Sexual selection and assortative mating: an experimental test

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Abstract

Mate choice and mate competition can both influence the evolution of sexual isolation between populations. Assortative mating may arise if traits and preferences diverge in step, and, alternatively, mate competition may counteract mating preferences and decrease assortative mating. Here we examine potential assortative mating between populations of *Drosophila pseudoobscura* that have experimentally evolved under either increased ('polyandry') or decreased ('monogamy') sexual selection intensity for 100 generations. These populations have evolved differences in numerous traits, including a male signal and female preference traits. We use a 2 males: 1 female design, allowing both mate choice and competition to influence mating outcomes, to test for assortative mating between our populations. Mating latency shows subtle effects of male and female interactions, with females from the monogamous populations appearing reluctant to mate with males from the polyandrous populations. However, males from the polyandrous populations have a significantly higher probability of mating regardless of the female’s population. Our results suggest that if populations differ in the intensity of sexual selection, effects on mate competition may overcome mate choice.

Keywords: *Drosophila*; experimental evolution; mate competition; female preference; sexual conflict; sexual isolation; speciation.
Introduction

Sexual selection is often thought to be an important force in the origin of sexual isolation between populations, although this is subject to much debate (Mayr, 1963; Coyne & Orr, 2004; Rundle & Nosil, 2005; Sobel et al., 2010; ITN Marie Curie Speciation, 2011). Intersexual selection may facilitate sexual isolation because coevolution of mating signals and associated preferences may lead to divergence between populations. This divergence would then have the potential to generate assortative mating (i.e. a higher likelihood of mating with an individual from the same population) if populations come into secondary contact (Lande, 1981; Kirkpatrick, 1982; Price, 1998; Kirkpatrick & Ravigné, 2002; Uyeda et al., 2009).

While divergence in preferences between populations is often matched by signal divergence (Rodríguez et al., 2013), strong preferences may theoretically decrease isolation if preference genes introgress between species (Servedio & Bürger, 2014). Likewise, strong sexual selection can influence mate competition, which may facilitate population divergence, for example by reinforcing the action of mating preference on a given mating signal. Strong mate competition may also constrain the expression of mating preferences by reducing the opportunities to mate with preferred, but less competitive, mates (Wong & Candolin, 2005; Hunt et al., 2009). Thus, it is difficult to predict the overall influence of sexual selection on sexual isolation.

Experimental sexual selection directly manipulates a species’ mating system to observe, in real time, the evolutionary consequences on sexual traits, mating patterns, and the evolution of reproductive isolation (Holland & Rice, 1999; Martin & Hosken, 2003, 2004; Wigby & Chapman, 2004, 2006; Crudgington et al., 2005, 2010; Rundle & Chenoweth, 2005; Snook et al., 2005; Rundle et al., 2006; Bacigalupe et al., 2007, 2008). We have implemented
experimental sexual selection in *Drosophila pseudoobscura* by either enforcing monogamy (1 male:1 female) or promoting polyandry (1 female:6 males) and found a variety of evolutionary responses. For example, divergence between monogamous and polyandrous populations in an important male courtship signal has occurred, with males from polyandrous populations singing a faster courtship song compared to males from monogamous populations (Snook *et al.*, 2005). There is also evidence for coevolution of female preference for song; in playback experiments, females from the polyandrous populations prefer polyandrous-like male song whereas females from monogamous populations preferred monogamous-like song (Debelle *et al.*, 2014). Other traits that are implicated in sexual selection, such as cuticular hydrocarbon profiles, have also diverged between the sexual selection treatments (Hunt *et al.*, 2012).

Here we conduct what is referred to as a “choice” experiment in which mating trials involve 2 males: 1 female (Dougherty & Shuker, 2014) from replicate polyandrous and monogamous populations to examine how the evolutionary history of these populations influences mating patterns. This type of design was chosen at it usually results in a stronger expression of mating preferences compared to no-choice designs (Dougherty & Shuker, 2014). Moreover, such a design allows mating patterns to be influenced by both male-male and male-female interactions, and is considered to be the most appropriate way to test for sexual isolation between populations (Coyne *et al.*, 2005).

If female choice predominates mating interactions, we predict to observe a significant effect of both male and female evolutionary history on mating patterns. These effects could potentially result in assortative mating occurring within each replicate population of each sexual selection treatment. However, we have previously found that there was little within-
treatment (i.e. between-replicate) variation in patterns of song-preference divergence between the sexual selection treatments (Debelle et al. 2014), suggesting that sexual selection treatment consistently influences the direction of signal-preference coevolution in our populations (and other traits that may have diverged between treatments). We thus predict that if female choice predominates mating interactions, then assortative mating by treatment will occur (i.e. polyandrous females with polyandrous males and monogamous females with monogamous males).

Alternatively, male-male competition could largely predominate mating interactions, resulting in finding no effect of female evolutionary history on mating patterns. Males from polyandrous populations present a higher courtship frequency (Crudgington et al., 2010), a trait that could be implicated in male-male competition (e.g. Shine et al. 2005; Kim and Velando 2014). Additionally, male-male interactions are common between rival males of this species placed in a choice design (e.g. chasing, courtship interruption, physical threats and attacks; see Figure S1 in Appendix 1). We would therefore further predict that polyandrous males, who continuously experience strong male-male competition, will win more matings than monogamous males, regardless of female evolutionary history.

We test these alternative predictions by examining the mating patterns between the experimental populations after 100 generations of experimental evolution. To standardise female response against selection males, we also conduct the same experiment using females from the ancestral population. Because these females do not discriminate between male songs from the polyandrous and monogamous treatments (Debelle et al., 2014), we expect to observe random mating patterns. However, if male-male competition influences mating outcome, then we expect ancestral females to show the same mating outcome.
patterns as that of selection line females. We test for body size differences between our populations and treatments, and include it as a covariate in our analyses, because body size is frequently targeted by sexual selection and has a large influence on male mating success (Blanckenhorn, 2000). In *Drosophila* species, larger males win more aggressive encounters with other males (Partridge & Farquhar 1983; Partridge et al., 1987a), deliver more courtship (Partridge et al., 1987a,b) and mate faster (Partridge & Farquhar, 1983). We discuss how sexual selection influences mating outcome and the implications of these results for population divergence and speciation.

**Material and Methods**

**Sexual selection treatments**

The selection lines are described in detail in Crudgington et al. (2005). Briefly, an ancestral wild-caught population of the naturally polyandrous species *Drosophila pseudoobscura* from Tucson (Arizona, USA) was used to establish the selection lines. Four replicate populations (replicate 1, 2, 3 and 4) of two different sexual selection treatments were established. Adult sex-ratio in vials is manipulated by either confining one female with a single male (‘monogamy’ treatment; M) or one female with 6 males (‘elevated polyandry’ treatment; E) in vials. Henceforth, reference to E or M refers to the experimental sexual selection treatment flies derive from. Effective population sizes are equalized between the treatments (Snook et al., 2009). At each generation, offspring are collected and pooled together for each replicate population, and a random sample used to constitute the next generation in the appropriate sex-ratios, thus proportionally reflecting the differential offspring production across families. In total, 8 selection lines (M1, M2, M3, M4 and E1, E2, E3, E4) are
maintained, in standard food vials (2.5mm x 80mm) and with a generation time of 28 days. The ancestral population (A) is also maintained, in bottles (57 mm x 132 mm) with an equal sex-ratio of adult flies. All populations are kept at 22°C on a 12L:12D cycle, with standard food media and added live yeast.

Experimental flies

To generate the experimental flies, 50 reproductively mature adults (25 males and 25 females) of each treatment (E and M) and replicate (1, 2, 3 and 4) were used as parents and kept in mass-cultures, providing a common social context for parents of both sexual selection treatments. The resulting larvae were raised in controlled density vials (100 first instar larvae per food vial). Flies from these vials were collected and sexed on the day of hatching using CO₂ anaesthetization. Virgin males and females were kept separate in yeasted food vials with a maximum of 20 individuals per vial, and used in mating experiments once they had reached sexual maturity (four to six days old; Snook & Markow, 2001). Experimental females from the ancestral population were also generated using the same method.

To identify the population of origin of males, we clipped a small corner off the right lower wing margin of half of the males, under CO₂ anaesthetization, two days before the experiment. Wing clipping has no effect on male mating success in D. pseudoobscura (e.g. Dodd, 1989) but, as a control, half the males from each treatment were clipped. The males were then stored in vials of 12 individuals of the same population until the experiment.

Assortative mating design
We tested for assortative mating between the different populations by placing one female (E or M) in a food vial with two males (one E and one M). Competing males always came from the same replicate (e.g., one E1 and one M1 male, or one E3 and one M3 male). All the female-male combinations between populations were tested: we crossed the 8 female populations (E1-4; M1-4) with the 4 possible pairs of males (E1 and M1; E2 and M2; E3 and M3; E4 and M4), for a total of 32 combinations. For each combination, the minimum sample size was 40 females (N=1280 trials in total). Reproductively mature males were loaded first into food vials, followed by reproductively mature females, and each vial was observed until mating occurred, or for 20 minutes. If mating occurred, then the identity of the mating male was recorded (E or M). If no mating occurred, then the trial was discarded (N=116 trials).

Both mating latency and mating outcome (i.e. the identity of the winning male: E or M) were measured. Mating latency, defined here as the time between introducing the female into the vial until the start of mating, is an important component of *Drosophila* male competitive success and female preference (e.g. Bacigalupe et al., 2007). Mating outcome was used to predict the probability of an E or an M male winning with the different female populations. The same design was used with females from the ancestral (A) population (one A female with one E and one M male).

To examine a potential role of body size on mating patterns in our experiment, the length of wing vein IV of each individual (male and female) was measured as an estimate of body size (Crudgington et al., 2005) and included in the statistical analyses. Wings were mounted in a 30% glycerol-70% ethanol medium, photographs taken using a Motic camera and Motic Images Plus 2.0 software (Motic Asia, Hong Kong), and wing vein length measured with ImageJ (v. 1.44e (Abramoff et al., 2004). To control for potential temperature effects on
courtship behaviors (O’Dell, 2003), we measured temperature during trials using a Testo 168-735-1 thermometer (Testo Limited, United Kingdom), and subsequently used temperature as a covariate in the analyses (mean temperature during the time of each trial). The experiment was performed in 2-hour sessions, when the incubator lights came on, to mimic the *D. pseudoobscura* activity pattern (Noor, 1998). The different crosses were randomly assigned across the different days. The generations of the sexual selection treatments used were: replicate 1= 102, 105 and 107; replicate 2= 101, 104 and 106; replicate 3= 100, 103 and 105; replicate 4= 98, 101 and 103. The generation of the ancestral population used was 124.

**Predictions and statistical analyses**

Our main objective was to distinguish between three alternative outcomes: assortative mating could occur between replicate populations (i.e. a polyandrous male is more likely to mate with a polyandrous female from its own replicate population), or between sexual selection treatments (i.e. a polyandrous male is more likely to mate with a polyandrous female regardless of their respective replicate population), or not occur at all (i.e. matings could be mostly won by polyandrous males). We expect the non-coevolved ancestral females to mate randomly, given that at least for song, they exhibit no distinct preference. However this population is also subject to sexual selection, so predicting mating outcome is more difficult than in the polyandrous and monogamous populations. Thus, results of mating patterns for the females from the ancestral population were analysed separately.

Mating latency is used to measure female preference in *Drosophila*, with shorter latencies usually implying a more preferred mate (see references in Bacigalupe *et al.*, 2007; Debelle *et
A simple prediction then would be that mating outcome patterns are reflected in the mating latency patterns. However, this prediction is complicated by the potential action of sexual conflict, that could lead to polyandrous (and/or bigger) females exhibiting more resistance to mating, thereby increasing mating latency (Arnqvist & Rowe, 2005), and male-male competition, that could also affect mating latency (Bretman et al., 2009).

To test these predictions, we scored the winners of the mating encounters and measured mating latency. For both mating outcome and latency, we also included ‘type of cross’ in the model to test whether populations experiencing sexual conflict/sexual selection show greater measures of sexual isolation (for review, see Gavrilets, 2014). The crosses involving a male from the same population as the female (i.e. “coevolved”; e.g., an E1 female with an E1 and a M1 male or M1 female with an E1 and a M1 male) were considered as ‘within population’ crosses and all the other combinations were ‘between populations’ crosses (e.g., an E1 female with an E2 and a M2 male). The category ‘within population’ was further divided into two subcategories, ‘within E population’ when the E male and the E female were from the same population (e.g., E1 female, E1 male, M1 male) and ‘within M population’ when the M male and the M female were from the same population (e.g., M2 female, M2 male, E2 male).

To examine any effect of male and female body size on male mating success, we first tested for differences in absolute body size of males and females between the sexual selection treatments. These were tested both within replicate (e.g., E1 males vs. M1 males, or E3 females vs. M3 females) and with all replicates combined (E males vs. M males, and E females vs. M females), using Wilcoxon rank sum tests as size was not normally distributed. P-values were adjusted using the Holm procedure for multiple comparisons (Holm, 2012).
Average body size differed significantly between the treatments, with both E males and females being overall larger than their M counterparts, either taking all replicates into account or across most replicates (Table 1). To disentangle the effect of body size on mating patterns from the action of other traits that responded to sexual selection manipulation, we ran statistical models analysing both mating outcome and latency either with absolute male and female body size as covariates (presented within the text) or without (Appendix S1).

We analysed mating outcome (whether E or M males win) using a generalized linear mixed model with a binomial distribution. We specifically investigated what variables influence the probability of the two possible mating events (‘E male wins’ versus ‘M male wins’; ‘E male wins’ was used as the reference event). Female treatment, male replicate, E and M male size, E and M male relative size difference, female size, the temperature and the type of cross were included as fixed effects in the model. The interaction between female treatment and male replicate was also tested. Male and female replicate were nested within their respective sexual selection treatment. This analysis models the probability of an E male winning. We ran the same model for A females, with the exception that ‘female treatment’, ‘type of cross’, and ‘female replicate’ were obviously not included as effects in the model.

To test the mating latency response, we first log-transformed mating latency and then analysed it using a linear mixed model with a Gaussian distribution. Female treatment (‘E’ was used as the reference level), winning male treatment (‘E’ was used as the reference level), absolute body sizes of both males and of the female, temperature and type of cross (‘between populations’ was used as the reference level) were included as fixed effects. In addition to absolute male and female body sizes, the relative body size difference between the E and the M male was also included in the model as a fixed effect (a factor with two
levels: ‘E larger than M’ or ‘E smaller than M’ than M; ‘E smaller than M’ was used as the reference level. The interactions between winning male and female treatment (to test for assortative mating within sexual selection treatment), and between type of cross and winning male treatment (to test for a difference between the treatments in assortative mating within population), were also tested. Male and female replicate were nested within their respective sexual selection treatment, to account for variation among the replicated populations (Garland & Rose, 2009). This analysis models the speed it takes males from the different selection lines to mate with females of the different selection lines. We ran the same model for A females, with the exception that ‘female treatment’ and ‘type of cross’ could not be included as main effects and ‘female replicate’ could not be included as a random effect in the model.

In all the mixed models described above, the significance of fixed effects was tested using likelihood ratio tests. Normality and homoscedasticity of the residuals were checked graphically. Model estimates were used in figures, adjusted for the effects of all the other variables not included in the figure. All statistical analyses were performed in R (R Development Core Team 2005). The lme4 library was used for mixed-models (Bates & Sarkar, 2007), and the glht function in the multcomp library was used for post-hoc analysis of the mixed-model results (Hothorn et al., 2008). Raw mating outcome and mating latency data are also shown in Appendix S1 (see Fig. S2 and S3).

Results

There is no effect of the type of cross (that is, whether the female and the mating male are from the same population or not) on either mating outcome or mating latency. Neither E nor
M males are faster to mate or more likely to mate when they are in the presence of a female from their own population (Table 2). Instead, E males win significantly more matings with all females and mate overall at least as quickly as M males.

In the case of mating outcome, E males win more matings than M males regardless of female treatment (for E females: E males = 377, M males = 146, $\chi^2 = 98.83, P<0.001$; for M females: E males = 360, M males = 136, $\chi^2 = 101.16, P<0.001$). The mixed-model approach confirms this pattern, finding a much higher mating success of E males in comparison to M males (i.e., E males have a mating probability greater than 0.5 regardless of their replicate population; Fig. 1a; Table 2), and no significant effect of female treatment on the mating outcome (Table 2). Neither the relative size difference between the males, nor male absolute body sizes, have a significant effect on mating outcome (Table 2), meaning that the higher mating probability of E males is not the result of their larger size. In contrast to males, female size significantly influences the probability of an E male winning: E males are less successful with larger females (Table 2; Fig. 2a). Running the model without male and female body size shows the same pattern of treatment effect on mating outcome (see Table S1 of Appendix S1).

For mating latency, there is a significant interaction between winning male treatment and female treatment (Fig. 1b; Table 2). E females mate faster with E males when E males win, and mate slower with M males when M males win. In contrast, M females mate as quickly with M males as they do with E males. That is, when M males win, it takes them longer to initiate copulation with E females than with M females. Male body size has a significant
effect on mating latency. The relative size difference between the E and the M male
influences mating latency, with mating latency being shorter when the E male is larger than
the M male (Fig. 2b; Table 2). M male absolute size is negatively associated with mating
latency; that is, as M male size increases, males start mating with females earlier (Fig. 2c; 
Table 2). Overall, these results suggest that larger males, particularly M males, start mating
earlier than smaller males. In contrast, female size has no significant effect on mating
latency (Table 2). Running the model without male and female body size shows the same
direction of treatment effects on mating latency (see Table S1 of Appendix S1).

Mating trials with ancestral females show that E males also have a higher probability of
winning matings than M males (Fig. 3a; Table 3) and that M males take longer than E males
to achieve matings with ancestral females (Fig. 3b; Table 3). Ancestral female body size have
no effect on mating outcome or latency, likely because these females exhibit less variation in
body size than selection lines females (Levene’s test: $F_1=39.57$, $P<0.001$). Running models
without body size shows the same pattern of treatment effects (see Table S2 of Appendix
S1).

Discussion

We used an experimental approach to understand how changes in sexual selection intensity
can influence assortative mating in a system in which we have quantified changes in traits
related to both intra- and inter- sexual selection. We find that assortative mating is not
observed, either between treatments, or within sexual selection treatments. Instead, males
from polyandrous populations, who evolved under mate competition, benefit from a much
higher mating success, winning about 4 times more often than M males, regardless of female selection history.

What might cause these mating patterns? Predictions of assortative mating largely derive from an expectation of greater male-female coevolution under strong sexual selection (Lande, 1981; Kirkpatrick, 1982; Price, 1998; Kirkpatrick & Ravigné, 2002; Uyeda et al., 2009). There is evidence in our populations for coevolved song and female song preferences (Debelle et al., 2014) which may generate assortative mating. Song in this species is used as a species-specific signal, suggesting it is important in determining mating success and in reproductive isolation (Williams et al., 2001). We have measured a variety of other male traits in these populations that are thought to potentially influence pre-mating sexual selection and found divergent responses between the treatments in some (cuticular hydrocarbon profiles; courtship frequency; Hunt et al., 2012;Crudgington et al., 2010) but not all (sex comb tooth number; Snook et al., 2013) traits. The extent to which female preferences has changed for non-song traits have not been measured.

However, because we find that E males equally win with all types of females, it seems unlikely that male-female coevolution can explain our patterns of mating success. Yet, this does not mean that coevolution between the sexes has not occurred. Patterns of mating latency may provide some evidence of coevolution. Most interestingly, while E males mate faster than M males with females from populations experiencing polyandry (E and A), this difference is not seen with M females. When M males do win matings with M females, this is achieved faster than when M males win matings with E females. Therefore M males do seem to benefit from a relative mating advantage with M, and only M, females. This advantage perhaps reflects M female mating preference for M male courtship song (Debelle et al.,}
However, E males mate as fast as M males with M females, implying that E males can override this female preference.

Varying the intensity of sexual selection will also have targeted traits that evolve under male-male competition. Mate competition can override female mating preference by reducing the ability of females from detecting, evaluating and/or mating with preferred mates (Wong & Candolin, 2005), for example by intensifying courtship (i.e. decreasing courtship latency or increasing courtship rate) to maximise their mating success. Courtship rate commonly increases in a competitive context, as shown in sticklebacks (Shine et al., 2005), garter snakes (Kim & Velando, 2014) or fiddler crabs (Milner, 2012). Other experimental evolution studies have found that males from monogamous populations evolve reduced competitive mating success (Kawecki et al., 2012). The fact that E males initiate courtship faster and court more frequently than M males (Snook et al., 2005; Crudgington et al., 2010) may then influence the ability of females to detect and evaluate between males (Shaw & Lugo, 2001). Another trait potentially associated with competitive mating success is body size (Blanckenhorn, 2000). We found that the relative size difference between the E and the M male influenced mating latency, such that mating latency was shorter when the E male was larger than the M male. Larger M males also experienced a mating benefit; we found that as M male size increased, mating latency decreased. Generally then larger body size, particularly of E males, may influence mating patterns. This result has been shown in other Drosophila species where larger males mate faster due to their increased locomotor activity (Partridge et al., 1987b; Long & Rice, 2007).

While male body size was important in determining mating latency, neither absolute male body size nor the relative difference in male body sizes influenced mating outcome. The role
of male body size in mediating mating success in *D. pseudoobscura* is unclear; in some studies, larger males are more likely to be paired with females than smaller males (Partridge *et al.*, 1987a) but in other studies this body size advantage was not observed (Markow, 1988; Markow & Ricker, 1992). Instead of a male effect, we found that female body size had an influence on what male won, with E males being more likely to win with smaller compared to larger females. This suggests sexual conflict over mating decisions (Clutton-Brock & Parker, 1995). Sexual conflict occurs in our polyandrous populations and is eliminated in our monogamous populations (Crudgington *et al.*, 2005, 2010). Increased male mating persistence can evolve under sexual conflict (Arnqvist & Rowe, 2005) and E males are more persistent than M males (Crudgington *et al.*, 2010). Smaller, less resistant M females, may be less able to resist such males. We did not observe an overall effect of female treatment on mating latency or outcome, but the mating latency and size effects on mating success described here suggest that subtle interactions influence the outcome of the mating trials.

Male-male competition and female preference are not mutually exclusive forms of selection. For example, rapid, vigorous courtship may be selected for when mate competition is high, but will also be indirectly targeted by female preferences. Females are likely to obtain indirect benefits from mating with males who can out-compete other males. In this sense separating sources of selection into intra- versus intersexual selection is simplistic. However, the fact that we see polyandrous males succeeding in mating trials, despite some evidence for coevolution between the sexes in the experiment, suggests that greater selection on male competitive courtship ability in the polyandrous populations has overwhelmed any selection likely to cause assortative mating between populations from the treatments (or between replicate populations within the polyandry treatment). Parker & Partridge (1998)
suggested that if sexual conflict over mating outcome was strong, competitive males could act as a force for gene flow and inhibit speciation (alternatively, if female choice predominates, sexual conflict could increase speciation by assortative mating). Our results are more compatible with the “males ahead” outcome of this model, with polyandrous males, evolving under strong sexual selection, winning out in mating competitions with males and females from different evolutionary histories. Overall, this suggests that sexual selection has the potential to inhibit, as well as to increase, assortative mating and speciation (Servedio, 2004).

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77.

Tables

**Table 1** Average body size values (in millimetres) between the sexual selection treatment, by sex and replicate. Standard deviation is given next to each average body size value. Wilcoxon rank sum tests were performed between E and M treatments for all replicates combined, and for each replicate, to compare body size differences between the sexual selection treatments. P is the p-value. The sample size is N = 1019. E = polyandry, M = monogamy, R = replicate.

<table>
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<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tr>
<td></td>
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<td>M</td>
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<tr>
<td>Mean size</td>
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<tr>
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<tr>
<td>R4</td>
<td>2.24 ±0.06</td>
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Table 2 Output of the mixed-model for mating outcome and mating latency analyses for selection line females, including model estimates and tests statistics. In the mating outcome model, the response variable was the probability of an E male winning the mating. In the mating latency model, the response variable was the mating latency of the winning male. Winner treatment is the sexual selection treatment of the winning male (E or M), female treatment is the sexual selection treatment of the female (E or M), type of cross distinguishes between ‘within E population’, ‘within M population’ and ‘between populations’ crosses, and E-M relative size difference is the relative size difference between the males (‘E larger’ or ‘E smaller’). The following elements are specified: the model estimate(s) of each variable (β), the likelihood ratio statistic used to test the main effect of each variable (LR) and the p-value of the likelihood ratio test (p). The sample size is N = 1019. E = polyandry, M = monogamy.
### MATING OUTCOME

<table>
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<th>MATING LATENCY</th>
<th>Parameters</th>
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<td></td>
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539
Table 3 Output of the mixed-model for mating outcome and mating latency analyses for ancestral females, including model estimates and tests statistics. In the mating outcome model, the response variable was the probability of an E male winning the mating with an ancestral female. In the mating latency model, the response variable was the mating latency of the winning male. Winner treatment is the sexual selection treatment of the winning male (E or M) and E-M relative size difference is the relative size difference between the males (‘E larger’ or ‘E smaller’). The following elements are specified: the model estimate(s) of each variable (β), the likelihood ratio statistic used to test the main effect of each variable (LR) and the p-value of the likelihood ratio test (p). The sample size is N = 179. E = polyandry, M = monogamy.

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Figures

Fig. 1 Mating outcome probability and mating latency of selection line females. (a) Mating outcome (probability of an E male winning). The letters represent the fitted mating probabilities estimated by the mixed-model of an E male winning depending on female sexual selection treatment (labels of the x-axis). As these probabilities are superior to 0.5, the figure shows that E males have overall a higher mating success than M males. (b) Mating latency depending on male and female sexual selection treatment. The letters represent the fitted mating latencies estimated by the mixed-model of a male winning depending on male sexual selection treatment (the plotted values) and female sexual selection treatment (labels of the x-axis). The figure shows that M males mate as fast as E males with M females. Post-hoc tests adjusted for multiple comparisons show that mating latency significantly differs between E and M males with E females, but not with M females (for E females: $z=-3.1, p=0.0038$; for M females: $z=0.3, p=0.95$). In both (a) and (b), M is for monogamy, E is for polyandry, and 95% confidence intervals around each predicted value are represented in dotted lines. The model outputs are given in Table 2. E = polyandry, M = monogamy.
Female sexual selection treatment

(a) Winning probability of E male

(b) Mating latency (s)
**Fig. 2** Body size effects on mating outcome probability and mating latency of selection line females. (a) Mating outcome depending on female body size. The letters represent the fitted mating probabilities estimated by the mixed-model of an E male winning depending on female body size. The figure shows that female size is negatively correlated with the probability of an E male winning. (b) Mating latency depending on the relative size difference between E and M males. The letters represent the fitted mating latencies estimated by the mixed-model depending on male relative size difference. The figure shows that mating latency is reduced when the E male is larger than the M male (representing 35% of the trials). (c) Mating latency depending on M male body size. The letters represent the fitted mating latencies estimated by the mixed-model depending on M male body size. The figure shows that mating latency is negatively correlated with M male body size. In all plots, M is for monogamous males and E is for polyandrous males, and 95% confidence intervals around predicted values are represented in dashed lines. The model outputs are given in Table 2. E = polyandry, M = monogamy.
Fig. 3 Mating outcome probability and mating latency of ancestral females. (a) Mating outcome (probability of an E male winning). The letter represents the fitted mating probability estimated by the mixed-model of an E male winning. As this probability is superior to 0.5, the figure shows that E males have a higher mating success than M males. (b) Mating latency depending on male sexual selection treatment. The letters represent the fitted mating latencies estimated by the mixed-model of a male winning depending on male sexual selection treatment (the plotted values). The figure shows that E males mate slightly faster than M males. In both (a) and (b), M is for monogamy, E is for polyandry, and 95% confidence intervals around each predicted value are represented in dotted lines. The model outputs are given in Table 3. A = ancestral, E = polyandry, M = monogamy.