

1 **Title:** Lifetime changes in phenotypic expression and evolutionary potential of female mating
2 traits in *Drosophila melanogaster*.

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15 **Abstract**

16 Recognition of the ubiquity of female multiple mating has evoked an important shift in
17 sexual selection research, emphasising the adaptive nature of female mating strategies. While
18 phenotypic changes in female mating traits have been previously studied, little is known
19 about the genetic basis of female mating behaviour and its potential to respond to selection at
20 different stages throughout an individual's life. Using a large quantitative genetic breeding
21 design, we observed lifetime female mating behaviour in *Drosophila melanogaster* to
22 examine the effect of female age and mating history on three key mating traits: courtship

23 latency, mating latency and copula duration. Courtship latency (time until males initiate
24 courtship) decreased with the cumulative number of females' previous matings. Mating
25 latency (defined here as the time between the beginning of courtship and the start of
26 copulation) increased with female age, and copula duration was found to decrease as females
27 aged. We calculated quantitative genetic estimates for mating traits in virgin females and at
28 the females' third mating to examine changes in the evolutionary potential of mating traits.
29 We found considerable additive genetic variation in courtship latency and mating latency
30 measured in virgin females. Copula duration displayed no heritable variation among females
31 across sire families, but male effects were consistent with the idea that this trait is under male
32 control. Heritability estimates differed significantly from zero in virgin females for courtship
33 latency and mating latency. Heritability estimates did not differ significantly from zero when
34 females were mating for the third time. However, overlapping 84% confidence intervals
35 between heritability estimates obtained from virgin and mated females suggest that female
36 mating strategies may have the potential to respond to selection at these different life stages.

37 *Introduction*

38 Males and females are characterised by a fundamental difference in reproductive
39 strategies. Due to differences in the size of gametes (anisogamy), it has traditionally been
40 assumed that female fitness is determined largely by the number of gametes produced and
41 much less by the number of mates, while male fitness depends on the male's ability to gain
42 access to multiple females (Bateman, 1948; Trivers, 1972). This results in higher variance in
43 reproductive success among males compared to females, and hence stronger sexual selection
44 acting on males (Bateman, 1948; Trivers, 1972). Consequently, much theoretical and
45 empirical work has focused on investigating the fitness consequences of variation in male
46 reproductive strategies (Andersson, 1994; Simmons, 2001), while less attention has been

47 given to the evolution of female mating strategies (Jennions & Petrie, 1997; Pomiankowski,
48 Iwasa, & Nee, 1991). In recent decades, the ubiquity of female multiple mating (polyandry)
49 has been recognised, promoting studies of the benefits of multiple mating to females (F
50 Garcia-Gonzalez & Simmons, 2005; Jennions & Petrie, 2000; Newcomer, Zeh, & Zeh, 1999;
51 Slatyer, Mautz, Backwell, & Jennions, 2012). Increasing evidence for such benefits
52 challenges the traditional view of sex roles (Rosvall, 2011) and is driving a shift in how we
53 view female sexual behaviour, with a greater focus on the adaptive function of female
54 behaviour and morphology (Pizzari & Wedell, 2013). Nevertheless, investigation of the
55 evolution of female mating traits remains much less intensely studied than male traits
56 (Bakker, 1993; Bakker & Pomiankowski, 1995; Jennions & Petrie, 1997; Narraway, Hunt,
57 Wedell, & Hosken, 2010; Qvarnström, Brommer, & Gustafsson, 2006; Sharma, Tregenza, &
58 Hosken, 2010; Wagner, 1998).

59 If female mating strategies are adaptive, differences in strategies are predicted because the
60 costs and benefits of mate choice can vary both between females and within individual
61 females over their lifetime (Kodric-Brown & Nicoletto, 2001). It is well known that many
62 environmental and developmental factors influence aspects of female mating behaviour, such
63 as predation risk (Atwell & Wagner, 2015; Forsgren, 1992; Godin & Briggs, 1996), diet
64 (Fox & Moya-Larano, 2009; Hebets, Wesson, & Shamble, 2008; Hunt, Brooks, & Jennions,
65 2005) and experience gained from previous interactions with males (Collins, 1995; Marler,
66 Foran, & Ryan, 1997; Stoffer & Uetz, 2015). One of the most studied influences on female
67 mating preference is age. Due to the decline in reproductive potential associated with
68 increasing age, life history theory predicts that current reproductive investment will increase
69 as life expectancy decreases (Charlesworth & Leon, 1976; Clutton-Brock, 1984; Stearns,
70 1992; Williams, 1966). Thus, age-related changes in reproductive potential may result in
71 phenotypic changes in mating strategies at different life stages. Studies across a number of

72 species including Mediterranean fruit flies (Anjos-Duarte, Costa, & Joachim-Bravo, 2011),
73 crickets (Gray, 1999; Mautz & Sakaluk, 2008; Prosser, Murray, & Cade, 1997), cockroaches
74 (Moore & Moore, 2001), wolf spiders (Wilgers & Hebets, 2012) and guppies (Kodric-Brown
75 & Nicoletto, 2001) have found that female choosiness declines with age. This supports the
76 life-history prediction of reduced selectivity in older females. In addition to age, previous
77 experience can influence features of current mating behaviour. Courtship experience can
78 affect a female's likelihood of accepting a mate (Collins, 1995; Dukas, 2005b; Stoffer &
79 Uetz, 2015) and female sexual receptivity commonly decreases after mating (Chapman,
80 2001; Gioti et al., 2012; Kubli, 2008; Manning, 1967; Ortigosa & Rowe, 2003; Peretti &
81 Carrera, 2005; Ringo, 1996).

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

91 Importantly, to understand the potential for and the constraints on the evolution of
92 sexually selected traits, knowledge of the extent of genetic variation in female mating
93 behaviour is essential. Findings from studies investigating phenotypic changes in female
94 mating traits over the lifespan demonstrate plasticity in female choosiness (Anjos-Duarte et
95 al., 2011; Gray, 1999; Kodric-Brown & Nicoletto, 2001). However, the influence of factors

96 such as age and mating history on the evolvability of female mating strategies is unknown
97 because genetic estimates of behavioural traits, and of their potential to respond to selection,
98 are often based on single time points (Hoffmann, 1999). Investigating the influence of age
99 and mating history on the evolvability of female mating traits is important to determine if the
100 response to selection changes over the lifespan. In species with repeated or continuous
101 reproduction, focusing on a single time point could lead to inaccurate extrapolation of
102 evolvability to different life stages.

103 Here, we investigate genetic and environmental sources of variation in female mating
104 behaviours in a population of *D. melanogaster* recently derived from the field. Specifically,
105 using longitudinal observations, we first investigate the effects of female age and previous
106 mating history on female mating behaviour. The traits we examine include courtship latency,
107 mating latency, and copula duration. We then investigate the genetic basis of these traits in
108 females by calculating quantitative genetic estimates for all mating traits for virgin females
109 and for females at their third mating. By doing so, we examine both phenotypic variation in
110 female mating behaviour over lifespan as well as changes in the evolvability of traits from
111 virgin to previously mated females.

112 **Methods**

113 *Breeding design*

114 Focal flies came from a laboratory population of sixth generation descendants of wild type
115 (*wt*) *D. melanogaster* collected near Innisfail in Northern Queensland, Australia. We used a
116 full-sib half-sib breeding design to quantify genetic variation in aspects of female mating
117 behavioural traits. Mating traits were recorded for 765 daughters distributed among 70 sire
118 families and 198 dam families. To produce parents of focal females, we collected larvae from
119 a population cage of *wt* flies and raised them at a standard density of 50 larvae per vial.

120 Virgin offspring were collected at peak eclosion and kept in single sex vials with 10 males
121 per vial and 5 females per vial. Each male was mated to three virgin females to generate
122 families of paternal half-siblings. 'Dam families' were comprised of 4 female offspring
123 produced by a sire with a single dam (full-sib). 'Sire families' were comprised of 12 paternal
124 half-siblings produced by a sire across three dams (half-sib). Four virgin female offspring
125 (daughters) from each full sibling dam family were randomly collected. We also collected
126 four additional females from each dam family that were frozen to later estimate full sibling
127 dam family averages for female body size. Egg, larvae, and adult flies were maintained at
128 25°C on a 12:12h light:dark cycle throughout the experiment.

129 **Ethical Note**

130 No ethical approval was required for the study.

131 *Female mating behaviours*

132 Mating opportunities for daughters began at 5 days of age. All daughters were kept in
133 individual vials with 10ml of sugar-maize medium and transferred to fresh food vials every
134 week. Each of these females was given a mating opportunity with a sexually naïve male from
135 an isogenic line (see below for details regarding the generation of isogenic lines) every
136 Monday, Wednesday and Friday over her entire lifespan (Figure 1). On each of these days,
137 half of the families were measured in the morning (10am) and half in the afternoon (2pm).
138 The time of day was alternated between mating opportunities for each family. All matings
139 were carried out in the same temperature and humidity controlled environmental chamber
140 which minimized variation in environmental conditions between mating opportunities. At the
141 beginning of each mating opportunity, males were carefully aspirated into the female's vial.
142 We then observed the time from the male's placement in a female's vial until the initiation of

143 courtship towards that female (courtship latency), the time between the beginning of
144 courtship until copula started (mating latency), and copula duration. Behaviours were
145 recorded by continuous scan sampling and all males were removed via aspiration from the
146 vial after 1 h. Female longevity was assessed before the beginning of each mating
147 opportunity and death was determined by lack of movement.

148 The empirical investigation of female mating traits is problematic because they are likely
149 to be influenced by male phenotype; both genotypic and environmental male effects can
150 influence the expression of female behaviour (Ahuja & Singh, 2008; Bacigalupe,
151 Crudginton, Jon, Moore, & Snook, 2008; Ferveur, 2005; Moore, Brodie, & Wolf, 1997;
152 Partridge, Hoffmann, & Jones, 1987; Wolfner, 1997, 2002). In order to account for male
153 effects on female mating traits, we standardized male identity in each mating opportunity by
154 using males from one of ten isogenic lines (Garcia-Gonzalez & Evans, 2011; Travers, Garcia-
155 Gonzalez, & Simmons, 2015; Travers, Simmons, & Garcia-Gonzalez, 2016). We generated
156 each isogenic line via a full sibling mating protocol which was initiated with one founder pair
157 of flies taken from a replica of an LH_M population (See Byrne & Rice, 2005 for details). First,
158 full sibling matings were conducted for 16 generations, followed by several generations of
159 within-line matings where approximately 15 individuals from each line were used to start
160 each new generation. We then conducted a further 21 generations of full sibling matings.
161 Each isogenic line was mass bred in to individual population cages before the beginning of
162 the experiment to allow the generation of sufficient number of flies for the mating trials. For
163 each mating opportunity, we collected larvae from population cages on grape agar plates and
164 transferred larvae into food vials. Sexually naive males were collected 9-11 days later and
165 kept in groups of ten in 10ml food vials. Males were 2-3 days old when used in mating trials.
166 In order to standardise males in each mating opportunity, we randomly selected males from
167 the same isogenic line for each mating opportunity (e.g. isogenic line 1 in the first

168 opportunity for all females, isogenic line 2, 3, 4, etc. for the 2nd, 3rd, 4th, etc. mating
169 opportunity).

170 *Statistical Analyses*

171 *Phenotypic variation in behaviour across lifespan*

172 Our analyses examined the effect of female age and number of previous matings on the
173 following: 719 females over 5,066 mating trials for courtship latency, 631 females over 1545
174 mating trials for mating latency and 572 females over 1,193 events for copula duration.
175 Linear mixed models (LMMs) using the square root transformed courtship latency, mating
176 latency and copula duration were fitted using the lme4 package (Bates et al., 2014) in R,
177 version 3.1.2 (R Core Team, 2015). Response variables were transformed to satisfy
178 assumptions of normal and homoscedastic residuals. We included female age (\approx mating
179 opportunity number), cumulative number of matings, body size, time of day of mating trial,
180 and the number of mating opportunities since the female last mated as covariates. We also
181 included the interaction between female age and cumulative number of matings. As a
182 consequence of including time since the female's last mating as a covariate, we excluded
183 virgin females from the phenotypic analysis as virgins do not have any previous matings. In
184 our analysis of courtship latency, we included an additional covariate to test whether
185 courtship latency differed between trials where females mated or not. To account for repeated
186 measures of the same females over multiple mating events, individual female ID was
187 included as a random effect, along with isogenic male line ID in all models. When testing for
188 interactions, we included individual-specific random slopes to avoid overconfidence in
189 interaction estimates (Schielzeth & Forstmeier, 2009). Significance of fixed effects was
190 tested using Wald chi-square tests implemented in the Anova function of the car package
191 (Fox & Weisberg, 2011). Non-significant fixed effects were excluded from the final models.

193 For the genetic analyses of the three mating traits in females, we first calculated
194 quantitative genetic estimates for virgin females using the first occurrence of each behaviour
195 in a female's lifetime. For the analyses of behaviours in previously mated females, we used a
196 subset that included behaviours measured at the females' third mating. Thus, the two subsets
197 differed not only in female mating history, but also with respect to average female age (see
198 Figure 1 for a schematic overview of the measurements obtained for genetic analyses).
199 Within the two subsets (virgin and previously mated), there was also variation in age, as not
200 all females were courted or mated in their first mating opportunity in the virgin subset, and
201 similarly, there was variation in the mating opportunity at which females mated for the third
202 time. The average female age of virgins was 5 days for courtship latency, and 6 days for
203 mating latency and copula duration. Previously mated females were aged 21 days on average
204 for all three behaviours. We investigated the genetic basis of the traits the third mating in
205 order to include females that had previous mating experience and to ensure a sufficient
206 number of individuals were still alive to obtain an adequate sample size. Sire and dam effects
207 were tested for all three behaviours both in virgin and previously mated females using LMMs
208 on square root transformed courtship latency, mating latency and copula duration. Visual
209 inspection of the residuals from all models using both untransformed and transformed
210 response variables revealed that the square root transformation satisfied the model
211 assumptions of normal and homoscedastic residuals while the raw data and log
212 transformations did not. The models included sire, dam nested within sire and isogenic male
213 line ID as random effects. Significance of all three random effects was determined using
214 likelihood-ratio tests. Female age, body size, mating time of day and number of opportunities
215 since females last mating were included as fixed effects in all models. Significance of the
216 fixed effects was tested using Wald chi-square tests as described above.

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

240 ***Results***

241 *Phenotypic variation across lifespan*

242 There was substantial variation in female longevity and thus in the number of mating
243 opportunities that females were offered (mean mating opportunities \pm SD = 14.96 ± 4.36 ;
244 range = 1–24; mean lifespan in days after start of experiment \pm SD = 39.49 ± 11.74 ; range =
245 1–62). Examination of the mating traits over female lifespan revealed that courtship latency
246 was significantly affected by cumulative number of previous matings ($\chi_1^2 = 7.62$, $p = 0.006$)
247 with lower courtship latency (males initiating courtship sooner) for females with more
248 previous matings (Figure 2a). There was no main effect of female age on courtship latency
249 ($\chi_1^2 = 0.07$, $P = 0.789$), but there was a significant interaction between female age and mating
250 experience ($\chi_1^2 = 4.14$, $P = 0.042$), meaning that the negative effect of female mating history
251 on courtship latency became weaker as females aged. We also found a significant effect of
252 number of opportunities since the female last mated, with longer courtship latencies for
253 females that mated more recently ($\chi_1^2 = 9.32$, $P = 0.002$). Finally, we found a significant
254 negative relationship between courtship latency and whether a female mated at that mating
255 opportunity ($\chi_1^2 = 5.30$, $P = 0.021$), meaning that mating did not occur when courtship was
256 initiated later.

257 While we found no significant effect of cumulative number of previous matings on mating
258 latency ($\chi_1^2 = 0.21$, $P = 0.647$) or time since last mating ($\chi_1^2 = 1.01$, $P = 0.315$), this trait was
259 significantly affected by female age, with older females taking longer to mate from the
260 initiation of courtship ($\chi_1^2 = 17.801$, $P < 0.001$; Figure 2b).

261 Copula duration was significantly affected by female age ($\chi_1^2 = 37.00$, $P < 0.001$), with
262 shorter copulation times for older females (Figure 2c). We also found a significant effect of
263 time since last mating ($\chi_1^2 = 4.32$, $P = 0.038$), with shorter copulation durations in females
264 that mated more recently. No effect of cumulative number of matings was found for copula

265 duration ($\chi_1^2 = 0.14$, $P = 0.706$). We did not find a significant effect of female body size or
266 mating time of day on any of the three traits (all $P > 0.1$). Not surprisingly, we found some
267 evidence for multicollinearity between age, mating history and time since last mating.
268 However, variance inflation factors were moderate, ranging between 1.40 and 3.60 in all
269 models, suggesting that multicollinearity was not an issue in the data analysis (Craney &
270 Surles, 2002).

271 *Quantitative genetics*

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

274 Courtship latency showed substantial phenotypic variation for both virgins (mean \pm SD =
275 1448 ± 1096 sec) and previously mated females at their third mating (mean \pm SD = $1135 \pm$
276 840 sec). We found substantial additive genetic variation and significant sire variance among
277 virgin females but low and non-significant additive genetic variation in courtship latency
278 among females at their third mating (see Table 1). However, both when females were virgin
279 and previously mated, courtship latencies were significantly affected by the isogenic line
280 from which males were drawn (virgin: $\chi_1^2 = 65.05$, $P < 0.001$; previously mated: $\chi_1^2 = 13.83$, P
281 < 0.001). Neither female age, body size or mating time of day had a significant effect on
282 courtship latency among virgins or previously mated females.

283 We found large phenotypic variation in mating latency among virgin (mean \pm SD = $712 \pm$
284 887 sec) and previously mated females (mean \pm SD = 771 ± 924 sec). Mating latency of
285 virgin females showed high levels of additive genetic variation and significant variance
286 across sire families (see Table 1). In contrast, mating latency among mated females exhibited
287 low additive genetic variance, and non-significant sire effects. Male line ID had a significant

288 effect on mating latency in virgins but not in previously mated females (Table 1). Female age
289 had a significant negative effect on mating latency among virgins ($\chi_1^2 = 7.308$, $P = 0.007$),
290 and mated females ($\chi_1^2 = 4.221$, $P = 0.039$), while female body size and mating time of day
291 had no significant effect.

292 Copula duration showed lower phenotypic variation among virgin (mean \pm SD = 1077 \pm
293 331 sec) and previously mated females (mean \pm SD = 1024 \pm 361 sec) than the other two
294 mating traits. In both virgins and mated females, copula duration showed low and non-
295 significant levels of additive genetic variation and non-significant sire effects (Table 1).
296 There was significant variation among male isogenic lines for copula duration with virgins
297 ($\chi_1^2 = 51.776$, $P < 0.001$), but not for copula duration with previously mated females ($\chi_1^2 = 0$,
298 $P = 1$). Female age, body size and mating time of day had no effect on copula duration in
299 either virgin or previously mated females.

300 ***Discussion***

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

306 *How does mating behaviour change across lifespan?*

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

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

318 The relationship between mating latency and female age suggests that a female's
319 resistance to mating increases as she ages. Studies in other species have found the opposite -
320 a decrease in female choosiness with age. For example, in guppies, Kodric-Brown &
321 Nicoletto (2001) found that younger females were more selective than older females. Moore
322 & Moore (2001) found decreased choosiness in female cockroaches (*Nauphoeta cinerea*)
323 when mated past the optimal mating age, and also found a correlation between the reduction
324 in choosiness and fertility, which suggests that a decrease in choosiness may be due to a
325 reduction in reproductive potential. The increase in female resistance to mating (or
326 choosiness) in later life may arise from a shift in the cost-benefit ratio of mating across
327 different life history stages. In *D. melanogaster* mating is costly due to harmful seminal fluid
328 proteins transferred during copulation (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995).
329 Thus, frequent remating by females incurs increasing costs as they suffer reduced survival
330 and hence reduced lifetime reproductive success (Chapman et al., 1995; Fowler & Partridge,
331 1989). Moreover, as older females are more likely to have mated previously and ensured
332 fertilisation of at least part of their lifetime egg supply, they have less to gain from further
333 matings. Therefore, the increased resistance of older females to male courtship attempts may
334 reflect reduced benefits from mating in later life that do not outweigh the costs associated

335 with additional matings. Due to collinearity between female age and mating history, the
336 effect of female age on mating latency may be somewhat driven by mating history. However,
337 we found no significant effect of mating history on mating latency. In order to disentangle the
338 effect of age and mating history on female mating latency, a control experiment measuring
339 mating latency in aged virgin females would be required.

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

360 invest less in older females (Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2011) as the
361 return on their investment will be lower due to reduced egg production in older females
362 (Boorman & Parker, 1976).

363 *Does additive genetic variance in female mating behaviour change throughout life?*

364 The presence of high levels of heritable variation in courtship latency in virgin females
365 reveals that female genotype affects mating behaviour. Markedly, the present study is the first
366 to document the quantitative genetic basis of female effects on courtship latency and
367 indicates that females vary genetically in their receptivity or attractiveness to males. Evidence
368 from previous studies suggests genetic variation in female attractiveness. Ratterman et al.
369 (2014) found that males ranked attractiveness among different female genotypes in the same
370 order across ten inbred lines of *D. melanogaster*. Male mate choice for phenotypic indicators
371 of fecundity (e.g. female abdomen width or body size) is predicted due to the associated
372 fitness gains of mating with large females (Ebert, 1993; Gromko, Briot, Jensen, & Fukui,
373 1991; Lefranc & Bundgaard, 2000). However, we found no effect of female body size on the
374 male's latency to initiate courtship, indicating that genetic variance in female receptivity or
375 attractiveness is not driven by female body size, at least in this population.

376 We also revealed evidence for a genetic basis for mating latency in virgin females which
377 supports prior findings in this species (Gromko, 1989; Narraway et al., 2010; Sgro, Chapman,
378 & Partridge, 1998). Moore (1989) found evidence for genetic variation in female preference
379 in cockroaches, and a quantitative genetic study in a wild population of flycatchers found
380 significant additive genetic variation in female preference (Qvarnström et al., 2006). Previous
381 studies have also found significant additive genetic variation in female resistance to harm
382 from males and that female reluctance to remate has a positive effect on lifetime fitness (Lew,
383 Morrow, & Rice, 2006; Linder & Rice, 2005). If resistance to mating above the female

384 optimum mating rate is beneficial, directional selection should erode genetic variation in
385 female resistance to male harm. What maintains genetic variance in female mating latency
386 remains unclear. One potential explanation is that variation in female resistance could be
387 maintained if selection acts on males to overcome female resistance. Sexually antagonistic
388 coevolution in mating frequency is well documented in *D. melanogaster*, and studies have
389 demonstrated selection on male ability to coerce females to mate above their optimum rate
390 (Holland & Rice, 1999; Pitnick, Brown, & Miller, 2001; Rice, 1996). Tennant et al. (2014)
391 found a negative covariance between female choosiness and male attractiveness in *D.*
392 *melanogaster*, consistent with sexual conflict theory. Thus, conflicting selection on mating
393 rates between the sexes could potentially explain the maintenance of high levels of additive
394 genetic variance in female mating latency.

395 The significant effect of male line on virgin mating latency suggests that males also differ
396 genetically in their attractiveness (Hoffmann, 1999; Hosgood & Parsons, 1965; Mackay et al.,
397 2005; Manning, 1961; Taylor, Wedell, & Hosken, 2007; Wedell & Tregenza, 1999), and
398 supports a previous study which demonstrated that both male and female genotype contribute
399 to variation in mating latency (Tennant et al., 2014; but see Ratterman et al., 2014).

400 Our genetic analyses of copula duration revealed a significant effect of male isogenic line
401 when mating with virgin females but no additive genetic variation among females, either
402 when virgin or previously mated. This finding is consistent with previous evidence for
403 predominantly male control of copula duration (Bretman, Lizé, Walling, & Price, 2014;
404 Bretman et al., 2013; Friberg, 2006; Tennant et al., 2014). Nevertheless, Edward et al. (2014)
405 suggested that both sexes might contribute to variation in copula duration in *D. melanogaster*,
406 while another study failed to detect significant additive genetic variation in males in the trait
407 across two different environments (Taylor, Evans, & Garcia-Gonzalez, 2013). However,

408 Taylor et al. (2013) were not able to separate male and female effects, and this could have led
409 to a failure to detect additive genetic variance in copula duration.

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

425 We found a significant effect of male line on all three traits when measured in virgin
426 females. Males in this species exert a strong influence on female remating rates through the
427 effects of sex peptide transferred during mating, which inhibits female remating (Wolfner,
428 1997). Our finding of a significant effect of male line suggests that male effects also
429 influence other components of female mating behaviour. Male effects from previous partners
430 may increase variation in female responses to subsequent potential mates. If so, increased

431 environmental variation in female behaviour may also hinder the ability to detect additive
432 genetic variance in mating behaviours in mated females.

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

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731 **Figure 1.** Full-sib half-sib breeding design and experimental mating design. 72 Sires were
732 mated to three dams each. From every full-sib family, four daughters were offered a mating
733 opportunity three times per week for their entire lifespan, starting at an age of 5 days. Two
734 daughters are shown for illustrative purposes. Courtship latency, mating latency and
735 copulation duration (see box inlet and main text for details) were recorded whenever
736 occurring. For the phenotypic analyses of mating behaviours, we included all recorded
737 behaviours. For the quantitative genetic analyses, we included behaviours when they first
738 occurred (virgin females; blue), or at a female's third mating (previously mated females;
739 brown). As a consequence, there was substantial variation both between and within the two
740 subsets. Fly illustrations taken from Sokolowski (2001). Adapted with permission.

741 **Figure 2.** Effects of cumulative number of matings on a) courtship latency, and of female age
742 on b) mating latency, and c) copula duration. Boxes and whiskers represent the raw data

743 while lines and shaded areas represent backtransformed LMM predictions for mean effects
744 and approximate 95% confidence intervals, respectively. Box plots show the median, the
745 lower and upper quartials with whiskers extending to 1.5 times the interquartial range and
746 open circles showing outliers.

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

Sires

$$N_{\text{Sire}} = 72$$

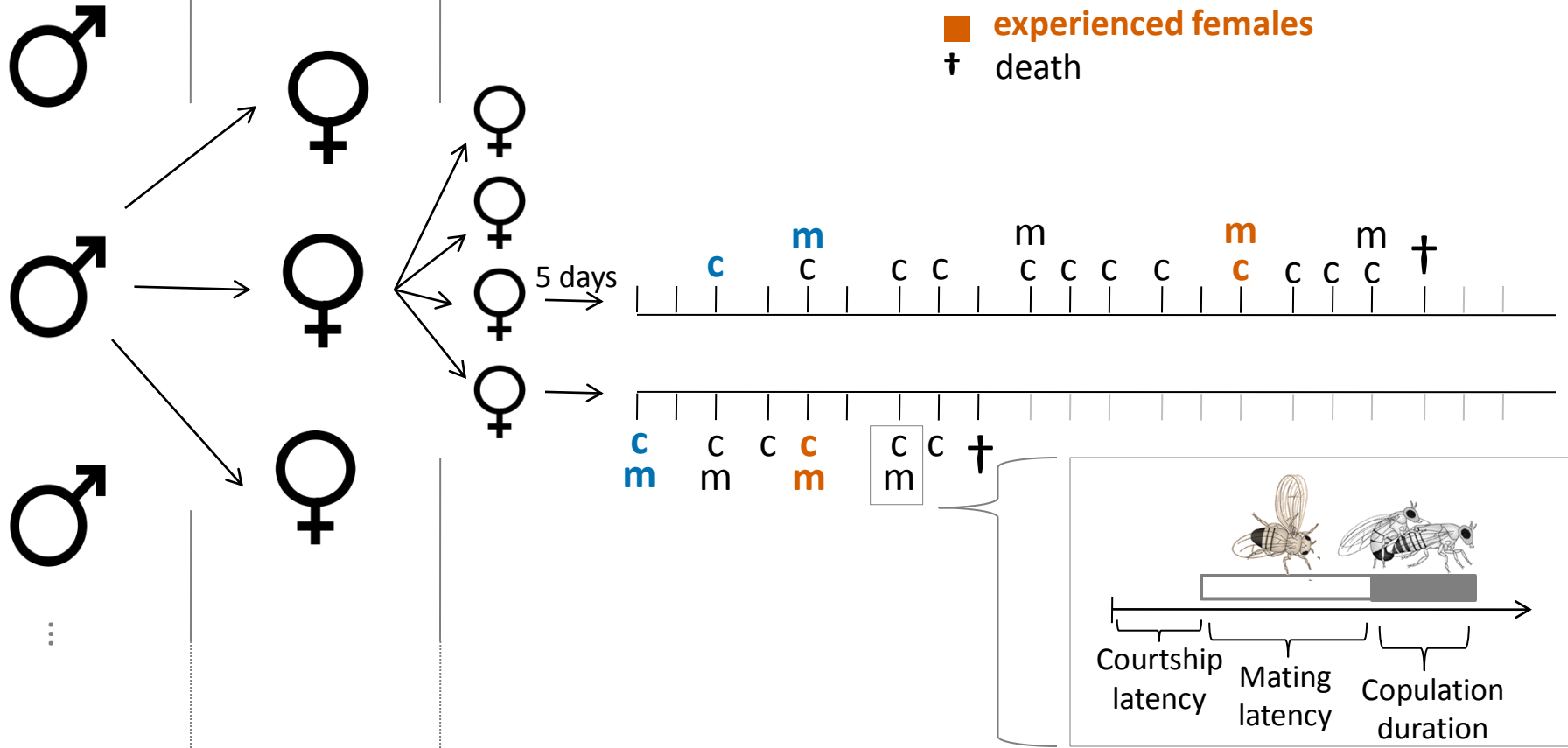
Dams

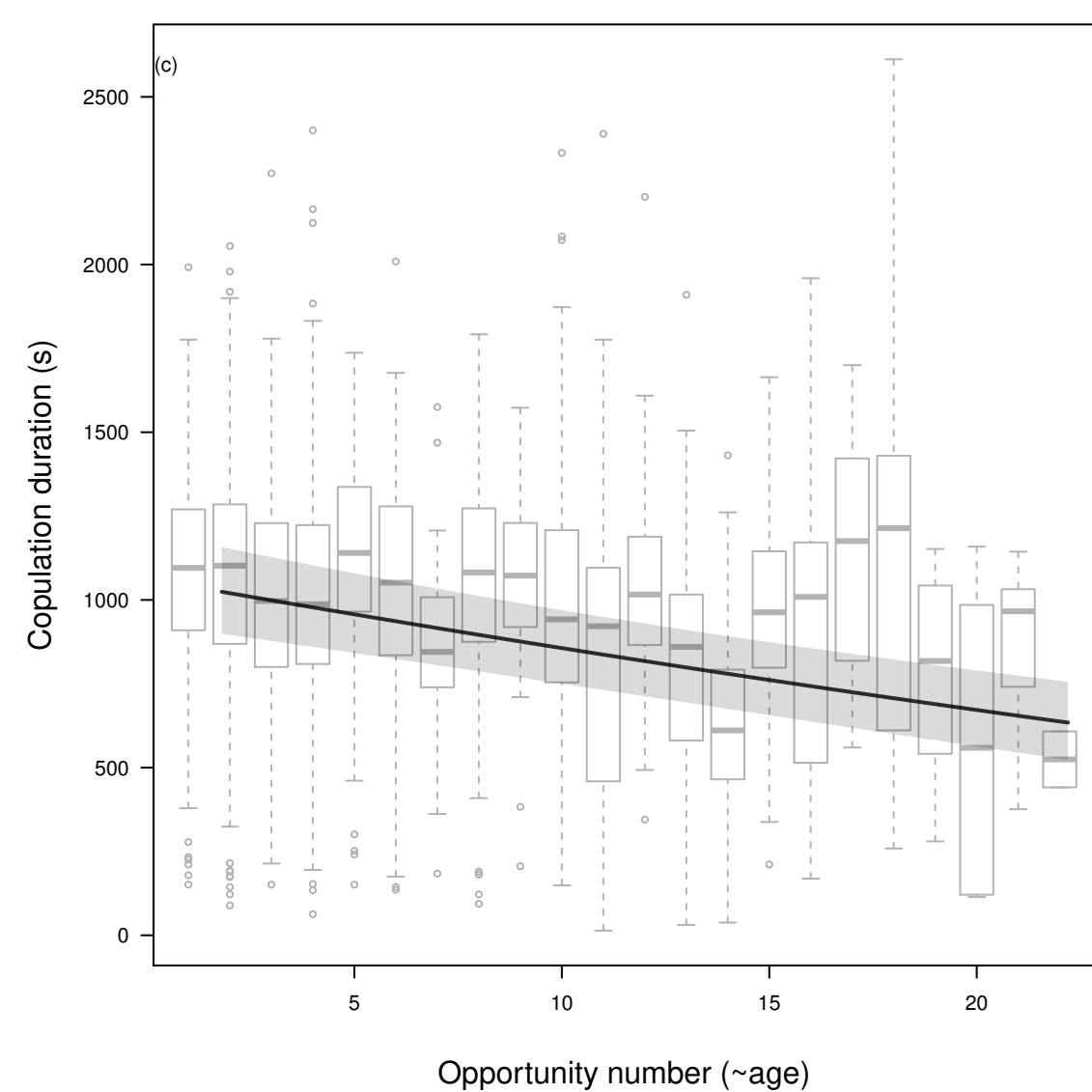
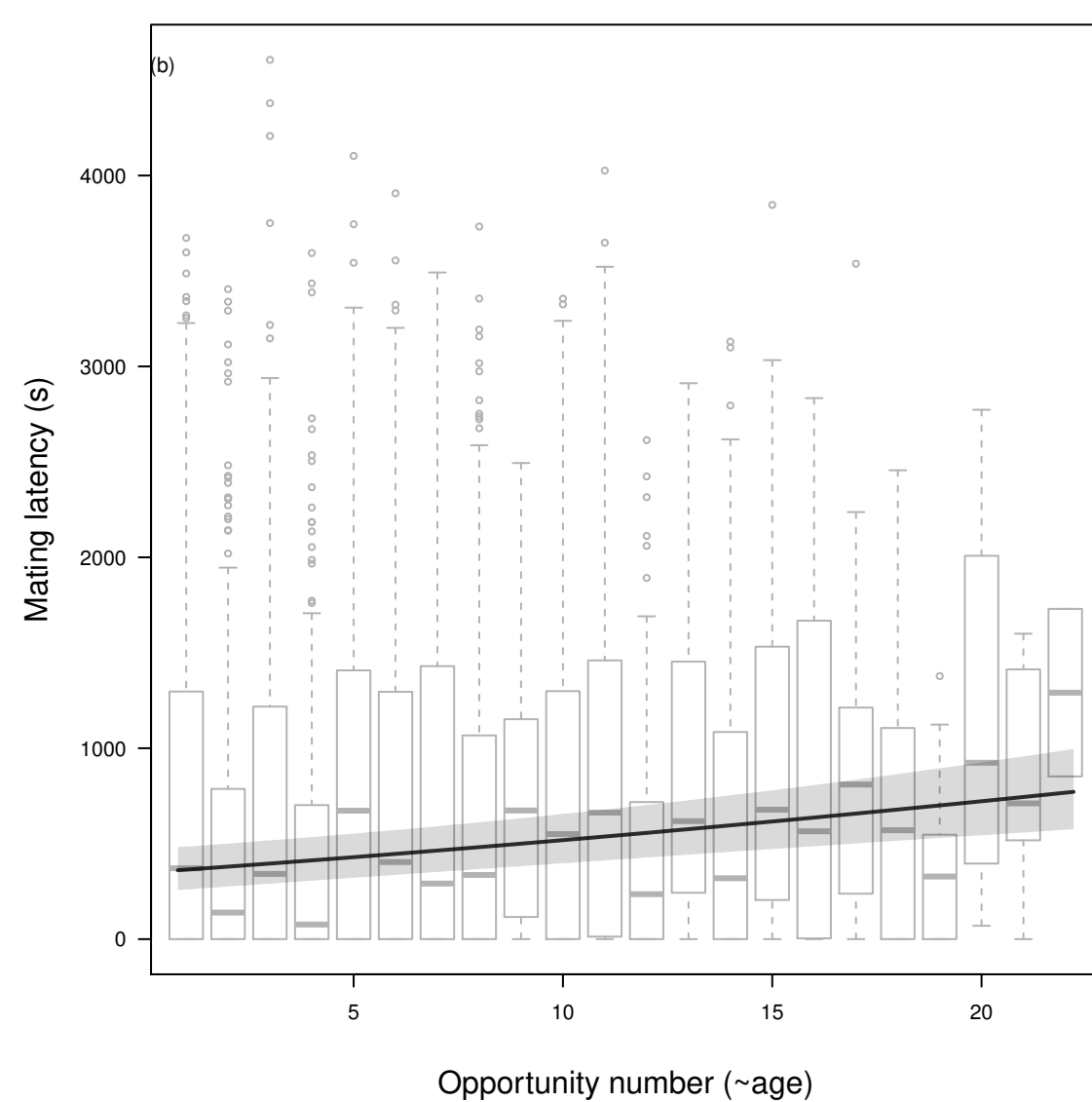
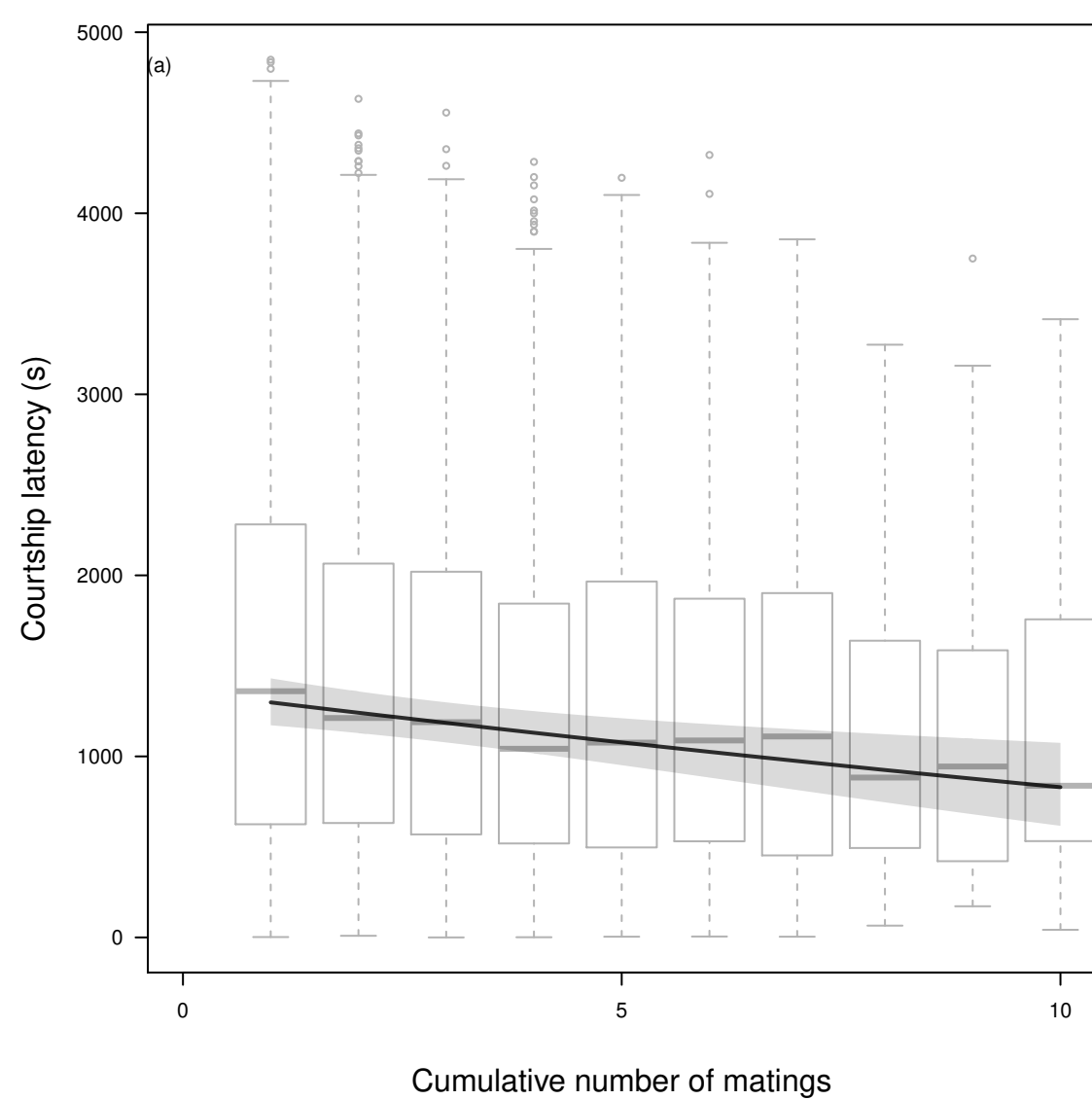
$$N_{\text{Dam}} = 3 \times N_{\text{Sire}}$$

Daughters

$$N = 4 \times N_{\text{Dam}}$$

- | mating opportunity
- c courtship latency
- m mating latency and copulation duration
- virgins
- experienced females
- † death





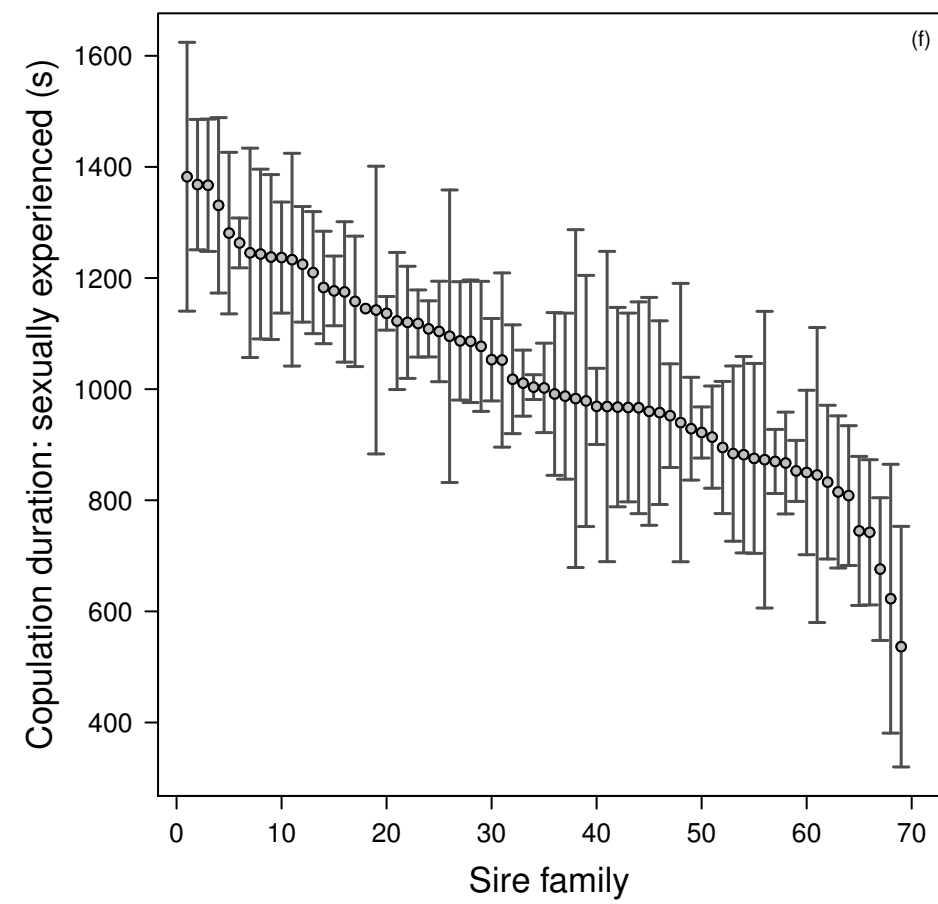
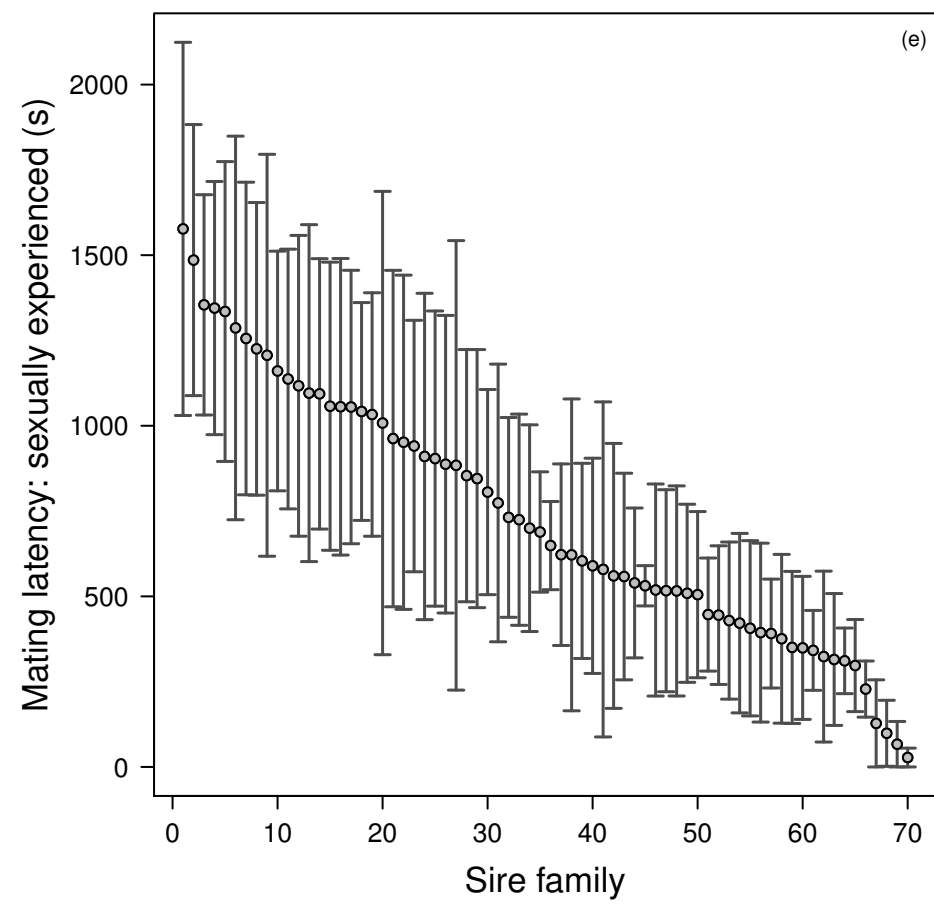
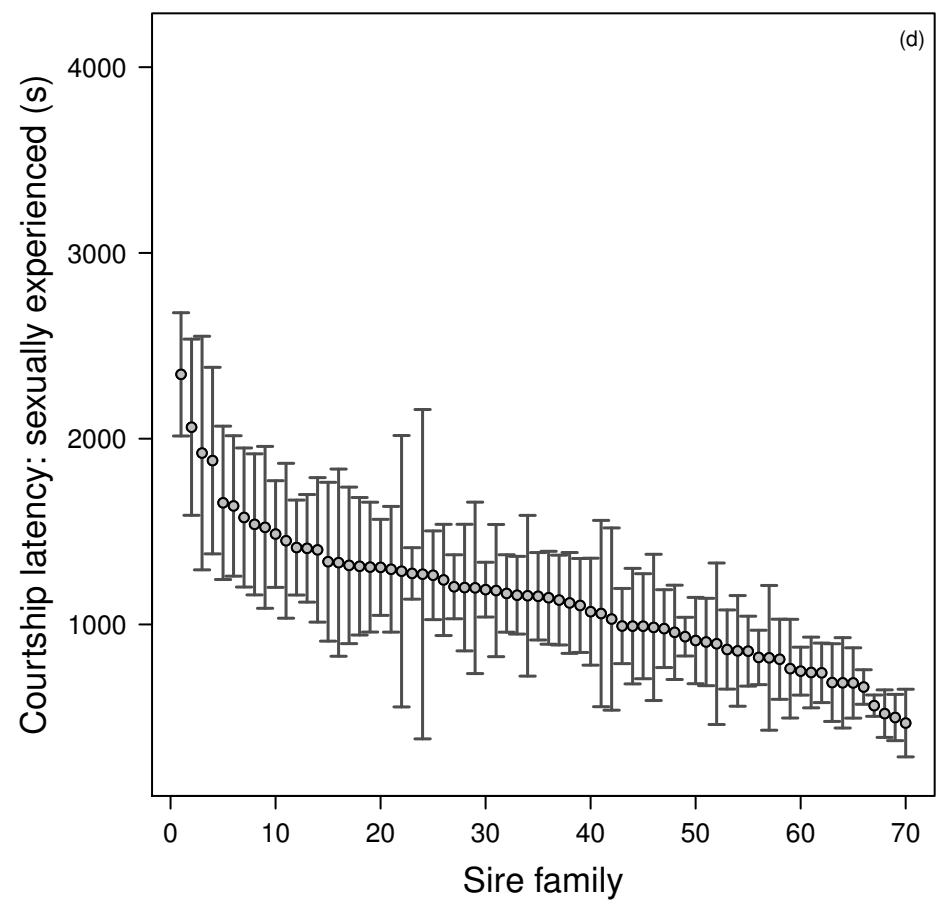
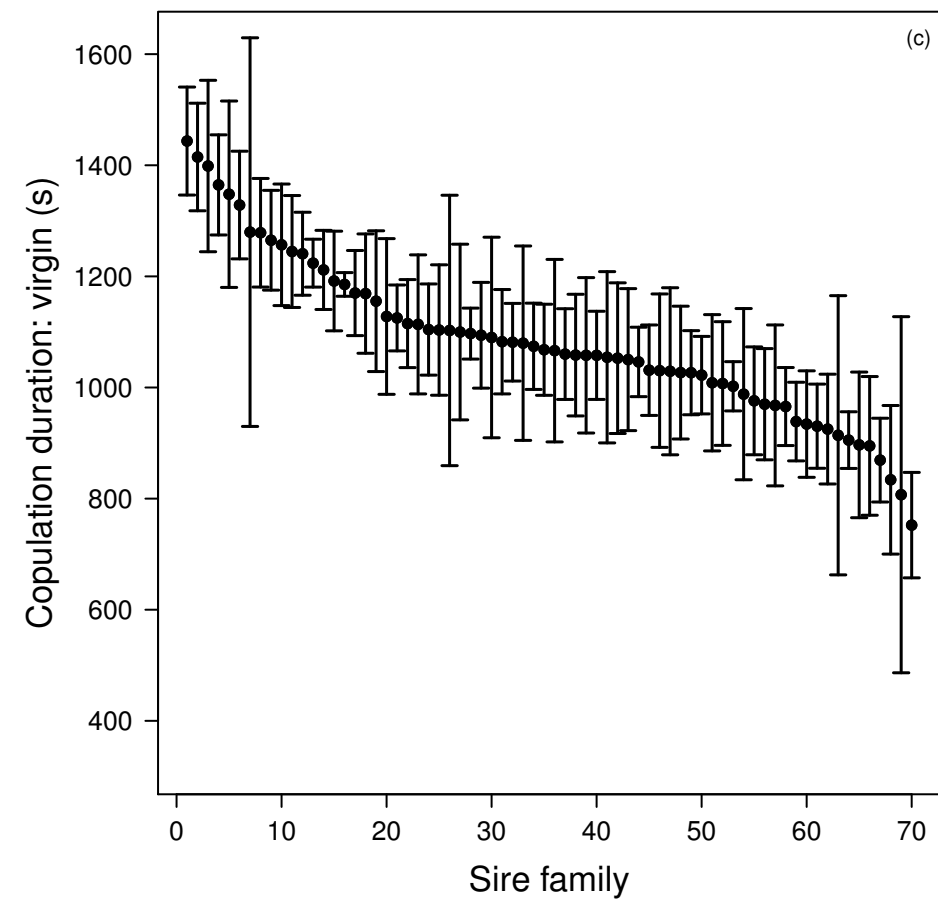
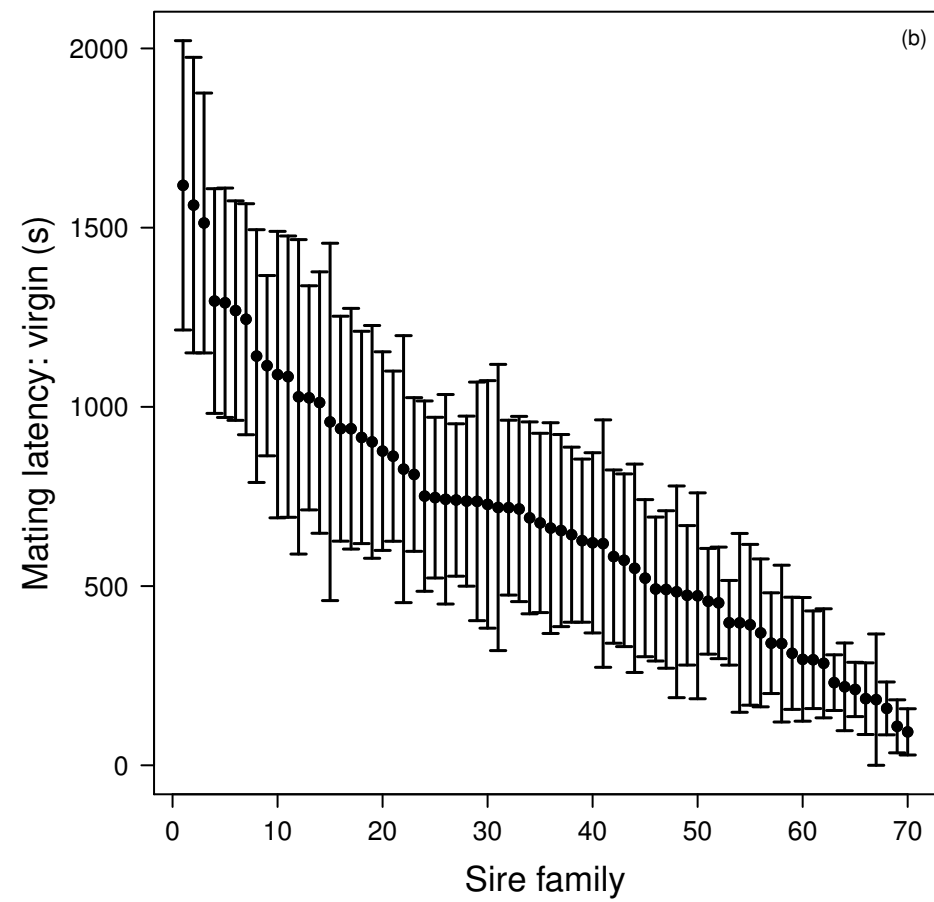
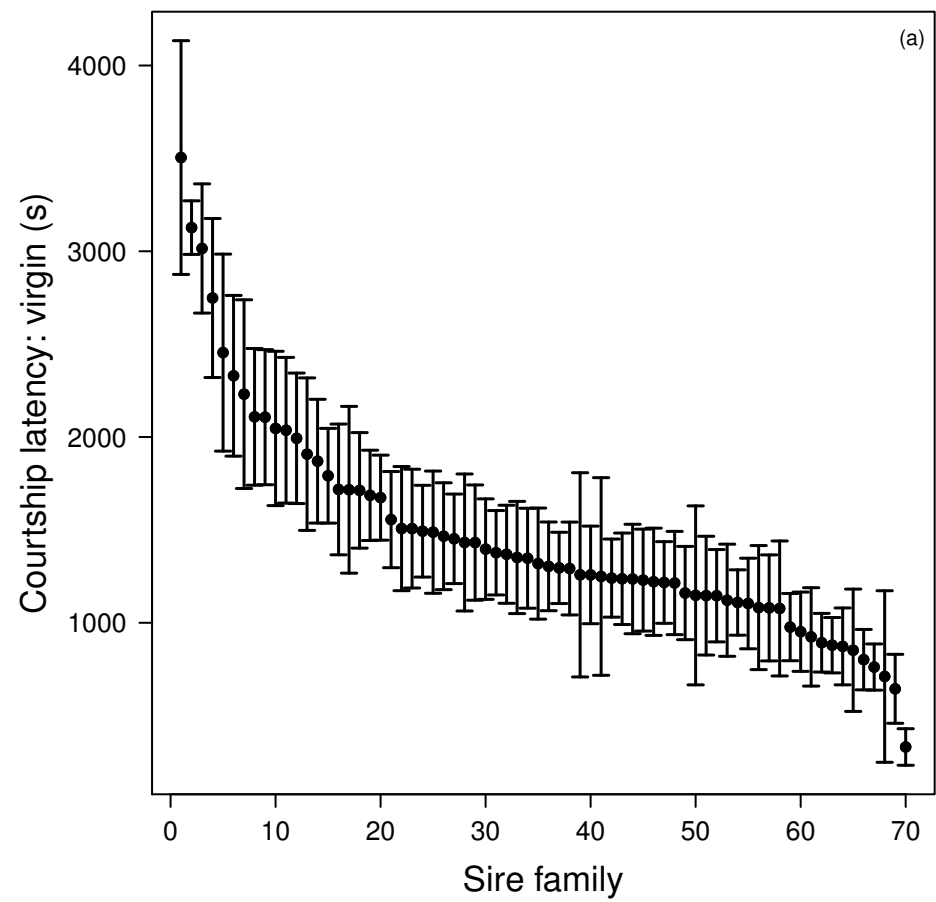


Table 1. Quantitative genetic parameters for mating traits in females

	N	Mean (SD) [secs]	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 (2787)	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 ($P < 0.05$).