

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

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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 can 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.

Keywords: brood size, clutch size, local mate competition, longevity, sex allocation, sex ratio.

INTRODUCTION

The relationship between body size and fitness is predicted to influence a large number of reproductive behaviours (Stearns, 1992). This relationship has attracted much attention in 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; Charnov *et al.*, 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 and Luck, 1995; Visser, 1995; West *et al.*, 1996; Ellers *et al.*, 1998; Ellers and

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Jervis, 2003; Rivero and West, 2002, 2005). In contrast, there is a much poorer understanding of how body size influences fitness in males (Van den Assem *et al.*, 1989; Heinz, 1991; Kazmer and Luck, 1995).

Several recent studies have emphasized 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, 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 local mate competition 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.*, in press). 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 evolutionarily stable 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 evolutionarily stable sex ratio is biased towards the sex that causes the smaller competitive effect.

Here we use three experiments to examine the fitness consequences of body size in male *Nasonia vitripennis*. First, we examined whether absolute male size influences mating success. We measured how variation in body size influenced the insemination ability of solitary males presented with ten females for a limited time. Although 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 and Parker, 1996; M.N. Burton-Chellew *et al.*, unpublished data). Consequently, in our second experiment, we 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, including: 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 our third experiment, we estimated male fitness by measuring the lifetime mating success of males provided with mating opportunities for the duration of their lives. Our field data suggest that the emergence period of females on a patch can range from 1 to 19 days [mean \pm standard deviation = 9.00 ± 2.36 , $n = 9$ (M.N. Burton-Chellew *et al.*, unpublished data)]. The mating success of a male will therefore be determined by his ability to inseminate females over time while withstanding the costs of mating in terms of both courtship and insemination. We varied the mating regime in this experiment to determine whether there was a cost of mating, and if this cost differentially affected males of different sizes. For instance, smaller males may suffer a greater reduction in longevity as a result of mating, limiting lifetime mating success.

METHODS

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 and Parker, 1990; Orzack *et al.*, 1991; Flanagan *et al.*, 1998; Reece *et al.*, 2004; Shuker *et al.*, 2004, 2005, 2006b, 2007b). Females typically mate once before dispersing to find new oviposition sites (Shuker *et al.*, 2007a; Burton-Chellew *et al.*, in press). 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).

Nasonia 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 an interval of 5–10 s (Van den Assem *et al.*, 1980b; Beukeboom and 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.*, 1980b; Van den Assem and Jachmann, 1999; Bordenstein *et al.*, 2000). Copulations are short, with a mean of approximately 14 s (Burton-Chellew *et al.*, in press), 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 and 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 and Jachmann, 1999).

Experimental strains and maintenance

The three experiments used two strains: HV7 and STDR. HV7 (hereafter referred to as 'wild-type') is a relatively outbred laboratory strain created by the mixing of seven previously inbred laboratory strains, all of which were originally collected from the Hoge Veluwe, by Professor L. Beukeboom (University of Groningen, Netherlands). The red-eye mutant strain STDR (hereafter referred to as 'red-eye'), which dates back to the 1950s (Whiting, 1954; Saul and 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 16 h–8 h 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 × 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 4 days before re-culturing were typically in excess of 500 individuals, with the aim of avoiding any inbreeding effects associated with small population size.

Size and mating success

We tested males of varying size for their ability to court, mate, and inseminate up to 10 females in 15 min. To generate a large range in male size, we 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 3 days. Only one male from any given foundress group was used in the experiment. Females all developed from a host that had been presented simultaneously to two mated females as potential foundresses for 3 days. Female size therefore spanned a smaller range. One virgin wild-type male was placed in a glass observation vial (75 × 10 mm) containing 10 virgin wild-type females. All females with a given male came from different mothers. All individuals were less than 3 days old when tested. After 15 min the male was removed and the females separated and given hosts to parasitize over a 48-h period (two batches of three hosts for 24 h each). Male mating and insemination success was measured as the proportion of the ten females that produced daughters (diploid offspring) in any of their six hosts. Pilot trials showed that 15 min is the optimum time to differentiate male success (i.e. there is variance in male success). To terminate each trial, the test vial was placed in a box of ice for 60 s, slowing down the wasps and allowing the males and females to be easily separated with a paintbrush. In total, 99 males were tested over 3 days. Males from hosts parasitized by one, two or three females were equally allocated randomly to each day. Male size was determined after death by measuring the length of the right hind tibia using a Leica dissecting microscope (×100) 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).

Size and mating success in competition

We placed two males into the same arena and tested for their competitive ability to inseminate up to 10 females in 15 min. 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 10 females that produced daughters (diploid offspring) with the wild-type eye colour phenotype. Females that produced daughters with the red-eye phenotype had mated the competing red-eye male. Given the generally low rate of multiple mating (Shuker *et al.*, 2007a; Burton-Chellew *et al.*, in press), we considered it unlikely that any females would have mated both males within the 15 min. However, some females (12 of 810)

produced daughters exhibiting both the wild-type and red-eye phenotype. These females were scored as having mated both males. In total, 81 trials were performed over 3 days.

Size, lifetime mating success, and the cost of mating

We measured the longevity and reproductive success of different sized males in response to varying lifetime mating opportunities. Again, to generate a large range in male size, we 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 = $723 \pm 3.2 \mu\text{m}$), or four females and one host (mean leg length = $664 \pm 5.5 \mu\text{m}$) (these leg lengths are significantly different: $F_{1,102} = 84.58$, $P < 0.0001$). Again, we only used one male per foundress group. We created three mating treatments: (a) solitary unmated males ($n = 28$); (b) solitary males presented with four females for the first 24 h of their life, and then kept alone ($n = 36$); and (c) solitary males kept with four females for their whole life, with the females being replaced every 24 h ($n = 41$). There was no female mortality within the 24 h. Unfortunately, because 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 we were often forced to provide them with only one female every 24 h. Also, it was not possible to give the males equal numbers of females. This added noise to our experiment but was random with respect to male size. All wasps were from the wild-type strain.

We measured longevity by recording the time of emergence and time of death, with checks being performed approximately every 6 h. 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 lifetime mating success was measured as the number of females that went on to produce daughters.

Statistical analyses

In Experiment 1 (size and mating success), some females laid only diapause offspring (offspring that are in suspended development and cannot be sexed easily as they are yet to develop adult morphologies), so we failed to determine if they had been inseminated or not. Therefore, male success for Experiment 1 was analysed as the proportion of those females laying non-diapause offspring that were thus known to have been inseminated. We 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 only the actual known proportions are presented. In Experiment 2 (size and mating success in competition), we 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 3 (size, lifetime mating success, and the cost of mating), we fitted general linear models, using stepwise regression, for longevity and lifetime mating success using the JMP IN software, version 5.1 (SAS Institute Inc.).

The proportions of females inseminated in all three experiments were analysed 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 while retaining maximum power, we analysed 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, a full model was fitted to the data, including all explanatory variables and their interactions. All continuous explanatory variables were assessed for non-linearity by fitting quadratic terms. Terms were then removed 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 chi-squared test. We checked the appropriateness of our 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, we rescaled the deviance by the heterogeneity factor, the ratio of the residual deviance to the degrees of freedom (McCullagh and Nelder, 1983). After correcting for over-dispersion, an *F*-test was used to test the significance of a term (Crawley, 1993). For the sake of consistency, Figures 1 and 2 show the number of females inseminated as proportion data and means are presented together with their standard errors (back-transformed from binomial estimates).

RESULTS

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 because, on average, 15% of the females in Experiment 1 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 ($\chi^2_{(1)} = 0.72$, $P = 0.40$, $n = 99$) (Fig. 1). A quadratic term for male size was also non-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 ($F_{1,95} = 1.93$, $P = 0.15$).

Size and mating success in competition

On average, the two males combined to inseminate 9.1 ± 0.12 of the 10 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. 2a), or his size relative to that of the other male ($\chi^2_{(1)} = 0.80$, $P = 0.37$) (Fig. 2b). 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 percentage of females inseminated was slightly higher for Experiment 1, with solitary males inseminating $53 \pm 2\%$, compared with $43 \pm 1\%$ for males in competition ($\chi^2_{(1)} = 15.43$, $P < 0.0001$, $n = 180$).

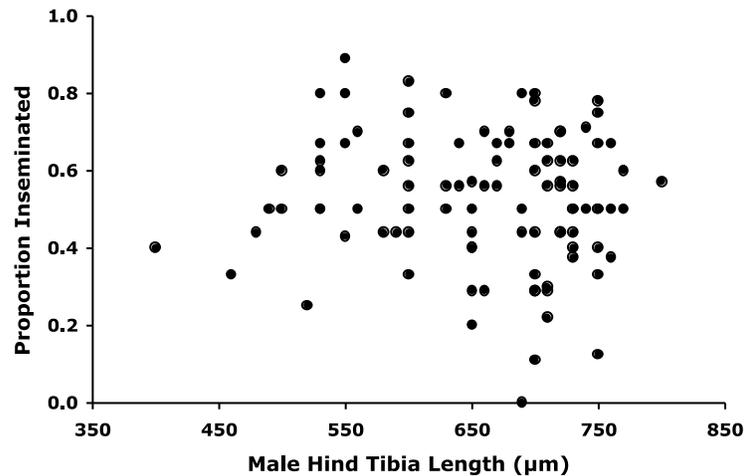


Fig. 1. The insemination success of solitary males. Body size did not influence the proportion of females that a male could inseminate, when placed with 10 females for 15 min (Experiment 1).

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). 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: $F_{1,34} = 3.40$, $P = 0.07$; 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 our experimental design, did not depend on male size ($F_{1,39} = 0.69$, $P = 0.41$) (Fig. 4). When we 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 ($F_{1,39} = 0.03$, $P = 0.86$), as was the interaction ($F_{2,39} = 0.95$, $P = 0.37$).

Although it is not an independent analysis, we 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 ($F_{1,39} = 1.22$, $P = 0.28$, heterogeneity factor = 1.82).

DISCUSSION

Male size had no significant effect on our measures of fitness in all three experiments. These experiments examined mating success when alone (Fig. 1), mating success when in competition (Fig. 2), and lifetime mating success when provided with daily access to mates (Fig. 4). Our final experiment also allowed us 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).

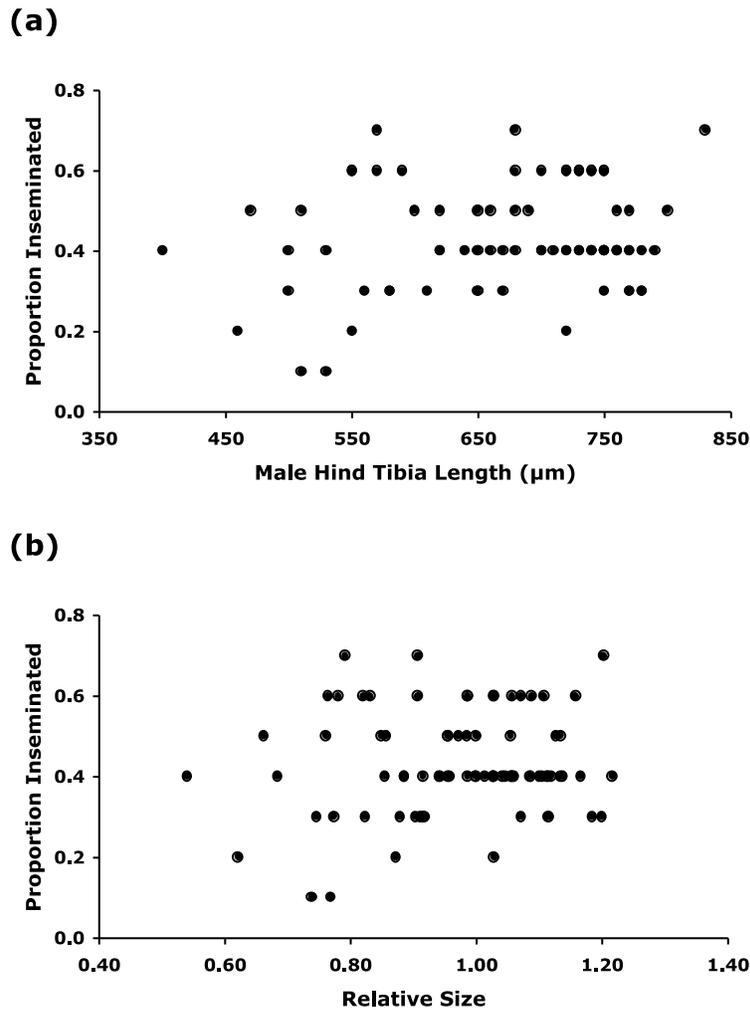


Fig. 2. The insemination success of males in competition. The proportion of females that a focal male inseminated, when placed with 10 females and one other male for 15 min, was not significantly influenced by either (a) the size of the focal male or (b) the relative size of the focal male to that of his competitor (ratio of focal male leg length to competitor male leg length) (Experiment 2).

Experiments 1 and 2 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 and Parker, 1996; M.N. Burton-Chellew *et al.*, unpublished data). Experiment 1 examined the speed at which males can court and copulate with multiple females. A possible limitation of our design was that the potential benefits of being large, such as increased energy reserves or sperm production, would only be relevant over periods longer than 15 min. However, this limitation was addressed in Experiment 3. Experiment 2 tested the influence of male size in competition. Although the design was the same as that of Experiment 1 but for the addition of another male, male size could be expected to be

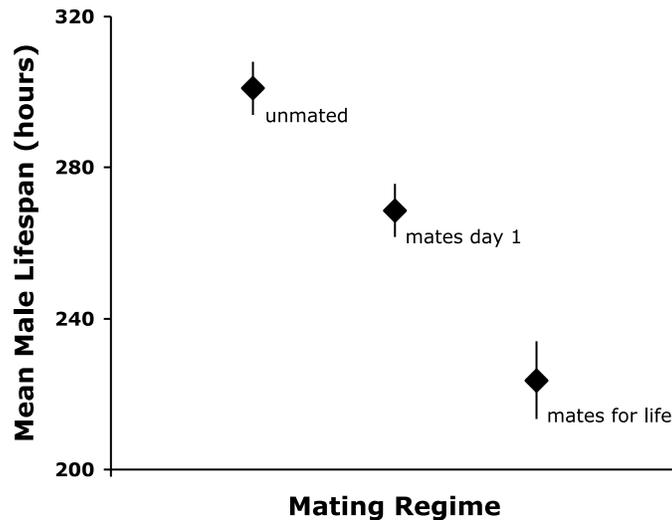


Fig. 3. The effect of mating on longevity (mean \pm standard error). Virgin males live significantly longer than mated males, which in turn live significantly longer than males that had mates for their whole lives (Experiment 3).

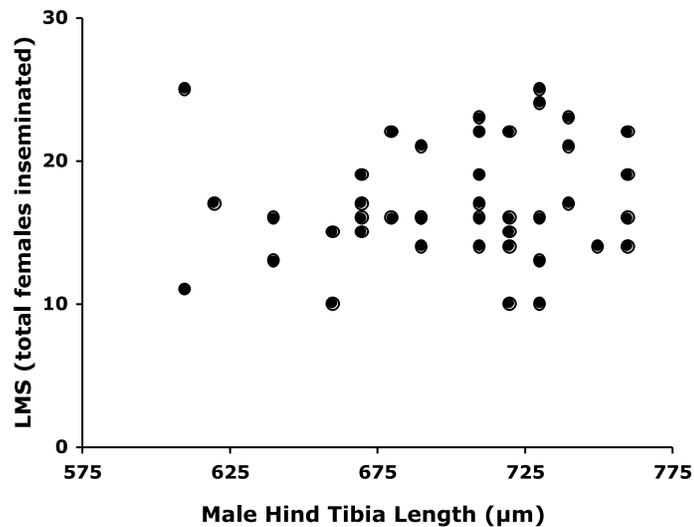


Fig. 4. The relationship between male size and total number of females inseminated (lifetime mating success, LMS). Male size had no effect on the total number of females inseminated, even when controlling for longevity or the number of females offered (Experiment 3; treatment C only).

more important in such a scenario. This is because males could compete for access to females (Van den Assem *et al.*, 1980a), or there could be female choice (Hughes and Hughes, 1985; Hardy *et al.*, 2005). However, again we 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 3 measured male lifetime mating success, longevity, and the cost of mating. The key result was that although mating reduced longevity significantly, this was equally costly for males of all sizes. The costs of mating for females have been well documented in insects (Fowler and Partridge, 1989; Chapman *et al.*, 1995; McLain and Pratt, 1999; Blanckenhorn *et al.*, 2002; Moore *et al.*, 2003; Shuker *et al.*, 2006a) but less attention has been given to the costs of mating for males (Cordts and Partridge, 1996; Prowse and Partridge, 1997; Cordero, 2000; Kotiaho and Simmons, 2003; Martin and Hosken, 2004; Sakaluk *et al.*, 2004; Perez-Staples and Aluja, 2006; Simmons and 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 our study, the costs to males could stem from either the increased energy demands of extra copulations and inseminations, or the continual efforts of courting. The effort of courting may well explain our cost of mating. As our males were confined with females for either 24 h or their whole lives, it is probable that they expended considerable energy in repeated courtship attempts, which necessitate the production and release of potentially costly pheromones (Van den Assem *et al.*, 1980b; for an example of costly pheromone production, see Johansson *et al.*, 2005). Despite a longer latency to courtship when males are presented with mated females as opposed to virgins, they do still typically court frequently (Burton-Chellew *et al.*, in press), and courtship is known to be costly in other insects (Cordts and 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.*, in press) [estimates of multiple mating in the wild: 4% (M.N. Burton-Chellew *et al.*, unpublished data)]. 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 and 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 our experiment and so our 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 our experiment could only resolve differences in terms of the success or failure of insemination. Thus we did not consider male ejaculate quality quantitatively. How male *N. vitripennis* invest in sperm is not known in detail. One could argue 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.*, in press). 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 and Boivin, 2006; Shuker *et al.*, 2006c). Finally, our 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 and Partridge, 1996), and the longevity costs of being small for female *N. vitripennis* only apply when food is not available (Rivero and West, 2002). The extent to which male *N. vitripennis* feed in the wild is as yet unknown.

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