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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

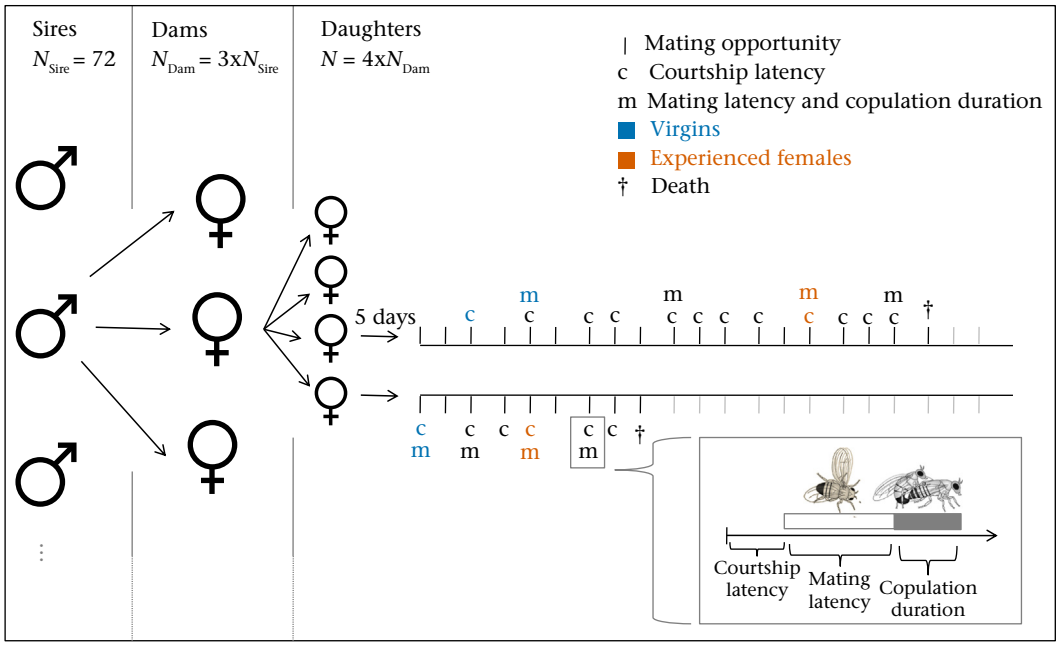

Figure 1. Full-sib half-sib breeding design and experimental mating design. Seventy-two sires were mated to three dams each. From every full-sib family, four daughters were offered a mating opportunity three times per week for their entire life span, starting at an age of 5 days. Two daughters are shown for illustrative purposes. Courtship latency, mating latency and copulation duration (see box inlet and main text for details) were recorded whenever they occurred. For the phenotypic analyses of mating behaviours, we included all recorded behaviours. For the quantitative genetic analyses, we included behaviours when they first occurred (virgin females: blue) or at a female's third mating (previously mated females: brown). As a consequence, there was substantial variation both between and within the two subsets. Fly illustrations adapted from Sokolowski (2001) with permission from Nature Publishing Group.

opportunity by using males from one of 10 isogenic lines (Garcia-Gonzalez & Evans, 2011; Travers, Garcia-Gonzalez, & Simmons, 2015; Travers, Simmons, & Garcia-Gonzalez, 2016). We generated each isogenic line via a full-sibling mating protocol which was initiated with one founder pair of flies taken from a replica of an LH_M population (see Byrne & Rice, 2005 for details). First, full-sibling matings were conducted for 16 generations, followed by several generations of within-line matings where approximately 15 individuals from each line were used to start each new generation. We then conducted a further 21 generations of full-sibling matings. Each isogenic line was mass bred in individual population cages before the beginning of the experiment to allow the generation of sufficient numbers of flies for the mating trials. For each mating opportunity, we collected larvae from population cages on grape agar plates and transferred larvae into food vials. Sexually naïve males were collected 9–11 days later and kept in groups of 10 in 10 ml food vials. Males were 2–3 days old when used in mating trials. To standardize males in each mating opportunity, we randomly selected males from the same isogenic line for each mating opportunity (e.g. isogenic line 1 in the first opportunity for all females, isogenic line 2, 3, 4, etc. for the 2nd, 3rd, 4th, etc. mating opportunity).

Statistical Analyses

Phenotypic variation across the life span

Our analyses examined the effect of female age and number of previous matings on the following: 719 females over 5066 mating trials for courtship latency, 631 females over 1545 mating trials for mating latency and 572 females over 1193 events for copula duration. Linear mixed models (LMMs) using the square root-transformed courtship latency, mating latency and copula duration were fitted using the lme4 package (Bates Maechler, Bolker, & Walker, 2014) in R, version 3.1.2 (R Core Team, 2015). Response variables were transformed to satisfy assumptions of normal and homoscedastic residuals. We included female age (≈mating opportunity number), cumulative number of matings, body size, time of day of mating trial and the number of mating opportunities since the female last mated as covariates. We also included the interaction between female age and cumulative number of matings. As a consequence of including time since the female's last mating as a covariate, we excluded virgin females from the phenotypic analysis as virgins do not have any previous matings. In our analysis of courtship latency, we included an additional covariate to test whether courtship latency differed between trials where females mated or not. To account for repeated measures of the same females over multiple mating events, individual female ID was included as a random effect, along with isogenic male line ID in all models. When testing for interactions, we included individual-specific random slopes to avoid overconfidence in interaction estimates (Schielzeth & Forstmeier, 2009). Significance of fixed effects was tested using Wald chi-square tests implemented in the Anova function of the car package (Fox & Weisberg, 2011). Nonsignificant fixed effects were excluded from the final models.

Quantitative genetic analyses

For the genetic analyses of the three mating traits in females, we first calculated quantitative genetic estimates for virgin females using the first occurrence of each behaviour in a female's lifetime. For the analyses of behaviours in previously mated females, we used a subset that included behaviours measured at the females' third mating. Thus, the two subsets differed not only in female mating history, but also with respect to average female age (see Fig. 1 for a schematic overview of the measurements obtained for genetic analyses). Within the two subsets (virgin and previously mated), there was also variation in age, as not all females were courted or mated in their first mating opportunity in the virgin subset, and similarly, there was variation in the mating opportunity

at which females mated for the third time. The average female age of virgins was 5 days for courtship latency, and 6 days for mating latency and copula duration. Previously mated females were aged 21 days on average for all three behaviours. We investigated the genetic basis of the traits the third mating in order to include females that had previous mating experience and to ensure a sufficient number of individuals were still alive to obtain an adequate sample size. Sire and dam effects were tested for all three behaviours both in virgin and previously mated females using LMMs on square root-transformed courtship latency, mating latency and copula duration. Visual inspection of the residuals from all models using both untransformed and transformed response variables revealed that the square root transformation satisfied the model assumptions of normal and homoscedastic residuals while the raw data and log transformations did not. The models included sire, dam nested within sire and isogenic male line ID as random effects. Significance of all three random effects was determined using likelihood ratio tests. Female age, body size, mating time of day and number of opportunities since females' last mating were included as fixed effects in all models. Significance of the fixed effects was tested using Wald chi-square tests as described above.

Genetic parameters for the three behavioural traits in virgins and previously mated females were calculated using restricted maximum likelihood (REML) from LMMs on standard nested mixed models for a paternal half-sibling design. LMMs on untransformed data were fitted with sire and dam nested within sire as random effects. We performed the analyses on untransformed data because many genetic parameters (e.g. CV_A and I_A) cannot be used for comparative purposes if variance components are extracted from transformed data (Garcia-Gonzalez, Simmons, Tomkins, Kotiaho, & Evans, 2012). Observational variance components were estimated from minimal models including only significant fixed effects and all the random effects. Narrow sense heritabilities (h^2) of the mating behaviours were estimated from the ratio of additive genetic variance (V_A: four times the sire variance component) to total phenotypic variance. Mean-standardized measures of evolvability were calculated, namely the coefficient of additive genetic variation ($CV_A = \sqrt{V_A}/\bar{x}$ where $\bar{x}$ is the phenotypic mean of the trait) and I_A ($V_A/(\bar{x}^2)$), an estimate of the expected proportional change under a unit strength of selection (Garcia-Gonzalez et al., 2012; Hansen, Pélabon, & Houle, 2011; Houle, 1992). Standard errors for all quantitative genetic parameters were calculated by jackknifing across sire families (Roff, 2006). To test whether heritabilities of mating behaviours in virgin and mated females were significantly different from each other, we calculated 84% confidence intervals around all heritability estimates. In doing so, significance of the difference between the subsets at the 0.05 significance (alpha) level could be detected based on whether the intervals of the virgin and mated females' estimates overlapped (see Goldstein & Healy, 1995). We obtained 84% confidence intervals by multiplying Student's t values for our sample sizes by the standard errors of the heritability estimates.

RESULTS

Phenotypic Variation Across the Life Span

There was substantial variation in female longevity and thus in the number of mating opportunities that females were offered (mean mating opportunities ± SD = 14.96 ± 4.36; range 1–24; mean life span in days after start of experiment ± SD = 39.49 ± 11.74; range 1–62). Examination of the mating traits over the female's life span revealed that courtship latency was significantly affected by cumulative number of previous matings ($\chi^2_1 = 7.62$, $P = 0.006$) with lower courtship latency (males initiating courtship sooner) for females with more previous matings (Fig. 2a). There was no main effect of female age on courtship latency ($\chi^2_1 = 0.07$, $P = 0.789$), but there was a significant interaction between female age and mating experience ($\chi^2_1 = 4.14$, $P = 0.042$), meaning that the negative effect of female mating history on courtship latency became weaker as females aged. We also found a significant effect of number of opportunities since the female last mated, with longer courtship latencies for females that mated more recently ($\chi^2_1 = 9.32$, $P = 0.002$). Finally, we found a significant negative relationship between courtship latency and whether a female mated at that mating opportunity ($\chi^2_1 = 5.30$, $P = 0.021$), meaning that mating did not occur when courtship was initiated later.

While we found no significant effect of cumulative number of previous matings on mating latency ($\chi^2_1 = 0.21$, $P = 0.647$) or time since last mating ($\chi^2_1 = 1.01$, $P = 0.315$), this trait was significantly affected by female age, with older females taking longer to mate from the initiation of courtship ($\chi^2_1 = 17.801$, $P < 0.001$; Fig. 2b).

Copula duration was significantly affected by female age ($\chi^2_1 = 37.00$, $P < 0.001$), with shorter copulation times for older females (Fig. 2c). We also found a significant effect of time since last mating ($\chi^2_1 = 4.32$, $P = 0.038$), with shorter copulation durations in females that mated more recently. No effect of cumulative number of matings was found for copula duration ($\chi^2_1 = 0.14$, $P = 0.706$). We did not find a significant effect of female body size or mating time of day on any of the three traits (all $P > 0.1$). Not surprisingly, we found some evidence for multicollinearity between age, mating history and time since last mating. However, variance inflation factors were moderate, ranging between 1.40 and 3.60 in all models, suggesting that multicollinearity was not an issue in the data analysis (Craney & Surles, 2002).

Quantitative Genetics

Quantitative genetic parameters for all traits for virgin and previously mated females are displayed in Table 1. Sire family means are displayed in Fig. 3.

Courtship latency showed substantial phenotypic variation for both virgins (mean ± SD = 1448 ± 1096 s) and previously mated females at their third mating (mean ± SD = 1135 ± 840 s). We found substantial additive genetic variation and significant sire variance among virgin females but low and nonsignificant additive genetic variation in courtship latency among females at their third mating (see Table 1). However, both when females were virgin and previously mated, courtship latencies were significantly affected by the isogenic line from which males were drawn (virgin: $\chi^2_1 = 65.05$, $P < 0.001$; previously mated: $\chi^2_1 = 13.83$, $P < 0.001$). Neither female age, body size or mating time of day had a significant effect on courtship latency among virgins or previously mated females.

We found large phenotypic variation in mating latency among virgin (mean ± SD = 712 ± 887 s) and previously mated females (mean ± SD = 771 ± 924 s). Mating latency of virgin females showed high levels of additive genetic variation and significant variance across sire families (see Table 1). In contrast, mating latency among mated females exhibited low additive genetic variance and nonsignificant sire effects. Male line ID had a significant effect on mating latency in virgins but not in previously mated females (Table 1). Female age had a significant negative effect on mating latency among virgins ($\chi^2_1 = 7.308$, $P = 0.007$), and mated females ($\chi^2_1 = 4.221$, $P = 0.039$), while female body size and mating time of day had no significant effect.

Copula duration showed lower phenotypic variation among virgin (mean ± SD = 1077 ± 331 s) and previously mated females (mean ± SD = 1024 ± 361 s) than the other two mating traits. In both virgins and mated females, copula duration showed low and nonsignificant levels of additive genetic variation and

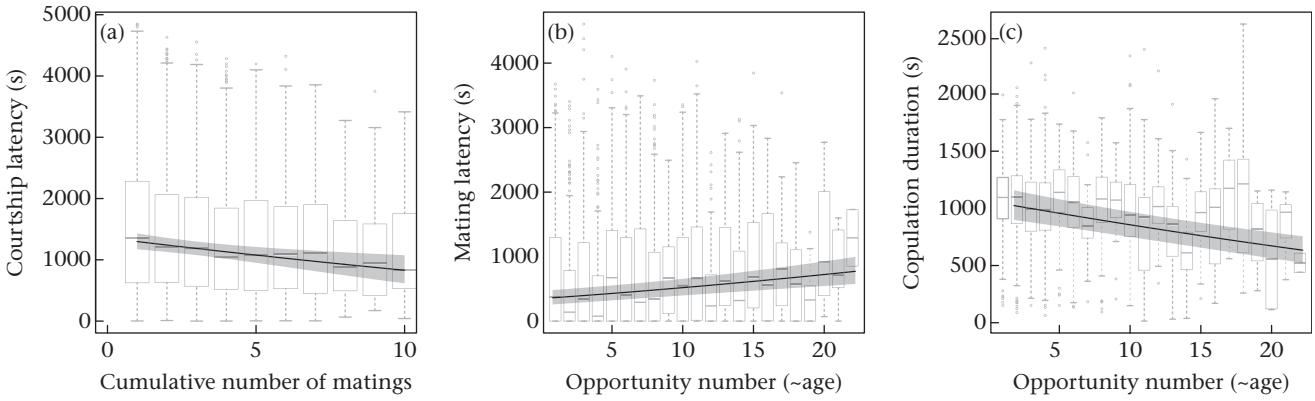

Figure 2. Effects of cumulative number of matings on (a) courtship latency, and of female age on (b) mating latency and (c) copula duration. Boxes and whiskers represent the raw data while lines and shaded areas represent backtransformed LMM predictions for mean effects and approximate 95% confidence intervals, respectively. Box plots show the median, the lower and upper quartiles with whiskers extending to 1.5 times the interquartile range and open circles showing outliers.

nonsignificant sire effects (Table 1). There was significant variation among male isogenic lines for copula duration with virgins ($\chi^2_1 = 51.776$, $P < 0.001$), but not for copula duration with previously mated females ($\chi^2_1 = 0$, $P = 1$). Female age, body size and mating time of day had no effect on copula duration in either virgin or previously mated females.

DISCUSSION

Using data on lifetime mating behaviour of females from a population of *D. melanogaster* recently derived from the wild, we have shown how female mating traits change throughout life in relation to age and mating history. We also examined the evolvability of mating traits in females and how levels of additive genetic variance change from virgin to previously mated females.

How Does Mating Behaviour Change Across the Life Span?

We found significant variation across the life span in all mating traits examined. Females with more previous matings were courted sooner. However, females were slower to accept matings as they aged. We also observed a decrease in copula duration in later life.

The reduction in courtship latency associated with female mating history suggests that male willingness to mate increases with female mating history. Previous studies that have investigated sources of variation in courtship latency have focused mainly on male effects (Dukas, 2005a; Eastwood & Burnet, 1977; Gromko, 1987; Hoffmann, 1999; Moehring & Mackay, 2004). The speed at which a male initiates courtship may reflect precopulatory male choice for variation in female willingness to mate. Here, we showed that a female's mating history can influence the speed at which a male initiates courtship, suggesting important female effects on this mating behaviour.

The relationship between mating latency and female age suggests that a female's resistance to mating increases as she ages. Studies in other species have found the opposite—a decrease in female choosiness with age. For example, in guppies, Kodric-Brown and Nicoletto (2001) found that younger females were more selective than older females. Moore and Moore (2001) found decreased choosiness in female cockroaches, *Nauphoeta cinerea*, when mated past the optimal mating age, and also found a correlation between the reduction in choosiness and fertility, which suggests that a decrease in choosiness may be due to a reduction in reproductive potential. The increase in female resistance to mating (or choosiness) in later life may arise from a shift in the cost–benefit ratio of mating across different life history stages. In *D. melanogaster* mating is costly due to harmful seminal fluid proteins transferred during copulation (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995). Thus, frequent remating by females incurs increasing costs as they suffer reduced survival and hence reduced lifetime reproductive success (Chapman et al., 1995; Fowler & Partridge, 1989). Moreover, as older females are more likely to have mated previously and ensured fertilization of at least part of their lifetime egg supply, they have less to gain from further matings. Therefore, the increased resistance of older females to male courtship attempts may reflect reduced benefits from mating in later life that do not outweigh the costs associated with additional matings. Owing to collinearity between female age and mating history, the effect of female age on mating latency may be somewhat driven by mating history. However, we found no significant effect of mating history on mating latency. To disentangle the effect of age and mating history on female mating latency, a control experiment measuring mating latency in aged virgin females would be required.

We also found that copula duration was shorter with older females. Previous studies have reported that copula duration differed depending on female mating status, but the findings have been inconsistent between studies (Bretman, Fricke, & Chapman, 2009; Singh & Singh, 2004; Sirot, Wolfner, & Wigby, 2011). Our quantitative genetic analysis suggests that copula duration is to a large degree under male control (Friberg, 2006; Lüpold et al., 2013; MacBean & Parsons, 1967); there were no sire effects on copula duration, which depended more on the isogenic line from which males were drawn. Males have been reported to adjust copulatory investments according to the risk and intensity of sperm competition (Bretman, Westmancoat, & Chapman, 2013). Our findings suggest that males may also adjust their copulatory investment according to female age. Specifically, males may invest less refractory-inducing and ovulation-stimulating seminal fluid proteins when mating with older females. An examination of the stages of copulation in *D. melanogaster* by Gilchrist and Partridge (2000) revealed that sperm transfer to the female is complete by the midpoint of copulation and that the remaining copulation time is dedicated solely to the transfer of seminal fluids. Evidence also suggests that males can tailor their ejaculate components to take advantage of ovulin (an ovulation-increasing protein) transferred by previous mates while maintaining investment in sex peptide to inhibit remating (Sirot et al., 2011). Thus, it is possible that males also alter their ejaculate when mating with older females to transfer less seminal fluid proteins (ovulin and/or sex peptide), as

Table 1
Quantitative genetic parameters for mating traits in females

	N	Mean (SD) [s]	n_{sires}	n_{dams}	V_{Sire} (SE)	V_{Dam} (SE)	V_A (SE)	V_P (SE)	V_R (SE)	h^2 (SE)	h^2 84% CI	CV_A (SE)	CV_P (SE)	CV_R (SE)	I_A (SE)	P_{Sire}	P_{Dam}	$P_{isogenic\ line\ male}$
Courtship latency virgins	747	1448 (1096.78)	70	198	64071 (37692)	47845 (36912)	256287 (150770)	982837 (63904)	870919 (57190)	0.261 (0.150)	0.048, 0.474	0.350 (0.103)	0.685 (0.028)	0.589 (0.067)	0.122 (0.070)	0.038*	0.055	<0.001*
Courtship latency third mating	458	1135 (840.06)	70	190	16136 (21430)	10987 (32549)	64545 (85718)	656930 (64938)	629807 (60039)	0.098 (0.132)	−0.089, 0.285	0.224 (0.163)	0.714 (0.026)	0.678 (0.050)	0.050 (0.068)	0.514	0.702	<0.001*
Mating latency virgins	661	712 (887.21)	70	198	39468 (20040)	0	157872 (80162)	743023 (52680)	703554 (52206)	0.212 (0.106)	0.06, 0.363	0.558 (0.0144)	1.211 (0.0435)	1.074 (0.082)	0.311 (0.157)	0.030*	1.000	<0.001*
Mating latency third mating	427	771 (923.53)	70	187	10395 (31137)	36279 (44945)	41582 (124549)	842470 (75739)	795796 (85064)	0.051 (0.150)	−0.162, 0.264	0.265 (0.508)	1.191 (0.049)	1.161 (0.102)	0.070 (0.212)	1.000	0.182	0.278
Copula duration virgins	520	1077 (331.849)	70	193	3437	3447 (5265)	13750 (11151)	93797 (7975)	86883 (9291)	0.147 (0.118)	−0.021, 0.315	0.109 (0.045)	0.284 (0.013)	0.263 (0.021)	0.012 (0.009)	0.565	0.749	<0.001*
Copula duration third mating	330	1024 (360.85)	69	170	10395 (5508)	0	41582 (22033)	842470 (13835)	795796 (14993)	0.050 (0.178)	−0.203, 0.303	0.0210 (0.060)	0.901 (0.021)	0.874 (0.044)	0.041 (0.021)	0.146	1.000	1.000

Number of offspring (N), trait means, number of sire (half-sib) and dam (full-sib) families (n), variance components for sires (V_{Sire}) and dams (V_{Dam}), additive genetic variation (V_A), total phenotypic variation (V_P), residual variation (V_R), narrow sense heritabilities (h^2) and their 84% confidence intervals, mean-standardized additive genetic variances (Evolvabilities: CV_A and I_A), coefficient of phenotypic variation CV_P, and the coefficient of residual variation CV_R are shown. All quantitative genetic parameters were obtained from untransformed LMMs. Significance values for Sire (P_{Sire}), Dam (P_{Dam}) and isogenic male lines ($P_{isogenic\ line\ male}$) effects were calculated from square root transformed LMMS. Standard errors (SE) are provided within brackets. Asterisks indicate significant p-values ($P < 0.05$).

older females are likely to have mated previously and the chance of the female remating decreases due to increased risk of extrinsic mortality. Males may also invest less in older females (Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2011) as the return on their investment will be lower due to reduced egg production in older females (Boorman & Parker, 1976).

Does Additive Genetic Variance in Female Mating Behaviour Change Throughout Life?

The presence of high levels of heritable variation in courtship latency in virgin females reveals that female genotype affects mating behaviour. Markedly, the present study is the first to document the quantitative genetic basis of female effects on courtship latency and indicates that females vary genetically in their receptivity or attractiveness to males. Evidence from previous studies suggests genetic variation in female attractiveness. Ratterman, Rosenthal, Carney, and Jones (2014) found that males ranked attractiveness among different female genotypes in the same order across 10 inbred lines of *D. melanogaster*. Male mate choice for phenotypic indicators of fecundity (e.g. female abdomen width or body size) is predicted due to the associated fitness gains of mating with large females (Ebert, 1993; Gromko, Briot, Jensen, & Fukui, 1991; Lefranc & Bundgaard, 2000). However, we found no effect of female body size on the male's latency to initiate courtship, indicating that genetic variance in female receptivity or attractiveness is not driven by female body size, at least in this population.

We also revealed evidence for a genetic basis for mating latency in virgin females which supports prior findings in this species (Gromko, 1989; Narraway et al., 2010; Sgro, Chapman, & Partridge, 1998). Moore (1989) found evidence for genetic variation in female preference in cockroaches, and a quantitative genetic study in a wild population of flycatchers found significant additive genetic variation in female preference (Qvarnström et al., 2006). Previous studies have also found significant additive genetic variation in female resistance to harm from males and that female reluctance to remate has a positive effect on lifetime fitness (Lew, Morrow, & Rice, 2006; Linder & Rice, 2005). If resistance to mating above the female optimum mating rate is beneficial, directional selection should erode genetic variation in female resistance to male harm. What maintains genetic variance in female mating latency remains unclear. One potential explanation is that variation in female resistance could be maintained if selection acts on males to overcome female resistance. Sexually antagonistic coevolution in mating frequency is well documented in *D. melanogaster*, and studies have demonstrated selection on male ability to coerce females to mate above their optimum rate (Holland & Rice, 1999; Pitnick, Brown, & Miller, 2001; Rice, 1996). Tennant, Sonser, and Long (2014) found a negative covariance between female choosiness and male attractiveness in *D. melanogaster*, consistent with sexual conflict theory. Thus, conflicting selection on mating rates between the sexes could potentially explain the maintenance of high levels of additive genetic variance in female mating latency.

The significant effect of male line on virgin mating latency suggests that males also differ genetically in their attractiveness (Hoffmann, 1999; Hosgood & Parsons, 1965; Mackay et al., 2005; Manning, 1961; Taylor, Wedell, & Hosken, 2007; Wedell & Tregenza, 1999), and supports a previous study which demonstrated that both male and female genotypes contribute to variation in mating latency (Tennant et al., 2014; but see Ratterman et al., 2014).

Our genetic analyses of copula duration revealed a significant effect of male isogenic line when mating with virgin females but no additive genetic variation among females, either when virgin or

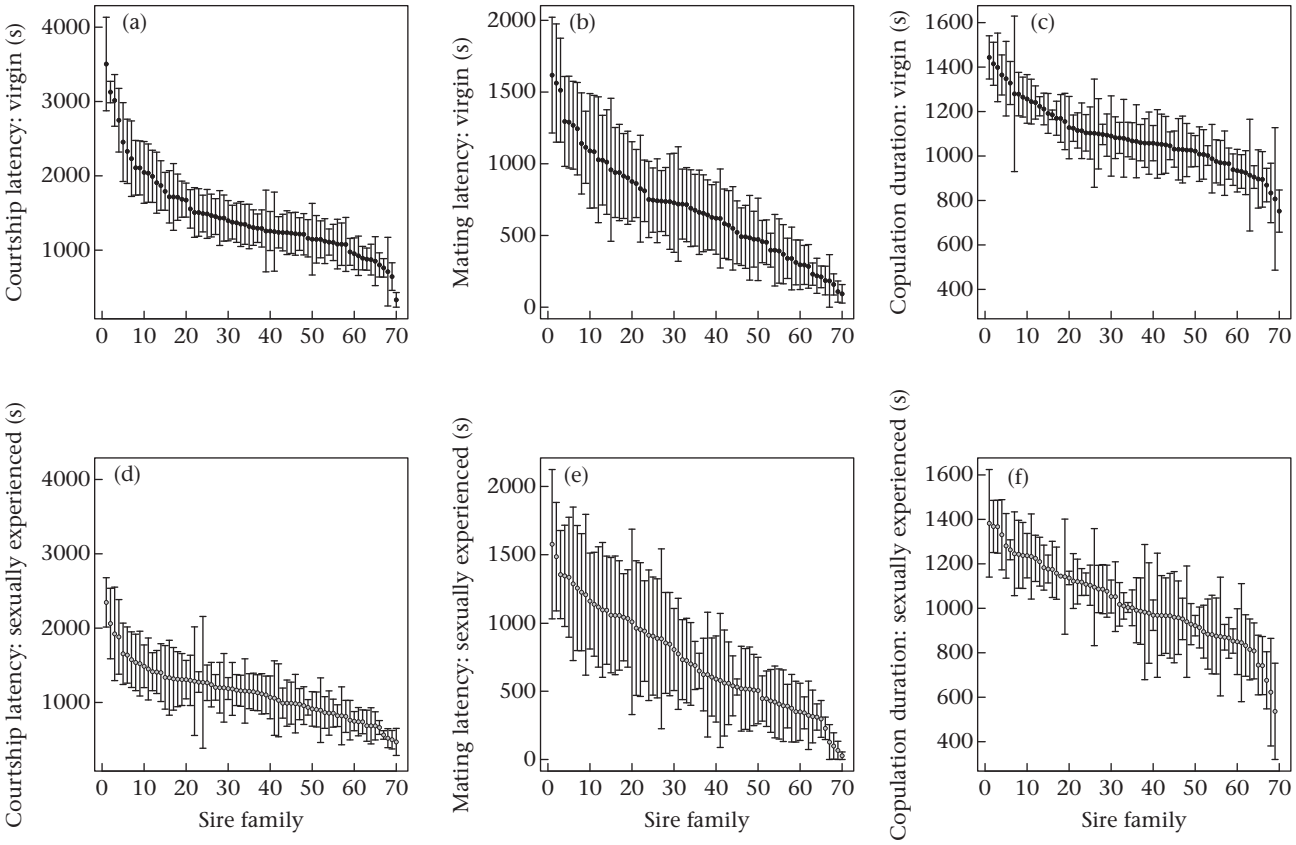

Figure 3. Raw sire family means ± SE of (a, d) courtship latency, (b, c) mating latency and (c, f) copula duration in virgins (black) and previously mated females (grey), respectively. Sire families were sorted by decreasing y values.

previously mated. This finding is consistent with previous evidence for predominantly male control of copula duration (Bretman, Lizé, Walling, & Price, 2014; Bretman et al., 2013; Friberg, 2006; Tennant et al., 2014). Nevertheless, Edward, Poissant, Wilson, and Chapman (2014) suggested that both sexes might contribute to variation in copula duration in *D. melanogaster*, while another study failed to detect significant additive genetic variation in males in the trait across two different environments (Taylor, Evans, & Garcia-Gonzalez, 2013). However, Taylor et al. (2013) were not able to separate male and female effects, and this could have led to a failure to detect additive genetic variance in copula duration.

We found no significant differences between sire families in any of the three traits when measured in the females' third mating. Nevertheless, we are unable to conclude that the levels of heritable variation in the three mating traits are lower for mated than virgin flies because the 84% confidence intervals on the heritability estimates between the two groups of females overlapped, indicating that the estimates do not differ significantly from each other. The failure to detect statistically significant variation among sire families in the females' third mating may have been caused by a loss of statistical power due to the reduction in sample size from virgin females. The statistical power to detect moderate levels of heritability (following Lynch and Walsh's (1998) power analysis for half-sibling breeding designs) for courtship and mating latency in virgins was high (>0.8), while the power to detect a smaller heritability, as found in virgin copula duration ($h^2 = 0.14$), was reduced (power ~ 0.6). Furthermore, the statistical power to detect low heritability estimates such as those found in the mated female subset was low (Lynch & Walsh, 1998). Therefore, we cannot rule out heritable variation in virgin copula duration or in the traits measured in the females' third mating.

We found a significant effect of male line on all three traits when measured in virgin females. Males in this species exert a strong influence on female remating rates through the effects of sex peptide transferred during mating, which inhibits female remating (Wolfner, 1997). Our finding of a significant effect of male line suggests that male effects also influence other components of female mating behaviour. Male effects from previous partners may increase variation in female responses to subsequent potential mates. If so, increased environmental variation in female behaviour may also hinder the ability to detect additive genetic variance in mating behaviours in mated females.

In conclusion, our findings show phenotypic plasticity throughout life in female mating traits. We also found high levels of additive genetic variation in courtship latency and mating latency in virgin females from a population recently derived from the wild, which suggests these traits can respond to selection. Overlapping confidence intervals on heritability estimates in these traits among young virgin females and older mated females suggest that female mating strategies have the potential to respond to selection irrespective of age or mating history.

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