

ORIGINAL ARTICLE

The quantitative genetic basis of polyandry in the parasitoid wasp, *Nasonia vitripennis*

DM Shuker, AJ Phillimore, MN Burton-Chellew, SE Hodge and SA West

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

Understanding the evolution of female multiple mating (polyandry) is crucial for understanding sexual selection and sexual conflict. Despite this interest, little is known about its genetic basis or whether genetics influences the evolutionary origin or maintenance of polyandry. Here, we explore the quantitative genetic basis of polyandry in the parasitoid wasp *Nasonia vitripennis*, a species in which female re-mating has been observed to evolve in the laboratory. We performed a quantitative genetic experiment on a recently collected population of wasps. We found low heritabilities of female polyandry (re-mating frequency after 18 h), low

heritability of courtship duration and a slightly higher heritability of copulation duration. However, the coefficients of additive genetic variance for these traits were all reasonably large ($CV_A > 7.0$). We also found considerable dam effects for all traits after controlling for common environment, suggesting either dominance or maternal effects. Our work adds to the evidence that nonadditive genetic effects may influence the evolution of mating behaviour in *Nasonia vitripennis*, and the evolution of polyandry more generally. *Heredity* (2007) 98, 69–73. doi:10.1038/sj.hdy.6800897; published online 20 September 2006

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Introduction

The evolution of female multiple mating is a puzzle in species in which males provide little or no resources apart from sperm (Ridley, 1988; Andersson, 1994). In many cases female fertility is maximized by a limited number of copulations (Arnqvist and Nilsson, 2000). However, the emerging picture from a wide range of species is that females mate more frequently than necessary for full fertility, risking elevated costs associated with numerous matings (Daly, 1978; Chapman *et al.*, 1995; Shuker *et al.*, 2006). Explanations for female multiple mating with different partners (polyandry) have concentrated on either the potential genetic benefits that become available to females by sampling multiple males via copulation (Andersson, 1994; Eberhard, 1996; Jennions and Petrie, 2000; Radwan, 2003; Simmons, 2005), or on the fact that the observed female mating rate might represent a sexual conflict of interests (Parker, 1979; Thornhill and Alcock, 1983; Brown *et al.*, 1997; Rice, 1998; Chapman *et al.*, 2003).

Understanding the evolution of polyandry is therefore important for understanding the role of females in sexual selection, in particular female choice, and for the life history consequences of reproduction. It is also important for understanding the scope of sexual conflict, since polyandry may arise as a consequence of sexual conflict over mating (e.g. Rowe *et al.*, 1994; Shuker and Day, 2001;

Shuker and Day, 2002), but may also be a cause of sexual conflict by forcing males into sperm competition and enabling females to make cryptic mate choices (Parker, 1970; Birkhead and Moller, 1998; Simmons, 2001). Despite the interest in the evolution and maintenance of polyandry, its genetic basis has received less attention. While the genetic basis of mating behaviour has been considered in detail in *Drosophila melanogaster* (e.g. Hall, 1994; Sokolowski, 2001; Mackay *et al.*, 2005), it is only relatively recently that polyandry has been examined genetically in a wider variety of insects (Orthoptera: Solymar and Cade, 1990; Lepidoptera: Torres-Vila *et al.*, 2001; Wedell, 2001; Torres-Vila *et al.*, 2002; Wedell *et al.*, 2002; Coleoptera: Harano and Miyatake, 2005; Hymenoptera: Kraus *et al.*, 2005).

We have recently observed polyandry evolving in laboratory populations of the parasitoid wasp, *Nasonia vitripennis* (Burton-Chellew *et al.*, unpublished data). Freshly collected females from the wild do not readily re-mate (Van den Assem *et al.*, 1980; Van den Assem and Jachmann, 1999), although they are not strictly monandrous. However, the propensity of females to re-mate evolves repeatedly in the laboratory, increasing over time (Van den Assem and Jachmann, 1999). Estimates of levels of polyandry in the wild have not been obtained, although traditionally females have been thought to mate only once, immediately on emergence from their host before dispersing to find oviposition sites (Whiting, 1967). The reasons for this increase in polyandry in *N. vitripennis* are currently not clear, although the changes in polyandry are associated with changes in female mating behaviour (Burton-Chellew *et al.*, unpublished data).

Here, we consider the quantitative genetic basis of polyandry in a recently collected population

Correspondence: Dr DM Shuker, Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, UK.

E-mail: david.shuker@ed.ac.uk

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of *N. vitripennis*, measuring polyandry as the likelihood of re-mating after 18 h. We show that female re-mating behaviour has a low heritability but is influenced by large, significant maternal or nonadditive genetic effects.

Materials and methods

Mating behaviour of *N. vitripennis*

N. vitripennis (Hymenoptera: Pteromalidae) is a gregarious parasitoid of large dipteran pupae (e.g. Calliphoridae and Sarcophagidae: Whiting, 1967). Courtship involves a stereotyped series of events (Barass, 1960). Following contact, the male mounts the female and positions his head over her antennae. Courtship begins with a series of head nods, which continue in a rhythmic display consisting of approx 4–7 nods per series, with a short pause between each series. The first head nod of each series is associated with the release of a mandibular pheromone (Van den Assem *et al.*, 1980). Virgin females generally display receptivity after a short bout of courtship, drawing their antennae forward and arching their abdomens. The male will back up, establish genital contact, and inseminate the female. Copulation typically lasts around 10–15 s after which the male moves back to perform a short bout of postcopulatory courtship before dismounting.

The study population

The laboratory population was generated from founding individuals collected from three different nest boxes from Hoge Veluwe forest, the Netherlands in June 2004. Reciprocal crosses between males and females from each nest box generated nine crosses, replicated 15 times. These 135 mated females were distributed between three stock tubes of hosts (fly pupae). Ten days postoviposition parasitized hosts were removed from and then redistributed back into the tubes at random. Three generations of random mating and host mixing ensued, with approximately 150 females founding each generation, producing an outbred population hereafter referred to as HV04. All cultures were maintained at 25°C with a 18:6 light:dark cycle. Under these conditions the generation time is approximately 14 days.

Quantitative genetic experiment

We performed a standard split-family sib analysis (Falconer and Mackay, 1996; Roff, 1997; Lynch and Walsh, 1998). Virgin wasps were collected from the population culture 2 days before emergence. A large number of parasitized hosts were opened and virgin male and female wasp pupae were mixed with a fine paintbrush. Full-sib/half-sib families were created by making mating groups of one male pupa (sire) with five female pupae (dams). Following emergence, we allowed females to mate for 2 days after which we placed each female individually into a tube with two host pupae, so that larval environments were split within full-sib families. After 2 days of oviposition we removed the female and placed the hosts in separate tubes. We randomly distributed these tubes across test tube racks, so that hosts from the same families were distributed across racks and shelves in the incubator. Additional virgin females from the population culture were maintained separately from the experimental families in order

to generate virgin males for mating trials. Approximately 20 females were placed into a culture tube with around 40 hosts. This was carried out every 3 days generating a continuous supply of inexperienced experimental males.

Two days before offspring emergence, we opened each host and collected five virgin females, yielding 10 experimental daughters per full-sib family. The day following emergence we fed the females with honey solution-soaked filter paper. Before both the primary and secondary mating trials we removed all experimental females and males from the incubator at least 30 min before the experiment took place to allow acclimatization. To standardize light conditions, both primary and secondary matings were observed on a white board illuminated by a Euromex Fiber Optic light source EK-1 at setting eight.

Mating trials

To test female polyandry, each female was given two mating trials, separated by approximately 18 h. We performed all primary matings between 1700 and 2000 hours at ambient temperature (median temperature 23°C). A female was extracted from the test tube and placed in an observation vial with a virgin male already present. We recorded the time in seconds to the onset of courtship, the onset of copulation and the end of copulation. Postcopulatory courtship was allowed after which we removed the female and placed her in a solitary test tube. Any female who had not been courted after 5 min was discarded. From these data we obtained the courtship duration and copulation duration for virgin females. As expected, acceptance rates of the virgin females were uniformly high (97.6% of females mated in the first mating trial).

The re-mating assay took place approximately 18 h later (again median temperature was 23°C). Each female was observed for 10 min or until copulation. For each female we recorded receptivity at first courtship (R_1) and receptivity within 10 min (R_{10}). Any females engaged in courtship at 10 min were allowed to finish courtship and commence copulation if they accepted the male. Any females who had not been courted within the 10 min were not included in the results.

To ensure that the observed mating events did involve sperm transfer, we repeated the mating trials with 46 virgin males and females from the stock culture, giving each female a tube containing three hosts after copulation. Forty-one of the females laid eggs, and all of these females produced daughters, confirming sperm transfer.

Quantitative genetic analysis

In total, 1005 individuals, representing 24 half-sib, and 106 full-sib families were analysed for our four mating traits (courtship duration, copulation duration, R_1 and R_{10}). We obtained variance components from linear mixed models with REML in S-Plus 7 (Insightful Corporation, Seattle, WA, USA). For haplodiploids (Liu and Smith, 2000), the sire variance component consists of additive genetic effects (such that $\sigma_{\text{sire}}^2 = V_A/2$) and the dam within sire component consists of additive, dominance and common environmental components ($\sigma_{\text{dam(sire)}}^2 = V_A/4 + V_D/2 + V_{EC}$). The two measures of polyandry (R_1 and R_{10}) were modelled as threshold characters, assuming continuous variation (liability) on

an underlying scale (Falconer and Mackay, 1996; Roff, 1997; Lynch and Walsh, 1998). The heritability was measured first on the observed (0,1) scale and then converted to an estimate on the underlying scale:

$$h^2 = h_{\text{obs}}^2 [p(1 - p)]/z^2$$

where h_{obs}^2 is the heritability on the observed scale, p is the proportion of females re-mating, and z is the ordinate on the standardized normal curve in the underlying scale corresponding to p , calculated as

$$z = [e^{(-0.5X^2)}]/\sqrt{2\pi}$$

where $X = [\text{sign}(0.5-p)][1.238c(1 + 0.0262c)]$, and $c = \sqrt{-\ln[4p(1-p)]}$.

The approximate standard error is obtained from:

$$\text{s.e.}(h^2) = \text{s.e.}(h_{0,1}^2)[p(1 - p)]/z^2$$

where $\text{s.e.}(h_{0,1}^2)$ is the standard error on the observed scale. The coefficient of additive genetic variation (CV_A) was calculated as $CV_A = 100\sqrt{V_A/\bar{x}}$ (on the observed scale for the polyandry measures) following Houle (1992).

Our heritability estimates for all traits were low, making interpretation of genetic correlations problematic given the necessarily large standard errors (Roff, 1997; Lynch and Walsh, 1998). We therefore only present the phenotypic correlation between courtship duration and copulation duration, and use logistic regression to explore the effect of copulation duration and courtship duration in the first mating trial on the likelihood of re-mating (as both R_1 and R_{10}).

Results

Descriptive statistics

The mean courtship duration was 10.6 ± 0.3 s and the mean copulation duration was 14.9 ± 0.1 s (both $N=1005$). The frequency of re-mating in the second mating trial in response to the first courtship was $R_1=0.46$, and within 10 min was $R_{10}=0.70$.

Quantitative genetic estimates

The genetics of female re-mating were dominated by dam effects (Table 1). The heritabilities for all traits were low ($h^2 < 0.15$), with only copulation duration having significant heritability, although the heritability of R_1 is close to significance. There were significant dam effects for all traits, while common environment variance components were small (Table 1). The coefficients of additive genetic variation, however, suggest there is a reasonable amount of additive genetic variation swamped by the nonadditive variation for all four traits ($CV_A = 7.01-19.98$; Table 2).

Phenotypic relationships between traits

Females that were courted for longer also tended to copulate for longer. There was a weak but highly significant positive phenotypic correlation between courtship duration and copulation duration; however, the correlation explained very little of the variation in the data ($r_{1003} = 0.104$, $P = 0.0009$, $R^2 = 1.1\%$). Aspects of the first mating trial had at best only weak influence on female re-mating. There were weak negative relationships between courtship or copulation duration and

Table 1 Variance components and estimates of the heritability through the sire and dam for the four traits with their standard errors ($N = 1005$ individuals, from 106 dams and 24 sires)

	Sire	Dam	Host	Residual
<i>Variance components</i>				
R_1	0.00829	0.02183	0.00464	0.21442
R_{10}	0.00452	0.02496	0.00001	0.18154
Courtship duration	1.22871	4.59652	2.97146	76.00258
Copulation duration	0.55618	0.99172	0.27165	5.57319
<i>Heritability</i>				
	Sire	s.e.	Dam	s.e.
R_1^a	0.105	0.060	0.553	0.168
R_{10}^a	0.074	0.052	0.820	0.206
Courtship duration	0.029	0.026	0.217	0.093
Copulation duration	0.150	0.063	0.537	0.126

Heritabilities significant at the 5% level are highlighted in bold.

^aHeritabilities presented on the underlying scale.

Table 2 Coefficients of additive genetic variation (CV_A : following Houle, 1992) for the four traits

Trait	R_1	R_{10}	Courtship duration	Copulation duration
CV_A	19.98	9.61	7.01	7.43

CV_A s calculated using the sire estimate of the additive genetic variance.

Table 3 Phenotypic relationships between female polyandry (R_1 and R_{10}) and courtship duration and copulation duration analysed via logistic regression

Polyandry	Behaviour	β (s.e.)	χ^2	P	R^2 (%)
R_1	Courtship	-0.021 (0.008)	8.06	0.005	0.6
R_1	Copulation	-0.006 (0.023)	0.06	0.81	0.0
R_{10}	Courtship	-0.015 (0.007)	4.09	0.04	0.3
R_{10}	Copulation	-0.056 (0.025)	5.09	0.02	0.4

β (s.e.) is the logistic regression coefficient (standard error). Significance tested via likelihood χ^2 -tests.

R_1 or R_{10} , all of which explained less than 1% of the variation in likelihood of re-mating (Table 3). The most convincing relationship was that between courtship duration and receptivity at first courtship (logistic regression coefficient: $\beta = -0.021 \pm 0.008$, $\chi^2 = 8.06$, $P = 0.005$, $R^2 = 0.6\%$).

Discussion

The genetic basis of polyandry in our experiment was dominated by nonadditive genetic or maternal effects. Despite nontrivial coefficients of additive genetic variation, heritabilities of female re-mating and copulation and courtship duration were therefore low. Behavioural traits typically exhibit low heritabilities (Mousseau and Roff, 1987; Merila and Sheldon, 2000; Boake *et al.*, 2002) and this is often attributed to the inherent variability of behaviours, especially when individuals are interacting. Here, we have both residual environmental variation (after experimentally and statistically limiting common environmental effects) and substantial nonadditive genetic effects (Table 1). These latter effects could include

both dominance and/or some other maternally inherited variation (Meffert *et al.*, 2002). While we have no specific estimates for the dominance variance component for these female mating behaviours, they would have to be larger than average for behavioural traits to explain all the nonadditive variation (e.g. Crnokrak and Roff, 1995; Roff, 1997; Stirling *et al.*, 2002), suggesting that the full-sib variances are influenced by some maternal effects as well. However, dominance effects have recently been shown to influence variation in polyandry in the beetle *Callosobruchus chinensis* (Harano and Miyatake, 2005) and aspects of courtship behaviour in houseflies (Meffert and Hagenbuch, 2005). Dominance may also play a part in maintaining heritable variation in re-mating rate in the wild (Crnokrak and Roff, 1995).

How might a maternal effect arise in *N. vitripennis*? Even though there is no parental care, females may influence variation in offspring phenotypes via their energetic investment in eggs or by influencing the degree of larval competition within a host via clutch size (Godfray, 1994). Our split-family design should have minimized the latter, but we do not know how females vary in their patterns of egg investment (although recent work suggests that egg size variation does not have a large effect on larval performance in *N. vitripennis*: Lalonde, 2005). In addition, female venom mobilizes host lipid reserves into the host's haemolymph (Rivers, 2004), providing another possible source of maternal effect. Other nonadditive genetic effects influencing *Nasonia* mating behaviour have also been uncovered in the context of the courtship behaviour of interspecific male hybrids (Beukeboom and Van den Assem, 2001; Beukeboom and Van den Assem, 2002). In a series of experiments, hybrid males tended to resemble their maternal grandfathers in terms of courtship phenotype (since males are haploid, they do not have paternal grandfathers, only maternal grandfathers). While the mechanistic basis for this effect is still unclear, one possibility is that grand-paternally imprinted genes influence grand-offspring behaviour. It remains to be seen whether any of our nonadditive effects could have a similar basis (see also Mills and Moore, 2004).

As with all heritability studies, specific estimates for polyandry will vary with the environment and also the explicit experimental protocol (Falconer and Mackay, 1996). However, our results are consistent with other recent work, despite the fact that most studies have not specifically controlled for nonadditive effects. For example, broad-sense heritabilities in excess of 0.4 have been found in crickets, butterflies and moths (Solymar and Cade 1990; Torres-Vila *et al.*, 2001, 2002; Wedell *et al.*, 2002) and our estimates of polyandry heritability via dams are likewise large and significant. Our data also confirm that species deemed to be for the most part monandrous can have segregating additive genetic variation in re-mating rate (i.e. the moth *Lobesia botrana*; Torres-Vila *et al.*, 2002). While the evolutionary changes in female re-mating frequency observed in the laboratory attest to this variation, our results suggest that nonadditive effects may have contributed to this response and influenced the rate of evolutionary response (Burton-Chellew *et al.*, unpublished data).

Phenotypically the four mating traits we scored were fairly independent of each other, with only weak correlations between them. While there seems to be a

general trend for females that took longer to court or copulate to be less likely to re-mate, it does not explain much of the variation in female polyandry. Such as it is, the trend may reflect overall female receptivity. However, there could also be a mechanistic or sexual selection component. For example, female re-mating could depend on some aspect of first or second male quality, such as the volume of sperm transferred. We have not explicitly considered the role of males here, but sperm can be an important limiting factor on female fitness in parasitoid wasps (e.g. Godfray, 1994; Hardy *et al.*, 1998; West *et al.*, 1998; West *et al.*, 1997). Males may vary in the numbers of sperm they transfer (Henter, 2004; Shuker *et al.*, 2006) and males of some species even continue to copulate when they are sperm-depleted (Damiens and Boivin, 2006). We have some evidence that female re-mating *per se* is mechanistically independent of male mating partner (Burton-Chellew *et al.*, unpublished data), but in broader terms we may expect aspects of male and female mating behaviour to coevolve, with female multiple mating becoming more likely if males are selected to mate beyond their ability to successfully inseminate females. We are currently exploring these aspects of male mating competitiveness in *Nasonia* and how they influence female behaviour and reproductive allocation.

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