40 fresh (less than one month old at 4°C) hosts. Population densities during the four days before re-culturing were typically in excess of 500 individuals, with the aim of avoiding any inbreeding effects associated with small population size.

3.3.3 Experiment on 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. Due to time constraints, only the size of focal males was measured but previous work has shown that offspring body size is strongly negatively correlated with foundress number (Chapter 4; Sykes *et al.* 2007). 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).

3.3.4 Experiment on 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 (4%; Burton-Chellew *et al.* 2007; Shuker *et al.* 2007), 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. Holmes (1974) suggests that to establish conservative estimates of multiple mating, one should double the proportion of mixed broods found as females may not use the sperm. This gives an estimate of 3% of females mating multiply in my experiment. In total, 81 trials were performed over three days.

3.3.5 Experiment on 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: $F_{1,102} = 84.58$, $P < 0.0001$). This was to create even more extremes in size and fits with behaviours found in the wild

(Burton-Chellew et al. submitted; Appendix C). 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.

3.3.6 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 (Charlesworth *et al.* 1994), 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).

3.4 Results

3.4.1 Experiment on 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. 3-1; $\chi^2_{(1)} = 0.72$, 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).

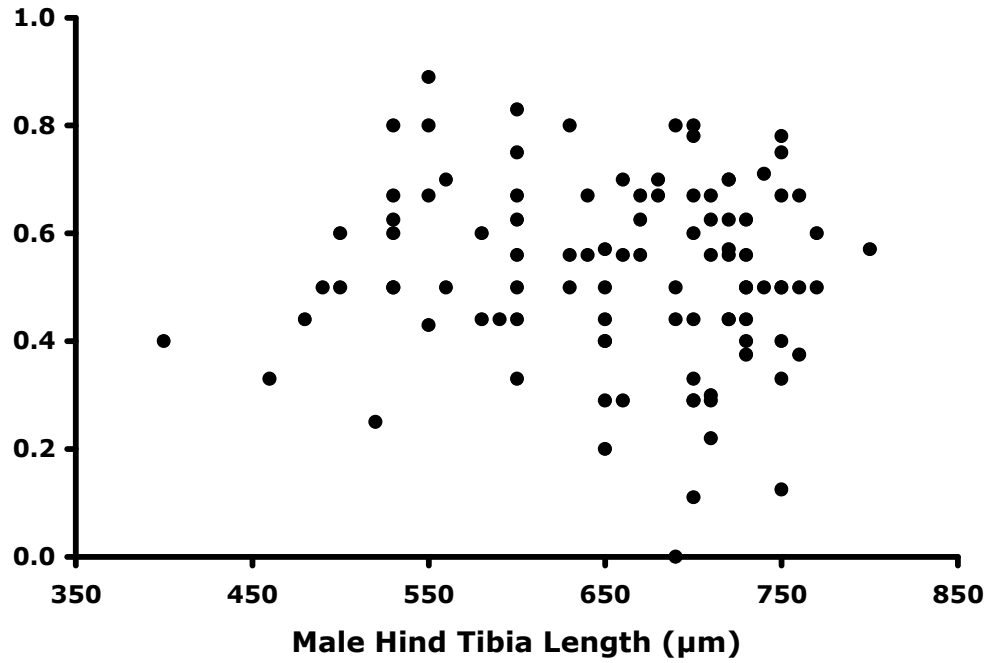
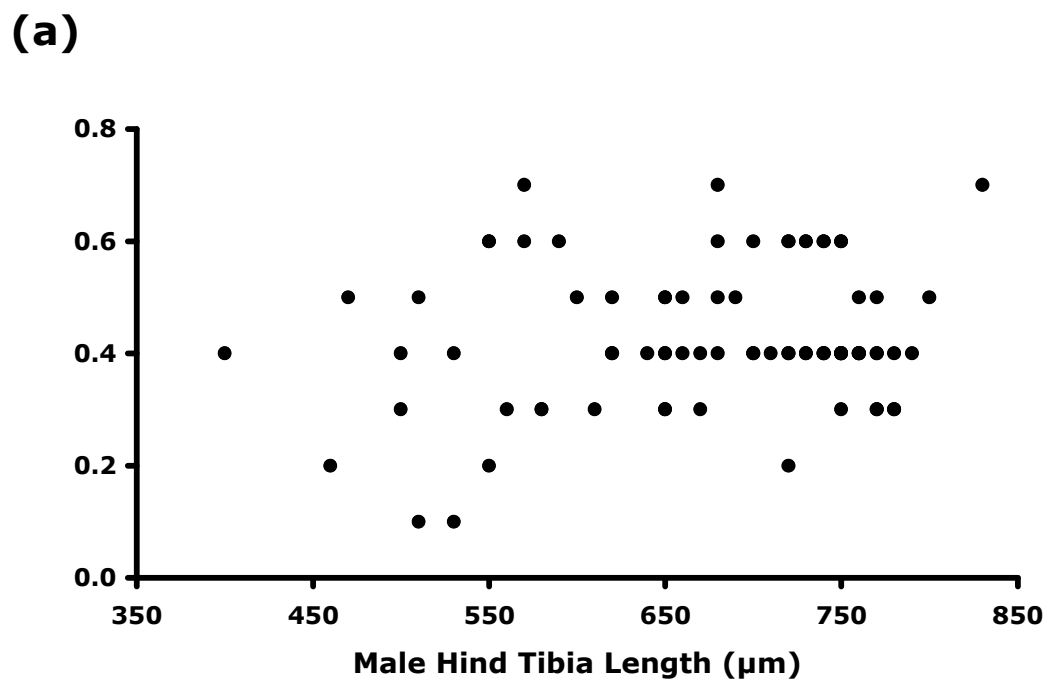


Figure 3-1. The insemination success of solitary males. Body size did not influence the proportion of females that a male could inseminate; when placed with 10 females for 15 minutes.

3.4.2 Experiment on 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. 3-2a), or his size relative to that of the other male ($\chi^2_{(1)}$ = 0.80, P = 0.37; Fig. 3-2b).



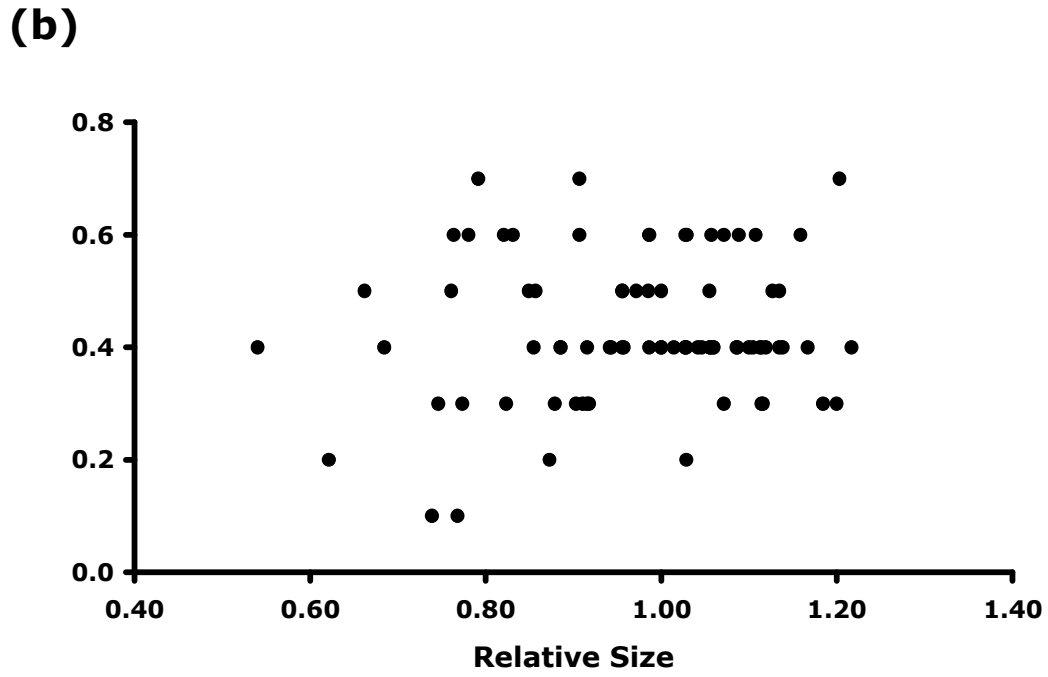


Figure 3-2 (a+b). The insemination success of males in competition. The proportion of females that a focal male inseminated, when placed with 10 females and one other male for 15 minutes, was not significantly influenced by either: (a) the size of the focal male; or (b) the relative size of the focal 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 inseminations, compared to only 0.43 ± 0.14 for males in competition ($\chi^2_{(1)} = 15.43$, P < 0.0001, N = 180).

3.4.3 Experiment on 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. 3-3).

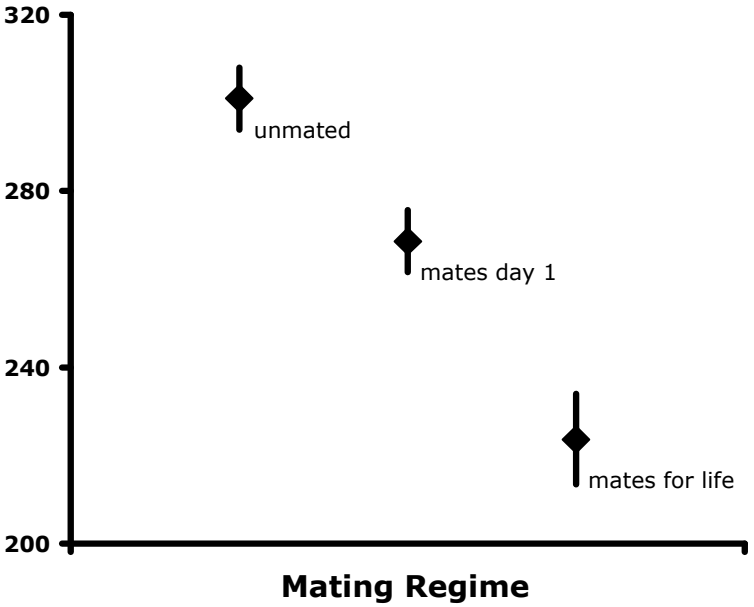


Figure 3-3. The effect of mating on longevity (mean ± SEM). Virgin males live significantly longer than mated males, which in turn live significantly longer than males that had mates for their whole lives.

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. 3-4). 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 3-4. 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 ($F_{1,39} = 1.22$, $P = 0.28$, $HF = 1.82$).

3.5 Discussion

Male size had no significant affect on my measures of fitness in any of the three experiments. These experiments examined mating success when alone (Fig. 3-1), mating success when in competition (Fig. 3-2), and lifetime mating success (LMS) when provided with daily access to mates (Fig. 3-4). 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. 3-3).

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). 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.* 1980), 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 1999; Blanckenhorn *et al.* 2002; Moore *et al.* 2003; Shuker *et al.* 2006) 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 2004; 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, it is possible that the costs to males derived from co-habiting

with females as they could have monopolised the food resource (honey-soaked paper discs). This is unlikely as the discs are frequently used in the lab to maintain far greater numbers of wasps and are large enough that numerous wasps can feed from one disc at the same time. It is more likely that, in this instance, 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.* 1980; for an example of costly pheromone production see Johansson, Jones *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.* 2007); 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.* 2007; only 2 of 49 females sampled in the wild were polyandrous; Grillenberger *et al.* 2007). 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 2001; Burton-Chellew *et al.* 2007; Shuker *et al.* 2007). 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.* 2006). 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 1996), 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 4. The role of asymmetric larval competition

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4.1 Abstract

Sex allocation theory offers excellent opportunities for testing how animals adjust their behaviour in response to environmental conditions. A major focus has been on instances of local mate competition (LMC), where female-biased broods are produced in order to maximise mating opportunities for sons. However, the predictions of LMC theory can be altered if there is both local competition for resources during development and an asymmetry between the competitive abilities of the sexes, as has been seen in animals ranging from wasps to birds. Here, I test the extent to which asymmetric larval competition alters the predictions of LMC theory in the parasitoid wasp *Nasonia vitripennis*. I found that the body size of both sexes was negatively correlated with the number of offspring developing within the host. Further, I found that when faced with high levels of competition, the body size of females, but not males, was influenced by the sex ratio of the competing offspring – females were smaller when a higher proportion of the brood was female. This asymmetric competition should favour less biased sex ratios than are predicted by standard LMC theory. I then develop a theoretical model that can be parameterised with our data, allowing me to determine the quantitative consequences of the observed level of asymmetric larval competition for sex allocation. I found that although asymmetric competition selects for less biased sex ratios, this effect is negligible compared to LMC. Furthermore, a similar conclusion is reached when I reanalyse existing data from another parasitoid species where asymmetric larval competition has been observed - *Bracon hebetor*. Consequently, I suspect that asymmetric larval competition will have its greatest influence on sex ratio evolution in species that have smaller clutches and where local mate competition is not an issue, such as birds and mammals.

4.2 Introduction

Sex allocation theory allows some excellent opportunities for testing how animals adjust their behaviour in response to environmental conditions (Charnov 1982; Hardy 2002; West & Sheldon 2002). Fisher (1930) showed that frequency dependent selection would favour the less common sex, selecting for equal investment into male and female offspring. Since then, it has been realised that this breaks down if there are competitive interactions between relatives, termed local resource competition (LRC, Hamilton 1967; Clark 1978). If competition for resources differs between the sexes, such that the production of one sex leads to greater LRC, then selection favours overproduction of the sex that leads to less LRC (Bulmer & Taylor 1980; Taylor 1981).

The form of LRC that has attracted the most attention is local mate competition (LMC; Hamilton 1967). Hamilton (1967) showed that when mating takes place between the offspring of a small number of mothers, before the daughters disperse, then a female biased sex ratio is favoured. This bias is favoured because it leads to reduced competition for mates between related males, and provides more mates for sons (Taylor 1981). In haplodiploid species an additional bias is favoured because inbreeding increases the relative relatedness of mothers to daughters (Hamilton 1972; Frank 1985; Herre 1985). There is a huge amount of experimental support for LMC theory, showing that species subject to LMC produce female biased sex ratios, and that individuals adjust their offspring sex ratio facultatively in response to the local level of LMC (Flanagan *et al.* 1998).

However, the predictions of LMC theory can be altered if there is also LRC for resources during larval development (Godfray 1986). Godfray (1986) examined the situation where offspring compete for resources, such as when a number of parasitoid wasps develop on a single host, and there can be sexual asymmetries in larval competition. In this case, when males and females differentially affect the level of competition experienced by other members of the clutch, the ESS sex ratio is biased towards the sex that causes the smaller competitive effect. Although such asymmetries have been observed in species subject to LMC such as parasitoid wasps (Ode *et al.* 1996; van Baaren *et al.* 1999), their

quantitative effects on sex allocation are unclear. In addition, such asymmetries have also been observed more widely in organisms where LMC does not occur, such as birds (Oddie 2000; Arnold *et al.* 2003; Uller 2006).

Here I test the extent to which asymmetric larval competition alters the predictions of LMC theory in the parasitoid wasp *Nasonia vitripennis*. *N. vitripennis* has proved to be an extremely useful organism for testing LMC theory, with a large number of experimental and field studies demonstrating that females adjust their offspring sex ratio in response to the extent of LMC (Werren 1980; Werren 1983; Orzack 1986; Orzack & Parker 1986; King & Skinner 1991; King 1992; King *et al.* 1995; Molbo & Parker 1996; Flanagan *et al.* 1998; Reece *et al.* 2004; Shuker *et al.* 2004a; Shuker *et al.* 2004b; Shuker & West 2004; Shuker *et al.* 2005; Shuker *et al.* 2006a; Shuker *et al.* 2006b). The possibility that asymmetric larval competition may be important in *N. vitripennis* has been suggested by a recent study showing that wasp size correlates with the sex ratio of wasps developing in a host (Rivero & West 2005). However, the sex ratio was not manipulated experimentally in this previous study, and so the result could be due to other correlated factors. Here, I test for asymmetric larval competition by experimentally manipulating both the number of offspring developing in a host and their sex ratio (proportion that are male). I then develop a theoretical model that can be parameterised with our data, and which allows us to determine the quantitative consequences of the observed level of asymmetric larval competition for sex allocation.

4.3 Methods

4.3.1. Study organism

Nasonia vitripennis is a small (females are approximately 2mm long), gregarious, parasitoid wasp that parasitizes large fly pupae (Whiting 1967). Females seek out the host puparia, paralyse the fly pupa within, and can oviposit over 60 eggs on the pupa's surface. Upon emergence the males wait outside the host for the females, who develop more slowly. They then mate and the females disperse to find new hosts. The flightless males remain behind and die. *N. vitripennis* has been used as a model organism for

studying local mate competition (LMC) and its relationship with sex allocation behaviour, with females producing highly female-biased sex ratios when ovipositing alone (sex ratio = 0.1-0.2), through to highly male-biased sex ratios (>0.9) if superparasitising previously parasitised hosts (e.g. as shown in above references). Once a host is parasitized there is a window of approximately 48 hours during which further females tend to parasitise the host (superparasitism). Superparasitism rarely occurs after this period because by then the first brood will have developed sufficiently to start utilising the host's resources, leaving too little food for later larvae (Werren 1984a; Shuker *et al.* 2005).

4.3.2. Laboratory stock

I used wasps from strain HV7 originally collected from bird boxes in Hoge Veluwe National Park in the Netherlands by Prof. L.W. Beukeboom in the summer of 2002. Approximately 50 female mated-wasps were placed in each of 6 glass vials (25 x 75 mm) plugged with a sponge bung. I added about 50 hosts (*Calliphora vomitoria* fly pupae) to each vial on which the females could feed and lay their eggs. The vials were kept in incubators (25 deg C, 16 hours light: 8 dark) and after approximately 14 days hundreds of new offspring emerged and mated for 3-5 days. I then again allowed approximately 50 mated-females per vial to climb into a new vial and thus repeat the whole process.

4.3.3. Experimental design

In order to test how siblings affect each other I created a range of competitive environments by manipulating the number (1-3) and mating status (mated or virgin) of females ovipositing on the host. This influenced both the number of offspring competing for resources in each host and the sex ratio of these offspring.

The experimental females were generated from a large sample of females collected from the stock culture as either mated (allowed to emerge and mate in mass culture for 24 hours, N = 500) or as virgin females (collected as 12-day old pupae from a random sample of hosts from the mass culture; N = 500). Each female was individually placed in

a 10 x 75 mm vial stopped with cotton wool. Each female was given a host for 24 hours and then a disc of honey-soaked filter paper for a further 24 hours; this period gave the wasps time to feed and then develop and mature their eggs. I then allocated females to one of 6 treatments (sample size of 40 – 50 per treatment): 1 x mated female; 1 x mated and 1 x virgin female; 2 x mated females; 1 x mated and 2 x virgin females; 2 x mated females and 1 x virgin female; 3 x mated females. These treatments successfully generated clutches with a wide range of clutch sizes and sex ratios (See Table 4-1). I excluded

Number of ovipositing females		Initial clutch sizes		Initial sex ratios		n
Mated	Virgin	Mean	St. Dev	Mean	St. Dev	
1	0	19.28	8.41	0.14	0.17	36
1	1	32.93	13.73	0.57	0.20	29
1	2	45.88	11.41	0.74	0.14	34
2	0	34.33	11.09	0.36	0.21	40
2	1	45.68	13.19	0.57	0.13	44
3	0	47.11	11.88	0.35	0.17	35

Table 4-1. The mean clutch sizes and sex ratios of broods produced in the varying treatments. The test offspring were collected from these broods.

clutches containing only diapause individuals (as occurred in 5.5% of cases) or ones where supposedly mated females produced all-male broods (as occurred in 15% of cases). This resulted in the removal of 60 clutches so that my treatments varied in sample size from 29 to 44 (a total of 218 samples). Each vial was labelled blind with the help of a colleague who allocated a unique symbol to each treatment, only revealing the allocations after the experiment. A single host was added to each vial for a period of 6 hours, after which the ovipositing females were removed and the hosts were placed in the incubator at 25 deg C (16l: 8d) until the focal offspring emerged.

4.3.4. Testing for asymmetric larval competition

I used three measures to assess the consequences of larval competition: body size, longevity and, in the case of females, fecundity. I scanned the tubes three times per day and recorded the time when 5 females had emerged from the host as our estimate of

emergence time to measure adult longevity. All wasps from a clutch normally eclose and then emerge from the host within 24 hours of each other (Whiting 1967) even when superparasitism occurs, (Werren 1980). Directly measuring eclosion times for individual wasps would have required invasively opening hosts, risking pupal mortality. After emergence, I allowed the wasps to mate for 24 hours and then individuals from each clutch were sorted into separate 10 x 75 mm vials and stopped with cotton wool. I collected up to five males and five females to measure lifespan in the absence of hosts and two females to record lifetime fecundity. The remaining wasps from each clutch were left to die, whereupon I counted the number of males, females and diapause larvae (I added this data to the number of each sex used for assessing fecundity and lifespan). I also measured the right-hind tibia of up to five males and five females from each clutch as an estimate of body size. Variation within a group decreases with greater sampling. Previous work indicates that sampling between three and five individuals from a group gives a high level of accuracy (unpubl. data). To do this I dissected each leg and used a Leica dissecting microscope with x100 magnification. Males have longer hind-tibias than females, even though they are smaller in other morphological traits (Whiting 1967).

When measuring the longevity of wasps in the absence of hosts, I supplied each individual with a fresh disc of honey-soaked filter paper every 48 hours until they died. I checked for and recorded deaths three times per day, and calculated the mean longevity of males and females from each clutch to avoid pseudo-replication. To assess lifetime fecundity of female wasps I gave them 6 fresh hosts every 48 hours until they died. These parasitised hosts were then placed in a new vial so that the offspring could emerge, be counted, sexed and, in a sample of cases, have their tibia measured as before. I recorded the number of males, females and diapause larvae found in each vial.

4.3.5. Analysis

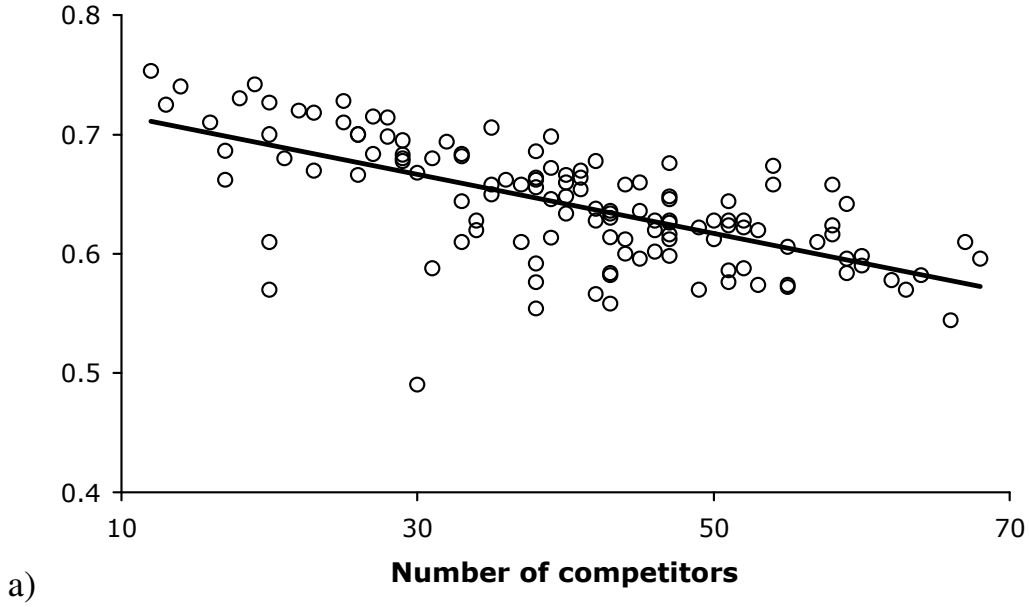
To assess the effects of larval competition and the sex ratio of the competing larvae, I collapsed the six treatments together to provide a continuous range of clutch sizes and sex ratios, using each as a main effect. I then assessed the significance of these main effects and their interaction on size, longevity and fecundity using general linear models with

model simplification (Crawley 2002). When assessing the causes of variation in longevity I considered the possible effects of body size along with the main effects of the experiment. The sex ratio data were taken as the proportion of males and were arcsine square root transformed prior to analysis. All analyses were undertaken with the statistical package JMP IN 5.1 (SAS Institute Inc.).

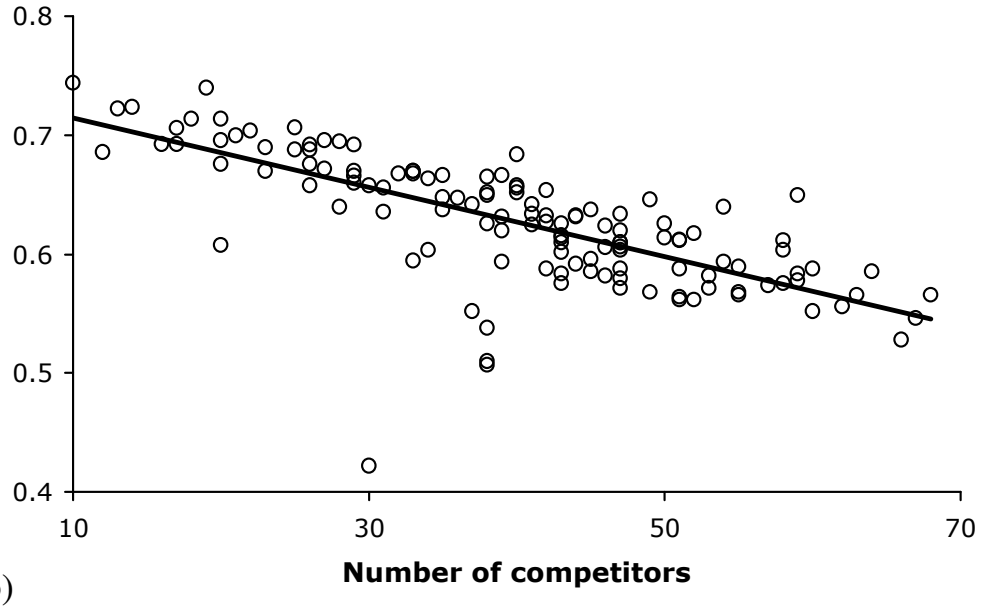
4.4 Results

4.4.1. Competition and body size

Male offspring were smaller when they had to share a host with more competitors, as might be expected from resource limitation (Fig. 4-1a; $F_{1,125}$=92.06, R^2=0.424, P<0.0001). However, the sex ratio of the larval competitors had no effect ($F_{1,124}$=1.26, P=0.26), and the interaction between sex ratio and clutch size was also not significant ($F_{1,123}$=2.17, P=0.14). Female offspring were also smaller when they had to share a host with more competitors (Fig. 4-1b; $F_{1,128}$=142.55, R^2=0.527, P<0.0001). However, the size of female offspring also varied with the sex ratio of the wasps developing in a host - in larger clutches females were smaller when sex ratios were female biased (Interaction between sex ratio and brood size: $F_{1,127}$=5.70, P=0.019), though sex ratio was not a main effect ($F_{1,127}$<0.01, P=0.98). I examined this further by splitting the dataset into individuals that came from large or small clutches, using the median of 40 individuals as the cut-off. In large clutches female offspring were smaller when they were competing with a higher proportion of females (Fig. 4-2; $F_{1,65}$ =8.91, R^2=0.113, P=0.004); in small clutches sex ratio had no effect ($F_{1,63}$ =1.86, P=0.18). Males had longer tibias than females ($F_{1,253}$=11.00, R^2=0.50, p=0.001) but when I analysed both sexes simultaneously I found qualitatively identical results as those above.



a)



b)

Figure 4-1. Larval competition influences tibia length. (a) Males: male body size decreases as the number of competitors increases (-0.25*brood size + 74.04) (b) Females: female body size also decreases as the number of competitors increases (-0.29*brood size + 74.40).

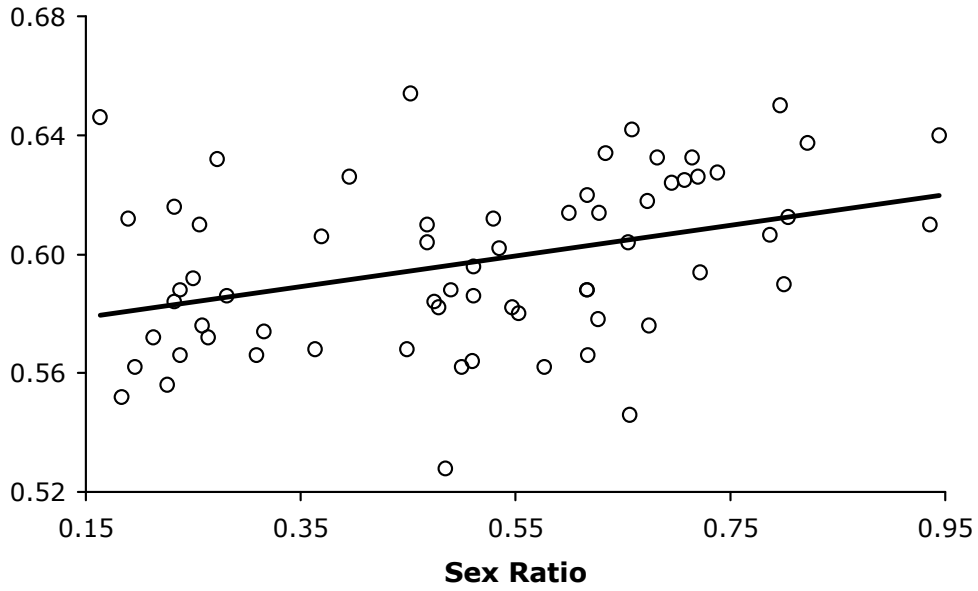


Figure 4-2. Larval competition interacts with larval sex ratio to influence female tibia length. For large clutches (above the median of 40 offspring) sex ratio is positively correlated with female body size such that females are larger the less females they are competing with (4.28*transformed sex ratio + 56.39).

4.4.2. Competition and longevity

I found no correlation between longevity and the level of larval competition or larval sex ratio. This result held when analyzing males (clutch size: $F_{1,88}$=18.09, P=0.48; sex ratio: $F_{1,88}$=1.14, P=0.29), females (clutch size: $F_{1,182}$=3.13, P=0.08; sex ratio: $F_{1,182}$=0.37, P=0.55), or both sexes together (clutch size: $F_{1,272}$=2.24, P=0.14; sex ratio: $F_{1,272}$=0.08, P=0.77). Larger wasps lived longer. This result held when analyzing males (Fig. 4-3; $F_{1,44}$=7.29, R^2=0.45, P=0.01), females (Fig. 4-3; $F_{1,119}$=11.65, R^2=0.090, P=0.001), or both sexes together ($F_{1,164}$=19.05, R^2=0.14, P< 0.0001). Males lived longer than females ($F_{1,164}$=5.23, P=0.023), and there was no significant interaction between sex and body size on longevity ($F_{1,156}$=0.35, P=0.55).

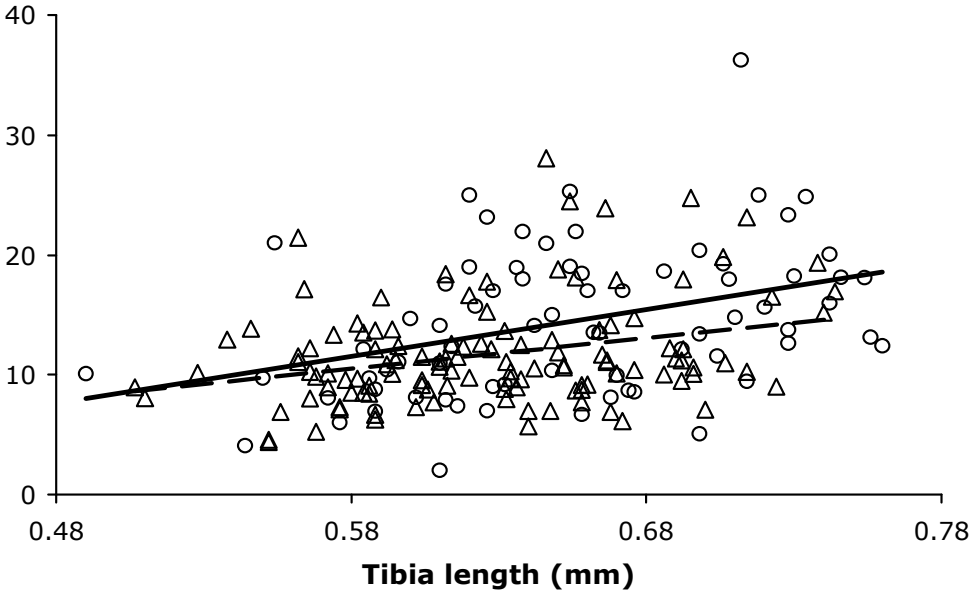


Figure 4-3. Larger wasps live longer and males live longer than females (males: circles, continuous line (0.42*body size -12.92); females: triangles, broken line (0.25*body size – 4.22)).

4.4.3. Competition and lifetime female fecundity

Females with more competitors have lower lifetime fecundity (Fig. 4-4; $F_{1,23}$=6.86, R^2=0.230, P=0.015) but the sex ratio of their competitors was not important ($F_{1,23}$=0.18, P=0.69) even when considered as an interaction with number of competitors ($F_{1,22}$=0.95, P=0.34). This effect of the number of competitors was via it s influence on body size, as shown by the fact that if body size was included in the model, body size was positively associated with lifetime fecundity ($F_{1,24}$=6.97, P=0.015), and competitor number is no longer significant ($F_{1,23}$=0.31, P=0.58).

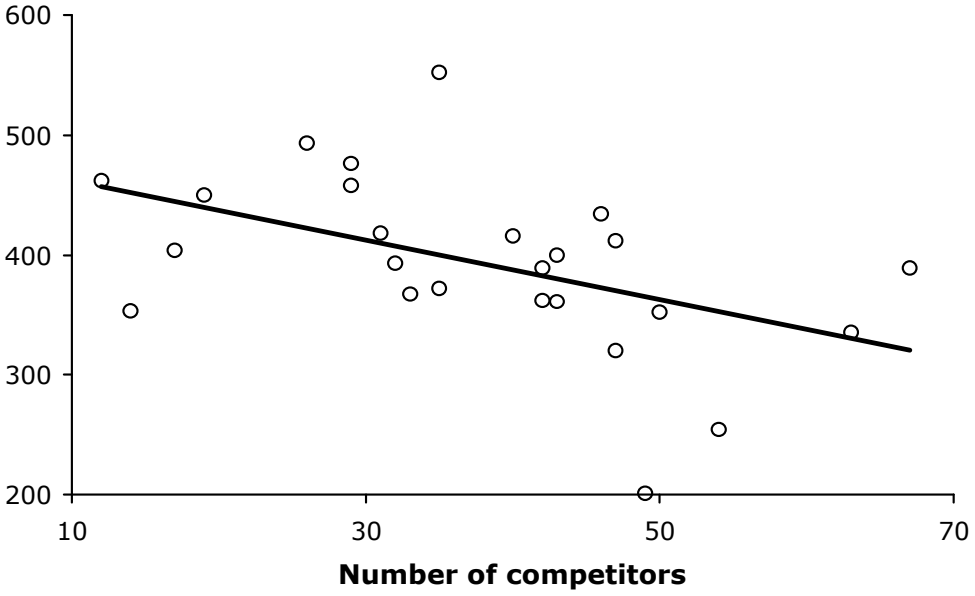


Figure 4-4. Larval competition is negatively correlated with female lifetime fecundity (-2.49*number of larval competitors + 486.99).

4.5. Discussion

I investigated how the number and sex of *N. vitripennis* offspring developing within a host influenced larval competition for resources. I found that body size of both male and female offspring was negatively correlated with the number of offspring developing within their host (Fig. 4-1). In addition, I found that size of females, but not males, was influenced by the sex ratio of wasps developing within the host. Specifically, females were smaller in hosts where a higher proportion of the offspring where female, and this effect was greater at larger clutch sizes (Fig. 4-2). This asymmetric larval competition would reduce the marginal benefit of producing females and so favour a less female biased sex ratio than predicted by standard LMC theory (see below; Godfray 1986). Body size was positively correlated with longevity in males and females (Fig. 4-3), and fecundity in females (Fig. 4-4; see also Flanagan *et al.* 1998; Rivero & West 2002; Rivero & West 2005). Males were found to live longer than females, though there was no interaction between sex and body size. Whilst competitor number affected body size and

body size was linked to longevity, I found no direct relationship between competitor number and longevity, presumably because we lacked statistical power to link these two effects in just one step.

What are the consequences of the observed level of asymmetric larval competition for sex ratio evolution in *N. vitripennis*? In the appendix I develop a theoretical model for the evolutionary stable (ES) sex ratio, which can be parameterised with my data. I assume the classic LMC structure of *N* mated females laying eggs per patch, the offspring of which then develop and mate, before only the females disperse (Hamilton 1967; Hamilton 1979). However, I also allow the fitness of females to depend upon the size and the sex ratio of the brood in which they develop (Godfray 1986). In order to parameterise this model it is necessary to do two things. First, I use the relationships provided in Table 4-2 to calculate how fitness depends upon brood size and sex ratio. It is thought the fitness of

Trait	Affected by	Intercept	Factors
Female body size	larval competition(c)*sex ratio(Aboul-Nasr 1981)	73.17	-0.27*c -0.03*sr + 0.2*c*sr
Female longevity	female body size(Burger and Hofbauer 1993)	-4.22	+ 0.25*fb
Female fecundity	female body size(Burger and Hofbauer 1993)	-63.62	+ 7.16*fb
Male body size	larval competition(c)	74.07	- 0.25*c
Male longevity	male body size(mb)	-12.92	+ 0.42*mb

Table 4-2 Parameters from the experiment used to quantify the expected influence on sex ratio of asymmetric larval competition in *Nasonia vitripennis*. Parameters are derived from the general linear model analysis.

a female parasitoid wasp will be limited by either the number of hosts that she can find (host limitation) or the number of eggs that she can produce (egg limitation), or some intermediate between these extremes (Driessen and Hemerik 1992; Rosenheim 1996; Sevenster, Ellers *et al.* 1998; Rosenheim 1999; Rosenheim 1999; West *et al.* 1999; West and Rivero 2000). I can investigate the two extreme cases by assuming fitness is proportional to longevity and fecundity. These correspond to host and egg limitation respectively. Second, I used field data on *N. vitripennis* from Molbo and Parker (1996) to

determine the range of parameter values that are useful to investigate. Their data gives a mean brood size of approximately 25 (range 7-51), which does not vary significantly with foundress number ($F_{1,11}$=1.9; P=0.19).

Putting the data into my theoretical model I find that asymmetric larval competition has a negligible effect on the ES sex ratio (Fig. 4-5). Figure 4-5 shows the predicted ES sex ratio for *N. vitripennis* assuming that fitness is determined by longevity, but practically identical results where obtained assuming fitness is determined by fecundity. The solid line shows the classic prediction provided by Hamilton's (Hamilton 1979) original equation. The dashed lines show the predictions for a brood size of 70. I have not shown

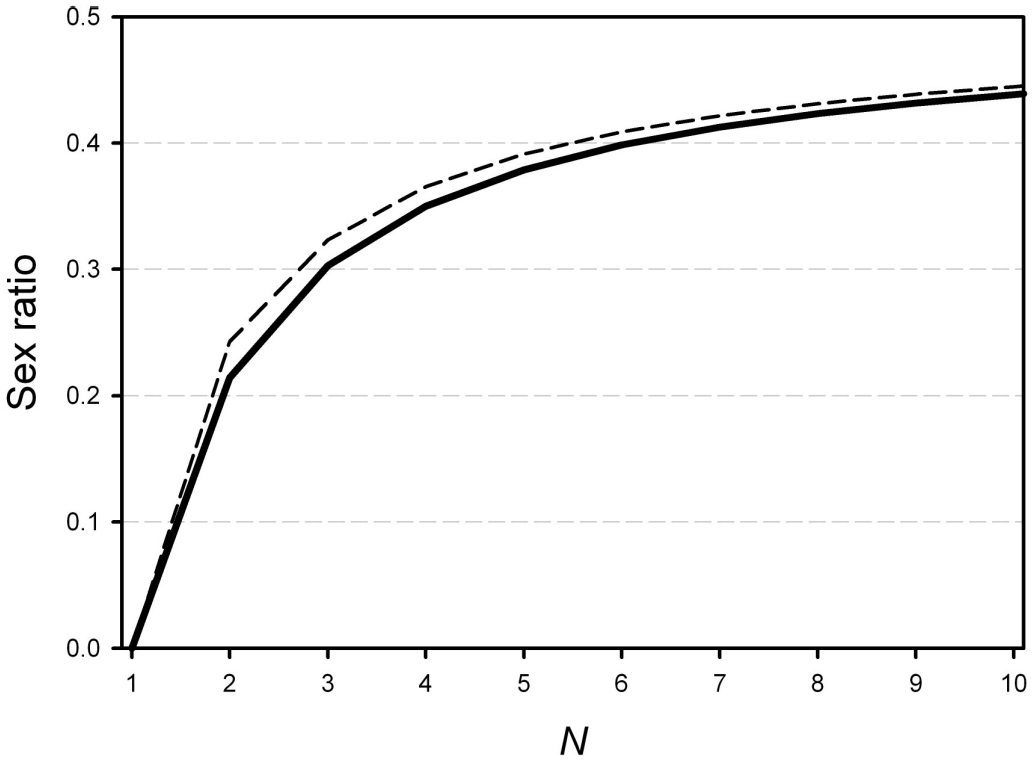


Figure 4-5. The predicted consequences of asymmetric larval competition for offspring sex ratios in *N. vitripennis* where *N* is the number of ovipositing females on a host. The solid line shows the classic prediction for haplodiploids (Hamilton 1979), and the dashed lines show the prediction from my model and data, for a brood size of 70 (the effect with smaller brood sizes was not distinguishable from the original LMC prediction). I predict that asymmetric larval competition leads to a less female biased sex ratio, but this effect is negligible compared to the consequences of LMC.

predictions for the range of brood sizes observed in nature, because these could not be distinguished from the original LMC curve. Consequently, while my model predicts that females should produce a sex ratio less biased than predicted by Hamilton's original model, the magnitude of this effect is negligible for the range of brood size observed in *N. vitripennis***.** The reason for this small effect is that any effect of asymmetric larval competition is dwarfed by the effect of LMC. My model also predicts that the sex ratio should vary with brood size, but this effect will also be negligible. Variable brood size is likely to be far more important through its influence on the extent of LMC (Werren 1980; Stubblefield & Seger 1990; Flanagan *et al.* 1998; Shuker *et al.* 2005).

My results show that females suffer from asymmetric larval competition, as suggested by Rivero and West (2005), but in the opposite direction to their prediction. A possible explanation for this difference is that our experiment was specifically designed to address this issue, and had greater power. I: (a) manipulated sex ratio experimentally, whereas they relied on natural variation; (b) produced more variable sex ratios and brood sizes. However, another possibility is that the previous experiment was assessing the impact of host feeding and that this may have different consequences for males and females (Rivero & West 2005). If male and female larvae require different nutrients, as occurs in other insect taxa (Stockhoff 1993; Telang *et al.* 2001; Dubois *et al.* 2002; Telang *et al.* 2002; Moreau *et al.* 2003; Telang *et al.* 2003) then numerous complications can emerge. For example, host feeding may use up resources that are preferentially used by one sex, leading to that sex facing greater competition, or having to switch its resource use. Further asymmetry in response could come from the fact that males develop more quickly than females, allowing them to use resources and develop while resources are relatively less limiting (Godfray 1986; Godfray 1994; Rivero & West 2005). This suggests the possibility that females could adjust their clutch size and sex ratio behaviour dependent upon the extent to which they feed upon a host. Another surprising result was that I found that males lived longer than females.

My model also allows me to use existing data to determine the quantitative effect of asymmetric larval competition in another species - the parasitoid *B. hebetor*. In this

species there is also asymmetric larval competition, in the same direction as I found with *N. vitripennis*, with females suffering an increased competition for resources when the brood is more female biased (Ode *et al.* 1996). I used the data collected by Ode *et al.* (Ode *et al.* 1996) and Antolin and Strand (1992) to parameterise my model (see Fig. 4-6 legend). As with *N. vitripennis*, this led to the prediction of a less female biased sex ratio (Fig. 4-6).

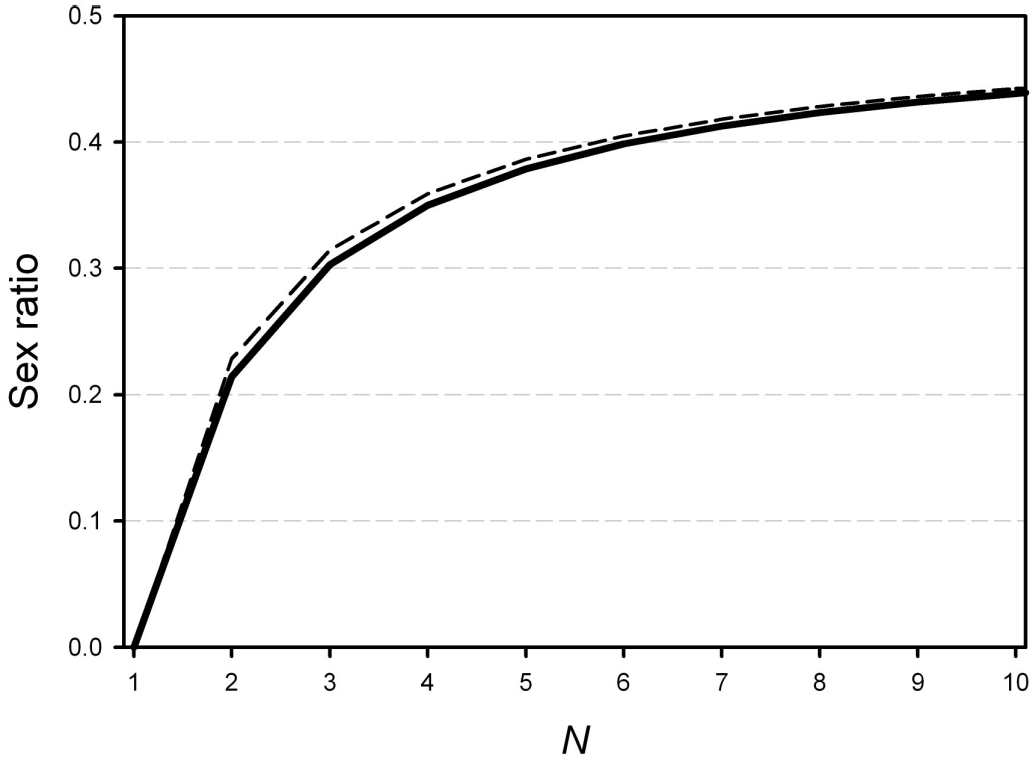


Figure 4-6. The predicted consequences of asymmetric larval competition for offspring sex ratios in *B. hebetor* where *N* is the number of ovipositing females on a host. The solid line shows the classic prediction for haplodiploids (Hamilton 1979), and the dashed lines show the prediction from my model, parameterised with data collected by Antolin and Strand (1992) and Ode *et al.* (1996). From Ode *et al.* (1996) I obtained the relationships: Body size = 0.5475 + (0.0046 x host weight) - (0.0099 x clutch size) + (0.043 x sex ratio); Longevity =-17.53 + (102.30 x size); Lifetime fecundity = -734.72 + (2509.55 x body size). From Antolin and Strand (1992) I obtained an average brood size of 9.1, and an average host size of 20mg. I predict that asymmetric larval competition leads to a less female biased sex ratio. Although the effect is greater than in *N. vitripennis*, it is still very small compared to the consequences of LMC.

Although the effect of asymmetric larval competition was again small, it was at least visible for the average brood size. The extent of LMC in *B. hebetor* is unclear. Whilst related males could compete for mates, and the sex ratio is female biased, inbreeding avoidance reduces the possible importance of LMC (Antolin & Strand 1992; Cook *et al.* 1994; Ode *et al.* 1995; Ode *et al.* 1996; Ode *et al.* 1998). Nonetheless, as illustrated by figure 4-6, the influence of asymmetric larval competition will be low, even when LMC is weak.

I conclude by considering the importance of asymmetric larval competition more generally. In other parasitoids and insects where LMC occurs (West *et al.* 2005), I suspect that effects of LMC will frequently be the overriding factor, as I have found with *N. vitripennis* and *B. hebetor*. Asymmetric larval competition will therefore be relatively unimportant. Consequently, I suspect that the possibilities for asymmetric larval competition to have significant influences on sex ratio evolution will be in species that have smaller clutches and where local mate competition is not an issue, such as birds and mammals (Oddie 2000; Arnold *et al.* 2003).

Footnote:

In this chapter I assessed the consequences of asymmetric larval competition on certain fitness traits including longevity and fecundity. In this experiment, both male and female size correlated with longevity when given honey every 48 hours. Female fecundity also correlated with body size. This result appears to be in contrast with some of the findings from previous chapters. In chapter 2, when females were starved of all food, body size correlated with longevity, but when food was present, in the form of hosts, body size had no effect. In contrast, in chapter 2, female body size had no impact on fecundity. In chapter 3, when males were provided with honey every 24 hours, longevity was independent of size.

These findings may be the result of different methodology. In the absence of food, or sufficient food (only every 48 hours), size appears to correlate positively with longevity in both males and females. When food is readily available (honey every 24 hours, fresh

hosts for females to feed on) size no longer correlates with longevity. In chapter 4, where females emerge from standard hosts with parasitized by 1-3 foundresses, female fecundity is positively correlated with body size. This is not the case in chapter 2 where 1-3 foundresses are given either standard hosts or ones that have previously been fed-upon. When the full model being tested includes larval competition, body size has no impact. However, if one assesses simply the correlation between body size and fecundity in females that came from either standard or poor quality hosts, then the situation changes. Body size is significant for females from poor quality hosts ($F_{1,81} = 12.44$, $P = 0.0007$) but not for females from standard hosts ($F_{1,85} = 0.447$, $P = 0.506$). Females from Chapter 4 standard hosts are developing in broods with higher sex ratios than females from Chapter 2 standard hosts. The results from Chapter 4 also showed that females suffer more from larval competition when the sex ratio of those competitors is increased. It may be that the act of host-feeding exaggerates the effect of male competitors. This may act in the same way as limiting food for adults; driving the increased importance of female body size.

Chapter 5. Inbreeding depression & *Nasonia vitripennis*

5.1 Abstract

Inbreeding depression is the reduction in fitness caused by mating close relatives. It is expected that inbreeding depression has less effect in haplodiploids than diploids because males express deleterious alleles each generation, enabling them to be purged. However, this may not be the case for traits that are (a) only expressed in females (b) not under selection in males. Little is known about inbreeding depression in parasitoid wasps, particularly gregarious species, which are expected to suffer from less inbreeding depression than solitary wasps because they experience inbreeding more frequently and so purge deleterious alleles at a higher rate. I assess the effect of inbreeding depression on development time, longevity and fecundity in female *Nasonia vitripennis* that have been inbreeding under low selection levels for many generations. I found a significant heterozygote advantage for development time, and an effect of inbreeding depression on longevity and fecundity. However, in most cases the variation between maternal genotypes was much greater than between in- versus outbreeding.

5.2 Introduction

When close relatives mate, their offspring have an increased chance of amassing homozygous alleles, which usually results in a decline in fitness (Roff 1997; Keller & Waller 2002). This phenomenon is known as inbreeding depression and is a central factor in the evolution of mating systems and life-history traits (Keller & Waller 2002; Roff 2002; Dolgin *et al.* 2007). There are two main theories for the occurrence of inbreeding depression: (a) heterozygotes have an advantage over homozygotes (over-dominance hypothesis; Roff 2002) and (b) increasing homozygosity increases the chance of having two deleterious recessive alleles (partial-dominance hypothesis; Charlesworth & Charlesworth 1999; Roff 2002).

However, despite the inherent costs, inbreeding does occur, particularly in gregarious species where the benefit of mating with siblings must have outweighed the detrimental effect of inbreeding depression (Henter 2003). For some organisms the cost of inbreeding may not be as great as it first seems, as considered by two predictions that arise through the currently more favoured partial-dominance hypothesis (Roff 2002). First, if inbreeding occurs over many generations then continued expression of deleterious recessive alleles should purge them from the population, reducing the effect of inbreeding depression over time (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Waller 1993). Second, purging can be at an increased rate, as in haplodiploid species where haploid males express, and purge, deleterious mutations each generation. This should result in haplodiploids suffering less from inbreeding depression (Bruckner 1978; Crozier 1985; Werren 1993; Peer and Taborsky 2004). However, whilst there is a relative scarcity of data in haplodiploids (Henter 2003), there is some evidence that female haplodiploids may suffer inbreeding depression up to levels experienced by *Drosophila* (Roff 2002) in traits that (a) are only expressed in females or (b) are not under selection in males. Henter (2003) discovered an effect of inbreeding in a solitary parasitoid wasp and, through meta-analysis, across haplodiploids as a whole. Gregarious wasps tend to use a single host for multiple offspring that mate before dispersing; this results in a higher

frequency of inbreeding and so they may be less susceptible to inbreeding depression because they purge alleles at a higher rate than solitary parasitoids.

Here, I have tested whether inbreeding depression does occur in a gregarious haplodiploid and whether the findings support the over- or partial-dominance hypothesis. The over-dominance hypothesis states that heterozygotes have a higher level of fitness than homozygotes and since inbreeding increases the frequency of homozygotes, that causes inbreeding depression (Roff 2002). Accordingly, successive generations of inbreeding will reduce fitness, but an outbreeding event will restore fitness to the original levels (Roff 2002). In contrast, the partial-dominance hypothesis posits that it is not the frequency of homozygotes, but the frequency of deleterious alleles being expressed through homozygosity that causes inbreeding depression. This results in continual purging of deleterious alleles so that once two inbred lines outbreed with each other again, they are only left with favourable alleles, resulting in higher fitness levels than originally (Roff 2002). These predictions have been modelled on numerous occasions (Barrett and Charlesworth 1991; Hedrick 1994; Wang, Hill et al. 1999). I tested for the occurrence of inbreeding depression in the gregarious parasitoid wasp *Nasonia vitripennis* by comparing the speed of development, starvation resistance and fecundity of outbred versus inbred wasps following many generations of inbreeding.

5.3 Method

5.3.1. Study organism

Nasonia vitripennis is a small (females are approximately 2mm long), gregarious, parasitoid wasp that parasitizes large fly pupae (Whiting). Females seek out the host puparia, paralyse the fly pupa within, and can oviposit over 60 eggs on the pupa's surface. Upon emergence the males wait outside the host for the females, who develop more slowly. They then mate and the females disperse to find new hosts. The flightless males remain behind and die. *N. vitripennis* has been used as a model organism for studying local mate competition (LMC) and its relationship with sex allocation behaviour, with females producing highly female-biased sex ratios when ovipositing

alone (sex ratio = 0.1-0.2), through to highly male-biased sex ratios (>0.9) if superparasitising previously parasitised hosts (e.g. as shown in above references). Once a host is parasitized there is a window of approximately 48 hours during which further females tend to parasitise the host (superparasitism). Superparasitism rarely occurs after this period because by then the first brood will have developed sufficiently to start utilising the host's resources, leaving too little food for later larvae (Werren 1984; West et al. 2004).

5.3.2. Inbred lines

I was kindly allowed the use of individuals from 12 lines of wasps that had been created from isogenic females by Prof. L. Beukeboom (Uni. of Groningen, Netherlands). Each isogenic line had been inbred for 16 generations using alternating single brother-sister and father-daughter matings, resulting in >99% homozygosity at all loci. These lines came from different localities and hence are likely to represent different genotypes. When I received them, I kept three replicate groups of each line, with each group housed in a 25 x 75 mm glass vial containing approximately 20 individuals and 30 hosts. The vials were stopped with sponge bungs and then placed in an incubator (25 ºC, 16 hours light: 8 dark) until the offspring had emerged from the hosts and mated. At this point a sample of approximately 30 individuals was placed in a new 25 x 75 mm vial with 20 fresh hosts and returned to the incubator. These 30 individuals that became parents of the next generation were under very low levels of selection due to laboratory conditions: there was no need for dispersal, foraging for mates, foraging for food or foraging for fresh hosts. This may mean that there was a lower selection level purging deleterious mutations. This process was repeated 12 times to ensure that my lines were sufficiently inbred.

5.3.3. Creating the crosses

A fully-factored set of crosses (with 3 replicates of each) was created with females from each line being crossed with males from each of the other lines, including a cross with the female's own line. Each crossed pair was placed in a labelled 10 x 75 mm glass vial

stopped with cotton-wool and kept in an incubator as before (25 ºC, 16 hours light: 8 dark). Each pair were given 6 hours in which to mate before the males were removed and replaced with 3 fresh hosts which the females were able to parasitise.

5.3.4. Offspring fitness

Many crosses proved to be non-viable and either did not maintain stocks over 12 generations or did not produce any offspring; 44 crosses were viable, 7 of which were homozygous. After approximately 10 days 30 pre-eclosion females were collected (morphological differences enable sex to be determined before eclosion) from each viable cross. Each female was placed in a separate 10 x 75 mm labelled glass vial stopped with cotton wool. 12 individuals were kept in an 18 ºC incubator (16 hours light: 8 dark). 6 of these individuals were regularly scanned to record the time they took to reach eclosion and 6 were scanned to assess their starvation resistance (adult longevity in the absence of food). A further 12 individuals were assessed in the same manner but were kept in a 25 ºC incubator (16 hours light: 8 dark). The remaining 6 individuals were each provided with a male to mate for 6 hours and 3 fresh hosts every 3 days for up to 6 oviposition periods. The parasitized hosts were placed in separate 10 x 75 mm labelled glass vials and stopped with cotton wool. The grandoffspring in these hosts were allowed to develop in an incubator (a 25 ºC, 16 hours light: 8 dark). Following their deaths, they were sexed and counted.

5.3.5. Analysis

I used a general linear modelling approach to assess the effect of inbreeding on each of my measures of female fitness: time to reach eclosion, adult longevity and fecundity. For each fitness component I first assessed how much variation could be explained using all relevant variables, including maternal genotype, female zygosity and, where appropriate, temperature. I fitted full models containing all the main effects and their interactions, and then manually simplified the models to the minimum adequate model through stepwise deletion of non-significant terms ($p > 0.05$ for main effects, $p > 0.01$ for second order interactions; (Crawley 2002) using the statistical package JMP IN 5.1 (SAS Institute Inc.). When appropriate, I also assessed the data within each temperature treatment. For

my measure of female fecundity, I first assessed the number of offspring produced by each female in her first three broods, which due to the large sample size amounted to 36,541 wasps. I then tested to see whether I could ascertain the same result by assessing just the first brood produced by a female. This was in case (a) inbreeding depression affected the level of resources amassed by emergence, which can vary greatly before host-feeding (Rivero & West 2002) or (b) the same result was also found when assessing single broods so that in future experiments I would be able to use the saved time to test larger numbers of replicates.

5.4 Results

5.4.1. The effect of inbreeding on time to eclosion

I assessed the impact of inbreeding on the time taken for adults to eclose. I assessed the variables of mother's genotype, female zygosity and the temperature at which the female developed (18 or 25 ºC) and established the minimum adequate model. The temperature at which the females developed was the most significant factor: females developing at 25ºC eclosed much earlier than those at 18 ºC (Means of 358.3 hours and 455.0 hours respectively; $F_{1, 282} = 4261.641$, $P < 0.0001$). The maternal genotype of each focal wasp was also highly significant ($F_{4, 282} = 26.808$, $P < 0.0001$) with the mean time to eclosion varying between genotypes from 395.2 to 415.7 hours. Further, there was an interaction between maternal genotype and temperature; for some genotypes the difference in development time at 25 ºC, opposed to 18 ºC, was higher whilst for others it was reduced (Fig. 5-1; $F_{4, 282} = 18.959$, $P < 0.0001$). There was also a significant effect of female zygosity; homozygous females took longer to eclose (mean of 408.6 hours) than heterozygous females (mean of 404.8 hours; $F_{1, 282} = 4.281$, $P = 0.0395$). There were no other significant interactions ($P > 0.01$ in all cases). The minimum adequate model explained 94.0 % of the variation.

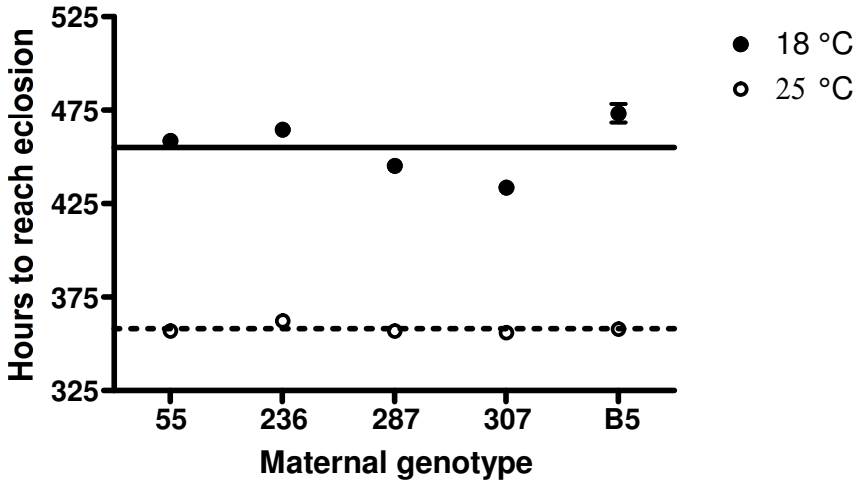


Figure 5-1. Relationship between a female's maternal genotype and the time she takes to reach point of eclosion at 18 and 25 ºC. The solid line represents the mean time taken at 18 ºC and the dashed one at 25 ºC. Standard error bars are present, but the values are so small that in most cases they are hidden within the data points.

Due to the interaction between maternal genotype and temperature, I then assessed the impact of inbreeding on the time taken for adults to eclose at 18 and 25 ºC separately. I first established the minimum adequate model for females developing at 18 ºC and found the same pattern as before. The maternal genotype of each focal wasp was highly significant ($F_{4, 137} = 24.717$, $P < 0.0001$) with the mean time to eclosion varying between genotypes from 436.2 to 475.8 hours. There was also a significant effect of wasp zygosity, as homozygous wasps took significantly longer to reach eclosion (Fig. 5-2; 461.47 hours) than heterozygous wasps (453.63 hours; $F_{1, 137} = 4.801$, $P = 0.030$). There was no significant interaction between maternal genotype and zygosity ($F_{4, 133} = 1.093$, $P = 0.363$). The minimum adequate model explained 43.3% of the variation. I then assessed the data for wasps developing at 25 ºC, again established the minimum adequate model but this time found a slightly different pattern. Whilst time to eclosion varied with maternal genotype (ranging from 356.0 to 362.6 hours; $F_{4, 145} = 6.694$, $P < 0.001$), zygosity was not a significant main effect ($F_{1, 144} = 0.001$, $P = 0.982$), nor was there any significant interaction between maternal genotype and zygosity ($F_{4, 140} = 0.079$, $P = 0.989$). The minimum adequate model explained 15.6% of the variation.

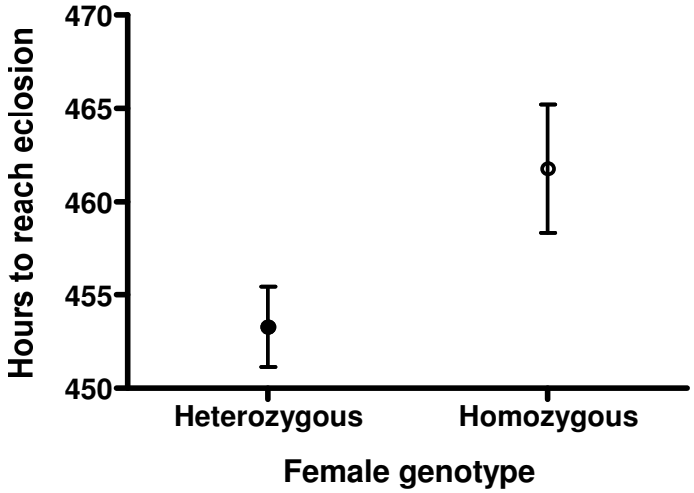


Figure 5-2. Relationship between female genotype and time taken to reach point of eclosion when developing at 18 °C.

5.4.2. The effect of inbreeding on longevity

I then assessed the impact of inbreeding on adult starved longevity. I assessed the variables of mother's genotype, zygosity of the offspring and the temperature at which the offspring were developing (18 or 25 ºC) and established the minimum adequate model. Again the largest explanatory factor was temperature; females living at 18 ºC survived longer than those at 25 ºC ($F_{1, 259}$ = 2913.774, P < 0.0001). Maternal genotype was the next most significant factor with longevity ranging between 560.0 and 633.0 hours; $F_{4, 259}$ = 22.900, P < 0.0001) and once again there was an interaction between maternal genotype and temperature; increasing temperature decreased longevity to a greater or lesser extent depending on maternal genotype (Fig. 5-3; $F_{4, 259}$ = 8.677, P < 0.0001). There was also a significant interaction between maternal genotype and female zygosity; for most, but not all maternal genotypes, heterozygous females lived longer than homozygous females (Fig. 5-4; $F_{4, 259}$ = 3.701, P < 0.006). Female zygosity was not a main effect ($F_{1, 259}$ = 0.012, P = 0.915), but was kept in the model because of its interaction with maternal genotype. No other interaction was significant (P > 0.01 in both cases). Overall, the minimum adequate model explained 92.3% of the variation.

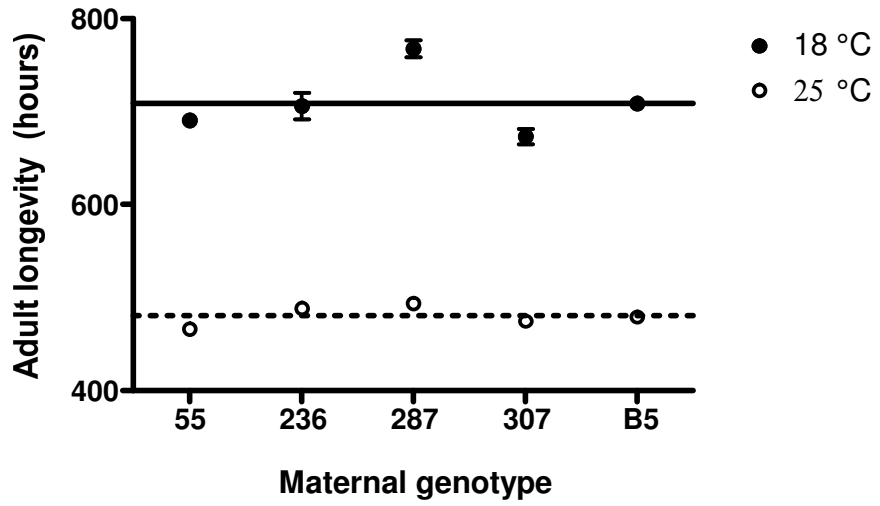


Figure 5-3. Relationship between a female's maternal genotype and her adult longevity at 18 and 25 ºC. The solid line represents the mean longevity of females living at 18 ºC and the dashed one at 25 ºC. Standard error bars are present, but the values are so small that in most cases they are hidden within the data points.

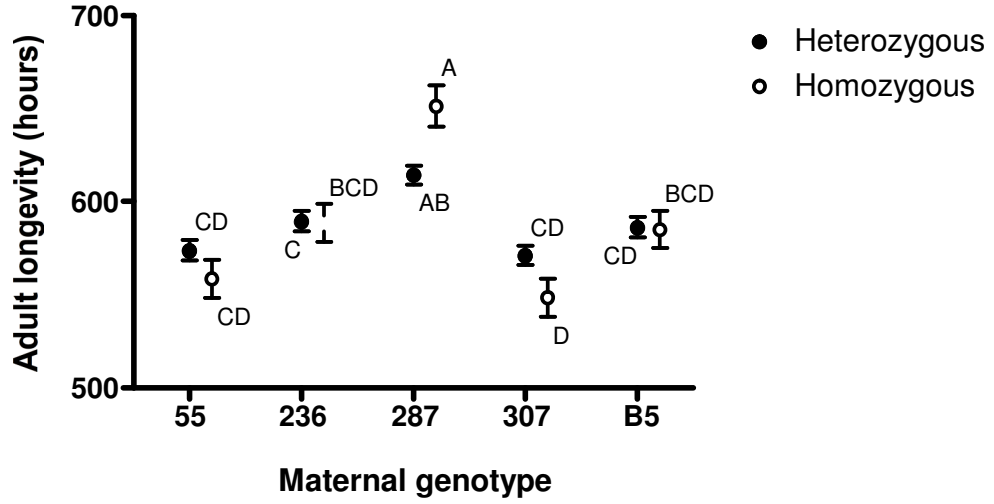


Figure 5-4. Relationship of interaction between a female's zygosity and her maternal genotype on adult longevity. Data points sharing a letter are not significantly different from each other.

Once again, due to the interaction between maternal genotype and temperature, I assessed the longevity data separately for wasps living at both 18 and 25 ºC. At 18 ºC the minimum adequate model indicated that longevity was only affected by the maternal genotype ($F_{4, 122} = 15.548$, $P < 0.0001$) with mean genotype longevity ranging from 673.4 to 767.5 hours. Zygosity was not significant ($F_{1, 121} = 0.264$, $P = 0.608$), nor was there any significant interaction between maternal genotype and zygosity ($F_{4, 117} = 3.109$, $P = 0.018$). The minimum adequate model explained 33.9% of the variation.

The same pattern was found in the wasps living at 25 ºC, where the minimum adequate model indicated that the only significant effect was maternal genotype ($F_{4, 142} = 8.957$, $P < 0.0001$) with mean longevity for each genotype ranging from 466.5 to 494.6 hours. Zygosity was not significant as a main effect ($F_{1, 141} = 0.388$, $P = 0.534$), nor was there any significant interaction between maternal genotype and zygosity ($F_{4, 137} = 0.953$, $P = 0.436$). The minimum adequate model explained 20.1% of the variation.

5.4.3. The effect of inbreeding on fecundity

I assessed data for effect of inbreeding on the total number of offspring produced during a female's first three broods. Maternal genotype was highly significant ($F_{3, 97} = 11.260$, $P < 0.0001$) and the mean brood sizes produced by females of each maternal genotype ranged from 183.7 to 274.6. There was also a significant interaction between maternal genotype and zygosity (Fig. 5-5; $F_{3, 97} = 5.406$, $P = 0.0018$). In some cases homozygosity increased brood size and in other cases heterozygosity did. Zygosity was not significant as a main effect ($F_{1, 97} = 1.382$, $P = 0.243$) but was maintained in the model by the interaction. Overall, the minimum adequate model explained 30.7% of the variation.

I then investigated whether this effect was also apparent during just the first brood produced by a female. In this instance whilst the minimum adequate model showed that maternal genotype was again extremely significant (mean brood size produced by each maternal genotype ranging from 45.0 to 58.8; $F_{6, 225} = 3.141$, $P = 0.0056$), zygosity was not a significant main effect ($F_{1, 224} = 0.017$, $P = 0.895$) nor was there a significant

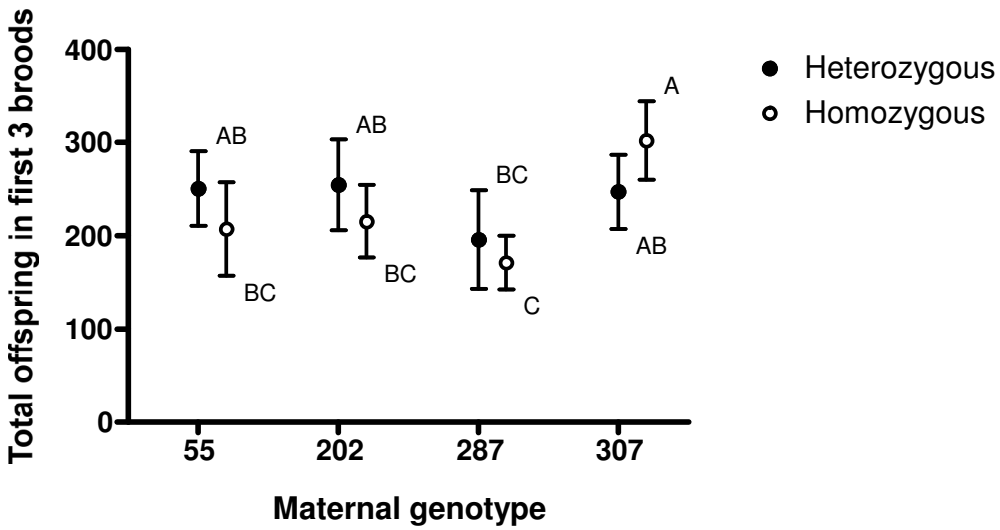


Figure 5-5. Relationship of interaction between a female's zygosity and her maternal genotype on the number of offspring she produced during her first three broods (three hosts available per brood; nine hosts in total). Data points sharing a letter are not significantly different from each other.

Interaction between maternal genotype and zygosity ($F_{6, 118} = 2.513$, $P = 0.023$). The minimum adequate model explained 7.7% of the variation.

5.5 Discussion

I discovered evidence of inbreeding depression, but only with regards development time. My results showed that maternal genotype had an extremely significant impact on all levels of fitness: time to eclosion, adult longevity and fecundity. I also discovered evidence of inbreeding depression in time to eclosion where heterozygotes developed more quickly, both when temperature was a factor and when I just looked at wasps developing at 18 °C. There was also a significant interaction between maternal genotype and female zygosity for both adult longevity and the number of offspring produced in the first three broods; whilst in most cases there was a heterozygote advantage, in some there was a homozygous one. In most instances, the temperature at which the wasps were housed, or the maternal genotype explained the greatest amount of variation.

My findings support the suggestion that gregarious parasitoids suffer from inbreeding depression less than solitary parasitoids do. There is very little data regarding inbreeding depression in haplodiploids and currently few conclusions can be made. Henter (2003) found that inbreeding depression caused a drop of 38% in longevity in the solitary wasp *Uscana semifumipennis*. In contrast, when looking at the difference in mean time to reach eclosion at 18 °C, I found that homozygotes were just 2% (8.62 hours) slower than heterozygotes. However, this may be because (a) I was using a gregarious wasp which therefore suffers less inbreeding depression (b) the laboratory selection pressures were low enough that deleterious mutations were not purged as frequently as normal in haplodiploids, meaning that there would be a less drastic difference between inbred and outbred crosses (c) development time may be under such high selection pressure anyway, due to competition for resources, that deleterious alleles are maintained less frequently in the population than those affecting adult longevity.

I found that there were instances of both homozygous and heterozygous advantage, but am unable to give significant support for the partial-dominance hypothesis. The over-dominance hypothesis posits that there should only be a heterozygous advantage, which is not supported here. In the case of partial-dominance, a homozygous advantage may exist if a purged deleterious allele for a trait has been superseded by a more beneficial allele in line 'A' than in line 'B'. In this instance, a homozygous cross within line 'A' may have a higher fitness level for a particular trait than a heterozygous cross with line B, depending on which allele is dominant. However, because I am unable to compare the fitness values (e.g. development time) following outbreeding with those preceding inbreeding, there is no strong support for the partial-dominance hypothesis.

The work is further hampered by the number of crosses that proved to be non-viable. Originally there were 12 lines crossed with each other, giving 144 combinations. However, so many crosses did not produce offspring that we had data from fewer than 40 combinations, limiting the power of the experimental design as variation between maternal genotype is so high. The nature of the crosses also meant that there were far more heterozygous crosses than homozygous ones. A more efficient method would be to

balance the crosses equally between heterozygous and homozygous to gain greater insight into the frequency of heterozygous advantage.

In this study I found evidence for a small, but significant effect of inbreeding depression in a gregarious parasitoid wasp, which adds to the growing body of work investigating both the mechanism of inbreeding depression (Charlesworth & Charlesworth 1999; Roff 2002) and its role in influencing behaviour and ecology (Keller & Waller 2002; Roff 2002; Dolgin *et al.* 2007). Further work with gregarious parasitoids may also help discover whether the frequency of inbreeding affects the impact of inbreeding depression (Henter 2003).

Chapter 6. General Discussion

I have included detailed discussions at the end of each data chapter. Here, I present a brief review of the main findings of the thesis and highlight where to take this work in the future.

6.1 Clutch size theory

Body size is strongly negatively correlated with number of larval competitors (Chapter 2; Chapter 3; Chapter 4) and as size is frequently correlated with fitness (Godfray 1994; Jervis *et al.* 2003; Chapter 2; Chapter 4), this could have a profound impact on clutch size decisions. In Chapters 2 and 3, I assessed the fitness impact of body size on both males and females. I first varied female size (Chapter 2) by manipulating clutch size and host condition. The limited larval resources ensured variation in adult size and I then measured the impact this variation had on starvation resistance, ovipositing lifespan and fecundity.

I found that female body size is strongly positively correlated with starvation resistance, supporting previous work (Visser 1994; Rivero & West 2002; Bezemer *et al.* 2005) that larval resources can be used to increase lifespan and that large-bodied individuals have greater reserves. This finding suggests that, in the absence of hosts when a female must rely on her lipid reserves, body size will correlate highly with fitness. I also found that starvation resistance is further affected, though to a lesser extent, by larval competition; the larger the size of the clutch the shorter lived the resulting females. However, although body size correlates with starvation resistance, it has no effect on reproductive lifespan, fecundity (but see Chapter 4; Sykes *et al.* 2007) or offspring sex ratio. In contrast, larval competition negatively influences female fecundity as well as longevity and is likely to play an important role in fitness even when hosts are not limited.

Body size can, therefore, be considered a useful indicator when predicting the cost of being host-limited, whilst larval competition can be used to predict the likelihood of being egg-limited. The correlation between body size and starvation resistance could lead

to a shift in optimal clutch size. In environments where future hosts are predicted to be scarce, females should be selected to reduce their clutch size so as to enable daughters to acquire sufficient reserves to disperse to new hosts. Similarly and perhaps counter-intuitively, females may also be selected to reduce clutch size when daughters will be in host-rich environments. Fecundity is negatively correlated with larval competition, so females may also be selected to reduce clutch size as the likelihood of offspring being egg-limited increases. This could result in the largest clutch sizes being produced by host-limited females that expect a less host-limited environment for their offspring and the smallest clutches being produced by egg-limited females that expect either extreme host or extreme egg-limitation for their daughters. These predictions are based on the assumption that females can predict future host frequency. However, if a female uses the same cues to predict current and future host environments then selection will probably favour the more reliable 'current information' than a 'future prediction of host frequency'. If a female uses separate cues, then it may be that she is able to tailor her clutch sizes accordingly.

The link between body size and fitness may, however, be greater than appeared here, as my study did not include a cost of dispersal. Hosts were readily supplied and it could be expected that the combined costs of dispersal, maintenance and reproduction could be greater even than the daily benefits of host-feeding, as found by Casas et al. (2005) in the parasitoid *E. vuilletti*. Visser (1994; *Aphaerta minuta)* and Kazmer and Luck (1995; *Trichogramma pretiosum*) found that larger females were better at finding new hosts, even though finding a patch and travel efficiency was independent of size (Visser 1994). Incorporating dispersal costs into laboratory experiments can be extremely difficult but they may be needed to gain a true understanding of the relationship between body size and fitness in females.

Clutch size decisions may also have implications for male size that in turn impact on fitness. In Chapter 3, I assessed the correlation between body size and mating success in males. By manipulating clutch size through foundress number, I was able to generate variation in male body size. I then tested the relationship between body size and mating

success for males (i) given a female for 15 minutes (ii) given a female and male competitor for 15 minutes and (iii) given fresh females throughout their lives.

I found no effect of size on male mating success or longevity, despite considerable variation in both traits. The presence of a competitor did reduce the number of matings achieved by the focal male, but it was independent of either his absolute size, or his size relative to his competitor. However, the effect of size may have been reduced for two reasons. First, males were provided with honey solution every 24 hours, which may have negated any benefit from larger males carrying more lipid reserves. Future experiments testing the fitness consequences of male size should either remove honey-solution altogether, or reduce it to feeding every 48 hours, as this latter treatment highlighted a correlation between body size and male longevity in Chapter 4 (Sykes *et al.* 2007). Secondly, this experiment may not have constituted a fair replication of male-male competition. In the wild, males gather at a monopolisable resource, namely the exit hole from which females emerge. This was not replicated in the vials, where many females were available at once and males may never have been in contact with each other.

The data does show a large cost to mating, even when males are just given mating opportunities for 24 hours. However, this mating cost is independent of male size. The results therefore suggest that there is not a cost to being small in males but there are factors that must be taken into consideration. First, copulation attempts and ejaculate cost were not measured. Secondly, as previously mentioned, males were given honey-solution in an effort to increase the variation between lifespans. Work has shown (Rivero and West 2002; Chapter 2) that females only display costs of being small when food is unavailable and that both male and female longevity can correlate positively with size when fed with honey-solution every 48 hours (Chapter 4; Sykes *et al.* 2007). It is therefore highly plausible that a similar effect exists for males. If this is the case, then it is possible that larger males use their lipid reserves for extra reproductive effort and their carbohydrates for somatic maintenance (Casas *et al.* 2005). There is no data for the amount of energy invested in copulation attempts, multiple matings, or ejaculate material, which could be screening the cost of being small. Larger males may be performing more copulation rituals, gaining multiple matings with the same female and investing more into

the ejaculate. The cost of repeated copulation attempts could be increased because females were unable to disperse. Mated females rarely accept second matings (Burton-Chellew *et al.* 2007) but males may still attempt copulation rituals. The design of the experiment may, therefore, have simply left larger males wasting more energy in fruitless copulation attempts. Repeating the experiment without food supplements and allowing females to disperse once mated, can readily test whether there is a cost to being small in males. If there is no cost, then male fitness would not limit clutch size but may lead to an increased clutch size when laying a very high sex ratio, as when superparasitising. Further, it may lead to an increase in a female's tendency to superparasitise.

6.2 Sex allocation theory

Theory predicts that unequal fitness returns from male and female offspring should select overproduction of the sex offering the greater return (Fisher 1930; Suzuki & Iwasa 1980; Hardy 1994). In Chapter 4, I assessed whether asymmetric larval competition (ALC) in *N. vitripennis* may select for overproduction of a particular sex. By manipulating the number (1-3) and mating status (virgin or mated) of ovipositing females I generated a range of competitive clutch sizes and sex ratios. This enabled me to test whether one sex coped better with increasing clutch sizes or sex ratios than the other. I measured fitness through body size, longevity and in the case of females, fecundity.

I found that both male and female body size is negatively correlated with the number of larval competitors. In turn, in both males and females, body size is positively correlated with longevity. This is interesting as, in the previous experiments, female body size only correlates with longevity in the absence of food, when females rely on lipid reserves. When lipids are available through host-feeding there is no longer correlation between body size and longevity. In this experiment, females were only given carbohydrates (honey solution) every 48 hours, so lipid reserves could have been required, correlating longevity with body size. In Chapter 3, male longevity was not associated with body size. In that experiment, males were provided with honey-solution every 24 hours, whereas in this one, honey-solution was provided merely every 48 hours. This suggests that honey-solution every 48 hours is not sufficient to sustain males and lipid reserves were being

used at a significant enough rate to detect a consequence of body size. The data from Chapter 4 (Sykes *et al.* 2007) show no direct effect of clutch size on longevity in either males or females.

In this experiment (Chapter 4; Sykes *et al.* 2007), whilst both male and female body size negatively correlate with clutch size, only female body size suffers from varying sex ratios. In large clutches, female body size negatively correlates with the proportion of females in the clutch. This sex ratio effect on female body size does not lead to a direct effect on longevity, which may be due to the combined effect of honey-solution every 48 hours, slightly minimising variation and a lack of statistical power. There was no effect of sex ratio on male body size or longevity.

Here, female fecundity is positively correlated with body size. This is in contrast to the result from Chapter 2 where there was no effect of body size but that may be a result of (i) sample size and (ii) experimental method. In this experiment, I counted the offspring from every host used by 25 females (totalling 9,823 offspring from 600 hosts), whilst the data from Chapter 2 compared offspring numbers from four oviposition periods of 177 females (totalling 49,803 offspring from 4,248 hosts). In both experiments, the focal females were given six fresh hosts every 48 hours, however, in Chapter 2 half of the females came from host-fed hosts, which increased the negative correlation between clutch size and fecundity at any given time.

The results show that in large clutches ALC can occur, with female fitness positively correlating with sex ratio. This differs from the findings of Rivero and West (2005) where female fitness correlates negatively with sex ratio. The difference may be because I (i) specifically tested for ALC (ii) had greater statistical power (iii) did not take host-feeding into account, whereas Rivero and West did. In their experiment, female body size interacted with treatment, either increasing (if previously starved) or decreasing (if newly-emerged) the amount a female host-fed. Larger females laid larger females and larger clutches, but female offspring body size declined with maternal host-feeding and with increased clutch sex ratio. Clutch sex ratio was negatively correlated with maternal body size. It may be that males and females utilise different nutrients from the host and

that host-feeding increases the competitive threat from males. The issues of reduced nutrients from host-feeding and decreased cost of size through feeding on honey-solution, could be investigated by repeating my experiment with these variables included.

I took account of the reduced marginal benefit of producing females and modelled the wider implications, finding that the effects of local mate competition (LMC) dwarf those of ALC. I assumed classic LMC structure but allowed the fitness of females to depend upon the size and sex ratio of the brood in which they developed. I then calculated the consequences of extreme host and egg limitation by assuming that fitness was proportional to longevity and fecundity respectively. The response showed that ALC has such a small effect, in comparison to LMC, that it is likely to be significant only when LMC does not occur such as in birds and mammals (Oddie 2000; Arnold *et al.* 2003).

Inbreeding depression

Inbreeding depression occurs when increased homozygosity leads to a decrease in fitness, either because of increased expression of recessive deleterious alleles (partial-dominance hypothesis) or because there is a heterozygous advantage (over-dominance hypothesis; Charlesworth & Charlesworth 1999; Roff 2002). Inbreeding depression should be reduced in haplodiploids because haploid males express and thus purge, deleterious alleles each generation (Bruckner 1978; Crozier 1985; Werren 1993; Peer & Taborsky 2004). However, it is possible that alleles expressed only in females will avoid this cull (Roff 2002; Henter 2003), facilitating inbreeding depression in female haplodiploids. The effect of inbreeding depression may be further reduced in species with high inbreeding rates, such as *N. vitripennis*, because frequent inbreeding will increase the frequency that deleterious alleles are expressed in females, thereby purging them more frequently and thus reducing the impact of inbreeding depression (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Waller 1993).

I assessed the impact of inbreeding depression on female fitness and found some evidence of inbreeding depression. Inbred lines were crossed and the resulting females were measured for their development time and longevity (in the absence of food) at 18 °C and 25 °C. Females that were being tested for the effect of inbreeding depression on

fecundity were offered three fresh hosts every three days for up to six oviposition periods. Maternal genotype and temperature influence development time, longevity and fecundity. The data show an effect of inbreeding depression on development time at 18 °C, with homozygous females taking longer than heterozygous females to reach eclosion. This effect did not occur when females were developing at 25 °C. There was also evidence for more specific inbreeding and outbreeding depression depending on the wasp's genetic strain. Both longevity and fecundity were influenced by interactions between maternal genotype and wasp zygosity. However, sometimes homozygosity decreased fecundity and lengthened development time, whilst sometimes it had the opposite effect.

Unfortunately, the experiment had a number of limitations that could be remedied. Sample size was greatly reduced by a high number of non-viable crosses, resulting in few homozygous crosses. Further, a more balanced design, with equal numbers of homozygotes and heterozygotes would have enabled greater insight into the possibility of heterozygous advantage. The two hypotheses of over and partial-dominance offer different quantitative predictions (described in detail in Chapter 5; Roff 2002). If outbreeding follows successive generations of inbreeding, the over-dominance hypothesis predicts a return to the pre-inbreeding mean fitness level. In contrast, the partial-dominance hypothesis predicts an increase in mean fitness levels due to the purging of deleterious alleles. However, the wasp lines used in this experiment had been under weak laboratory selection for many generations. This could have reduced the frequency at which deleterious mutations were purged, thereby reducing any potential difference between a pre and post-inbreeding fitness mean and reducing any cost of in- or outbreeding. Further, I did not have the resources to compare pre-inbreeding fitness traits with post-inbreeding fitness traits, which makes it more difficult to distinguish between the two hypotheses' predictions.

The results from this work support the findings of Luna and Hawkins (2004) who found that *N. vitripennis* experienced modest inbreeding depression, although they did not observe any cost of outbreeding. They found that lifespan and fecundity increase when females are paired with non-sibling males from the same strain, or males from different

strains. Whether *N. vitripennis*, a chronic but not obligate inbreeder (Luna & Hawkins 2004), should selectively outbreed when given the option, may be controlled less by the cost of inbreeding, or indeed outbreeding, but by the ability to distinguish relatives. Neither Reece *et al.* (2003) nor Shuker *et al.* (2004) found any evidence that *N. vitripennis* is able to determine the relatedness of individuals whether directly or indirectly. So regardless of the extent of inbreeding depression in *N. vitripennis*, it may be that they are unable to adjust their behaviour accordingly except by the frequency with which they superparasitise.

6.3 Concluding remarks

The factors affecting clutch size and sex allocation decisions in *N. vitripennis* are numerous and interlinked. Clutch size appears to have important consequences for body size, which in turn impacts on longevity and fecundity, at least in females. Increased female size allows for greater flexibility, whether being food-limited when hosts are rare, or having sufficient eggs when hosts are numerous. However, clutch size may also have an influence on fecundity above and beyond that shown through body size, particularly when taking into account the effects of host-feeding and altered sex ratios. Sex allocation is largely governed by the limitations of local mate competition. However, there is still variation that may be explained through interactions between maternal condition and larval competition. Further work is required on the costs of female dispersal, male mating behaviour and host nutrient use to enable the separate elements influencing fitness to be brought together.

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Appendices

Appendix A:

A commentary on the evolution of sexual reproduction published in Current Biology (Sykes & West 2005) – I was first author.

Appendix B:

A study on male influence over sex allocation in *N. vitripennis* (Shuker *et al.* 2006) in which I carried out experiment three.

Appendix C:

A microsatellite analysis of mating behaviour in natural populations (Burton-Chellew *et al.* submitted) – I worked in both the field and laboratory, assisted with molecular analysis and commented on the manuscript.

Appendix D:

A molecular analysis of the population structure of natural populations (Grillenberger *et al.* 2007) – I worked in the laboratory and assisted with molecular analysis.

Appendix E:

An experimental test of whether humans adjust their level of cooperation in response to population structure (West *et al.* 2006) – I helped carry out the experimental data collection for this paper.

information at the level of single neurons (see [5] for an elegant review of this work). These quantity-sensitive neurons tend to have a 'preferred numerosity', firing most strongly when presented with a specific number of visual objects. Unfortunately, the level at which these cells represent numerical information is at present unclear. While it is possible that number-sensitive cells reflect abstract numerical processing, it is also possible that such cells process only visual numerical content, and thus may be tied to a single sensory modality.

The findings of Jordan *et al.* [13], however, suggest that the macaque brain is capable of integrating multi-sensory information about quantity, and thus raise the possibility that previously identified prefrontal number-sensitive cells may underlie this processing. Future work could therefore profit from developing cross-modal tasks that can be used in conjunction with neurophysiological recordings. In this way, the results of Jordan *et al.* [13] pave the way for a broader comparative investigation of modality-independent number representations; their approach will undoubtedly lead to new insight into both the nature of and mechanisms underlying numerical representations.

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Evolution: Revenge of the Clones!

Recent work on ants shows both extraordinary patterns of reproduction and a new type of sexual conflict, leading to the remarkable scenario where females have no father and males have no mother.

Edward M. Sykes and Stuart A. West

Why do we have sex, and why so often, when many species do without it? This question still poses a major problem for biologists [1,2] and is raised once again with the recent discovery [3,4] that two species of ant produce workers sexually but queens and sons (reproductives) asexually.

If the main aim of reproduction is to create copies of our genes, then why don't we simply produce clones of ourselves, as asexual organisms do? Our gene combinations have been selected over time as successful, yet we pass on only half of them and mix these up with our partner's during meiosis — twisting fully working gene combinations into ones that may not function as well; this is the so-called 'recombination load'. This cost alone may seem bad enough, but in species such as humans with separate sexes, there is the added cost of producing males to fertilise the females, effectively cutting the number of reproducing individuals by half. This is known as the two-fold cost of sex. A variety of theories have been put forward to explain why, despite these costs, sexual reproduction is widespread in animals and plants [1,2].

The two most favored hypotheses explaining sex and recombination are, first, that they provide an advantage in coevolutionary arms races, especially with parasites; and second, that they facilitate the purging of deleterious mutations [1,2]. The parasite hypothesis relies on the idea that parasites will evolve to infect common genotypes in a population, providing an advantage to the production of rare genotypes by sex [5]. This explanation has been termed the Red Queen theory, because it suggests that, just like Alice, one has to run just to stay in the same place — mixing the successful genes from the last generation to stop the parasites infecting the offspring in the next. The mutation hypothesis relies on the idea that sex allows you to lose deleterious mutations in a few low quality offspring. This can make up for a two-fold cost of sex, as long as there are at least one or two mutations per genome per generation, and the fitness cost of each additional deleterious mutation is greater than the last, a phenomenon termed synergistic epistasis [6].

A common theme with most theories that provide an advantage

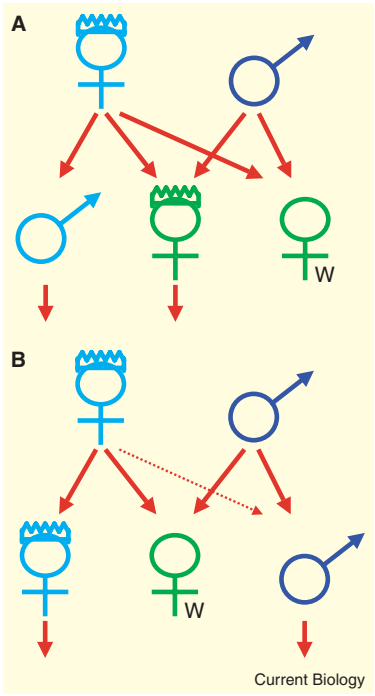

Figure 1. Reproduction in eusocial insects.
(A) Typical haplodiploid reproduction: unfertilised eggs become haploid males, while fertilised eggs develop into diploid females, either future queens or workers, (W). (B) A unique form of reproduction has been found in the ant *Wasmannia auropunctata*. Queens and males form reproductives through asexual reproduction, while sterile workers are the product of sexual reproduction. The dotted line shows how sons are induced as haploids containing only the father's genes, despite initially being produced sexually. In this way the gene pools of the two sexes are effectively separate except when they meet in sterile workers (W).

to sex is that nearly as much benefit can be obtained from only occasional sex [1,2]. Recent studies [3,4] have shown that two species of ant seem to get the best of both worlds, by switching between sexual and asexual reproduction, depending on what kind of offspring they are producing. In both species, the queens of the colonies produce future queens (gynes) asexually with all the benefits of cloning, but they produce workers sexually.

Why do they do this, and can this tell us anything about the benefit of sex? One possibility could relate to the desirability of variation to increase resistance against parasites [7,8]. If a single queen produced gynes and workers asexually, the entire colony would have the same genotype, which could make them extremely vulnerable to parasite infection. Producing workers sexually increases the number of genotypes in the colony. As workers are more numerous than reproductives, gynes are always of the less common genotype. making them safer from parasites even when produced asexually.

Does this mean that these ants provide unambiguous support for the parasite driven Red Queen explanation of sex? Sadly not, as an explanation can also be given that depends upon deleterious mutations. The colonies expand through a process known as 'budding', whereby new queens and workers simply pick up and move to a new location, taking larvae with them. At no point are the queens exposed to life 'outside' the colony without the aid of a fully developed workforce [3,4]. This may have reduced the selection pressure on the queens so that fewer mutations reduce fitness, enabling the queens to produce gynes asexually.

This illustrates the general problem that observational data on the pattern of reproduction within or across species can always be potentially explained by competing theories [2].

There is, of course, another problem with the queens producing asexually — the males don't get to pass on any genes to future reproductives. In one of the species, *Cataglyphis cursor*, the workers can reproduce, so there is still some opportunity for males to sire grandoffspring [3]. In the little fire ant *Wasmannia auropunctata*, however, the workers are sterile [4]. Somehow the males have got round this, as in some fertilised eggs the female's genes are ignored (how is not known) leaving haploid males instead of diploid workers (Figure 1). In this species we therefore have ended up with the remarkable scenario that females have no father and males have no mother!

This leads to another question: why do the males not make sure more eggs become sons? As there are multiple queens to a colony, there would always be other daughters for their sons to mate with, potentially even leading to a dichotomy of females, some producing only females and others producing only males. This evidently does not happen, and it seems male control only occurs rarely as there are a great many more workers than sons.

Perhaps the most exceptional aspect of this biology is that each sex is almost its own species, as the two gene pools only interact in the non-reproductive workers. This means that the two sexes are unfettered from the possibility of sexually antagonistic alleles, where selection acts in different directions in the two sexes [9]. Selection can still act on the alleles present in the workers though as, if they fail, the colony will not be able to support the production of reproductives. However, selection could be pushing the workers in a third direction, and so they don't necessarily reduce the antagonism. The importance of this antagonism is likely to vary across traits — for example, in some traits such as mandible size the separate groups could all have their own optimum, whereas in other traits, such as hormone production, the workers may have no optimum and could exist in an androgenous form.

Whether the two sexes no longer have to compromise or if this male control of reproduction is just a snapshot in an arms race, it seems that relaxed natural selection has allowed females to reproduce without sex, while sexual conflict has forced the males to follow suit. A major question is whether the workers are maintained sexually because of parasite load, mutation accumulation or a mixture or both?

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Neurotransmission: Emerging Roles of Endocannabinoids

Postsynaptic release of endocannabinoids can inhibit presynaptic neurotransmitter release on short and long timescales. This retrograde inhibition occurs at both excitatory and inhibitory synapses and may provide a mechanism for synaptic gain control, short-term associative plasticity, reduction of synaptic crosstalk, and metaplasticity.

Anatol C. Kreitlzer

Endocannabinoids are a class of lipophilic signaling molecules that are synthesized and released by postsynaptic neurons in response to increases in intracellular calcium levels or activation of metabotropic receptors. As their name implies, endocannabinoids activate the same G protein-coupled receptors as the active compounds in *Cannabis sativa* (marijuana). The primary neuronal subtype of this receptor, known as CB1, is widely distributed in the mammalian brain and is expressed in presynaptic terminals, where it can inhibit neurotransmitter release. The endocannabinoid system is thus well suited for rapid retrograde signaling across activated synapses. Recent studies are beginning to elucidate the physiological roles of this signalling.

Retrograde signaling by endocannabinoids was first observed in the cerebellum and hippocampus as a phenomenon termed depolarization-induced suppression of inhibition (DSI) [1,2]. DSI is a short-term depression of neurotransmitter release that can be elicited by postsynaptic depolarization sufficient to activate voltage-sensitive calcium channels. Increases in intracellular calcium levels stimulate the production of endocannabinoids, perhaps via phospholipase D (PLD) [3], which can then diffuse to adjacent presynaptic terminals and suppress neurotransmitter release for tens of seconds [4,5]. A similar phenomenon has been observed at excitatory synapses and is known as depolarization-induced suppression of excitation (DSE) [6].

In addition to depolarization-mediated calcium entry, activation of metabotropic glutamate and acetylcholine receptors can drive endocannabinoid release through a separate biosynthetic pathway [7,8]. Receptor-mediated endocannabinoid production requires phospholipase Cβ (PLCβ) [9], an enzyme which is activated by G protein signaling and modulated by calcium. Thus, increases in intracellular calcium can directly stimulate endocannabinoid production via PLD, while at the same time increasing the efficacy of receptor-driven PLCβ-mediated biosynthesis. This receptor-driven release is critical for endocannabinoid-mediated long-term depression (LTD) of neurotransmitter release at both excitatory and inhibitory synapses [10–12]. Although LTD can be elicited by short (1 second) presynaptic bursts sufficient to activate postsynaptic metabotropic glutamate receptors, the subsequent receptor-driven endocannabinoid release may feature slower kinetics since LTD induction requires a sustained (5–10 minute) activation of presynaptic CB1 receptors [12,13].

Modulation of synaptic transmission by endocannabinoids was initially studied using non-physiological methods, such as seconds-long depolarization or application of high-affinity metabotropic receptor agonists, to evoke endocannabinoid release. But the modulatory role of endocannabinoids during normal synaptic activity was not known. Brenowitz and Regehr [14] found that depolarization-evoked endocannabinoid release from cerebellar Purkinje cells requires high levels of intracellular calcium, suggesting that depolarization alone may not play a prominent role in the release of endocannabinoids under normal physiological conditions.

Another study, in the cerebellum, by Maejima *et al*. [7] found that 50–100 Hz activation of excitatory parallel fiber synapses onto a Purkinje cell could yield a transient 10–15% heterosynaptic inhibition of neurotransmitter release at excitatory climbing fiber synapses on the same Purkinje cell. This synaptically evoked inhibition was mediated by endocannabinoids and required activation of postsynaptic metabotropic glutamate receptors, an early indication that receptor-driven endocannabinoid release is critical under more physiological conditions.

The first systematic study of synaptically evoked endocannabinoid release was that of Brown *et al*. [15], again in the cerebellum. Following brief trains of parallel fiber stimulation, they observed a transient ~50% inhibition of neurotransmitter release from parallel fibers, which

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ORIGINAL ARTICLE

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Male influence on sex allocation in the parasitoid wasp *Nasonia vitripennis*

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Abstract Sex allocation is an important reproductive decision for parents. However, it is often assumed that females have substantial control over sex allocation decisions, and this is particularly true in haplodiploid insects, in which females apparently determine sex by deciding whether to fertilise an egg (and produce a diploid daughter) or not (and produce a haploid son). Mechanisms by which males may influence sex allocation are not so straightforward, and their potential influence on sex ratios has been somewhat neglected. Here, we test whether males influence offspring sex ratios in the parasitoid wasp *Nasonia vitripennis*. We show that some of the variation in observed sex ratios can be attributed to males when comparing the affect of male strain on sex ratio. We did not find among-male variation in sex ratio with a less powerful experiment using males from only one strain or an effect of male mating environment. Our data suggest that males can influence female sex ratios and contribute to the variation around the sex ratios optimal for females. However, the influence is not large, suggesting that females have more influence on sex allocation than do males. We conclude by considering whether male influences on sex ratio represent differences in male reproductive competence or deliberate attempts by males to increase their fitness by influencing daughter production.

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Introduction

Sex allocation is an important reproductive decision for parents, and the study of sex ratios has proven to be one of the most productive areas in evolutionary biology (Charnov 1982; Frank 2002). Deciding the sex of the offspring, and how much to invest in those offspring, has received a great deal of theoretical and empirical attention (Charnov 1982; Hardy 2002). Our understanding of the evolution of sex ratios and sex allocation is helped enormously by the conceptually straightforward trade-off between the fitness obtained through sons and that obtained through daughters, a trade-off that is often very easy to measure empirically. Sex allocation theory is therefore one of the few areas in which evolutionary theory can be realistically tested in a quantitative fashion (West et al. 2000, 2002). This means that meaningful attempts to identify constraints on adaptive evolution can be made (Herre 1987; West and Sheldon 2002; Shuker and West 2004). Here, we consider one such constraint on adaptive female sex ratio behaviour, the role of males.

In many organisms, females have been assumed, explicitly or otherwise, to be in control of sex allocation. For example, the controversy over adaptive sex allocation in birds has been fuelled by the lack of an obvious mechanism by which females could shift sex ratios (Pike and Petrie 2003). In haplodiploid insects such as Hymenoptera on the other hand, females have been assumed to determine the sex ratio by the apparently simple mechanism of deciding whether or not to fertilise the eggs, producing either female or male offspring respectively. Males have been considered to have little influence on the sex ratio because it was not clear how males could influence sex ratio (e.g. Werren and Beukeboom 1998), although this view is perhaps the result of a lack of detailed studies. This has meant that attention on sex ratio evolution in the Hymenoptera has tended to concentrate on the female, although it has been argued that this assumption is unjustified (Orzack 1993, 2002).

Males could influence female sex allocation in a number of ways in haplodiploids (Henter 2004). First, males may differ in fertilisation ability, with some producing sperm that were unable to successfully fertilise eggs. This would lead to an increased production of sons relative to daughters. Second, fertilisation may take place, but the paternal and maternal genomes could be incompatible, leading to the embryonic death of daughters. Third, males may actively attempt to influence sex allocation because males only pass genes into the next generation via daughters (Hawkes 1992). Here, we address these issues in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Chalcidoidea). Females of this species have been repeatedly shown to facultatively alter their offspring sex ratios in the direction predicted by the local mate competition (LMC) theory of Hamilton (1967, 1979) (see also Suzuki and Iwasa 1980; Taylor and Bulmer 1980; Werren 1980). Females alter their sex ratios in response to (1) the presence of other females and (2) laying eggs on a host already parasitised by another female (e.g. Werren 1980, 1983; Orzack 1990; Orzack et al. 1991; King et al. 1995; Molbo and Parker 1996; Flanagan et al. 1998; Shuker and West 2004). In both cases females increase their offspring sex ratio with increasing numbers of offspring from other females on a patch because the level of local mate competition between their own sons is reduced as more unrelated individuals on a patch interact. However, for all the support of LMC theory in *N. vitripennis*, there remains unexplained variation around the optimal sex ratios. We consider whether males contribute in some way to this variation.

We used two approaches to explore whether males can influence the sex ratio females produce. The first was to partition the variance in female sex ratio amongst males either from one outbred strain ("Experiment 1") or from six strains established from collections from one natural population ("Experiment 2"). A significant effect of male mating partner would suggest that males influence the sex ratio, assuming that they vary in their ability to do so. We screened males for their effect on female sex ratio with females ovipositing either alone on fresh hosts (in which case females should produce large, highly female-biased clutches) or on parasitised hosts (leading to smaller, less female-biased clutches). In the third experiment, we used a different approach, experimentally manipulating the mating and oviposition environment of males and females, to see whether any effect of males depended on whether males had to inseminate one or several females in a short time period, as might be predicted if sperm limitation constrains female sex allocation. Females then either oviposited alone or together in groups.

Methods

Study organism

Nasonia vitripennis is a gregarious parasitoid of pupae of numerous species of large cyclorraphous Diptera (Whiting 1967). Males have small wings and are unable to fly, typically remaining close to the site of adult emergence to compete with each other for matings with emerging females. Females on the other hand mate before dispersing to parasitise fresh hosts. Females lay clutches of 20–40 eggs and limit oviposition in previously parasitised hosts (superparasitism) if possible (e.g. Werren 1980, 1984; Charnov and Skinner 1984; Shuker et al. 2005). Since *N. vitripennis* is haplodiploid, females are assumed to be able to facultatively alter the sex ratio by choosing whether or not to fertilise an egg (producing diploid females or haploid males, respectively). We used the outbred wild-type laboratory strain HV7 for experiments 1 and 3, derived from wasps from bird boxes collected from the field in 2001 and 2002 (Hoge Veluwe, Netherlands). For experiment 2 we used the strains C378, C289, C349, C194, C222A and C130, again collected from Hoge Veluwe (in 2003). We also used the red-eye mutant laboratory strain STDR to allow us to identify the broods of individual wild-type females when superparasitising (experiments 1 and 2) or in multi-foundress groups (experiment 3). All wasps were maintained prior to the experiment in mass culture, with *Calliphora vomitoria* pupae as hosts, at 25°C, 16:8 h light/dark conditions. Under these conditions, males emerge after 13 or 14 days, with females emerging soon after.

Experiment 1: sex ratio variation and male mating partner

We obtained mated HV7 females from mass culture and gave each one a separate host to parasitise. Before emergence of these broods (about 12 days post-oviposition), we opened the hosts and collected male and female pupae. Using these pupae, we set-up mating groups of one male and up to ten unrelated females in 25×75-mm glass tubes. Wasps emerged and mated for 2 days, and then we isolated individual females and pretreated them for 24 h with access to a fresh host followed by 24 h with honey-solution-soaked filter paper. This pretreatment allows the female to host-feed and mature eggs prior to the experiment. Females were then given either two fresh hosts to parasitise (treatment A), or two hosts that had been parasitised during the previous 24 h by similarly pretreated STDR females (treatment B). After 1 h, one-way escape tubes were fitted to the tubes to allow females to disperse from the experimental patch to allow females to limit the extent of superparasitism (Werren 1980; Godfray 1994). After 48 h all females were removed, and the hosts were incubated at 25°C. Following emergence of the brood, the number, sex and eye colour of all individuals were recorded. Broods where only males were produced, indicating female virginity, were discarded. In addition, the number of females emerging successfully from the collected pupae was unexpectedly low in some replicates, so sample sizes varied across males. In total, 134 females from 42 males provided data. Due to these emergence problems, treatment A was performed across two blocks.

There was no significant difference between blocks in sex ratio so the data were pooled for subsequent analysis (general linear mixed model $F_{1,102}$=0.02, P=0.89).

Experiment 2: sex ratio variation and male strain

We used six strains of recently collected *N. vitripennis*: C378, C289, C349, C194, C222A and C130. Randomly chosen virgin females from C378 were individually mated to males from one of the six lines and then either given a single fresh host to parasitise (treatment A) or one STDR pre-parasitised host (treatment B) following pretreatment as before. Again, we fitted one-way escape tubes and removed the females after 48 h. Females mated to males from each strain were split between racks and between shelves to reduce confounding positional effects in the incubator. The only difference between females was the strain identity of the male with which they mated.

Females who produced all-male broods in treatment A were considered virgins and were excluded from the sex ratio analysis. We checked whether females in treatment B who produced male-only broods were virgins by subsequently giving a fresh host to oviposit upon to check virginity status (since optimal sex ratios when ovipositing may include very small, male-only clutches; Werren 1980). From 39 putative virgins, 29 produced females when ovipositing on a fresh host. In total, eight females from treatment B could not be assigned virgin/non-virgin status as they died before ovipositing in the virgin-check treatment. Inclusion or not of these females did not influence the results (so results with them are presented below). Three females who produced more than ten diapause larvae were also excluded (again, results did not change if they were included). In total, 412 females provided oviposition data (N=26–40 for each treatment and strain combination), with 292 of these providing sex ratio data (N=9–34 for each treatment and strain combination).

Experiment 3: sex ratio variation and the mating environment

In the third experiment we checked whether male effects are influenced by the mating environment. All males and females were collected as pupae from a large sample of hosts parasitised by HV7 females and were therefore virgins prior to the mating treatments. We kept them individually in 10×75-mm glass tubes and fed them with honey-soaked filter paper for 2 days. We then randomly allocated them to mating treatments, putting males and females together in 25×75-mm tubes. We used a mating treatment with two levels: (1) one male and one female, with three fresh hosts present; and (2) one male and five females, again with three fresh hosts present. The presence of hosts substituted for the pretreatment of experiment 1. We allowed the wasps to mate for 48 h, after which we placed females in one of two oviposition treatments: (1) oviposition alone with three fresh hosts; or (2) oviposition with four red-eye STDR females of the same age and mating status, again with three fresh hosts. Only one of the five females from each replicate from the multi-female mating treatment, chosen at random, was used for oviposition to avoid pseudoreplication. After 1 h in the oviposition tubes, we again fitted one-way escape tubes to allow females to disperse away from the patch. All females were removed after 48 h, and the hosts were then incubated at 25°C. As before, we sexed and counted all wild-type and red-eye offspring that emerged. Broods with no females, indicating virginity, were discarded, as were a limited number of broods with ten or more diapausing larvae or undeveloped pupae. In total, we used broods from 175 females, with sample sizes per treatment ranging from 38 to 50.

Statistical analysis

We defined sex ratio as the proportion of offspring that were male. Sex ratio data are characterised by binomially distributed data, and are most appropriately analysed with generalized linear models (GLMs), with binomial error structures and logit link functions (Crawley 2002; Wilson and Hardy 2002). However, problems in significance testing can arise if the data are overdispersed; dispersion is measured by calculating the so-called dispersion parameter by dividing the residual deviance by the residual degrees of freedom following model fitting (Crawley 2002). The data from the first and second experiments were somewhat overdispersed (the dispersion parameter from the full model was >4), so we used general linear models with arcsine–square root transformed sex ratios weighted by clutch size. For experiment 1 we used a mixed model with male as a random factor, and we tested the significance of the male effect by comparing the change in deviance explained by models with or without the random factor using a likelihood ratio test (Pinheiro and Bates 2000). For the fixed factors (treatment and clutch size), model simplification was carried out following Crawley (2002) to test the significance of main effects and interactions. In the third experiment, the dispersion parameter was close to 4. We therefore analysed experiment 3 using both generalized linear models with binomial errors and general linear models with transformed data. The results are similar, so only the former are reported here, except when considering multi-foundress groups alone, as the data were more overdispersed in this subset of the data. Linear modelling was performed with S-Plus 6 (Insightful Corporation, Seattle, WA, USA) and Genstat 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

Results

Experiment 1: sex ratio variation and male mating partner

There was no significant effect of male mating partner on female sex ratio (likelihood ratio test LR=0.50, df=1,

P=0.48). This was also true if only superparasitising females were considered (LR<0.01, df=1, P>0.9). There was a highly significant effect of oviposition treatment on sex ratio, with females ovipositing on fresh hosts producing a more female-biased sex ratio (sex ratio=0.18) than superparasitising females (sex ratio=0.41; $F_{1,132}$= 12.56, P=0.0005). There was no significant effect of clutch size on sex ratio ($F_{1,131}$=2.20, P=0.14), and amongst superparasitising females there was no effect of the red-eye clutch size ($F_{1,26}$<0.01, P =0.98). There was one outlier in the data set, where the female produced a clutch of 82 offspring, but it only included one female (giving a sex ratio of 0.987; this female was clearly not a virgin, although she may have been sperm-limited). Removal of this data point did not change the results.

Experiment 2: sex ratio variation and male strain

There was a significant effect of male strain on the sex ratio produced by a female ($F_{5,273}$=2.34, P=0.04; Fig. 1), but the proportion of variance explained by male strain was small (R^2=0.023). There was also a significant effect of treatment on sex ratio ($F_{1,273}$=171.26, P<0.0001; Fig. 1) as expected from theory. This GLM included female clutch size to control for both wild-type and STDR clutch sizes in this analysis (the latter is not useful for an analysis of both treatments because it is 0 in treatment A, but wild-type and STDR clutch sizes in treatment B are significantly negatively correlated: P<0.0001, R^2=0.31). Clutch size was not significant as a main effect on sex ratio ($F_{1,273}$=1.84, P=0.18), but there was a significant interaction between treatment and clutch size ($F_{1,273}$=19.86, P<0.0001). Other interactions were not significant (all P>0.1). The final model explained 45.6% of the variance in sex ratio.

In terms of specific contrasts, within treatment A (females ovipositing alone), males from strain C289 produced a significantly more female-biased sex ratio than males from the other five strains (controlling for clutch size $F_{1,186}$=7.38, P=0.007). Within treatment B (females superparasitising), males from strains C289, C194 and C349 produced sex ratios significantly more female-biased than males from the other three strains (controlling for STDR and wild-type clutch sizes $F_{1,98}$=6.53, P=0.01). It is worth noting that females mated to males from their own strain (C378) produced sex ratios among the least female-biased in the experiment (Fig. 1).

There was no effect of male strain on clutch size ($F_{5,280}$=1.35, P=0.24; Fig. 2) so that differences in sex ratio are not due to differential female mortality attributable to parental strain. As expected, there was a significant effect of treatment on clutch size ($F_{1,280}$=345.48, P<0.0001), with superparasitising females producing smaller clutches (Fig. 2). There was also a non-significant interaction between male strain and treatment ($F_{5,280}$=2.17, P=0.06). However, within each treatment separately there was no effect of male strain [treatment A $F_{5,185}$=1.34, P=0.25; treatment B (controlling for STDR clutch size) $F_{5,95}$=1.55, P=0.18].

Male strain did influence whether or not a female oviposited, at least when superparasitising. Using logistic regression, there was a significant effect of treatment (i.e. fresh vs already-parasitised hosts) on the probability that a female would oviposit in the first place ($\chi^2_{1,410}$=54.67, P<0.001), with females being more likely to oviposit on fresh hosts (88.7 vs 57.1% of females, respectively). There was no significant effect of male strain ($\chi^2_{5,405}$=9.05, P=0.11) and no interaction between treatment and strain ($\chi^2_{5,400}$=8.79, P=0.11). However, if we take STDR clutch size into account in treatment B, there was a significant effect of male strain on the probability of ovipositing ($\chi^2_{5,176}$=16.92, P=0.005). The influence of STDR clutch size differed between male strains ($\chi^2_{5,170}$=11.31, P=0.05).

We can also ask whether females were more likely to mate with males from some strains more than others. Virginity, or possible virginity, was generally low (2–12% of females). Taking females across both treatments, there

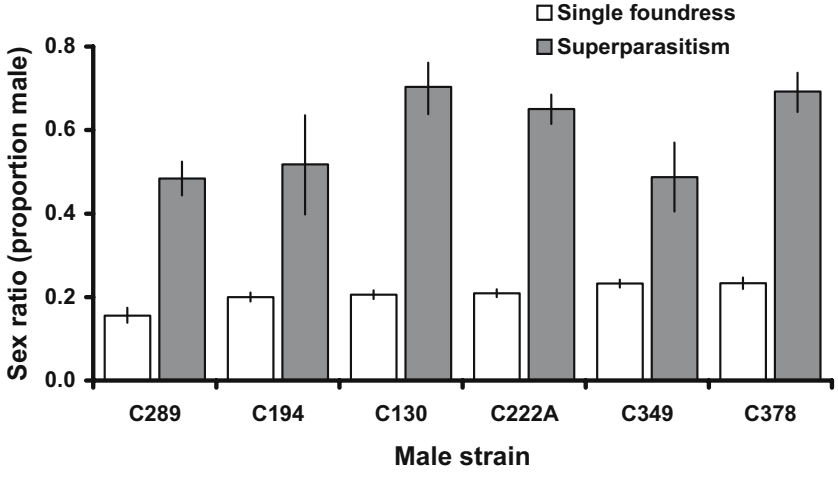

Fig. 1 Sex ratio with respect to paternal strain for females ovipositing as single foundresses or as superparasites. Error bars are binomial standard errors

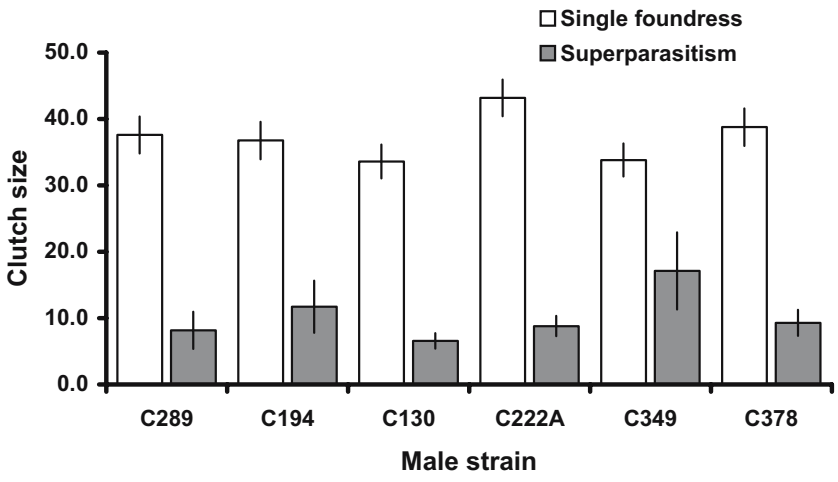

Fig. 2 Clutch size with respect to paternal strain for females ovipositing as single foundresses or as superparasites. Error bars are standard errors

was no significant effect of male strain on likelihood of a female remaining virgin (G test including the eight "possible virgins" G_5=3.95, P=0.56), so females did not seem to prefer one strain to another.

Experiment 3: sex ratio variation and mating environment

There was a highly significant effect of oviposition treatment, with females ovipositing alone producing a much more female-biased sex ratio than females in multi-foundress groups as expected (GLM $F_{1,173}$=137.52, P<0.0001; Fig. 3). There was no significant effect of mating group ($F_{1,172}$=2.21, P=0.14) and no significant effect of wild-type clutch size ($F_{1,171}$=0.03, P=0.87). Importantly, there were no significant interactions between the main effects (all removed from the model with P>0.39). For the females in multi-foundress oviposition groups, there was no effect of number of red-eye brood on sex ratio (GLM with arcsine–square root transformed data $F_{1,84}$=0.58, P=0.45). Within this subset of the data, again, there was no significant effect of wild-type clutch size ($F_{1,83}$=0.03, P=0.87) or mating group treatment, although this was close to significance at the 5% level ($F_{1,85}$=3.60, P=0.06).

Discussion

The role of males in influencing sex allocation has been neglected, particularly in haplodiploid organisms in which females seem to have a clear mechanism with which to allocate sex (Henter 2004). Here, we have considered what role males may play in explaining variation in patterns of sex allocation in *N. vitripennis*. In the first two experiments we used variation amongst males from either the same outbred strain or from several different strains to assay

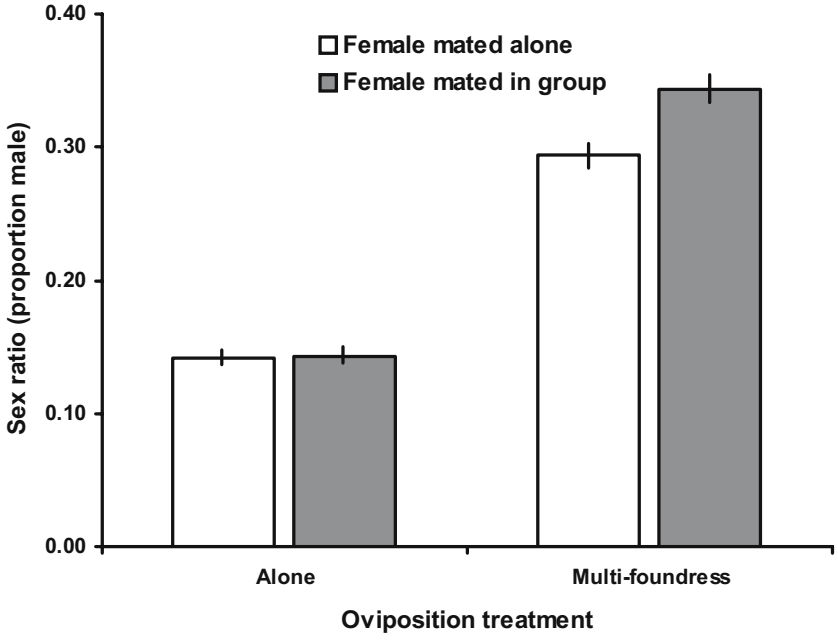

Fig. 3 Male influence on sex ratio with respect to mating environment. Females mated males either alone (*open bars*) or in groups of five (*shaded bars*), and then females either oviposited alone or in groups. Error bars are binomial standard errors

male influence on sex ratio. In the first experiment we failed to resolve any variation associated with males, although the sample sizes were small. In the second experiment we looked to see whether male strain influenced sex ratio, providing a more powerful test. Variation in sex ratio was associated with male strain as predicted, although the effect was small. In our third experiment we found no evidence that the number of females a male mated with influenced sex allocation, whether females oviposited alone or in groups.

What are the possible causes of the male strain effect we saw in experiment 2? The most straightforward is that male strains vary in their fertilisation success, for instance with males in some strains passing sperm of either insufficient quantity or quality to fertilise as many eggs as the females attempt to (Eberhard 1996). In terms of quantity, since we excluded females that produced only sons (and so could have been virgin in the sense of not having copulated), all the males in the experiments passed at least some sperm. However, if there was variation in the quantity of sperm between males, we would only expect to have seen a male effect in the situation where females produced large clutches, in which sperm was needed to produce 20 or more daughters and so might have been limiting, such as when females oviposited alone. We would perhaps not have expected to see such an effect when females produced the smaller, less female-biased clutches they did when superparasitising. On average, females in treatment A produced 29.53 daughters, and in treatment B they produced 3.98 daughters. Since we saw no treatment × strain interaction, this suggests the strain effect was similar when females oviposited alone or when superparasitising, and thus argues against males not passing sufficient numbers of sperm. More generally, there was no male strain × clutch size interaction that would again have been expected if sperm quantity was the cause. In addition, in experiment 3, females mated to males that could mate with four other females did not produce more males, indicative of sperm limitation, when compared with females who mated with just one male alone.

In terms of quality, males might have passed sperm that proved less capable of fertilising eggs, for instance through a lack of sperm viability or motility, leading to reduced fertilisation. This would lead to more males being produced than a female attempted to allocate. Again, if males had passed sperm completely incapable of fertilising any eggs, then they would have been excluded because we could not distinguish them from cases in which the male and female just did not copulate (since 100% males would be produced in both cases), so all males in the experiments passed some sperm capable of fertilising eggs. However, variation between strains in male sperm characteristics might explain the data, with male ejaculate quality thus being a potentially important constraint on facultative sex allocation.

Alternative explanations involve one or more side effects of the interaction between the genotypes of individuals from different strains. However, male strain did not influence the number of offspring produced, as we might expect if the results were due to differences in genetic compatibility between male and female strain influencing the survival of fertilised eggs or larvae. Females could also vary in their preference for males of different strains (Andersson 1994). In terms of pre-copulatory choice, there should have been variation in the number of females who remained unmated (i.e. virgin) with respect to male strain, but there was no such effect. In terms of post-copulatory choice, females could have chosen to either limit or not use sperm from males from certain strains, but it is worth noting that the most male-biased sex ratios were produced by females mated to males from their own strain, with which they are presumably compatible. Instead, more eggs were fertilised when females mated with males from strains different to their own. It is also worth remembering that whilst each strain was initiated from wasps from a different bird nest, they were all collected in the same locality. Therefore it would actually be quite surprising to find incompatibility between males and females from different strains.

One other possibility is that males are attempting to deliberately influence sex ratio to maximise their genetic contribution to the next generation by increasing daughter production (Hawkes 1992). In haplodiploids, daughters are the only way for males to pass on genes, and so they could be selected to increase fertilisation rate. In species with LMC, when females oviposit alone they produce highly female-biased sex ratios, but these sex ratios become increasingly less biased as more females oviposit together or as the relative brood size of a superparasitising female decreases (Hamilton 1967; Werren 1980). In these latter situations, optimal male and female sex ratios may differ, leading to a sexual conflict over sex allocation (Hawkes 1992). We are currently exploring to what extent male effects are the result of reproductive incompetence, or male attempts are to deliberately shift female sex ratios. One of our most intriguing results was that males may influence the likelihood of females ovipositing, at least when faced with an already parasitised host (females with fresh hosts oviposit very readily). Oviposition rate is one of the female reproductive traits males are known to influence via seminal products in some insects (Eberhard 1996; Chapman 2001).

The importance of clarifying the effect of males on the sex ratio is threefold. First, possible male effects represent a further class of constraint on the production of adaptive sex ratios. We have been examining constraints on adaptive sex allocation in *N. vitripennis* as part of a long-term research program, revealing in particular how information processing constrains female sex ratio behaviour (Reece et al. 2004; Shuker et al. 2004a,b; Shuker and West 2004). That males can passively or actively influence female sex ratio behaviour adds significantly to our understanding of sex allocation in *N. vitripennis*. Second, models of LMC assume, often implicitly, that females are the sole arbiters of sex ratio decisions (Orzack 1993, 2002). Whilst this may seem a reasonable assumption, evidence from *Muscidifurax raptorellus* suggests that male genotype can influence female oviposition behaviour (Legner 1988, 1989, al-

though recent data question this result, Legner and Stouthamer, personal communication), and surprisingly this has yet to be tested more widely (Hawkes 1992; Orzack 1993, 2002; but see Henter 2004). Given the often cryptic influence males can have on a variety of female reproductive behaviours (Eberhard 1996; Chapman 2001), we need to be sure whether males influence females when testing LMC models. The third reason for investigating hidden male effects on sex ratio is that they could provide an unexpected source of error in quantitative genetic studies of sex allocation. Male influence via sperm quality or seminal products therefore represents another possible environmental effect that may confound attempts to dissect genetically sex ratio behaviour (Antolin 1992).

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Facultative sex ratio adjustment in natural populations of wasps: cues of local mate competition and the precision of adaptation

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ABSTRACT

Sex ratio theory offers excellent opportunities for examining the extent to which individuals adaptively adjust their behaviour in response to local conditions. Hamilton's theory of local mate competition (LMC), which predicts female biased sex ratios in structured populations, has been extended in numerous directions to predict individual behaviour in response to factors such as relative fecundity, time of oviposition and relatedness both between foundresses and their mates. These extended models assume that females use different sources of information and have generally been either not tested or only tested in the lab. We use microsatellite markers to describe the oviposition behaviour of individual females in natural populations of the parasitoid wasp *Nasonia vitripennis*, and hence test 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.

INTRODUCTION

Sex ratio theory has provided excellent opportunities for examining the precision of adaptation (Boomsma et al. 2003; Charnov 1982; Hardy 2002; Herre 1987; Shuker and West 2004; West and Sheldon 2002). 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 1985b; 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 (Abe et al. 2003; Frank

1985b; Frank 1987; Reece et al. 2004; Shuker et al. 2005; Stubblefield and Seger 1990; Suzuki and Iwasa 1980; Taylor and Crespi 1994; Werren 1980; Yamaguchi 1985). For example, if one female produces a relatively smaller brood, then she should lay a less female biased, or even male biased, sex ratio (Frank 1987; Stubblefield and Seger 1990; Suzuki and Iwasa 1980; Werren 1980; Yamaguchi 1985). Table 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.

Here we address this problem by using microsatellite markers to trace the behaviour in the field of individual females of the parasitic wasp *Nasonia vitripennis*. *N. 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 (Grillenberger et al. 2007; Molbo and Parker 1996; Orzack et al. 1991; Shuker and West 2004; Werren 1983). *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 (Flanagan et al. 1998; Orzack and Parker 1986; Orzack and Parker 1990; Reece et al. 2004; Shuker et al. 2006; Shuker et al. 2007; Shuker et al. 2004a; Shuker et al. 2004b; Werren 1980). Here we use the power of molecular techniques to test these extensions to LMC theory in the wild. Specifically, we (1) test to what extent females adjust their sex ratio in response to predicted environmental parameters (Table 1), and (2) test which models of LMC best approximate sex allocation in

the wild. By genotyping more than 3500 offspring at four microsatellite loci, we were able to reconstruct the parental genotypes and hence determine the sex ratios produced by 49 females, in 350 broods across 18 natural patches. Our results provide the first detailed analysis of individual sex allocation under LMC in the wild.

MATERIALS AND METHODS

Study organism. *Nasonia vitripennis* is a gregarious parasitic wasp, 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. Males are brachypterous and unable to fly, and are typically the first to emerge. They then mate with the emerging females. Females are fully winged females and disperse away from the host. The mating system typifies that assumed by LMC, and *N. vitripennis* has long been an outstanding model organism for the study of sex ratios (21).

Sampling. We 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*. (Grillenberger et al. 2007)**.** That paper also describes the patterns of oviposition on the patches and the population genetics of the two study populations. Briefly, we 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 we 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. We checked for any un-emerged individuals by opening the fly puparia a month after the last emergence from that host. We 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 S1. Throughout we 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), we do not know the impact of larval mortality in the wild.

Molecular genetic analysis. We 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 we 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. (Pietsch et al. 2004) but the primers were redesigned for this study (Table S2). We 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).

We 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). We 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.

We 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 (Queller and Goodnight 1989). We used the Relatedness 5.0.8 program (developed by Goodnight; 2001) to generate relatedness values on a scale from -1.0 to 1.0. We 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, we attributed a value of zero relatedness (i.e. the average relatedness of an individual to the population).

Statistical Analyses. We performed two analyses. First we tested explanatory variables at the host and patch level. For the second analysis we 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. The difference in fecundity between a focal female and the other females on the host (or patch) was calculated by subtracting the number of offspring produced by other foundress females from the number produced by the focal female. This allowed us 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 we 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 the total number of hosts (parasitised plus unparasitized) was not known due to a recording error. 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 and Hardy 2002). Since females could contribute multiple clutches, for the first analysis we 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. We used restricted penalised quasi-likelihood (REPQL) as provided by the glme function in the Correlated Data library in S-Plus 7 (Pinheiro and 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 our data were slightly over-dispersed (dispersion parameter = 1.555). The fixed effects were tested using marginal t tests with approximate degrees of freedom (Pinheiro and Chao 2005). Given that several of the explanatory variables associated with different models of LMC are likely to be correlated with each other, we also tested variables alone in individual models.

For our second analysis, since GLMMs do not yield true likelihoods, we were 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 we 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).

RESULTS

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.

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 ($t_{270} = 5.00$, $P < 0.0001$; Figure 1). 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 2).

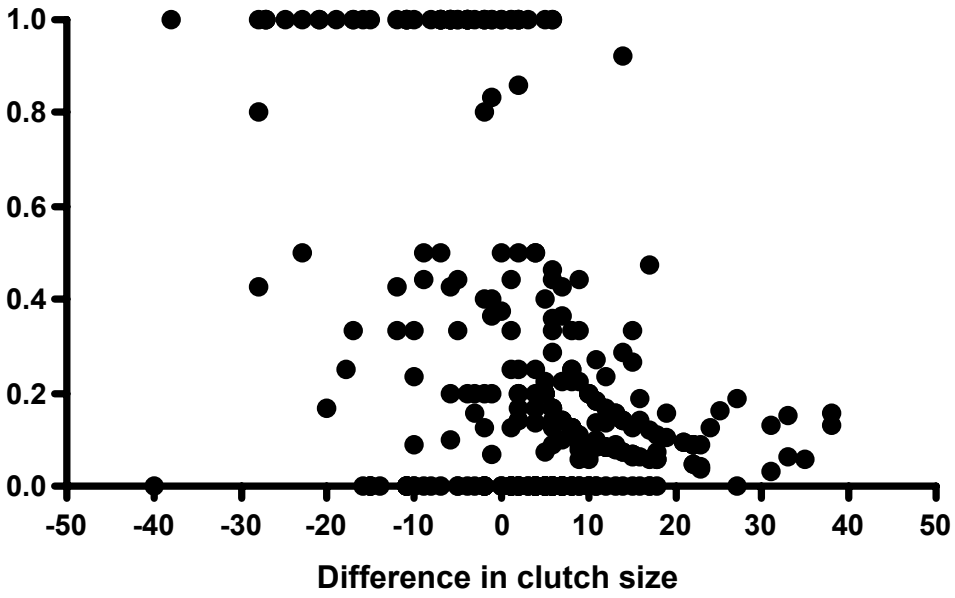

Figure 1. Sex ratios are negatively correlated with the difference in clutch size between females ovipositing on a host (including females ovipositing alone).

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 ($t_{282} = 6.34$, $P < 0.0001$; Figure 2) and the number of females laying eggs on a patch ($t_{282} = 2.74$, $P = 0.007$; Figure 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 ($t_{282} = 2.05$, $P = 0.04$).

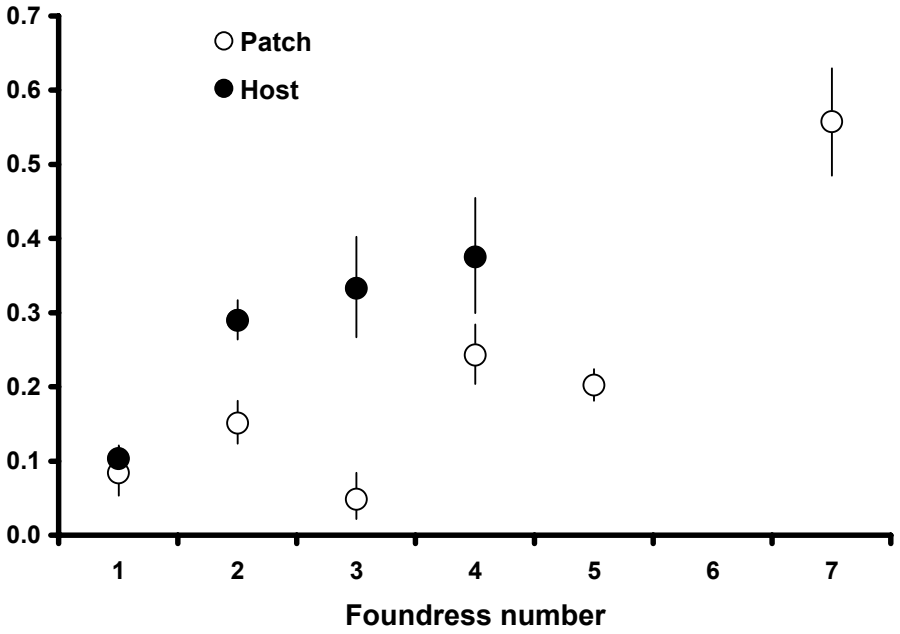

Figure 2. Sex ratios vary with the number of foundresses using the patch (open circles) or a particular host (filled circles). Error bars are 95% binomial confidence intervals.

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 Methods), with sex ratios declining with increasing relative clutch size as expected by theory (Figure 3). 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. 2007)**,** the Werren model also predicts a highly significantly proportion of the variance in sex ratio ($t_{152} = 4.04$, $P < 0.0001$; Figure 3 and Table 3).

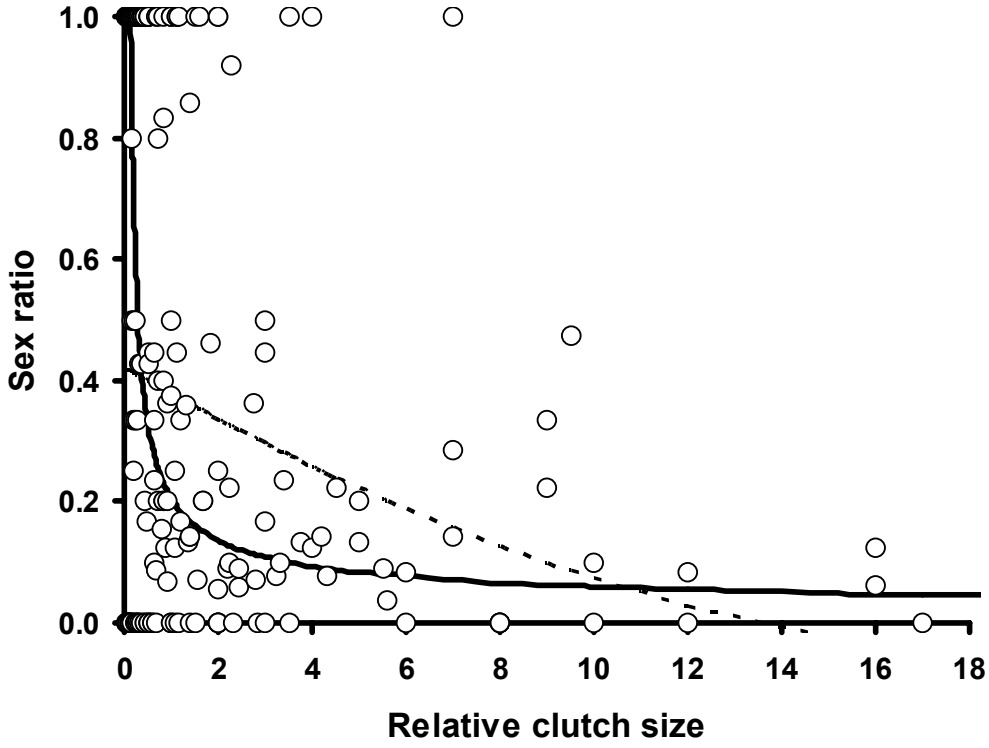

Figure 3. Sex ratios vary with relative clutch size when two or more females lay eggs on the same host (superparasitism). The dashed line is the relationship between sex ratio and relative clutch size (RCS) obtained from the analysis (sex ratio ~ 0.4211 – 0.0448*(RCS) + $0.0010*(RCS)^2$). The solid line 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).

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 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 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 (Werren 1980).

DISCUSSION

We used microsatellite markers to determine the sex ratio behaviour in the field of individual *N. vitripennis* females. We 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 (Figure 1). When this effect was included in the model, no other factors were significant (Table 2).We also tested the extent to which different LMC models could explain variation in sex ratio. We 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 and Seger 1990), were statistically significant, they did not fit the data as well as models based on relative fecundity at the host level (Shuker et al. 2005; Table 3; Suzuki and Iwasa 1980; Werren 1980).

Our 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 and West 2004). We 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 (King et al. 1995; Shuker et al. 2005; Shuker et al. 2006; Werren 1984), 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; Shuker et al. 2006; Shuker et al. 2007). 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 (Shuker et al. 2005; Van den Assem et al. 1980a; Van den Assem et al. 1980b). 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 our 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. 2007). 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 (Frank 1985a; Frank 1985b; Hamilton 1979; Herre 1985; Herre 1987) should lead to relatively random mating within the patch.

What information do females actually use to produce our observed negative correlation between offspring sex ratio and the relative clutch size that a female lays on a host (Figures 1 and 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 (Orzack and Parker 1990; Werren 1980; Werren 1984). 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 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 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 our 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, we do not know the order in which females laid eggs. Consequently, the first females to visit each host are also included in our 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 we 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. 2006) 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 our 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.

Our 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 1985b; Frank 1998; Greeff 1996; Reece et al. 2004; Taylor and Crespi 1994). 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 1985b; Greeff 1996; Herre 1985; 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 1985b; Frank 1986; Taylor and 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 and Roze 2007).

Conclusion

Our 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 us a great deal about what limits adaptive behaviour. Our results also emphasise two general points about the extent to which we 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 (Boomsma et al. 2003; Shuker and West 2004; West and Sheldon 2002). Here, we 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.

Acknowledgements

We should like to dedicate this paper to the memory of Chris Barnard. Thank you to Christof Pietsch for providing the German samples and two of the primers used. We are extremely grateful to The Royal Society, the Biotechnology and Biological Sciences Research Council, and the Natural Environment Research Council for funding. L.W.B. acknowledges a Pioneer grant of the Netherlands Organisation for Scientific Research.

Table 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 our 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 and 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 1985b; Herre 1985; Stubblefield and 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] (Suzuki and Iwasa 1980; Werren 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] (Suzuki and	Relative clutch size (focal female	Relative clutch size of focal female on a patch (as

Iwasa 1980; Werren 1980)	relative to co-foundresses) across the patch	difference in clutch sizes between focal and co-foundress females)
Asymmetrical LMC (Nunney and 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 1985b; Frank 1998; Shuker et al. 2004a; Taylor and Crespi 1994)	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. We use it here in a general sense to consider sex allocation based on relative clutch size.

Table 2. Analysis of sex ratio variation.

	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

Note: 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.

Table 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).

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**

Note: 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 our empirical analysis. For full details of the models see Table 1.

1. The specific version of the Werren (1980) model adjusted for haplodiploidy (Greeff 2002; Suzuki and Iwasa 1980) and parameterised using the single foundress sex ratio, relative clutch sizes, and inbreeding coefficient from this paper and Grillenberger *et al*. (2007)**.**

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).

SUPPLEMENTARY INFORMATION

Table S1. 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 S2. 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	194-222	HEX
		R) AAG ACC AGC TAG GGA AGA GGA TA		
Nv-23 (58°C)	II	F) ATA CTC AAG CAA GCC ACA GCA TA	235-257	FAM
		R) GCG TAC CAA TCC ACA GAA AAT AG		
Nv-41 (52°C)	V	F) GTC AGA CGT GGG CTT TGT C	326-358	NED
		R) TTA TGC GCC ACA CAC ACC		
Nv-46 (58°C)	IV	F) TTA CGT CAA GGT ATA GCT GC	235-267	FAM
		R) GAA TAA GTG GCT GAA AGT TCC		

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

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Genetic structure of natural *Nasonia vitripennis* populations: validating assumptions of sex ratio theory

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Running title: Natural population structure of *N. vitripennis*

Abstract

The parasitic wasp *Nasonia vitripennis* has been used extensively in sex allocation research. Although laboratory experiments have largely confirmed predictions of Local Mate Competition (LMC) theory, the underlying assumptions of LMC models have hardly been explored in nature. We genotyped over 3500 individuals from two distant locations (in the Netherlands and Germany) at four polymorphic microsatellite loci to validate key assumptions of LMC theory, in terms of both the original models and more recent extensions to them. We estimated the number of females contributing eggs to patches of hosts and the clutch sizes as well as sex ratios produced by individual foundresses. In addition we evaluated the level of inbreeding and population differentiation. Foundress numbers ranged from 1 to 7 (average 3.0 ±0.46 SE). Foundresses were randomly distributed across the patches and across hosts within patches, with few parasitizing more than one patch. Of the hosts, 40% were parasitized by more than one foundress. Clutch sizes of individual foundresses (average 9.99 ±0.51 SE) varied considerably between hosts. The time period during which offspring continued to emerge from a patch or host correlated strongly with foundress number, indicating that sequential rather than simultaneous parasitism is the more common. Genetic differentiation at the regional level between Germany and the Netherlands, as estimated by Slatkin's private allele method (0.11) and Hedrick's corrected G'_{LT} (0.23), indicates significant substructuring between regions. The level of population inbreeding for the two localities (F_{IL} = 0.168) fitted the expectation based on the average foundress number per patch.

Introduction

Local mate competition (LMC) theory (Hamilton 1967) is the basis for a large amount of research into adaptive sex ratio adjustment (Werren 1983; Herre 1985; Orzack 1986; King and Skinner 1991; Hardy 1994; Godfray and Werren 1996; Antolin 1999; Courteau and Lessard 2000; West *et al.* 2000; Shuker *et al.* 2004a; 2005). It assumes that a female has control over the sex ratio of her offspring and can maximize her fitness by reducing the competition between her sons. This is an evolutionary stable strategy if males are not the dispersing sex and if mating only takes place at the natal patch (Hamilton 1967). In such a mating system all males are competing to mate with the females that are available at the patch. If the patch population consists of only a single family, the males are brothers and it is beneficial for the foundress female to shift the offspring sex ratio strongly towards daughters to reduce competition among her sons. With increasing foundress number, competition between unrelated males increases and therefore selection favors females that produce more males to increase the chance that their sons mate with daughters of other females as well. This leads to a less female-biased sex ratio. The resulting prediction is that the offspring sex ratio in a patch is a function of the number of females ovipositing on that patch (Hamilton 1967).

A central assumption of LMC theory is that the population is highly subdivided in terms of mating. In the case of parasites this is thought to be due to the patchy distribution of hosts. Hamilton (1967) assumes that clutch sizes are equal and that there is random mating among the offspring of one patch. A patch could be for example all the fly pupae on a carcass or in a bird nest. The resulting population inbreeding F_{IT} follows $F_{IT} = 1 / (4n\text{-}3)$ with n being the mean number of foundresses per patch (Hamilton 1979).

Hamilton's LMC model has been further extended by several authors in various ways. The concept that females can have different clutch sizes and sex ratios has been incorporated by Werren (1980). Inbreeding has also been considered in several ways (Frank 1985; Herre 1985). Nunney and Luck (1988) modeled the combined effects of male dispersal, inbreeding and asynchronous parasitism on sex allocation, whilst Courteau and Lessard (2000) in turn developed several different scenarios of dispersal, i.e. before or after mating and dispersal probability for haploid, diploid and haplo-diploid organisms. Shuker *et al.* (2005) recently extended Nunney and Luck's (1988) model of asynchronous parasitism by considering two foundresses parasitizing hosts on a patch sequentially but allowing females to use either the same or different hosts. In species such as the parasitic wasp *Nasonia vitripennis* asynchronous parasitism on a single host is thought to have little

effect on the timing of emergence, as *N. vitripennis* larvae speed up their development to achieve a synchronous emergence of all individuals from a host (Werren 1980). In contrast asynchronous parasitism of several hosts in a patch leads to asynchronous emergence of the offspring. As such, males of an early foundress have a chance to mate with females of a later foundress, whose sons do not have access to the daughters of the early foundress. Such asymmetric LMC leads to a shift of the optimal sex ratio towards more males for the second foundress (Shuker *et al.* 2005). Like most models, Shuker *et al.*'s (2005) model has been confirmed under laboratory conditions (see also Shuker *et al.* 2006b), but few field studies have been performed to test these models.

The parasitoid wasp *Nasonia vitripennis* has been widely used for laboratory experiments regarding sex ratio adjustment and behavioral genetics (Werren 1984; Drapeau and Werren 1999; Beukeboom and van den Assem 2001; van den Assem and Beukeboom 2004; Shuker *et al.* 2005; 2006b). Laboratory experiments and two field studies (Werren 1983; Molbo and Parker 1996) have shown that *N. vitripennis* modulates the sex ratio of its offspring largely consistent with LMC theory. As Molbo and Parker (1996) used allozymes, which have a rather low variability, it is possible that they underestimated foundress number. In addition the level of superparasitism might also have been underestimated, as they themselves acknowledged. Werren (1983) on the other hand used the offspring number per patch as an indirect measure of the foundress number and found a strong positive correlation between patch offspring number and sex ratio, leveling off at 50% males.

Other genetic studies on parasitoid Hymenoptera have considered the level of the population rather than the level of individual patches, and have produced varying results on the population substructuring and the level of inbreeding. De Leon and Jones (2005) found for *Gonatocerus ashmeadi* a pronounced genetic structure between samples from the American East- and West coast (G_{ST} = 0.38), while Kankare *et al.* (2005) found differing results for *Cotesia melitaearum* and *Hyposoter horticola*. F_{ST} for *C. melitaearum* was much higher than for *H. horticola* (0.378 vs. 0.063), and both species showed significant isolation by distance. These differences between parasites of the same host species reflect their differences in mobility (Kankare *et al.* 2005). In a study on *Trichogramma pretiosum* (Antolin 1999), a rather high degree of population inbreeding (F_{IT} = 0.246) was found but no significant differentiation between three subpopulations within California. These different findings regarding the population structure of various parasitoid wasps do not allow any generalizations, and do not specifically test assumptions of LMC. In this study we use four polymorphic microsatellites to estimate the level of inbreeding, foundress numbers, timing of parasitism and individual sex allocation in two field populations of *N. vitripennis* in Europe in

order to test how well natural populations represent the idealized conditions assumed in models of LMC.

Material and Methods

Sampling

Nasonia vitripennis is a gregarious pupal parasitoid of a wide range of cyclorraphous flies. Like all Hymenoptera, *N. vitripennis* has a haplodiploid reproduction mode: fertilized eggs develop into diploid females, unfertilized eggs into haploid males. In *N. vitripennis* females usually mate at their place of birth and disperse after mating. Males have reduced wings and cannot fly (Whiting 1967).

Fly host pupae were collected from bird nests obtained from 95 nest boxes in a 1.4 km x 2.5 km field site in the Hoge Veluwe National Park (The Netherlands) (referred to as HV) and from baits placed in all HV nest boxes. A second plot consisted of 28 nest boxes along a straight ~600 m long road near Schlüchtern (Hessen, Germany) (referred to as Schl), where only baits were used. The collected host pupae were incubated individually at room temperature (~20˚C) and the emerging wasps, after being identified as *N. vitripennis*, were counted, sexed and stored directly in 90% ethanol for molecular analysis. For the HV-samples we kept record of the first and last day of emergence for every host pupa. Unfortunately we could not record the data per individual wasp. For baiting (in Schl), 25 laboratory hosts (*Calliphora vicina*) were placed in a mesh bag and left inside the nest box for approximately one week to allow parasitism. As the nest boxes are cleaned out every year and we did not find any host pupae that showed signs of emergence, we assume that our sample represents all offspring that emerged from these nest boxes.

Parentage analysis

DNA isolation followed a standard high salt-chloroform protocol (Maniatis *et al.* 1982). For genotyping we used four polymorphic microsatellites (dinucleotide repeats) (Table 1). Nv-22 and Nv-23 have originally been developed by Pietsch et al. (2004) but the primers have been redesigned in our laboratory. Primer sets for the other two microsatellites have been developed in our laboratory using the technique described by Rütten et al. (2001). The length of the amplified fragments was determined on an ABI Prism 377 DNA sequencer (Perkin-Elmer Applied Biosystems).

The genotypes of the females (here called foundresses) that oviposited on each host were determined from the genotypic data of the offspring following these simple rules: (1) A female can

maximally provide two alleles per locus. (2) The father can only provide one allele per locus (being haploid) that is shared by all full sisters. (3) Sons can only have an allele from their mother, as they develop from unfertilized eggs. If several foundress genotypes were possible based on the microsatellite profile, we always preferred the solution with the lowest number of foundresses. We allowed the foundresses to be multiply mated in our paternity analysis. This foundress assignment has been done independently by three of the authors (BKG, TK and MNB-C) to validate the assignment process. It yielded data on the number of foundresses per nest box and per host, as well as on the individual clutch sizes and sex ratio of every foundress.

Population structure analysis

Some sex allocation models use the population inbreeding coefficient F_{IT} as a measure of relatedness to estimate the optimal strategy for a foundress (e.g. Frank 1985 and see citations in introduction), assuming that females (and patches) have equal productivity. Furthermore, information about the population genetic structure allows estimations about gene flow and migration rates among populations. For this analysis we divided the samples into the two geographic regions (HV and Schl) which contain several nest boxes, each of which can be considered as a patch (in the LMC terminology) or a subpopulation (in the F-statistical sense). As the individuals emerging from one nest are the members of only a few families (Molbo and Parker 1996), the relatedness among these is very high. We therefore decided to use each foundress genotype once, rather than use the genotypes of all the offspring. In this way the sample size was reduced considerably, but we avoided multiple non-independent samples. The most common method for determining population differentiation and inbreeding involves *F*-statistics, which were originally designed for diploid organisms (Wright 1931; Weir and Cockerham 1984; Slatkin 1987; Cockerham and Weir 1993). As we only use the diploid females in our analysis, we can apply F-statistics. However, in their review on population genetics of X-linked genes and haplodiploids, Hedrick and Parker (1997) find that a major effect of haplodiploid inheritance is a reduced effective population size compared to diploids. Hence, care should be taken in comparing quantitative results with data of diploid organisms.

Hedrick (1999) cautioned against the use of conventional F-statistics on microsatellite data, as the high mutation rate and the high number of alleles of such markers can lead to a severe underestimation of the genetic differentiation. New mutations in separated populations can produce identical alleles that are not identical by descent and therefore mask the differentiation (Nauta and

Weissing 1996). He recommended the use of Slatkin's private allele method (Barton and Slatkin 1985; Slatkin 1985). Later Hedrick (2005) developed a standardized measure of F_{ST}, called G'_{ST} which is standardized for the maximal value that G_{ST} (a multi allelic version of F_{ST}) can reach, given a certain genetic diversity in a population. Here we apply all three methods and compare the results.

In the following we will use the F-statistical terminology as used by Hartl and Clark (1997), with the subpopulation (index *S*) being the individual nest box, or patch in the LMC sense, and the sampling areas (Schl or HV) being localities (index *L*). The total population (index *T*) represents the pooled data set of both localities. A classical F-statistical analysis within regions was not possible, as 5 out of the 18 nest boxes were parasitized by one female only (leading to very localised mating prior to female dispersal). As a substitute for F_{ST} we used Rousset's distance *a* (Rousset 2000) between pairs of individuals within a region, within and between patches to test for isolation by distance. The expectation is a linear positive correlation between genetic distance and the logarithmic geographic distance (Rousset 1997).

Population statistics were calculated using FSTAT 2.9.3 (Goudet 2001), GENEPOP (http://genepop.curtin.edu.au/) (Raymond and Rousset 1995), and SPAGeDi 1.2 (Hardy and Vekemans 2002). Statistical tests were performed with SPSS 13.0 or R 2.4.1 (R Development Core Team 2006). All mean values are given as arithmetic mean ± SE unless indicated differently.

Results

Foundress numbers and pattern of parasitism

From the 95 nests that were inspected at the Hoge Veluwe, 15 (16%) contained fly pupae of which 9 (9.5% of total) yielded *Nasonia vitripennis* emerging from at least one host. The baits in the HV nest boxes only yielded *Nasonia* in one case (HV 288). From the 28 baited nest boxes in Schlüchtern, 8 (29%) yielded *N. vitripennis*. The total number of natural hosts found per nest box ranged from 6 to 82. The number of parasitized hosts per nest box ranged from 1 to 79 (Table 2).

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We genotyped a total of 3550 individuals emerging from 9 natural nests (HV) and 9 baits (8 Schl and 1 HV) (the complete data can be found as Supplementary Data online). We could identify a total of 49 foundresses (arithmetic mean per patch: overall 3.0 ±0.46, in HV 2.9 ±0.74, in Schl 3.1 ±0.55; harmonic means: overall 1.9, HV 1.6, Schl. 2.4, Figure 1A). Assuming that the allele frequencies measured in our sample represent the genetic makeup of the whole population (HV and

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Schl combined), the chance that two unrelated individuals share the same allele is equal to the frequency of the particular allele in the population. Due to the high allelic variation of our markers, the chance of encountering two or respectively three females that have identical genotype in all four markers is < 0.001. The number of offspring per foundress per host varied between 1 and 39 (mean = 9.99 ±0.41), while the number of hosts being parasitized by a single foundress varied between 1 and 27 (mean = 7.17±1.02). The total number of offspring per foundress across all hosts varied between 1 and 346 (mean = 72.5± 9.46). The total number of offspring per host varied between 1 and 55 (mean = 14.77±0.64). The observed level of superparasitism is high. In 39.5% (N = 241) of all hosts we found evidence for more than one foundress and in 5.5% more than two foundresses (Figure 1). In Schlüchtern we found six foundresses parasitizing hosts in two nest boxes each (three on S11 and S21, one on S13 and S22, and one on S20 and S22; Table 2). We found no significant difference in the distribution of foundresses across patches or hosts between the natural nests (HV samples) and baits (SCHL samples) (Kolmogorov-Smirnov test: patch-level D = 0.29 n.s, host-level D = 0.5 n.s.).

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There was no evidence for female preference for or against patches or hosts used by other females. We found no significant deviation from a random distribution of the foundresses across used patches (dispersion test for a Poisson distribution following Grafen and Hails (2002), χ^2 = 69.03, df = 237, p = 1) or across the hosts within a patch (χ^2-test against Binomial distribution using pooled data of all patches and combining the low represented classes, χ^2 = 12, df = 9, p = 0.213). The total number of hosts present in a patch and the number of foundresses parasitizing also showed no significant correlation (adj. R^2 = -0.024, $F_{1,17}$ = 0.582, p = 0.456). Although there is a large variation in the clutch sizes per foundress, there was no significant correlation between foundress number per host and the clutch size per foundress (adj. R^2 = -0.003, $F_{1,342}$ = 0.07, p = 0.798). The mean coefficient of variation within clutch sizes of a particular host is 0.69 ±0.04, and therefore not negligible.

Although the data were not specifically collected to test for synchrony of parasitism, we can obtain some information from our data. The time window in which wasps emerged from a single host ranges from 1 to 10 days and for all hosts of a patch from 1 to 19 days (Figure 2). There is a strong positive relationship between foundress number and emergence window of a patch (adj. R^2 = 0.7161, $F_{1,7}$ = 21.18, β = 2.71 ±0.59, p = 0.0024; Figure 2A) and of a host (adj. R^2 = 0.093, $F_{1,139}$ = 15.4, β = 0.95 ±0.24, p < 0.001; Figure 2B).

Our data strongly suggest multiple mating in two cases (4%, N = 49). Among the HV-foundresses we found one female that was doubly mated (HV 267, foundress #14) and one that was mated three times (HV 330, foundress #26). An alternative explanation would be a genotyping error, but the decision on additional mates is supported by more than one marker, and doubtful individuals have been genotyped twice, which makes genotyping errors unlikely. The high level of inbreeding in the population (see data below) however, increases the relatedness of individuals in the populations and therefore the chance of highly related individuals parasitizing the same patch, which could then lead to the impression of multiple mating.

One nest produced only male offspring (HV 8), which can most easily be explained by a single unmated foundress. We excluded this progeny from further sex ratio analysis. Without this nest the sex ratio (proportion male) of the emerged offspring varied between 0.05 and 0.56 across the nests. The nest and host sex ratios as a function of foundress number roughly fit the theoretical predictions of basic LMC models (Figure 3).

Population genetic analysis

Due to the fact that *N. vitripennis* is mating in the natal patch, and the small size (~3 mm) of the dispersing females, the population of this parasitoid is expected to be highly subdivided. Random mating within the regions (Schl and HV) and the total population can be tested indirectly by comparing measured and expected heterozygosity among the foundress females. The mean F_{IL}= 0.168 ± 0.016 indicates a heterozygote deficiency and therefore non-random mating (inbreeding) within the regions; F_{IT} = 0.197 ± 0.014 shows more or less the same for the whole population (HV and Schl pooled) (Hartl and Clark 1997). The differentiation index F_{LT} = 0.035 ± 0.011 indicates a low differentiation among the regions in general (averaging over loci). Slatkin's private allele method results in N_m = 2.62 which corresponds to F_{LT} = 0.11 (following $F_{RT} = 1/(1+3N_m)$), a threefold higher value. Hedrick's standardized G'_{LT} = 0.23 is even higher (Hedrick 2005).

We found no positive correlation between geographic (ln (geographic distance)) and genetic distance (Rousset's *a*) within a locality (Mantel's test: Schl r^2 = 0.0047, n.s.; HV r^2 = 0.0043, n.s., Figure 4). The mean genetic distance between foundress females of one patch was not different from the mean genetic distance of foundress females from different patches within one region (HV: within patches 0.19 ± 0.04, between patches 0.21 ± 0.02, 2-sided t-test: t = -0.3833, df = 49.825, n.s.; Schl: within patches 0.16 ± 0.07, between patches 0.14 ± 0.02, 2-sided t-test: t = 0.1928, df = 17.09, n.s.).

Discussion

Herre (1985) found that species of fig wasp that are more likely to encounter a conspecific on a patch are more likely to shift sex ratios as predicted by LMC. *N. vitripennis* is known to have a very strong response to LMC in the laboratory. Here we have shown that foundress numbers vary across hosts and patches in the wild, with a high superparasitism rate of 40% at the host level and 63% at the patch level. Therefore we can conclude that conditions favouring facultative sex allocation in *N. vitripennis* are frequent enough for LMC theory to be relevant to our field populations. Given these data, *Nasonia* should have evolved as a result of LMC selection and be an ideal model organism to test assumptions of LMC. Table 3 gives an overview of the most important LMC model assumptions and the results of this study.

Fragmented populations?

A general assumption in LMC theory is that the population is highly subdivided in terms of mating. This is usually thought to be a consequence of the patchy distribution of hosts. Our data confirm that patches are often parasitized by only one female, leading to very localized mating. LMC theory then assumes that mated females then disperse randomly from their natal patch. Consistent with this, the individual based test for isolation by distance did not show an increase of genetic differentiation with geographic distance within localities (Figure 4). This lack of differentiation between patches is also shown by the equal level of genetic distance within and between patches of one locality. Using the conventional F-statistic as developed by Weir & Cockerham (1984) to compare the localities, we find a rather low degree of differentiation between the two sampling localities (F_{LT} = 0.035). The private allele method estimates the number of migrants per generation between the populations and can be interpreted as a F_{LT} of 0.11. This resembles more considerable differentiation and is in the same range as Hedrick's G'_{LT} of 0.23. Together, these data indicate that there is high dispersal within the scale of the localities and that the composition of foundresses parasitizing a patch represents a random genetic sample of the local population. Therefore the relatedness among the foundresses of a patch can be expected to be similar to that within a locality. Between the two localities (HV and Schl) however, gene flow seems to be very limited, as expected by the large distance of about 300 km. The low differentiation indicated by the conventional F-statistics can easily be explained by the high variation of the used markers (Hedrick 2005). Therefore, the variation independent measurements G'_{LT} and the private allele method should be

more informative. This leads to the conclusion that the relevant scale for LMC is the hierarchical level of localities, and not the total sample.

A common measure of the level of relatedness in a population is the population inbreeding coefficient F_{IT}. Hamilton (1979) predicted that, under the assumptions of random mating within a patch and equal foundress productivity, the population inbreeding under LMC should follow $F_{IT} = 1/(4n-3)$, with n being the harmonic mean number of foundresses per patch. For our study n is 1.9, resulting in an expected population inbreeding coefficient of 0.22 which is very close to the observed value of $F_{IL}= 0.168 \pm 0.016$. We use F_{IL} rather than F_{IT}, as the relevant level for LMC is the local population rather than the total sample as discussed above. However, the assumption of equal productivity is clearly violated and mating within a patch might not be random (as a consequence of asynchronous parasitism; see below). Therefore Hamilton's prediction can only be seen as a rough estimate.

Molbo and Parker (1996) calculated a population inbreeding coefficient F_{IT} of 0.312 for a Swedish population, which is considerably higher than our study. However, Molbo and Parker used all genotyped individuals for a calculation of F_{IT}, in contrast to our study (Molbo, personal communication). A recalculation of F_{IT} in our study using all individuals results in 0.272 ± 0.042 which more closely resembles the value of Molbo and Parker (1996). Moreover as Molbo and Parker (1996) used allozymes, the probability of underestimating the real number of foundresses due to limited variation in the marker is much higher than with the microsatellites we used (~10% Molbo and Parker 1996, < 1 % this study). In addition they estimated 1.5 foundresses per patch, while our estimate is 1.9. We also found a higher level of superparasitism (41%) than Molbo and Parker (23%). These differences could be explained by the higher resolution of our microsatellite markers, or by ecological differences between their Swedish population and our Dutch and German populations (such as population densities of parasites and hosts). An overall inbreeding coefficient F_{IL} of 0.168 corresponds to 45% sibmating (using $S = 4F_{IT}/(1+3F_{IT})$, Werren 1987). This is in the same range as the proportion of sibmating that has been found for *Trichogramma pretiosum* (56.6%, Antolin 1999), a gregarious parasitoid of Lepidoptera.

Equal clutch sizes and random mating within patches?

Hamilton (1967) assumed in his original LMC model that there is random mating among all the offspring on a patch and that all females in a patch lay equally sized clutches. Unsurprisingly,

females lay varying clutch sizes, and there is a large coefficient of variation in clutch sizes per pupa across the patches (0.69 ± 0.04). This variation could be a consequence of sequential parasitism where the first female usually lays the largest clutch and later females lay reduced clutches (Werren 1980).

Unfortunately we cannot measure deviations from random mating on patch level using our data. One way to do that would be to measure the relatedness between foundresses and their mates. As *Nasonia* males are haploid and we have only information from four microsatellite loci, such measurements would be rather limited in this context, and we therefore did not present such analysis here. However, we can draw some conclusions from our other findings. The data strongly suggest that parasitism of hosts on a patch is asynchronous (see next section for details), which leads to a bias in the opportunities for individuals from different hosts to mate with each other, as the daughters of early foundresses might have already left when the sons of late foundresses emerge. The sons of early foundresses on the other hand will have the chance to mate with their early sisters as well as with the daughters of late foundresses, as they stay on the patch. This obviously leads to the conclusion that mating among the offspring of a patch cannot be completely random, but only among the offspring that are present at the same time. (Shuker *et al.* 2006a).

Synchronous parasitism?

If all foundresses parasitized hosts at the same time, one would expect no increase in the emergence window with foundress number. As the emergence window on patch and host level is strongly positively correlated with foundress number (Figure 2), synchronous parasitism is perhaps the exception rather than the rule (Werren 1980, Hamilton 1967, Frank 1985). However, alternative explanations for the emergence window exist, including delayed developmental time due to crowding in the hosts, or individual foundresses parasitizing the same host several times, which might occur given the large variation in emergence time of the offspring of single foundresses (Figure 2B). Multiple parasitism by a single foundress on the same host may change the optimal sex ratio towards more males, if the female parasitized other hosts in between, as found by King (1992). Werren (1980) found that asynchronously laid clutches are synchronized by a speed up of development of the later clutches. Such a behavior would lead to a weaker correlation between foundress number and emergence window, than is evident from our data. However, we only collected data on the emergence window per host. To be able to resolve parasitism strategies of individual foundresses we would need data on the emergence time of individual offspring.

Nevertheless, LMC models for species such as *Nasonia vitripennis* should incorporate asynchronous parasitism, as is the case in some more recent models (Nunney and Luck 1988; Shuker *et al.* 2005).

Additional parameters

In addition to the assumptions from existing LMC theory (Table 3) that were tested, we also considered some other parameters. Although the total number of hosts may intuitively be considered as a good predictor of patch quality, we did not find a significant correlation between foundress number and the total number of hosts in a patch. One reason for this might be variation in individual host quality across patches. Also, variation in age of the hosts might play a role in the attractiveness of a patch. Hosts can only be parasitized by *N. vitripennis* if they are at a certain stage of development. If a patch has a large number of hosts suitable for parasitism for a longer period of time due to variation in host age, it might attract more wasps than a patch with an equal number of hosts that are all the same age. This would also explain the inferred patterns of sequential oviposition.

As superparasitism constitutes direct resource competition for a particular host, one may expect that the foundresses have evolved ways to avoid each other when parasitizing the same patch, as has already been shown in several studies (e.g. Shuker et al. 2005). Such a behavior would lead to an underdispersed pattern of parasitism. However, our results do not indicate a significant deviation from a random pattern of parasitism. We should mention though that our sample sizes, especially on patch level, are rather low and that the goodness of fit test that was applicable for our data is not very powerful. Hence, at patch level, we have no strong evidence for preference or avoidance of superparasitism.

The estimated percentage of unmated females (2%) is in the range of what has previously been reported: Beukeboom and Werren (2000) found 2.99% ± 2.32% in a larger field sample from the US. This frequency of so-called constrained females should not have a strong effect on the expected optimal sex ratio at the level of the population (Godfray 1990; Hardy and Godfray 1990). We assumed that the all-male family in a one-foundress patch in our study was due to an unmated female. We also found some all-male families among superparasitized hosts. In these latter cases family sizes were small and the assigned female also produced daughters in other hosts. Hence, such small all-male families can be considered as the outcome of superparasitism as predicted by LMC (Werren 1984).

Although previous studies indicated that single mating appears to be the rule in *Nasonia* (Azab *et al.* 1967; van den Assem and Visser 1976; van den Assem 1977), we found evidence that a small proportion (2 out of 49, ~ 4%) of foundresses are multiply mated. Genotyping errors can almost be ruled out, as we genotyped doubtful individuals at least twice, but the high level of inbreeding indicates that there is a high chance of highly related foundresses that have similar genotypes. If there would be the tendency that highly related females parasitize the same patch, there should be a correlation between genetic and geographical distance on a local scale. Our isolation by distance analysis however did not show any indication of such a correlation (Figure 4).

In general, polyandry reduces relatedness among the female offspring of a particular female. Unlike inbreeding, which would lead to selection for a more female biased sex ratio (Reece 2004; Shuker et al. 2004b), polyandry does not change the relatedness of a mother to her offspring and should therefore have no influence on sex allocation. It has been shown that multiple mating in *N. vitripennis* increases with time cultured in the lab (van den Assem and Jachmann 1999; Burton-Chellew *et al.* 2007). Furthermore, van den Assem and Visser (1976) showed that females are willing to mate a second time when they have already laid eggs. Therefore, it is conceivable that a previously mated female encounters a male that was born on the patch where she is ovipositing and mates a second time outside her natal patch. Nevertheless multiple mating seems to be rare in *N. vitripennis* and the effect of this behavior on the population genetic structure is likely to be negligible.

Finally, as predicted, we found a strong positive correlation between sex ratio and number of foundresses per patch, although there were large quantitative deviations from the predictions of Hamilton (1967) and Frank (1985). We consider sex allocation in more detail elsewhere (Burton-Chellew *et al.*, in prep).

To summarize our findings we can state that a suitable model of LMC for species such as *Nasonia vitripennis* should make the following assumptions: (1) large variation in clutch sizes, (2) non-random mating within the offspring of a patch, (3) asynchronous parasitism, (4) regular encountering of competitors, (5) highly structured mating populations (within localities) followed by (6) a random distribution of foundresses across the patches, and across hosts within patches. More recent models of LMC have started to take such factors into account (Nunney and Luck 1988; Shuker *et al.* 2005). Our findings provide empiric values for these factors and this will help to develop more realistic and precise LMC models, and hopefully also stimulate much needed studies of sex allocation in the wild for a wider range of parasitoid species.

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Tables and Figures

Figure 1: Frequency distribution of the number of foundresses per patch (A, B) and host (C, D) under natural conditions (A, C) and baits (B, D).

Figure 2: Emergence window per patch (A) and per host (B) in days as a function of the foundress number per patch (A) and per host (B). The circle surface is proportional to the sample size. The regression lines are highly significant (see text for details).

Figure 3: Sex ratio (proportion of males) ± SE as a function of foundress number per nestbox (all hosts pooled) (A) and per host (B), compared to the expectation of Hamilton (1967) (dotted line) and Frank (1985) assuming $F_{IL} = 0.168$(solid line).

Figure 4: Genetic differentiation in *N. vitripennis*. Shown is pairwise genetic differentiation in the form of Rousset's *a* (Rousset 2000) against logarithmic geographic distance. The upper graph shows the HV data (R^2 of regression line 0.0043), the lower graph Schl (R^2 of regression line 0.0047). All pairs of estimated foundresses from different nest boxes are shown, as well as the regression lines.

Table 1: Chromosomal location, primer sequences, number of alleles, Nei's overall gene diversity ($\mathbf{H_t}$) (Nei 1987) and annealing temperatures of four microsatellites used.

Primer	Chromosome*	Sequence	Allele no.	H_t	Ann. temp.	GenBank accession no.
Nv-22	I	5' GAC TGC GTA CCA CTC CAA AAA TA 3' 5' AAG ACC AGC TAG GGA AGA GGA TA 3'	16	0.90	58˚C	AY262041
Nv-23	II	5' ATA CTC AAG CAA GCC ACA GCA TA 3' 5' GCG TAC CAA TCC ACA GAA AAT AG 3'	13	0.39	58˚C	AY262044
Nv-41	IV	5' GTC AGA CGT GGG CTT TGT C 3' 5' TTA TGC GCC ACA CAC ACC 3'	11	0.85	52˚C	EU155141
Nv-46	V	5' TTA CGT CAA GGT ATA GCT GC 3' 5' GAA TAA GTG GCT GAA AGT TTC C 3'	27	0.87	58˚C	EU155142

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

Table 2: Number of foundresses estimated, total number of hosts, and total number of parasitized hosts at 2 field sites. HV = Hoge Veluwe National Park (The Netherlands); Schl = Schlüchtern (Hessen, Germany);

Nest box no.	No. of foundresses	Total hosts	Hosts used
HV 8	1	15	1
HV 13	5	27	27
HV 220	5	NA	9
HV 267	7	16	16
HV 288	1	25	11
HV 306	1	6	1
HV 323	2	8	6
HV 330	5	82	79
HV 344	1	43	4
HV 365	1	35	1
Schl 11	4 (a)	25	15
Schl 13	2(b)	25	3
Schl 16	2	25	4
Schl 20	2(c)	25	25
Schl 21	7(a, d)	25	9
Schl 22	4(b, c, d)	25	14
Schl 23	2	25	1
Schl 28	3	25	15
Total	49 (6 double visits)	466	241

a = 3 foundresses found in nest box Schl 11 that also parasitized nest box Schl 21

b = foundress that parasitized nest box Schl 13 and Schl 22

c = foundress that parasitized nest box Schl 20 and Schl 22

d = foundress that parasitized nest box Schl 21 and Schl 22

NA = the number of total hosts in these nest boxes was not recorded; for the total number of hosts the number of hosts parasitized (9) was assumed

Table 3: Overview of assumptions made by several models on local mate competition theory and the results of this study

Reference	Assumption	Found in this study?
General assumptions	(1) Localized mating within patches	Yes
	(2) Random dispersal of mated females	Yes
Hamilton 1967	Equal clutch sizes	No
Hamilton 1967	Random offspring mating within patches	No
Werren 1980, Hamilton 1967, Frank 1985	Synchronous parasitism	No
Nunney and Luck 1988, Shuker *et al.* 2005	Asynchronous parasitism	Yes

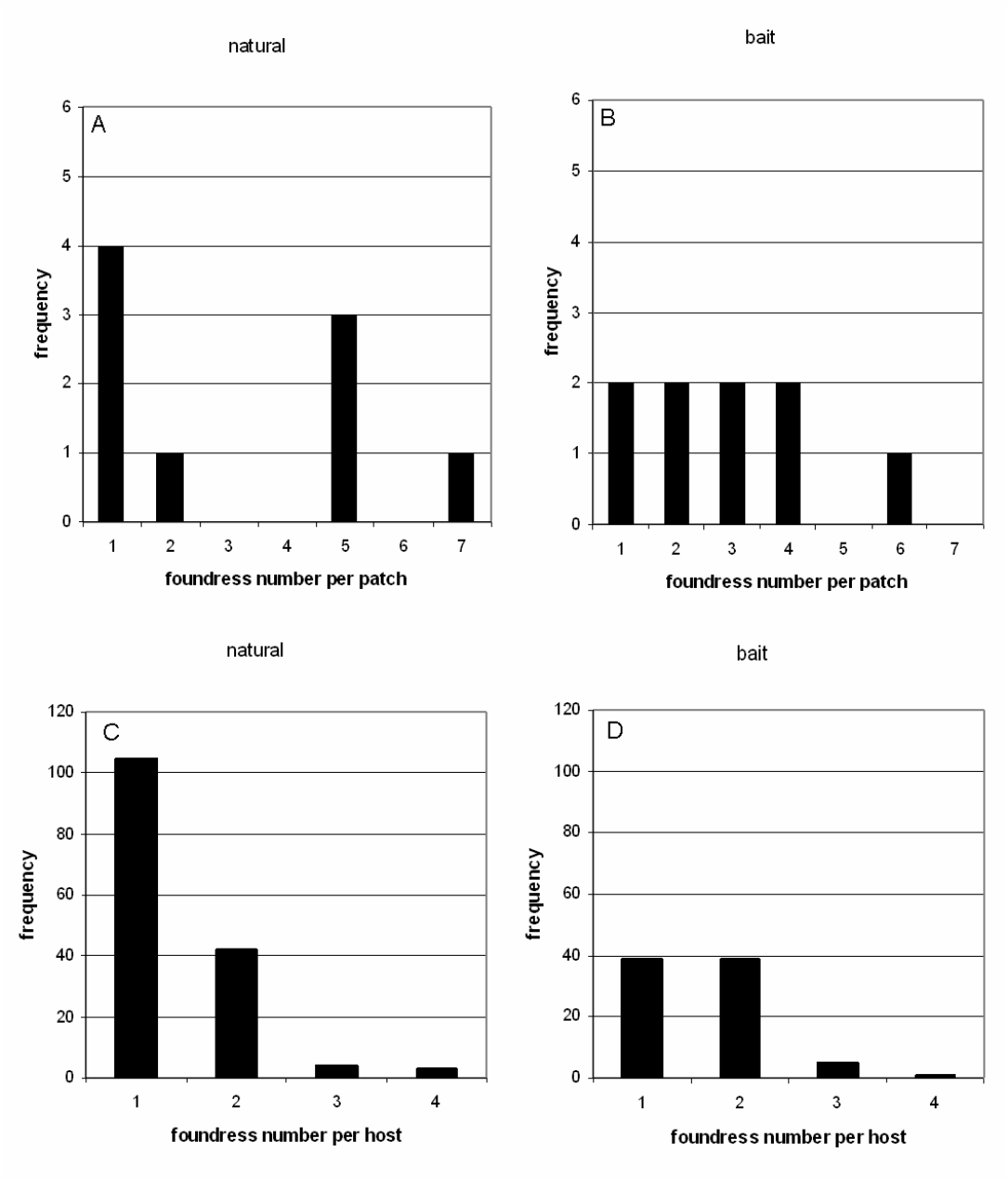

natural
A
frequency
foundress number per patch
bait
B
frequency
foundress number per patch
natural
C
frequency
foundress number per host
bait
D
frequency
foundress number per host

Figure 1

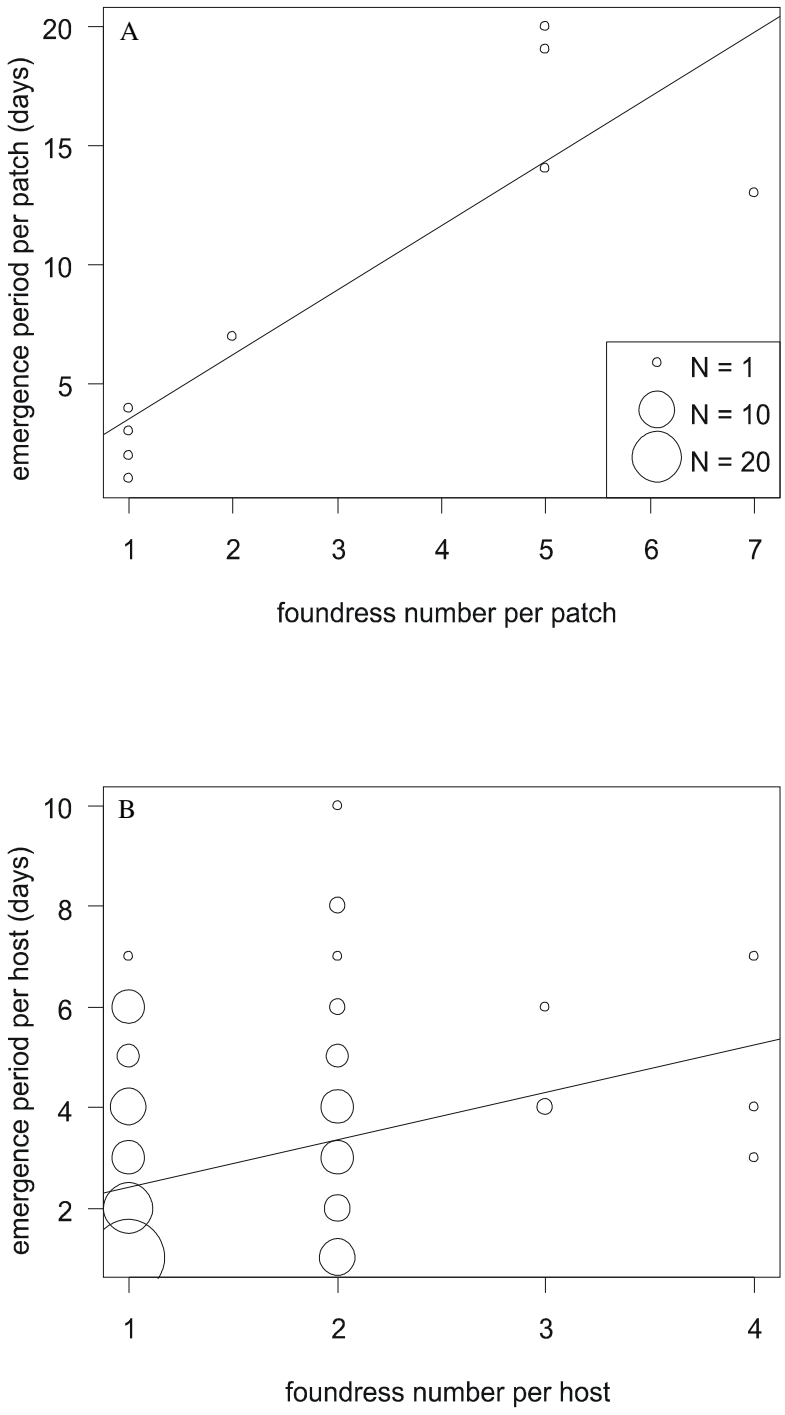
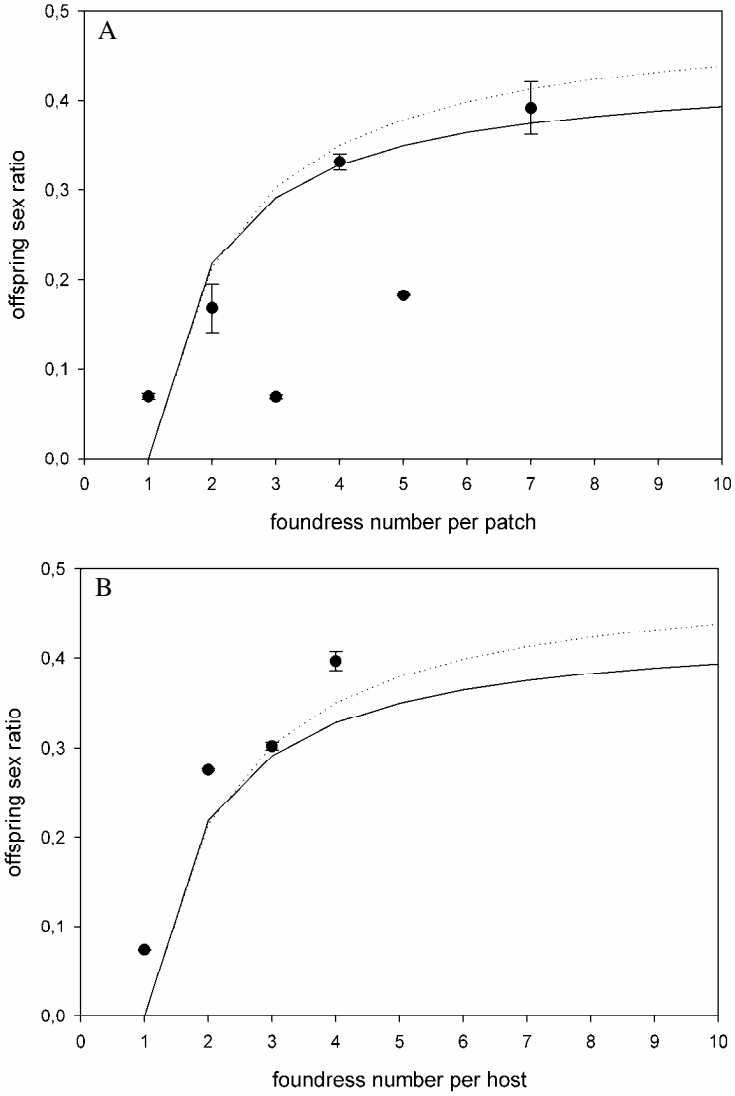

A
emergence period per patch (days)
20
15
10
5
1
2
3
4
5
6
7
foundress number per patch
N = 1
N = 10
N = 20
B
emergence period per host (days)
10
8
6
4
2
1
2
3
4
foundress number per host

Figure 2


A
offspring sex ratio
0,0
0,1
0,2
0,3
0,4
0,5
0 1 2 3 4 5 6 7 8 9 10
foundress number per patch
B
offspring sex ratio
0,0
0,1
0,2
0,3
0,4
0,5
0 1 2 3 4 5 6 7 8 9 10
foundress number per host

1

2 **Figure 3**

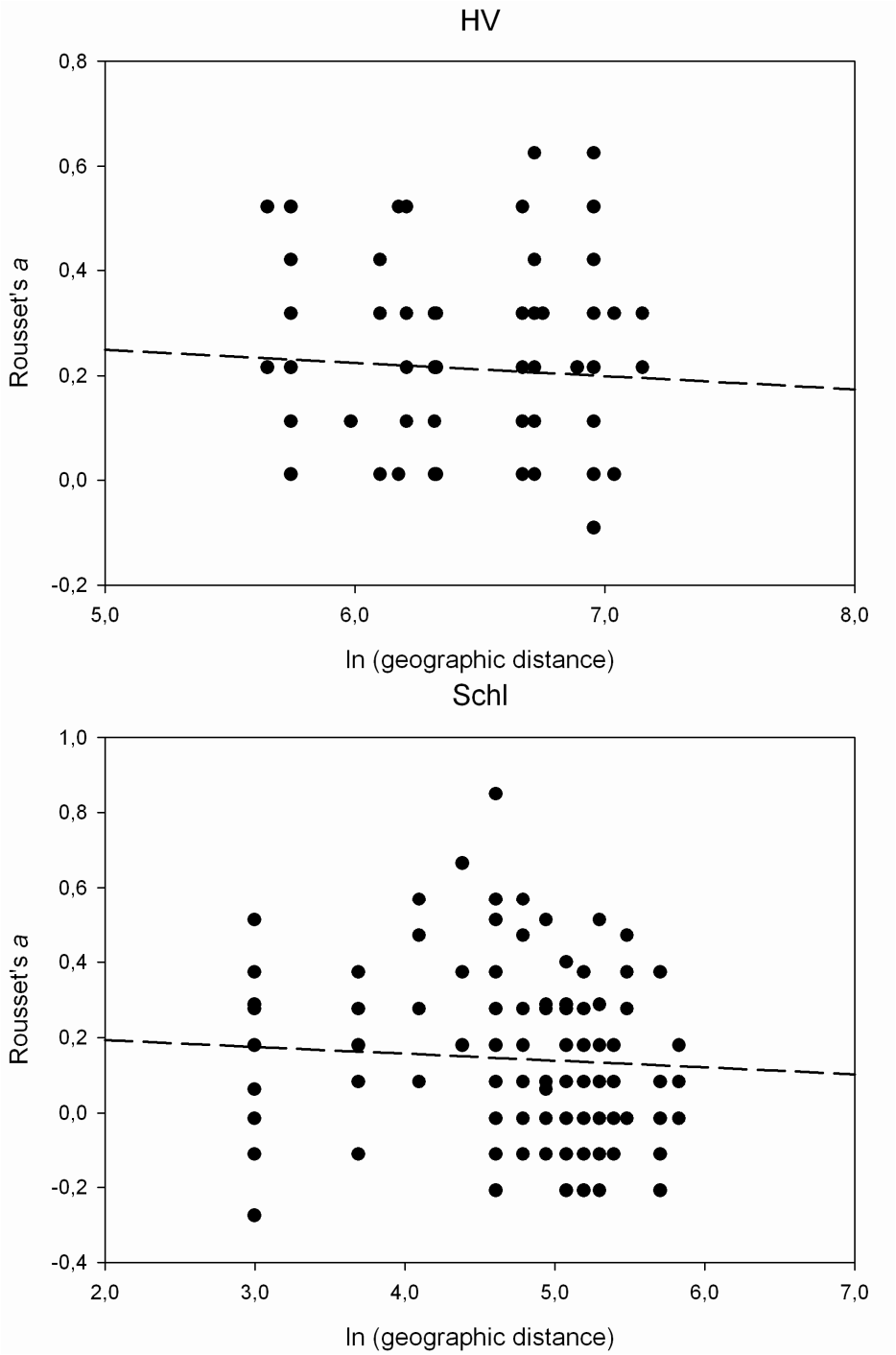

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Figure 4

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Report

Cooperation and the Scale of Competition in Humans

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Summary

Explaining cooperation is one of the greatest challenges for evolutionary biology [1–3]. It is particularly a problem in species such as humans, where there is cooperation between nonrelatives. Numerous possible solutions have been suggested for the problem of cooperation between nonrelatives, including punishment, policing, and various forms of reciprocity [3–14]. Here, we suggest that local competition for resources can pose a problem for these hypotheses, analogous to how it can select against cooperation between relatives [15–21]. We extend the prisoner's dilemma (PD) game to show that local competition between interacting individuals can reduce selection for cooperation between nonrelatives. This is because, with local competition, fitness is relative to social partners, and cooperation benefits social partners. We then test whether nonrelated humans adjust their level of cooperation facultatively in response to the scale of competition when playing the PD for cash prizes. As predicted, we found that individuals were less likely to cooperate when competition was relatively local. Cooperation between humans will therefore be most likely when repeated interactions take place on a local scale between small numbers of people, and competition for resources takes place on a more global scale among large numbers of people.

Results

Incorporating Local Competition into the PD

In the PD game, two individuals interact with each other and choose whether to cooperate or not cooperate (defect or cheat) [22]. The payoffs assumed for different strategies lead to the focal player doing better if they defect, irrespective of whether the other player cooperates or defects, but both parties defecting gives a lower payoff than if both had cooperated (Table 1). The dilemma is that from a selfish perspective each will decide that the best course is defect, thus making both worse off than if they had both cooperated. The most famous solution to this problem occurs when players interact a number of times (multiple rounds), termed the iterated PD [22]. In this case, cooperation can be favored by a "Tit-For-Tat" (TFT) strategy, which is to cooperate in the first round and in every subsequent round copy the partner's play from the previous round [22]. TFT is a strategy of facultative (reciprocal) cooperation, which punishes defectors by not cooperating with them.

We introduced the possibility of local competition for resources into the PD (Supplemental Data available with this article online). Specifically, we introduced a scale of competition parameter, a, that denotes the proportion of competition that occurs locally (i.e., at the level of the pair of social partners engaged in an evolutionary game) as opposed to globally (i.e., at the level of the whole population). Our results showed that as competition became more local, cooperation was selected against (Figure 1). Specifically, the expected number of interactions between players (rounds per game), required for TFT to be stable against invasion by always defect, increased with more local competition (higher a). Higher numbers of interactions favor altruism because they allow greater opportunities for reciprocal altruism [4, 22].

As competition becomes more local, the payoff of the focal individual relative to their partner becomes more important. With global competition ($a = 0$), fitness is determined by the classical payoff structure (absolute values). However, as competition becomes more local, the relative payoff becomes more important, and the absolute payoff becomes less important. In the extreme, with completely local competition ($a = 1$), the focal individual is competing only with their partner, and so fitness is determined by how the focal individual does relative to their partner. Cooperation never results in an increase in relative payoff, whereas defection does (Table 1). Consequently, the dilemma disappears, as defection always leads to a higher payoff, and both players cooperating does not lead to a higher payoff than both players defecting (Table 1).

Testing with Humans

We then tested our model by examining whether nonrelated humans adjust their level of cooperation in response to the scale of competition. We made students play the PD game in groups of three. Each student played each of the other two students for an unknown number of repeated interactions (an average of seven rounds). We varied the scale of competition by having five groups play at a time (a class) and giving cash rewards to the top five scores in the class for relatively global competition or the top score in each group for relatively local competition. Each student played once with

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Table 1. The Payoffs for Player A with Different Strategies in the Prisoner's Dilemma

		Player B	
		Cooperation	Defection
Player A	Cooperation	R = 3 (1), rewards for mutual cooperation	S = 0 (0), sucker's payoff
	Defection	T = 5 (∞), temptation to defect	P = 1 (1), punishment for mutual defection

The dilemma is that defection always leads to a higher payoff (T > R and P > S) but that both cooperating leads to a higher payoff than both defecting (R > P). Exact values given are for illustration. Given in parentheses are the relative payoffs, which is the payoff for player A divided by the payoff for player B. Local competition leads to the relative payoffs being the crucial score, in which case both cooperating does not lead to a higher payoff than both defecting (R = P).

relatively local competition and once with relatively global competition.

We found that students showed higher levels of cooperation when competition was relatively global than when competition was relatively local (Figure 2). This result held irrespective of whether analysis was carried out at the level of the individual ($t = 7.34$, $n = 57$, $p < 0.0001$; 52/57 individuals showed higher levels of cooperation with relatively global competition), group ($t = 7.80$, $n = 9$, $p < 0.0001$; 9/9 groups), or class ($t = 10.97$, $n = 4$, $p = 0.002$; 4/4 classes). Overall, students were more than twice as likely to cooperate with relatively global competition (44% of the time) than they were with relatively local competition (18%; Figure 2).

Discussion

Theoretical Explanations for Cooperation

We have shown that local competition for resources between nonrelatives selects against cooperation

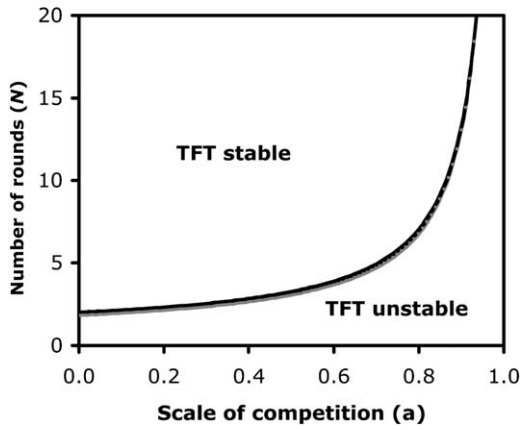

Figure 1. Local Competition Selects against Cooperation in the Iterated PD

The threshold expected number of rounds per game (N^*) required for Tit-For-Tat (TFT) to be evolutionarily stable is a monotonically increasing function of the scale of competition (a). Assuming the payoffs $T = 5$, $R = 3$, $P = 1$, and $S = 0$, then when competition is global ($a = 0$) TFT is stable when $N^* > 2$, and this threshold increases to infinity as competition becomes increasingly local (higher a).

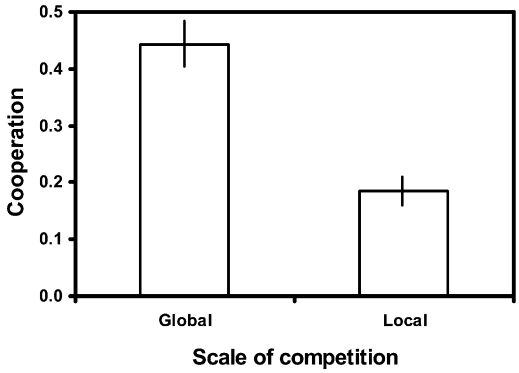

Figure 2. Human Cooperation and the Scale of Competition

The mean proportion of cooperative decisions made by students with respect to the scale of competition. Error bars are back-transformed 95% confidence intervals with individual students as independent data points. As predicted (see Figure 1), individuals were less likely to cooperate when competition was relatively local.

(Figure 1). As competition becomes more local, the fitness of an individual becomes more dependent upon how they do relative to the partners that they interact and potentially cooperate with. In this case, cooperation is selected against because it never leads to an increase in payoff relative to the beneficiary of cooperation (Table 1). Another way of conceptualizing this is that local competition leads to the increased fitness of the beneficiary of altruism coming at the cost of the provider of the cooperation and so selects against cooperation.

We used the framework provided by the PD, because it is a commonly used tool for demonstrating the problem of cooperation between nonrelatives [4, 22], and because we were able to test these predictions experimentally with humans. Several authors have argued that the PD, and the ability of reciprocal altruism to solve the problem of cooperation, require a large number of very specific assumptions and are likely to be of limited importance outside of humans [8, 23]. However, reciprocal altruism is just one of many mechanisms that can provide a direct fitness benefit to cooperation—alternatives include group augmentation, policing, sanctions, and punishment [7–14, 24]. We suggest that the consequences of local competition for these different mechanisms will depend upon how cooperation is favored. Although local competition makes relative fitness more important and, hence, selects against cooperation, it can also increase the advantage of punishing or spiteful behaviors [25, 26], which can be used to enforce cooperation.

Our results also illustrate a problem that needs to be solved in the theoretical literature. Several simulation studies, based on the PD, have suggested that limited dispersal favors cooperation [27, 28]. In contrast, analytical studies have suggested this will not necessarily be true because limited dispersal can also increase local competition. In the simplest case, the effect of increased competition exactly negates any benefit of limited dispersal [17, 29]. More complex analytical models can be constructed in which limited dispersal does not lead to such an increase in local competition, and hence, cooperation can be favored, depending upon biological details [3, 16–19, 29–35]. This raises the question of what

assumptions have removed this problem in the simulation studies and allowed the benefits of limited dispersal to outweigh the cost of increased competition.

Cooperation in Humans and Other Animals

Our results showed that humans were more likely to cooperate in the PD when competition was more global. This will happen when individuals tend to have repeated interactions (with the potential for cooperation) with a small number of other individuals (or within groups), but competition for resources occurs with a larger number of people (or between groups). Humans are a useful study organism for testing this hypothesis, as their cognitive abilities could allow the scale of competition to be assessed. In addition, determining the cognitive tools involved in cooperation between humans is key to resolving the debate over how the data from experimental games are to be interpreted [36–39]. We suspect that with most other organisms individuals will not be able to assess variation in the scale of competition, and so the response will be evolutionary rather than behavioral (i.e., fixed not facultative) [20, 21].

An implication of our results is that if the perceived scale of competition is manipulated, then this will alter the level of cooperation among humans. This could occur in numerous ways in all forms of society. One way is to create a common enemy, who must be competed against relatively globally. A famous example of this is in *Nineteen Eighty-Four*, where The Party uses a poster with a "monstrous figure of a Eurasian soldier" to unite the proles into "one of their periodical frenzies of patriotism" [40]. As Hamilton [15, 41] pointed out, the same competitive issues that can favor cooperation within groups, can also favor the evolution of hostility (or war) between groups. An alternative possibility is to reward local cooperation. For example companies (or any form of institution) could provide productivity rewards (or evaluations) to individuals that depend in part upon the performance of the workers that interact with that individual, rather than just the performance of the individual or the company as a whole.

Experimental Procedures

We carried out the following experiment on four undergraduate classes, three containing 15 students and one containing 12 students. Two of these classes were in 2004 and two were in 2005. This class was prior to a course on social evolution theory, which means it was before the students were taught relevant evolutionary biology. Each class was split up into five groups of three students (four groups in the class with 12 students). The PD was then explained to the students, who were then allowed to play approximately seven interactions to familiarize themselves with it.

We then made the students play for cash rewards. Cash rewards were given out anonymously. In two classes, the students played the relatively local competition session first and then the relatively global competition session second. In the other two classes, the students played the relatively global competition session first. For each class, we did the following. Within each group of three students, each student played each other for one game. Each game was for an average of seven repeated interactions of the PD, with the number of interactions unknown to the students (who were only told that there was an equal likelihood of the game finishing after each interaction). For each interaction, both players made their decisions simultaneously, without knowledge of the other's decision. After each interaction, the students were told the strategy that they had each chosen, and the points pay off, before the next interaction.

We varied the scale of competition by how cash prizes were awarded. The details of the cash rewards were given to the students at the start of each session (global or local). For relatively global competition, the five students in the class who scored the most points won £10 (top four students in the class with 12 students). For relatively local competition, the top score in each of the five (or four) groups of three students won £10. This procedure meant that the total amount awarded was the same for both local and global competition. An equally valid way of conceptualizing this is by considering within and between group competition: within group competition is relatively greater with local competition.

Our aim with this experiment was to test whether individuals adjusted their level of cooperation in response to the scale of competition. We tested for this by comparing the average proportion of interactions in which they cooperated, when playing the same two players, with relatively local and global competition. Data were arcsin square root transformed prior to analysis to remove the problem of nonnormal errors that can arise with proportion data. It can be argued that the results from the three individuals in the same group are not independent, because they arise from the same games. Consequently, we examined the robustness of results by analyzing our data at a number of levels: individuals (n = 57), groups (n = 9), and classes (n = 4). We calculated the proportion of times the individual (or group or class) cooperated (arcsin square root transformed) with relatively global competition minus the proportion of times the individual (or group or class) cooperated with relatively local competition. These values were then tested against the null hypothesis that the average value did not differ significantly from zero (i.e., there was no difference in the likelihood of cooperation between relatively local and relatively global competition) with a t test. All analyses were carried out with the package GLMStat 6.0 (http://www.glmstat.com). By comparing the incidence of cooperation in the relatively local and global games, we provide a qualitative test of our theory.

Cooperation in humans has been much investigated, and the importance of a range of other factors has been demonstrated, including sanctions, image scoring, and indirect reciprocity [4–7, 36, 39, 41–44]. In our experiment, the students were split by boards when playing the game and were not allowed to talk or signal at any point. Despite this, we cannot eliminate the possibility that their behavior was influenced by prior knowledge of each other, such as image scoring. However, this would only influence the average level of cooperation between players, at both local and global competition, and so does not alter our ability to test for differences between these two experimental treatments. A possible influence of time or experience (number of games played) was controlled for by making the students play local first in two classes and global first in two classes. Our results therefore show that when all else is equal, variation in the scale of competition leads to different levels of cooperation, suggesting that the importance of these other factors will be mediated by the scale of competition.

Supplemental Data

Supplemental Data include Supplemental Results and Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/11/1103/DC1/.

Acknowledgments

The experimental part of this paper was inspired by the Wedekind and Braithwaite (2002) study. We thank all the students who took part in the experiment, S. Frank, K. Foster, and two anonymous referees for comments, and K. Foster for providing the *Nineteen Eighty-Four* quote. We are funded by the Royal Society, Natural Environment Research Council, and Biotechnology and Biological Sciences Research Council.

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Supplemental Data

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Stuart A. West, Andy Gardner, David M. Shuker, Tracy Reynolds, Max Burton-Chellow, Edward M. Sykes, Meghan A. Guinnee, and Ashleigh S. Griffin

Supplemental Results and Experimental Procedures

We assume an infinite population of individuals, engaged in a large number of fitness-altering activities during their lifetime, of which at least one includes participation in a game of the prisoner's dilemma form. Each game lasts an indeterminate number of rounds, N, with a fixed probability of the game terminating at the end of each round. In each round, the payoff for each individual is determined as outlined in Table 1. Within any round, each player may cooperate (C) or defect (D); however, there is an infinite range of different strategies that individuals may adopt from game to game. We will consider two strategies: Tit-For-Tat (TFT) and Always-Defect (AD) (see main text for references). TFT is a facultative cooperation strategy, whereby individuals cooperate in the first round, and in subsequent rounds, they mirror the decision of their partner in the previous round. In this way, an individual adopting TFT enjoys the benefits of mutually cooperative play whenever she encounters a fellow TFT strategist. However, TFT will not suffer continued exploitation by defecting individuals, as cooperation is employed only insofar as the social partner is also cooperating. AD is a simpler strategy, leading the individual to defect in every round of the game. Under certain conditions, such as allowing for probabilistic strategies, or heterogeneous populations, more complicated strategies can beat TFT, but we focus on TFT to make our qualitative point most simply and clearly.

We will assume that payoffs combine additively over the rounds in each game, so that total payoff (p) for the game can be described according to the focal individual's strategy and the strategy of their partner. The payoff for TFT facing a fellow TFT strategist is $p_{\mathrm{TFT,TFT}} = NR$, because these two will cooperate with each other in all N rounds, accruing a payoff of R in each round. Note that, because N is a random variable, the total payoff for the game is also a random variable. The payoff for TFT facing AD is $p_{\mathrm{TFT,AD}} = S + (N-1)P$, because here there is a sucker's payoff, S, in the first round followed by $N-1$ rounds of mutual defection, each giving a payoff of P. The payoff for AD facing TFT is $p_{\mathrm{AD,TFT}} = T + (N-1)P$, and the payoff for AD facing AD is $p_{\mathrm{AD,AD}} = NP$. Total lifetime payoff is calculated by adding the payoff for this game to a baseline of 1, and we will assume that $p << 1$. Lifetime reproductive success is then simply one's lifetime payoff expressed relative to the average lifetime payoff of one's competitors, and we will define a scale of competition such that a proportion, a, of competition occurs locally within the pairing for the focal game and a proportion, $1-a$, of competition is global. In other words, if one adopts a strategy of $\mathrm{X} \in \{\mathrm{TFT, AD}\}$ and one's social partner adopts a strategy of $\mathrm{Y} \in \{\mathrm{TFT, AD}\}$, then one's fitness is given by:

$$w_{\mathrm{X,Y}} = \frac{1 + p_{\mathrm{X,Y}}}{1 + a(p_{\mathrm{X,Y}} + p_{\mathrm{Y,X}})/2 + (1-a)\overline{p}} \approx 1 + p_{\mathrm{X,Y}} - a(p_{\mathrm{X,Y}} + p_{\mathrm{Y,X}})/2 - (1-a)\overline{p} \quad (1)$$

where $\overline{p}$ is the average payoff for a game in the whole population, and where the approximation is made on the basis that $p << 1$. Because $p_{\mathrm{X,Y}}$ and $p_{\mathrm{Y,X}}$ are random variables, $w_{\mathrm{X,Y}}$ is also a random variable. The expectation for lifetime reproductive success, as a function of own and partner strategies, is

$$\overline{w}_{\mathrm{X,Y}} \approx 1 + \overline{p}_{\mathrm{X,Y}} - a(\overline{p}_{\mathrm{X,Y}} + \overline{p}_{\mathrm{Y,X}})/2 - (1-a)\overline{p} \quad (2)$$

where $\overline{p}_{\mathrm{X,Y}} = p_{\mathrm{X,Y}}|_{N=\overline{N}}$ and $\overline{p}_{\mathrm{Y,X}} = p_{\mathrm{Y,X}}|_{N=\overline{N}}$, and $\overline{N}$ denotes the expected number of rounds in any game.

We now consider the stability of a population playing one or the other of these two strategies. An evolutionarily stable strategy (ESS) is one that cannot be invaded by a variant strategy when the former is fixed in the population. The variant invades when its reproductive success is higher than average. In other words, for the strategy X to be an ESS requires that $\overline{w}_{\mathrm{Y,X}} < 1$ when $\overline{p} = \overline{p}_{\mathrm{X,X}}$. AD is an ESS when

$$\overline{w}_{\mathrm{TFT,AD}} = 1 + (1-a)(S-P) + a(S-T)/2 < 1. \quad (3)$$

Because $S < P$ and $S < T$, the above condition is always satisfied: AD is always evolutionarily stable. TFT is an ESS when

$$\overline{w}_{\mathrm{AD,TFT}} = 1 + T + (\overline{N}-1)P - a((S+T)/2 + (\overline{N}-1)P) - (1-a)\overline{N}R < 1. \quad (4)$$

Depending on the parameters, this may or may not be satisfied. In general, there is a threshold-expected number of rounds that must be exceeded in order for Tit-For-Tat to be evolutionarily stable:

$$N^* = \frac{T - (1-a)P - a(S+T)/2}{(1-a)(R-P)}. \quad (5)$$

In the limit of global competition ($a \to 0$), where there is only vanishing competition with one's social partner, this is given by $N^* \to \frac{T-P}{R-P}$. Because $T > R > P$, this threshold is greater than unity, i.e., the threshold-expected number of rounds that must be exceeded is some value greater than unity (for a numerical example see Figure 1). In the limit of local competition ($a \to 1$), where all competition is with one's social partner, then $(1-a)N^* \to \frac{1}{2}\frac{T-S}{R-P}$. Because $T > S$ and $R > P$ then $N^* \to \infty$ as $a \to 1$, i.e., as the intensity of competition between social partners increases to the extreme, the expected number of rounds per game required in order for TFT to be an ESS approaches infinity. In the limit of completely local competition, Tit-For-Tat cannot be evolutionarily stable. In general, the threshold-expected number of rounds is a monotonically increasing function of the intensity of local competition, because the derivative $\frac{dN^*}{da} = \frac{T-S}{2(1-a)^2(R-P)}$ is positive for all a, because $T > S$ and $R > P$.

Asymmetric larval competition in the parasitoid wasp *Nasonia vitripennis*: a role in sex allocation?

Edward M. Sykes, Tabitha M. Innocent, Ido Pen, David M. Shuker, Stuart A. West

APPENDIX

Here we extend a standard model of sex ratio evolution under local mate competition (LMC) to take into account effects of clutch size and sex ratio on female fecundity or longevity, as we have observed in our experiments. We will then parameterize the model with empirical estimates of these relationships to generate quantitative predictions of sex ratios.

The model is built around the standard life cycle of N mated females breeding in a patch. The mated females produce offspring that mate randomly among themselves within the patch, followed by dispersal of the newly mated females to random patches.

We use the direct fitness approach as developed by Taylor and Frank (1996) and Frank (1998), as we have done in previous papers on *Nasonia* sex ratios (Reece et al. 2004, Shuker et al. 2005).

A mother's total fitness is given by

$$W = c_f W_f + c_m W_m \qquad \text{(A1)}$$

where W_i is her fitness through sex-i offspring ($i = m, f$) and c_i is the class reproductive value of sex i. For haplodiploids, the reproductive value of females is twice that of males ($c_f = 2c_m$), which is another way of saying that a random gene from a population in the far future is twice as likely to be present today in a female as in a male (Taylor 1988). A focal female with sex ratio x (proportion sons) and clutch size k has fitness through daughters proportional to

$$W_f = (1-x)F \qquad \text{(A2)}$$

where $F = F(\bar{x}, k)$ is a daughter's expected reproductive success, which may depend on the average sex ratio $\bar{x}$ produced by the females in the patch and their clutch size k. We assume that k is fixed, not being under selection, unlike in the models of Godfray (1986), where simultaneous selection on sex ratio and clutch size was studied. We will consider two extreme situations, one where F is estimated by a female's fecundity and one where F is estimated by her life expectancy. However, it turns out that the numerical predictions for both scenarios are virtually identical, and we will therefore show only the results for fecundity.

The focal female's fitness through sons is given by

$$W_m = x\frac{1-\bar{x}}{\bar{x}}F \qquad \text{(A3)}$$

The ratio $(1-\bar{x})/\bar{x}$ is the expected number of mates per son and $F = F(\bar{x},k)$ is the expected reproductive success of those mates.

The direction and strength of selection on the sex ratio is given by the selection gradient

$$\frac{\mathrm{d}W}{\mathrm{d}x} = r_f c_f \frac{\partial W_f}{\partial x} + R_f c_f \frac{\partial W_f}{\partial \bar{x}} + r_m c_m \frac{\partial W_m}{\partial x} + R_m c_m \frac{\partial W_m}{\partial \bar{x}} \qquad \text{(A4)}$$

where r_i is the relatedness of sex-i offspring to the focal female and R_i the relatedness to the focal female of random sex-i offspring born in the same patch. The relatedness coefficients can be calculated from standard population genetic recursions (Taylor 1988, Reece et al. 2004). For haplodiploids with random dispersal of mated females, the results are

$$r_f = 1/(2-1/N),\ R_f = r_f/N,\ r_m = 1,\ R_m = r_m/N. \qquad \text{(A5)}$$

Working out the partial derivatives gives the selection gradient

$$\frac{\mathrm{d}W}{\mathrm{d}x} = -r_f c_f F + R_f c_f (1-\hat{x})F_x + r_m c_m \frac{1-\hat{x}}{\hat{x}}F - R_m c_m \left[\frac{F}{\hat{x}} - (1-\hat{x})F_x\right] \qquad \text{(A6)}$$

where all expressions on the right are evaluated at $x = \bar{x} = \hat{x}$. F_x represents the partial derivative of F with respect to the average patch sex ratio $\bar{x}$, again evaluated at $x = \bar{x} = \hat{x}$.

Female reproductive success F as a function of body size y was estimated with a linear model of the form

$$F = \beta_0 + \beta_1 y. \qquad \text{(A7)}$$

Body size y, in turn, was estimated as a function of sex ratio x and clutch size k by a model of the form

$$y = \gamma_0 + \gamma_1 k + \gamma_2 \arcsin(\sqrt{x}) + \gamma_3 k \cdot \arcsin(\sqrt{x}). \qquad \text{(A8)}$$

Plugging this relationship into (A7) and taking the derivative with respect to x yields the following estimate for F_x:

$$F_x = \frac{\beta_1(\gamma_2 + \gamma_3 k)}{2\sqrt{x(1-x)}}. \qquad \text{(A9)}$$

Predicted sex ratios were calculated numerically with Maple 9.5 as the $\hat{x}$ roots of the right-hand side of (A6) after plugging in (A7) and (A9) and estimates of the various parameters. Figure 5 shows the standard predicted sex ratio $N(2N\text{-}1)/[N(4N\text{-}1)]$ (Hamilton 1979) and the predicted sex ratios from our model for *Nasonia vitripennis* (parameter estimates from table 2), assuming a total number of competitors of k=70. For smaller clutch sizes, the deviation from the standard model is even smaller. Figure 6 shows the very similar predictions for *Bracon hebetor* (data in the figure legend).