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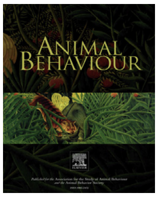

Lifetime changes in phenotypic expression and evolutionary potential of female mating traits in *Drosophila melanogaster*

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Recognition of the ubiquity of female multiple mating has caused an important shift in sexual selection research, emphasizing the adaptive nature of female mating strategies. While phenotypic changes in female mating traits have been previously studied, little is known about the genetic basis of female mating behaviour and its potential to respond to selection at different stages throughout an individual's life. Using a large quantitative genetic breeding design, we observed lifetime female mating behaviour in *Drosophila melanogaster* to examine the effect of female age and mating history on three key mating traits: courtship latency, mating latency and copula duration. Courtship latency (time until males initiate courtship) decreased with the cumulative number of females' previous matings. Mating latency (defined here as the time between the beginning of courtship and the start of copulation) increased with female age, and copula duration was found to decrease as females aged. We calculated quantitative genetic estimates for mating traits in virgin females and at the females' third mating to examine changes in the evolutionary potential of mating traits. We found considerable additive genetic variation in courtship latency and mating latency measured in virgin females. Copula duration displayed no heritable variation among females across sire families, but male effects were consistent with the idea that this trait is under male control. Heritability estimates differed significantly from zero in virgin females for courtship latency and mating latency but not when females were mating for the third time. However, overlapping 84% confidence intervals between heritability estimates obtained from virgin and mated females suggest that female mating strategies may have the potential to respond to selection at these different life stages.

Males and females are characterized by a fundamental difference in reproductive strategies. Owing to differences in the size of gametes (anisogamy), it has traditionally been assumed that female fitness is determined largely by the number of gametes produced and much less by the number of mates, while male fitness depends on the male's ability to gain access to multiple females (Bateman, 1948; Trivers, 1972). This results in higher variance in reproductive success among males than females, and hence stronger sexual selection acting on males (Bateman, 1948; Trivers, 1972). Consequently, much theoretical and empirical work has focused on investigating the fitness consequences of variation in male reproductive strategies (Andersson, 1994; Simmons, 2001), while less attention has been given to the evolution of female mating strategies (Jennions & Petrie, 1997; Pomiankowski, Iwasa, & Nee, 1991). In recent decades, the ubiquity of female multiple mating (polyandry) has been recognized, promoting studies of the benefits of multiple mating to females (Garcia-Gonzalez & Simmons, 2005; Jennions & Petrie, 2000; Newcomer, Zeh, & Zeh, 1999; Slatyer, Mautz, Backwell, & Jennions, 2012). Increasing evidence for such benefits challenges the traditional view of sex roles (Rosvall, 2011) and is driving a shift in how we view female sexual behaviour, with a greater focus on the adaptive function of female behaviour and morphology (Pizzari & Wedell, 2013). Nevertheless, investigation of the evolution of female mating traits remains much less intensely studied than male traits (Bakker, 1993; Bakker & Pomiankowski, 1995; Jennions & Petrie, 1997; Narraway, Hunt, Wedell, & Hosken, 2010; Qvarnström, Brommer, & Gustafsson, 2006; Sharma, Tregenza, & Hosken, 2010; Wagner, 1998).

If female mating strategies are adaptive, differences in strategies are predicted because the costs and benefits of mate choice can vary both between females and within individual females over their lifetime (Kodric-Brown & Nicoletto, 2001). It is well known that many environmental and developmental factors influence

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aspects of female mating behaviour, such as predation risk (Atwell & Wagner, 2015; Forsgren, 1992; Godin & Briggs, 1996), diet (Fox & Moya-Larano, 2009; Hebets, Wesson, & Shamble, 2008; Hunt, Brooks, & Jennions, 2005) and experience gained from previous interactions with males (Collins, 1995; Marler, Foran, & Ryan, 1997; Stoffer & Uetz, 2015). One of the most studied influences on female mating preference is age. Owing to the decline in reproductive potential associated with increasing age, life history theory predicts that current reproductive investment will increase as life expectancy decreases (Charlesworth & Leon, 1976; Clutton-Brock, 1984; Stearns, 1992; Williams, 1966). Thus, age-related changes in reproductive potential may result in phenotypic changes in mating strategies at different life stages. Studies across a number of species including Mediterranean fruit flies (Anjos-Duarte, Costa, & Joachim-Bravo, 2011), crickets (Gray, 1999; Mautz & Sakaluk, 2008; Prosser, Murray, & Cade, 1997), cockroaches (Moore & Moore, 2001), wolf spiders (Wilgers & Hebets, 2012) and guppies (Kodric-Brown & Nicoletto, 2001) have found that female choosiness declines with age. This supports the life history prediction of reduced selectivity in older females. In addition to age, previous experience can influence features of current mating behaviour. Courtship experience can affect a female's likelihood of accepting a mate (Collins, 1995; Dukas, 2005b; Stoffer & Uetz, 2015) and female sexual receptivity commonly decreases after mating (Chapman, 2001; Gioti et al., 2012; Kubli, 2008; Manning, 1967; Ortigosa & Rowe, 2003; Peretti & Carrera, 2005; Ringo, 1996).

Judge, Tran, and Gwynne (2010) examined the relative effects of age and mating on female choosiness in field crickets, and found that female mating status had a stronger effect on female selectivity than age, with virgin females being less choosy. Thus, social experience and environmental variables are likely to be important determinants of variation in female mating traits and could influence their potential to respond to selection. When female age and previous mating experience interact, there could be trade-offs between the expected decrease in choosiness arising from lowered reproductive potential and the potential increase in choosiness due to previous matings or when previous sperm are stored and still available for future fertilizations.

Importantly, to understand the potential for and the constraints on the evolution of sexually selected traits, knowledge of the extent of genetic variation in female mating behaviour is essential. Findings from studies investigating phenotypic changes in female mating traits over the life span demonstrate plasticity in female choosiness (Anjos-Duarte et al., 2011; Gray, 1999; Kodric-Brown & Nicoletto, 2001). However, the influence of factors such as age and mating history on the evolvability of female mating strategies is unknown because genetic estimates of behavioural traits, and of their potential to respond to selection, are often based on single time points (Hoffmann, 1999). Investigating the influence of age and mating history on the evolvability of female mating traits is important to determine whether the response to selection changes over the life span. In species with repeated or continuous reproduction, focusing on a single time point could lead to inaccurate extrapolation of evolvability to different life stages.

Here, we investigated genetic and environmental sources of variation in female mating behaviours in a population of *Drosophila melanogaster* recently derived from the field. Specifically, using longitudinal observations, we first investigated the effects of female age and previous mating history on female mating behaviour. The traits we examined include courtship latency, mating latency and copula duration. We then investigated the genetic basis of these traits in females by calculating quantitative genetic estimates for all mating traits for virgin females and for females at their third mating. By doing so, we examined both phenotypic variation in female mating behaviour over the life span as well as changes in the evolvability of traits from virgin to previously mated females.

METHODS

Breeding Design

Focal flies came from a laboratory population of sixth generation descendants of wild type (*wt*) *D. melanogaster* collected near Innisfail in Northern Queensland, Australia. We used a full-sib half-sib breeding design to quantify genetic variation in aspects of female mating behavioural traits. Mating traits were recorded for 765 daughters distributed among 70 sire families and 198 dam families. To produce parents of focal females, we collected larvae from a population cage of *wt* flies and raised them at a standard density of 50 larvae per vial. Virgin offspring were collected at peak eclosion and kept in single-sex vials with 10 males per vial and five females per vial. Each male was mated to three virgin females to generate families of paternal half-siblings. ‘Dam families’ comprised four female offspring produced by a sire with a single dam (full-sib). ‘Sire families’ comprised 12 paternal half-siblings produced by a sire across three dams (half-sib). Four virgin female offspring (daughters) from each full-sibling dam family were randomly collected. We also collected four additional females from each dam family that were frozen to later estimate full-sibling dam family averages for female body size. Egg, larvae and adult flies were maintained at 25 °C on a 12:12 h light:dark cycle throughout the experiment.

Ethical Note

No ethical approval was required for the study.

Female Mating Behaviours

Mating opportunities for daughters began at 5 days of age. All daughters were kept in individual vials with 10 ml of sugar–maize medium and transferred to fresh food vials every week. Each of these females was given a mating opportunity with a sexually naïve male from an isogenic line (see below for details regarding the generation of isogenic lines) every Monday, Wednesday and Friday over her entire life span (Fig. 1). On each of these days, half of the families were measured in the morning (1000 hours) and half in the afternoon (1400 hours). The time of day was alternated between mating opportunities for each family. All matings were carried out in the same temperature- and humidity-controlled environmental chamber which minimized variation in environmental conditions between mating opportunities. At the beginning of each mating opportunity, males were carefully aspirated into the female's vial. We then observed the time from the male's placement in a female's vial until the initiation of courtship towards that female (courtship latency), the time between the beginning of courtship until copula started (mating latency) and copula duration. Behaviours were recorded by continuous scan sampling and all males were removed via aspiration from the vial after 1 h. Female longevity was assessed before the beginning of each mating opportunity and death was determined by lack of movement.

The empirical investigation of female mating traits is problematic because they are likely to be influenced by male phenotype; both genotypic and environmental male effects can influence the expression of female behaviour (Ahuja & Singh, 2008; Bacigalupe, Crudgington, Jon, Moore, & Snook, 2008; Ferveur, 2005; Moore, Brodie, & Wolf, 1997; Partridge, Hoffmann, & Jones, 1987; Wolfner, 1997, 2002). To account for male effects on female mating traits, we standardized male identity in each mating