



Laboratory evolution of polyandry in the parasitoid wasp *Nasonia vitripennis*

MAXWELL N. BURTON-CHELLEW*, LEO W. BEUKEBOOM†, STU A. WEST* & DAVID M. SHUKER*

*Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh

†Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen

(Received 30 June 2006; initial acceptance 11 August 2006;
final acceptance 30 September 2006; published online 1 November 2007; MS. number: 9023)

The evolution and maintenance of multiple mating in females (polyandry) is an adaptive puzzle since females typically obtain all the resources they need from males in only one or a few matings. Females should therefore limit superfluous copulations to avoid the well-documented costs of mating. Previous studies have tended to focus on the maintenance of polyandry, and have examined the wide range of costs and benefits to females of multiple mating in species with varying levels of polyandry. There is much less empirical work charting the evolution of female mating behaviour and the origins of the polyandry trait itself. We investigated a potential increase of female remating rate in the parasitoid wasp *Nasonia vitripennis*. We screened the mating and remating rates of females from 20 strains, 18 of which were collected from the same locality across 3 years and varied in the number of generations that they had spent in the laboratory. Females from strains that had spent longer in the laboratory were increasingly likely to be polyandrous, confirming a previous study based on one laboratory strain. Experimental crosses between strains at opposite ends of the spectrum for time spent in the laboratory showed that heritable changes in the females were predominantly responsible for the change in mating behaviour, as males did not appear to influence the likelihood of a female remating.

© 2007 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Keywords: copulation duration; courtship; female receptivity; multiple mating; *Nasonia vitripennis*; parasitoid wasp; remating

The evolution of female multiple mating (polyandry) remains a puzzle in many taxa. Typically, females can obtain all the male-derived resources they need, especially sperm, from a limited number of copulations (Arnqvist & Nilsson 2000). Yet it is now apparent that females of many species mate more than is required to fertilize a full complement of their eggs (Birkhead & Møller 1995; Fedorka & Mousseau 2002). Furthermore, multiple mating appears to be costly in a number of ways, including increased risk of predation, exhaustion of time and energy, risk of injury, parasites and diseases, and harmful effects of the male

ejaculate (Daly 1978; Fowler & Partridge 1989; Chapman et al. 1995; Stockley & Seal 2001; Stutt & Siva-Jothy 2001; Blanckenhorn et al. 2002; Martin & Hosken 2003; Maklakov & Lubin 2004).

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

Correspondence: M. N. Burton-Chellew, Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, U.K. (email: m.burton@sms.ed.ac.uk).
L. W. Beukeboom is at the Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

(Rowe et al. 1994; Shuker & Day 2001, 2002) leading to convenience polyandry, as females act to reduce the costs of not mating rather than seeking gains from additional mates and copulations (Thornhill & Alcock 1983).

Studies have tended to concentrate on which of the above benefits can be identified in already polyandrous species (Tregenza & Wedell 1998, 2002; Evans & Magurran 2000; Konior et al. 2001; Worden & Parker 2001; Fedorka & Mousseau 2002; Kamimura 2003). An alternative approach is to examine the origin and spread of polyandry, in response to a change in the selection environment, either in the laboratory or in the wild (Torres-Vila et al. 2002; Harano & Miyatake 2005). Although the origin of polyandry is closely linked to its maintenance, the selection pressures can differ. This is due to a change in the costs and benefits of mating as a result of the coevolutionary dynamics of sexual conflict (Arnqvist & Rowe 2005). We investigated the significance of an anecdotal report by Van den Assem & Jachmann (1999) of a potential increase of female mating rate in one laboratory population of the parasitoid wasp *Nasonia vitripennis*. Believed to mate only once in the wild, *N. vitripennis* females are very unlikely to remate when presented with additional males in the laboratory, despite the eagerness of virgins to mate under identical conditions (Holmes 1974; Van den Assem & Feuth-de Bruijn 1977; Grant et al. 1980). Microsatellite data from 3500 wild-caught offspring indicate that only two of 49 foundresses had mated more than one male (M. N. Burton-Chellew et al., unpublished data). However, Van den Assem & Jachmann (1999) discovered that a laboratory-maintained strain (Leiden Lab II) of *N. vitripennis* had incidentally evolved an increased likelihood to remate, along with a number of other behavioural differences, including an increased amount of courtship necessary for females to mate in the first place, although their methods may have led to an inflated remating rate because males were prohibited from performing their postcopulatory courtship, which may act to 'turn off' female receptivity. An evolved increase in mating rate has also been reported in laboratory-maintained *Drosophila melanogaster* (Sgro & Partridge 2000). These observations suggested that mating behaviour had evolved over many generations of laboratory culture, and perhaps that laboratory culture could provide the opportunity to study the evolution of polyandry as it occurs.

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

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). The sex ratio is often very low as a response to local mate competition (Hamilton 1967). Females typically mate once before dispersing to find new oviposition sites. 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. Typically males compete to guard exit holes in the hosts, whereby they secure copulations with the virgin females as they exit the host (Van den Assem et al. 1980a).

Courtship and Mating

Nasonia vitripennis males have a stereotyped courtship consisting of mounting the female in response to volatile compounds that signify a female's presence and performing multiple series of four to seven head-nods, with each series separated by an interval of 5–10 s (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. 1980b; Van den Assem & Jachmann 1999; Bordenstein et al. 2000). Copulations are short ($\bar{X} \pm \text{SE} = 13.9 \pm 0.1$ s, $N = 1212$; this study) and males are unable to force unreceptive females into copulating. After copulation, the male performs a stereotyped postcopulatory courtship that serves to reduce future receptivity. When males are prevented from performing the postcopulatory courtship, the female is more likely to mate with a subsequent courting male (Van den Assem & Jachmann 1999).

Experimental Strains

We studied 18 strains that were collected from the same locality over consecutive summers from 2001 to 2003. Collections were made from bird nestboxes at Hoge Veluwe, the Netherlands, with no two strains originating from the same nestbox in the same year, so that each strain represents an independent replicate sample of the population. This is because each strain was derived from one or more mated female offspring that emerged from the parasitized pupae found in only one nest; the emerging wasps were likely to have been the offspring of only one or a few females. The wild-caught strains used were: B5; HV55; HV287; HV395 (collected 2001); HV202; HV236; HV307 (collected 2002); and C51; C61; C62; C80; C130; C189; C194; C222/a; C223; C349; C378 (collected 2003). All strains were collected in June, July or August. Strains from the same year were not screened at the same

time, providing a greater spread for the age of the strains. To compare these strains with long-established laboratory strains, we also used the strain Leiden Lab II, which is the same one used by Van den Assem & Jachmann (1999) to show evolved changes of mating behaviour in response to laboratory conditions. This strain was created from an individual *N. vitripennis* female caught in the *Calliphora* stocks of the Leiden University Physiology Department in 1971. We also used the red-eye mutant strain STDR, which dates back to 1950 (Whiting 1950, 1954; Saul & Kayhart 1956).

We maintained wasps in mass culture, generally at 25°C, under 16:8 h, light:dark conditions. Under this regime, males start to emerge after 13–14 days and mate with females which emerge soon after. All wasps were reared on *Calliphora vomitoria* hosts. Stock cultures were maintained in replicate transparent glass vials (10 × 75 mm). Typically on the fourth day after adult emergence, approximately 40 females were transferred to each of several new replicate vials of identical proportions and incubated with around 40 fresh *C. vomitoria* hosts. Population densities during the 4 days before reculturing were typically in excess of 800 individuals and females surrounded by several courting males were often observed.

For the experiments, approximately 30 parasitized *Calliphora* pupae (hosts) from the replicate stock vials were used to collect virgin *Nasonia* from each strain. We isolated individuals as pupae from the host puparia just prior to eclosion to ensure male and female virginity. Inexperienced males from the hosts were pooled before being randomly assigned to storage vials at a density of five males per glass vial (75 × 10 mm). Females were treated the same way but stored at a density of 10 females per vial. We fed all individuals by using filter paper soaked with a honey solution at least once before any experiments and at least once every 48 h. Individuals were used at an age of 1–3 days after emergence at the start of any experiment and to handle wasps we used an aspirator throughout. We removed all experimental subjects from the culture incubators and placed them at the observation locality (the same seat in the laboratory) at least 60 min prior to the observations to allow the wasps to acclimatize to laboratory conditions.

Polyandry Experiment

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

We placed each virgin female into her own glass vial (50 × 10 mm). Males remained in their storage vials of five individuals until they were extracted individually for the mating trials. For each preliminary mating, we introduced

a male to the female and observed them continuously for 3 min or until copulation terminated, after which we removed the male. This prevented any female from copulating more than once before the remating test. If a male was still courting or copulating at the end of the 3 min we continued the observation either until the male ceased courtship or a successful copulation was completed. Any female that copulated within the 3-min trial remained isolated in the observation vial and was returned to the culture incubator overnight, prior to her remating test the next day. We sampled 1366 female wasps in total (range 36–106 per strain, with an arithmetic mean of 68.3).

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

Our measure of polyandry for each strain was a measure of how many mated females from each strain mated during this 15-min remating trial. This is different to the measure of increased female receptivity reported in Van den Assem & Jachmann (1999), because their methods did not allow males to initiate and terminate postcopulatory courtship, which is an important determinant of female receptivity, limiting the likelihood that females remate (Van den Assem & Jachmann 1999). To ensure the copulations we were scoring involved sperm transfer, we checked, in two ways, whether putatively mated females produced female offspring, indicating successful insemination (owing to haplodiploid sex determination). For the first check, we used 76 females that copulated in the preliminary mating trial, but then did not copulate in the remating screen. We gave each female three fresh hosts to parasitize. Seventy-two females oviposited, although three produced only diapause larvae, which cannot easily be sexed, and these were excluded from further analysis. Of the remaining 69 broods, all contained female offspring. For the second check, we took 50 virgin females (not used in the polyandry experiment) and paired each with a virgin male as above. Forty-six females mated, and of these 41 laid nondiapause broods, all of which contained female offspring. Observed copulations are therefore clearly associated with at least some sperm transfer.

Effect of Males

We carried out an experiment to determine whether variation in the rate of polyandry was due to variation in male or female behaviour. We compared the remating behaviour of females when given males from their own strain (results from above) or from another strain. The mating trials were carried out as before, only in this case

we paired females with males from different strains for both the first and second mating trial. We used an old strain with a high score for polyandry (Lab II) and two young strains both with low scores for polyandry (C223 and C349). Females from strains C223 and C349 were presented with males from Lab II, and vice versa, in a reciprocal behavioural test.

Statistical Analyses

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

RESULTS

Evolution of Polyandry

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

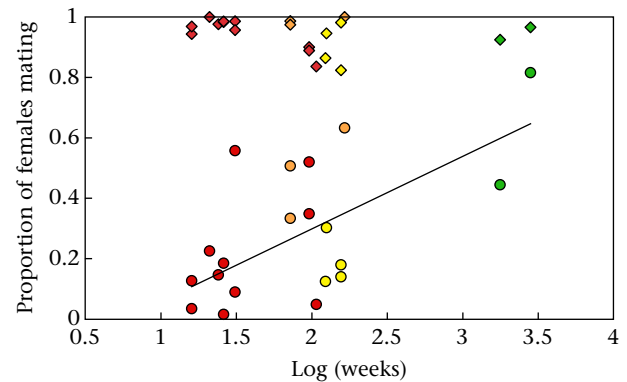


Figure 1. The relation between female receptivity to a courting male and her strain's time in the laboratory (log (weeks)). Females were virgin (filled diamonds, nonsignificant) or once mated (filled circles; $Y = 0.04 + 0.26X$) before each test. Red: collected 2003; orange: 2002; yellow: 2001; green: 1971 (Lab II) and 1950 (STDR).

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

Behavioural Changes

Mating behaviour varied in terms of both whether or not females were virgins and how long strains had been maintained in the laboratory (Table 1, Fig. 2). Virgin females copulated sooner than mated females (GLM: $F_{1,36} = 60.98$, $R^2 = 0.65$, $P < 0.0001$), and a positive interaction between mating status and the age of the strain showed that mated females remated sooner when their strains had spent more time in the laboratory ($F_{1,36} = 4.58$, $R^2 = 0.05$, $P = 0.04$; Fig. 2a). This was also true for mated females only ($F_{1,16} = 5.88$, $R^2 = 0.28$, $P = 0.03$).

Table 1. Comparisons of mating behaviour between virgin and mated females when presented with a virgin male

	Virgin female		Mated female	
	$\bar{X} \pm SE$	N	$\bar{X} \pm SE$	N
Courtship latency (s)	66.6 \pm 1.3	1319	83.3 \pm 2.2	524
Copulation latency (s)	92.6 \pm 1.4	1260	156.5 \pm 6.3	247
Courtship duration (s)*	23.6 \pm 0.5	1211	62.6 \pm 6.8	121
Copulation duration (s)	13.9 \pm 0.1	1212	13.4 \pm 0.2	106

*The courtship duration for mated females includes only females that signalled receptivity to the first courtship bout for equal comparison with the virgin females.

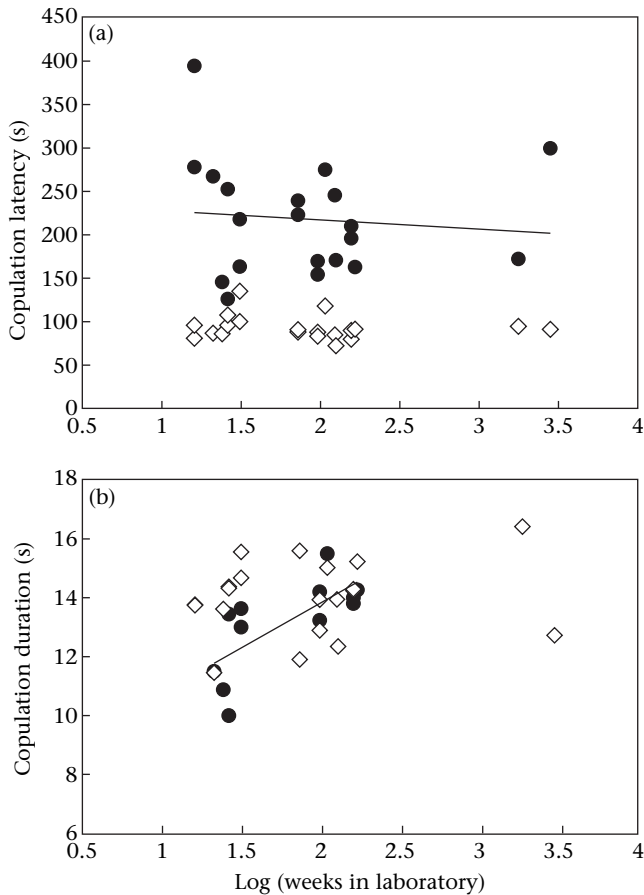


Figure 2. The relation between (a) copulation latency and (b) copulation duration and the strain's time in the laboratory (log (weeks)). Females virgin (\diamond ; both nonsignificant) or once mated (\bullet ; (a) $Y = 219.41 - 29.41X$; (b) $Y = 1.84X + 10.18$) before each test.

The copulation duration did not differ between virgin and mated females ($F_{1,31} = 0.04$, $P = 0.84$), but already mated females copulated for longer if their strain had spent longer in the laboratory ($F_{1,11} = 5.97$, $R^2 = 0.37$, $P = 0.03$; Fig. 2b). Virgin females were also courted sooner than mated females ($F_{1,33} = 5.64$, $R^2 = 0.15$, $P = 0.02$), regardless of how long they had been in the laboratory ($F_{1,33} = 0.15$, $P = 0.70$), and they also required less courtship to become receptive ($F_{1,33} = 11.35$, $R^2 = 0.22$, $P = 0.002$), again regardless of how long they had been in the laboratory ($F_{1,33} = 1.24$, $P = 0.27$).

Effect of Males

Female mating rate was consistent regardless of the strain identity of her mating partner (Table 2). Lab II females consistently showed a relatively high degree of polyandry whether they were with males from the same strain or one of the two younger strains (Fisher's exact test: $P = 0.77$). C223 females consistently showed a low degree of polyandry whether they were with males from the same strain or the older Lab II strain ($P = 0.20$). C349 females also consistently showed a low degree of polyandry whether they were with males from the same strain or

Table 2. The effect of males upon female remating

	Lab II males	C223 males	C349 males
Lab II females	0.47 (87)	0.48 (46)	0.38 (53)
C223 females	0.00 (52)	0.05 (43)	—
C349 females	0.10 (30)	—	0.10 (88)

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

the older Lab II strain ($P = 1.00$). This meant that male success depended upon the female genotype such that males courting mated Lab II females were more likely to mate than those paired with mated C223 or C349 females (Fisher's exact tests: all $P < 0.001$; Table 2).

DISCUSSION

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

While there has been little focus on the evolution of polyandry compared to its maintenance, there has been a history of artificial selection studies exploring female receptivity more generally, particularly in *Drosophila*, and a rapid evolutionary response to artificial selection and laboratory selection has been shown a number of times. For example, *Drosophila ananassae* was artificially selected for both short and long remating intervals (Singh & Singh 2001): in just 10 generations selection produced a rapid divergence in remating times with high realized heritabilities ranging from 0.23 to 0.33 among replicate strains. Piñeiro et al. (1993) found that female receptivity in *D. melanogaster* responded to selection for both increased and reduced latency to copulation; however, the level of receptivity remained constant despite 42 subsequent generations of relaxed selection, suggesting that there was

little selection pressure on receptivity in their laboratory conditions. Females from high receptivity lines hybridized more frequently with *Drosophila simulans* males, a result that mirrored the earlier finding that artificial selection for hybridization between these two species resulted in *D. melanogaster* females showing increased receptivity (Carracedo et al. 1991). Another study on *D. melanogaster* found an asymmetric response to selection on mating speed, in 29 generations, with females from the slow lines reducing their receptivity (Mackay et al. 2005). Estimates of realized heritability averaged 7%. The whole genome transcriptional response to selection was assayed and a large number of genes showed differential expression between the fast and slow replicate lines indicating substantial pleiotropy. Lastly, female receptivity also increased among laboratory populations of *D. melanogaster* in response to males with reduced courtship ability (McRobert et al. 1995); the stocks contained only males with a mutation (raised) that raises their wings and prevents them from performing their full courtship repertoire so the females may be increasing their receptivity to maintain their optimal mating rate (although selection at the level of the stock might have been the driving force in this unique situation). An analogous situation may be driving the change in our laboratory cultures, where male courtship is perhaps impaired by the high density of competing males (Van den Assem et al. 1980a). These examples are evidence that female mating behaviour can respond quickly to selection, although artificial selection is arguably stronger than any selection resulting from laboratory culture.

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

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

Why does polyandry evolve under laboratory conditions in this species? The mass culture environment of the laboratory is one in which high densities of individuals (several hundred) emerge within a short period of time and where females are unable to disperse after an initial mating (at least until after 3–4 days when some females are taken to found the next generation). During this time, females are subjected to high levels of repeated courtship, often with two or three males scrambling for access to one female. This is in contrast to the situation in the wild where females are free to disperse and forage for hosts as soon as they have mated. There are three straightforward explanations for the evolution of female multiple mating under this laboratory-induced change in mating system, which we are currently testing. First, polyandry has evolved because females have a much greater opportunity to sample and obtain indirect genetic benefits from the large number of potential mates now available to them (Jennions & Petrie 2000). Second, given the high levels of male harassment under laboratory conditions, multiple mating may have evolved as a way of limiting costly harassment, as envisaged by the convenience polyandry hypothesis (Thornhill & Alcock 1983). Third, the evolution of polyandry may be a pleiotropic effect of some other laboratory-induced change, and so not under direct selection at all (Halliday & Arnold 1987; Grant et al. 2005).

To conclude, studying the evolution of polyandry as it happens provides three advantages to complement the alternative of examining the selection regime in natural populations. First, we can trace the behavioural changes as they happen in replicate populations. This means we can identify causal agents of the evolution of polyandry, rather than the possible agents of the maintenance of polyandry, including benefits that have arisen after polyandry itself evolved (see above). Second, we can explore the genetic basis of polyandry. For polyandry to evolve, the trait must show heritable variation, as seen in *N. vitripennis* (Shuker et al. 2007). Although heritability was low for female polyandry and courtship duration, it was slightly higher for copulation duration. All three traits had high coefficients of additive genetic variance ($CV_A > 7.0$), suggesting that the additive effects are swamped by the nonadditive variation. Considerable dam effects were also found for all three traits, suggesting either dominance or maternal effects. Future experiments should investigate the number of loci involved and the mutability of the polyandry trait. Heritable variation in polyandry has also been shown in a number of insect species (Torres-Vila et al. 2002; Wedell et al. 2002; Harano & Miyatake 2005), including the honeybee, *Apis mellifera* (Kraus et al. 2005). Third, there is also a growing awareness of the link between mating behaviour, polyandry and speciation (Parker & Partridge 1998; Gavrilets 2000; Gavrilets et al. 2001; Gavrilets & Waxman 2002; Martin & Hosken 2004). *Nasonia vitripennis* is sympatric with two congeners that are endemic to North America: *Nasonia longicornis* in the west and *Nasonia giraulti* in the east. Partial prezygotic reproductive isolation occurs in all three species as a result of behavioural differences in courtship (Van den Assem & Werren 1994; Bordenstein et al. 2000; Beukeboom & Van den Assem 2001, 2002) and mating system (Drapeau & Werren 1999; Leonard & Boake 2006). This

raises the possibility of testing how varying levels of polyandry in *N. vitripennis* influence the degree to which it is prezygotically reproductively isolated from *N. giraulti* and *N. longicornis*.

Acknowledgments

We are very grateful to the BBSRC, NERC and the Royal Society for their kind funding. We are also very grateful to Marcel Visser for allowing us to use his nestboxes to collect samples and to Joop Prijs for his substantial help in collecting and maintaining the strains. Many thanks also to Aleta Graham and Gavin Ballantyne for their technical support, to our Honourable Honours Students, N. Bessos and A. J. Phillimore, for their enthusiastic involvement, and to the venerable Dr A. Hoyahoy.

References

- Andersson, M. B. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Arnold, S. J. & Duvall, D. 1994. Animal mating systems: a synthesis based on selection theory. *American Naturalist*, **143**, 317–348.
- Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, **60**, 145–164.
- Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton, New Jersey: Princeton University Press.
- Bernasconi, G. & Keller, L. 2001. Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. *Journal of Evolutionary Biology*, **14**, 186–193.
- Beukeboom, L. W. & Van den Assem, J. 2001. Courtship and mating behaviour of interspecific *Nasonia* hybrids (Hymenoptera, Pteromalidae): a grandfather effect. *Behavior Genetics*, **31**, 167–177.
- Beukeboom, L. W. & Van den Assem, J. 2002. Courtship displays of introgressed, interspecific hybrid *Nasonia* males: further investigations into the 'grandfather effect'. *Behaviour*, **139**, 1029–1042.
- Birkhead, T. R. & Møller, A. P. 1995. Extra-pair copulation and extra-pair paternity in birds. *Animal Behaviour*, **49**, 843–848.
- Blankenborn, W. U., Hosken, D. J., Martin, O. Y., Reim, C., Teuschl, Y. & Ward, P. I. 2002. The costs of copulating in the dung fly *Sepsis cynipsea*. *Behavioral Ecology*, **13**, 353–358.
- Bordenstein, S. R., Drapeau, M. D. & Werren, J. H. 2000. Intraspecific variation in sexual isolation in the jewel wasp *Nasonia*. *Evolution*, **54**, 567–573.
- Carracedo, M. C., Casares, P., Izquierdo, J. I. & Piñeiro, R. 1991. Receptivity and sexual maturation of *Drosophila melanogaster* females in relation to hybridization with *D. simulans* males: a populational analysis. *Animal Behaviour*, **42**, 201–208.
- Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*, **373**, 241–244.
- Daly, M. 1978. Cost of mating. *American Naturalist*, **112**, 771–774.
- Drapeau, M. D. & Werren, J. H. 1999. Differences in mating behaviour and sex ratio between three sibling species of *Nasonia*. *Evolutionary Ecology Research*, **1**, 223–234.
- Drnevich, J. M., Papke, R. S., Rauser, C. L. & Rutowski, R. L. 2001. Material benefits from multiple mating in female mealworm beetles (*Tenebrio molitor* L.). *Journal of Insect Behavior*, **14**, 215–230.
- Evans, J. P. & Magurran, A. E. 2000. Multiple benefits of multiple mating in guppies. *Proceedings of the National Academy of Sciences, U.S.A.*, **97**, 10074–10076.
- Fedorka, K. M. & Mousseau, T. A. 2002. Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour*, **64**, 361–367.
- Fowler, K. & Partridge, L. 1989. A cost of mating in female fruitflies. *Nature*, **338**, 760–761.
- Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature*, **403**, 886–889.
- Gavrilets, S. & Waxman, D. 2002. Sympatric speciation by sexual conflict. *Proceedings of the National Academy of Sciences, U.S.A.*, **99**, 10533–10538.
- Gavrilets, S., Arnqvist, G. & Friberg, U. 2001. The evolution of female mate choice by sexual conflict. *Proceedings of the Royal Society of London, Series B*, **268**, 531–539.
- Grant, B., Burton, S., Contoreggi, C. & Rothstein, M. 1980. Outbreeding via frequency-dependent mate selection in the parasitoid wasp, *Nasonia* (= *Mormoniella*) *vitripennis* Walker. *Evolution*, **34**, 983–992.
- Grant, C. A., Chapman, T., Pomiankowski, A. & Fowler, K. 2005. No detectable genetic correlation between male and female mating frequency in the stalk-eyed fly *Cyrtodiopsis dalmanni*. *Heredity*, **95**, 444–448.
- Halliday, T. & Arnold, S. J. 1987. Multiple mating by females: a perspective from quantitative genetics. *Animal Behaviour*, **35**, 939–941.
- Hamilton, W. D. 1967. Extraordinary sex ratios. *Science*, **156**, 477–488.
- Harano, T. & Miyatake, T. 2005. Heritable variation in polyandry in *Callosobruchus chinensis*. *Animal Behaviour*, **70**, 299–304.
- Holmes, H. B. 1974. Patterns of sperm competition in *Nasonia vitripennis*. *Canadian Journal of Genetics and Cytology*, **16**, 789–795.
- Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, **75**, 21–64.
- Kamimura, Y. 2003. Effects of repeated mating and polyandry on the fecundity, fertility and maternal behaviour of female earwigs, *Euborellia plebeja*. *Animal Behaviour*, **65**, 205–214.
- King, B. H., Grimm, K. M. & Reno, H. E. 2000. Effects of mating on female locomotor activity in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Environmental Entomology*, **29**, 927–933.
- Konior, M., Radwan, J. & Kolodziejczyk, M. 2001. Polyandry increases offspring fecundity in the bulb mite. *Evolution*, **55**, 1893–1896.
- Kraus, F. B., Neumann, P. & Moritz, R. F. A. 2005. Genetic variance of mating frequency in the honeybee (*Apis mellifera* L.). *Insectes Sociaux*, **52**, 1–5.
- LaMunyon, C. W. 1997. Increased fecundity, as a function of multiple mating, in an arctiid moth, *Utetheisa ornatrix*. *Ecological Entomology*, **22**, 69–73.
- Leonard, J. E. & Boake, C. R. B. 2006. Site-dependent aggression and mating behaviour in three species of *Nasonia* (Hymenoptera: Pteromalidae). *Animal Behaviour*, **71**, 641–647.
- Lopez-Arroyo, J. I., Tauber, C. A. & Tauber, M. J. 1999. Intermittent oviposition and remating in *Ceraeochrysa cincta* (Neuroptera: Chrysopidae). *Annals of the Entomological Society of America*, **92**, 587–593.
- Mackay, T. F. C., Heinsohn, S. L., Lyman, R. F., Moehring, A. J., Morgan, T. J. & Rollmann, S. M. 2005. Genetics and genomics of *Drosophila* mating behavior. *Proceedings of the National Academy of Sciences, U.S.A.*, **102**, 6622–6629.
- McRobert, S. P., Schnee, F. B. & Tompkins, L. 1995. Selection for increased female sexual receptivity in raised stocks of *Drosophila melanogaster*. *Behavior Genetics*, **25**, 303–309.
- Maklakov, A. A. & Lubin, Y. 2004. Sexual conflict over mating in a spider: increased fecundity does not compensate for the costs of polyandry. *Evolution*, **58**, 1135–1140.

- Martin, O. Y. & Hosken, D. J.** 2003. Costs and benefits of evolving under experimentally enforced polyandry or monogamy. *Evolution*, **57**, 2765–2772.
- Martin, O. Y. & Hosken, D. J.** 2004. Reproductive consequences of population divergence through sexual conflict. *Current Biology*, **14**, 906–910.
- Parker, G. A. & Partridge, L.** 1998. Sexual conflict and speciation. *Philosophical Transactions of the Royal Society of London, Series B*, **353**, 261–274.
- Piñeiro, R., Carracedo, M. C., Izquierdo, J. I. & Casares, P.** 1993. Bidirectional selection for female receptivity in *Drosophila melanogaster*. *Behavior Genetics*, **23**, 77–83.
- Rowe, L., Arnqvist, G., Sih, A. & Krupa, J.** 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in Ecology and Evolution*, **9**, 289–293.
- Saul, G. B. & Kayhart, M.** 1956. Mutants and linkage in *Mormoniella*. *Genetics*, **41**, 930–937.
- Sgro, C. M. & Partridge, L.** 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *American Naturalist*, **156**, 341–353.
- Shuker, D. M. & Day, T. H.** 2001. The repeatability of a sexual conflict over mating. *Animal Behaviour*, **61**, 755–762.
- Shuker, D. M. & Day, T. H.** 2002. Mate sampling and the sexual conflict over mating in seaweed flies. *Behavioral Ecology*, **13**, 83–86.
- Shuker, D. M., Phillimore, A. J., Burton-Chellew, M. N., Hodge, S. E. & West, S. A.** 2007. The quantitative genetic basis of polyandry in the parasitoid wasp, *Nasonia vitripennis*. *Heredity*, **98**, 69–73.
- Singh, S. R. & Singh, B. N.** 2001. Female remating in *Drosophila ananassae*: bidirectional selection for remating speed. *Behavior Genetics*, **31**, 361–370.
- Stockley, P. & Seal, N. J.** 2001. Plasticity in reproductive effort of male dung flies (*Scatophaga stercoraria*) as a response to larval density. *Functional Ecology*, **15**, 96–102.
- Stutt, A. D. & Siva-Jothy, M. T.** 2001. Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 5683–5687.
- Thornhill, R. & Alcock, J.** 1983. *The Evolution of Insect Mating Systems*. Cambridge, Massachusetts: Harvard University Press.
- Torres-Vila, L. M., Gragera, J., Rodriguez-Molina, M. C. & Stockel, J.** 2002. Heritable variation for female remating in *Lobesia botrana*, a usually monandrous moth. *Animal Behaviour*, **64**, 899–907.
- Tregenza, T. & Wedell, N.** 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution*, **52**, 1726–1730.
- Tregenza, T. & Wedell, N.** 2002. Polyandrous females avoid costs of inbreeding. *Nature*, **415**, 71–73.
- Vahed, K.** 1998. The function of nuptial feeding in insects: review of empirical studies. *Biological Reviews*, **73**, 43–78.
- Van den Assem, J. & Feuth-de Bruijn, E.** 1977. Second matings and their effect on sex-ratio of offspring in *Nasonia vitripennis* (Hymenoptera-Pteromalidae). *Entomologia Experimentalis et Applicata*, **21**, 23–28.
- Van den Assem, J. & Jachmann, F.** 1999. Changes in male perseverance in courtship and female readiness to mate in a strain of the parasitic wasp *Nasonia vitripennis* over a period of 20+ years. *Netherlands Journal of Zoology*, **49**, 125–137.
- Van den Assem, J. & Werren, J. H.** 1994. A comparison of the courtship and mating behavior of 3 species of *Nasonia* (Hymenoptera, Pteromalidae). *Journal of Insect Behavior*, **7**, 53–66.
- Van den Assem, J., Gijswijt, M. J. & Nubel, B. K.** 1980a. Observations on courtship strategies and mating strategies in a few species of parasitic wasps (Chalcidoidea). *Netherlands Journal of Zoology*, **30**, 208–227.
- Van den Assem, J., Jachmann, F. & Simbolotti, P.** 1980b. Courtship behavior of *Nasonia vitripennis* (Hym, Pteromalidae): some qualitative, experimental evidence for the role of pheromones. *Behaviour*, **75**, 301–307.
- Wedell, N. & Karlsson, B.** 2003. Paternal investment directly affects female reproductive effort in an insect. *Proceedings of the Royal Society of London, Series B*, **270**, 2065–2071.
- Wedell, N., Wiklund, C. & Cook, P. A.** 2002. Monandry and polyandry as alternative lifestyles in a butterfly. *Behavioral Ecology*, **13**, 450–455.
- Whiting, A. R.** 1967. Biology of parasitic wasp *Mormoniella vitripennis* [= *Nasonia brevicornis*] (Walker). *Quarterly Review of Biology*, **42**, 333–406.
- Whiting, P. W.** 1950. Mutant types in *Mormoniella*. *Genetics*, **35**, 140.
- Whiting, P. W.** 1954. Comparable mutant eye colours in *Mormoniella* and *Pachycrepoideus*. *Evolution*, **8**, 135–147.
- Worden, B. D. & Parker, P. G.** 2001. Polyandry in grain beetles, *Tenebrio molitor*, leads to greater reproductive success: material or genetic benefits? *Behavioral Ecology*, **12**, 761–767.