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Title page

Male *Drosophila melanogaster* show adaptive mating bias in response to female infection status

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Abstract

Given the non-trivial cost of reproduction for males and substantial variation in female quality, males have been predicted to show mating bias as an evolved strategy. Using a large outbred population of *Drosophila melanogaster*, we test this prediction and show that males may adaptively bias their mating effort in response to the infection status of females. Given a simultaneous choice between females infected with pathogenic bacteria and sham infected females, males preferentially mated with the latter, who had a higher reproductive output compared to infected females. This may provide evidence for pre-copulatory male mate choice. Assessment of the reproductive behaviour ensured that the observed pattern of mating bias was not due to differences in receptivity between females infected with pathogenic bacteria and sham infected females. Further, there was no evidence for post-copulatory male mate choice measured in terms of copulation duration.

Keywords: Copulation duration, Female receptivity, Male mate choice, Reproductive output, *Serratia marcescens.*

1. Introduction

Theory predicts that males will be less discriminating towards their mates because of lower investment in reproduction (Bateman, 1948; Trivers, 1972). However, several studies suggest that males can incur non-trivial costs of reproduction (Dewsbury, 1982; Andersson, 1994; Gems and Riddle, 1996; Galvani and Johnstone, 1998; Judge and Brooks, 2001; Kotiaho and Simmons, 2003; Martin and Hosken, 2004; Perez-Staples and Aluja, 2006; Byrne and Rice 2006; Friberg, 2006, Burton-Chellew et al., 2007; Long et al., 2009; Papadopoulos et al., 2010; Edward and Chapman, 2011; Nandy et al., 2012; Wegener et al., 2013). For males that contribute significantly to parental care or provide nuptial gift, costs of reproduction can be significantly large. In a polygynous species like *Drosophila melanogaster*, where there is no parental care involved or exchange of nuptial gifts, cost of reproduction can still be high due to high costs of ejaculate production and courtship related behaviour (Lefevre and Johnson, 1962; Partridge and Farquhar, 1981; Gromko et al., 1984; Cordts and Partridge, 1996; Edward and Chapman, 2011; Nandy et al., 2012). Further, if the cost of reproduction in males is sufficiently high and variation in female quality is substantial, male mate choice is likely to evolve as an adaptive strategy (Trivers, 1972; Bonduriansky, 2001; Edward and Chapman, 2011). Accordingly, mating effort and sperm investment of males are often biased towards females of higher quality (Bonduriansky and Brooks, 1998; Gage and Barnard, 1996; Katvala and Kaitala, 2001; Pitafi et al., 1995; Wedell and Cook, 1999). Some recent studies manipulated female quality in *D. melanogaster* and assessed the ability of males to show adaptive mate choice. In one set of studies, when offered a choice, males of *D. melanogaster* preferentially mated with larger, more fecund females compared to smaller, less fecund females (Byrne and Rice, 2006; Long et al., 2009; Edward and Chapman, 2011). Nandy et al. (2012) generated female quality variation in *D. melanogaster* by manipulating age and nutrition status of females and found that males preferentially mated with high fitness

69 females. More interestingly, the degree of male mate choice was positively correlated with
70 the variance in female fitness (Nandy et al., 2012). Thus in fruit flies, males seem to be
71 sensitive to signals of female quality variation and adaptively bias their reproductive efforts.

72 One of the most important determinants of an individual's quality is its parasitic load
73 (Hamilton and Zuk, 1982; Mckean et al., 2008). In a previous study, it was shown that
74 infecting *D. melanogaster* with pathogenic bacteria, *Serratia marcescens* resulted in
75 decreased reproductive output of females while male reproductive fitness remained
76 unaffected (Imroze and Prasad, 2011). Hence, it is possible that *Drosophila* males bias their
77 mating or show cryptic choice based on the infection status of the females. However, these
78 possibilities have rarely been addressed to date (but see Sullivan and Jaenike, 2006).

79 In the present study, we address this issue using a population of *Drosophila melanogaster*
80 that harbours substantial genetic variation as the host and a gram negative bacterium, *Serratia*
81 *marcescens* as the pathogen. We created males and females of three different infection
82 statuses (infected, sham infected and non-infected/ unhandled controls). We combined them
83 factorially and assessed their reproductive output and reproductive behaviour. We measured
84 mating latency (ML) as an indicator of female receptivity to mate and copulation duration
85 (CD) as an indicator of male ejaculate investment and post-copulatory cryptic male mate
86 choice (Friberg, 2006; Bretman et al., 2009; Nandy and Prasad, 2010; Nandy et al., 2012).
87 We then examined whether males bias their mating when offered a simultaneous two way
88 choice between infected and sham infected females.

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2. Materials and methods

2.1 Fly maintenance

We used a laboratory population of *Drosophila melanogaster* (called LH) that harbours considerable amount of genetic variation for this study (Chippindale and Rice, 2001). Larry Harshman founded this population with 400 wild caught inseminated females from central California in 1991 and since then it has been maintained as a laboratory population with an effective population size of >5000. Flies are maintained on a 14-day discrete generation cycle, at 25°C, 50% RH and 12:12 h light: dark cycle on cornmeal–molasses diet. Flies are cultured in vials (9.5cm height × 2.5cm diameter) with standard cornmeal-molasses food medium. Each generation, approximately 150 eggs are cultured per vial with 8-10 ml of food. A total of 60 such vials are set up. After 12 days when almost all flies have eclosed, adults from different vials are mixed and redistributed into 60 fresh food vials at a density of 16 pairs (16 males and 16 females) per vial. In addition to standard cornmeal-molasses food medium (identical to the one that the larvae fed on), these vials are provided with a limiting quantity of live yeast supplement. After two days of interaction, the flies are transferred to fresh food vials and allowed to oviposit for 18 hours, following which the adult flies are discarded. Egg density in these oviposition vials is then adjusted to ~150 per vial to start the next generation.

2.2 Generation of experimental males and females

To generate all experimental males and females, eggs were collected from adult flies of the stock population (LH) and dispensed into vials (9.5 cm height × 2.5cm diameter) containing 8-10 ml of cornmeal-molasses food at the density of ~150 per vial. The vials were incubated at 25°C, 50% RH and 12:12 h light: dark cycle. During peak eclosion (10^{th} day post egg

115 collection) males and females were collected as very young virgins (<4 hrs post eclosion).
116 Virgin flies were then housed for 2 days at a density of 10 per vial in single sex groups for the
117 experiments described below.

118 **2.3 Micro-organism and infection protocol**

119 The strain of bacteria used for all the experiments was *S. marcescens* ATCC 13880 (Lazzaro
120 et al., 2006). It is a pathogen that has a wide range of hosts. It has also been isolated from
121 *Drosophila* (Flyg et al., 1980; Flyg and Xanthopoulas, 1983; Cox and Gilmore, 2007).
122 Consequently, several studies of evolutionary ecology of immune response have used *S.*
123 *marcescens* as a model bacterial pathogen (Lazzaro et al., 2004; Lazzaro et al., 2006; Khan
124 and Prasad, 2013).

125 On the evening before infection, we inoculated a fresh bacterial culture in Luria Broth and
126 allowed it to grow overnight at 37°C to OD 1.0. We then centrifuged the resulting population
127 of bacteria and made it into slurry. Experimental flies (see section 2.2) under light CO_2
128 anaesthesia were infected by pricking at the lateral side of the thorax with a 0.1 mm minutien
129 pin (Fine Science Tools, Foster City, CA) dipped into the bacterial slurry. For sham infection,
130 we pricked the flies with a pin dipped in *Drosophila* Ringer solution (McKean and Nunney,
131 2001).

132 **2.4 Assay for reproductive behaviour and reproductive output**

133 On day 2 post eclosion, the vials (containing virgin males and females, see section 2.2) were
134 randomly divided into 3 groups. Flies in the first group of vials were subjected to infection
135 with live bacteria (I), flies from the second group of vials were subjected to sham infection
136 (SI) and flies from the third group of vials were used as non-infected (un-handled) controls

(NI). Following the treatment (infection/ sham-infection), flies were held for one day in single sex groups of 5 per vial.

On day 3 post eclosion, we constituted mating vials in an orthogonal 3 male types ×3 female types design. Five females of given treatment type (i.e., I or SI or NI) were combined with five males of given treatment type (i.e., I or SI or NI) in a fresh food vial without using anaesthesia and were allowed to interact for 45 minutes. Since in this experiment, the males in a vial were combined with only one type of female (i.e., the males could not choose between different types of females), we call this condition as 'no-choice' condition. Females were then separated (under light CO_2 anaesthesia) and transferred individually into test tubes (12mm × 75mm) containing corn meal-molasses medium (one female per test tube) to oviposit for 18 hours. Following this, females were discarded and test tubes were incubated at 25˚C. The number of progeny emerging from each test tube was counted 13 days later and averaged across the number of females that produced progeny from each vial to obtain a vial mean of reproductive output. We also calculated "Mating success" for each vial by taking the ratio of the females that produced progeny (and hence were mated) to the total number of females present in each vial. This yields a measure of the proportion of females that were inseminated within 45 minutes under "no-choice" condition within each vial (Nandy et al., 2012). The 45 minutes interaction time between males and females ensures single mating in our fly system and has already been standardised as a protocol (Nandy et al., 2012). During the interaction time, mating behaviour of the flies was closely observed at 25˚C under uniform overhead illumination. In each of the vials, we recorded the number of mating pairs over time which gave start and end time of copulation. Observations were continued till all mating pairs disengaged (this meant that for a small number of pairs, the observation continued beyond the 45 minute window). Using this data, we calculated the mean time to

begin copulation and the mean time to end of copulation for each vial (Nandy et al., 2012). Mean time to begin copulation gave us the mating latency (ML). The difference between the two values yielded the copulation duration (CD). Therefore, from each vial we obtained one value of ML and CD. Previous studies using LH population and its derivatives have followed similar protocol to determine ML and CD and this protocol has been standardised for our experimental system (Nandy and Prasad, 2011; Nandy et al., 2012).

The assay for reproductive behaviour (ML and CD) and reproductive output were repeated twice in two independent replicate experiments. In replicate experiment 1, we had 8-9 replicate vials per mating combination while in replicate experiment 2, we had 4-5 replicate vials per mating combination. In replicate experiment 1, due to an experimental error, food in some of the test tubes dried up. Hence, we could not get reproductive output data from the corresponding vials. The details of the exact number of replicate vials used in each of the replicate experiments have been provided in Table S2.

2.5 Assay for male mating bias

The experimental protocol to detect male mating bias was designed after those of earlier studies (Byrne and Rice, 2006; Nandy et al., 2012). Experimental males and females were collected as described above in section 2.2 and virgin flies were housed at a density of 10 per vial in single sex groups. On day 2 post eclosion, females were randomly divided into two groups. The females in the first group were subjected to bacterial infection (I) while those in the second group were subjected to sham infection (SI). They were then held in single sex groups of 10 individuals for one more day after which they were combined with males to assess male mating bias. The males used in this experiment did not receive any treatment and hence remained 'unhandled' till they were combined with females.

184 On day 3 post eclosion, we tested for male mating bias. In a fresh food vial, we combined 10
185 infected females and 10 sham-infected females with 10 unhandled males without using
186 anaesthesia. The two types of females in the vial were identified by colouring their abdomen.
187 This was done by feeding the females with dietary yeast suspension (7mg yeast/ 100µl)
188 combined with commercially available non-toxic food colours (green and red) for 3 hours
189 prior to the beginning of the mating bias experiment on day 3 post-eclosion. Further, we
190 replicated the experiments with reciprocal colouring of females to eliminate the effect of
191 abdomen-colours on mating bias. After combining the two types of females with males, the
192 vials were left undisturbed for 45 minutes. This protocol has been standardised in our
193 laboratory for our fly system and ensures a single mating per fly. Following this, mating was
194 stopped by mechanically disturbing the vials and females were separated under mild CO_2
195 anaesthesia. Females were then transferred individually to test tubes (12 mm × 75 mm)
196 containing corn meal-molasses medium to lay eggs for 48 hours, after which they were
197 discarded. The test tubes were then incubated at 25^{o}C and after 4 days they were examined
198 under a dissecting microscope to determine the presence of larvae (Byrne and Rice, 2006;
199 Nandy et al., 2012). If an oviposition tube contained larvae, then the resident female was
200 scored as 'mated' and in those with no larvae, the resident female was scored as 'unmated'
201 (Nandy et al., 2012). Therefore, from each vial we obtained the numbers of infected and
202 sham infected females that mated. In each vial, a mating bias score was calculated by taking
203 the ratio of the number of infected females mated to the total number of females mated.

204 The experiment was performed during the light phase of 12:12 LD cycle of flies at 25^{o}C
205 under uniform overhead illumination. Male mating bias experiment was repeated four times
206 in four independent replicate experiments. Within each replicate experiment, two sets of

female body colour combination (red: infected vs. green: sham infected and vice versa) were handled and each of these sets was composed of three replicate vials.

2.6 Data analysis

To assay the reproductive behaviour and reproductive output, we first, analysed the data with three factor mixed model ANOVA with treatments as the fixed factors crossed with replicate experiments. However, to account for the fact that the two replicate experiments had different sample sizes and which might lead to complications of unbalanced ANOVA, we further analysed data from each replicate experiment separately using a two-factor ANOVA treating male and female infection status as fixed factors.

We used a Wilcoxon rank sum test to analyse the mating success of females of all three types within each replicate experiment since this data was not normally distributed (verified by Goodness -of- fit test by Shapiro-Wilk W test).

While assessing male mating bias, in each vial, mating bias scores were calculated by taking the ratio of the number of infected females mated to the total females mated. If there is no mating bias, then a mating bias score of 0.5 is expected. A deviation from this score would suggest mating bias. For instance, if there is a bias towards the sham infected females, mating bias score would range between 0.5 and 0, with lower values indicating stronger bias. If the bias is towards infected females, mating bias score should range between 0.5 and 1, with higher values indicating stronger bias. Data were analysed with a two-way ANOVA with body colour as the fixed factor and the replicate experiments as random factor to check for the effect of female body colour on mating bias.

3. Results

3.1 Reproductive behaviour and reproductive output

As stated before, we analysed the data in two different ways (a) data from two replicate experiments were analysed together in a three-way mixed model ANOVA (see Table 1-3) and (b) data from the two replicate experiments were analysed separately using a two-way ANOVA (see supplementary material for Table S 1). However, the two analyses yielded similar results (see Table 1-3 and Table S 1). Hence, we present results from the three-way mixed model ANOVA in this manuscript.

Mating latency was not affected by the male or female infection status (Fig 1a, Table 1) indicating that infection did not change male or female receptivity to mating (Tukey's HSD). When housed with unhandled or non-infected control males, the mating latency of infected and sham infected females was not significantly different (mean mating latency± SE; *Replicate Experiment 1*: Infected= 6.58± 0.912, sham infected= 6.22± 0.641; p=0.75; *Replicate Experiment 2:* Infected= 7.94± 1.002, sham infected= 7.8± 0.896; p=0.921). Copulation duration was also not significantly affected by male or female infection status (Fig 1b, Table 2; Tukey's HSD). The mating success of the females from the three treatments (I, SI, NI) did not differ significantly in either of the replicate experiments (Table 4). This indicates that the proportion of the females inseminated (Mating success) within 45 minutes under "no-choice" condition was not significantly different across treatments.

There was no significant effect of male infection status on reproductive output (Fig 1c, Table 3). However, infected females showed approximately ~40% reduction in reproductive output (Fig 1c, Table 3). Females from the I treatment of each replicate experiment, produced significantly less progeny compared to females from SI and NI treatments (Tukey's HSD). Reproductive output did not differ significantly between SI and NI females (Tukey's HSD).

3.2 Male mating bias

We found no significant main effect or interaction of body colour and replicate experiment on mating bias scores. Hence we pooled data across body colour within each replicate experiment. To test whether the observed mean mating bias scores are significantly different from the expected mean value of 0.5, we did a one-sample t-test (two tailed, $\alpha = 0.05$). Values for the upper limit and the lower limit of the 95% CI did not overlap with 0.5 in any of the four replicate experiments (Table 5). Thus, mating bias scores within each of the four replicate experiments were significantly less than 0.5 indicating a bias in favour of the sham infected females (see Table 5). Proportion of the infected females fertilised was ~15.2% less than that of the sham infected females.

4. Discussion

In the present study, bacterial infection resulted in a reduction in the reproductive output of females whereas male reproductive ability remained unaffected. We found that males preferentially mated with sham infected females (which have relatively higher reproductive potential) when subjected to a simultaneous two-way choice between infected and sham infected females. However, infection status did not change female receptivity towards mating or male ejaculate investment (in terms of copulation duration).

The observed sexual dimorphism in the fitness consequence of *S.marcescens* infection in the present study not only confirms our previous report (Imroze and Prasad, 2011) but also does so in a very different context of fitness. In the present study, (a) reproductive fitness was measured under non-competitive condition instead of competitive condition (see Prasad et al., 2007) as in the previous study, (b) flies were exposed to a single mating instead of two days of male-female interaction as in the previous study and (c) flies were allowed to oviposit for one day immediately after infection while in the earlier experiment there was a 3-day gap

277 between the day of infection and the oviposition. Together, these findings point to the
278 remarkable robustness of the sex specific fitness effect of *S. marcescens* infection.

279 Based on the results presented here and in our previous report (Imroze and Prasad, 2011) it is
280 clear that female fitness in *D. melanogaster* is greatly reduced by bacterial infection. Further,
281 if the cost of reproduction for males is substantial (Dewsbury, 1982; Andersson, 1994;
282 Galvani and Johnstone, 1998; Judge and Brooks, 2001; Martin and Hosken, 2004; Byrne and
283 Rice, 2006; Friberg, 2006; Edward and Chapman, 2011; Nandy et al., 2012; Wegener et al.,
284 2013), they might be expected to show at least some degree of mate preference for uninfected
285 females over the infected ones. Indeed, our data indicated that when offered a simultaneous
286 two-way choice, males preferentially mate with sham infected females over infected females.

287 There are at least three potential explanations for the observed mating bias, including
288 adaptive male mate choice. First, it is possible that bacterial infection can potentially reduce
289 female receptivity to mating because of changes in and/or damage to physiology. Therefore,
290 infected females might be less eager to mate compared to the sham infected females and the
291 bias in mating pattern observed in our study can be explained in terms of higher receptivity of
292 the sham infected females compared to the infected females. However in the present study,
293 bacterial infection did not cause any change in the receptivity measured in terms of ML
294 among any given pair irrespective of the female or male infection status. We found no
295 significant difference in the receptivity, measured in terms of ML, of infected and sham
296 infected of females when housed with non-infected control males (See results; section 3.1).
297 Therefore, observed mating bias is unlikely to be due to greater receptivity of sham infected
298 females.

299 Second, the result can be an artefact of our experimental design. We scored a female as mated
300 if it produced eggs that hatched into larvae. Since pathogenic invasion interferes with host

physiology, it can potentially affect female egg production, leading to sterility in females. Therefore, it is possible that there might be no larval presence in the oviposition tubes even if the resident female receives mating during the two way choice experiment. However, our data of female mating success indicated that the mean proportion of fertilized females across treatments (I, SI, NI) did not differ significantly from each other (under 'no-choice' condition) in any of the replicate experiments (see Table 4).

Hence, it seems very likely that the mating bias observed in our experiment was due to the third possibility, i.e., adaptive male mate choice. Males might preferentially mate with sham infected females because they have higher reproductive output. Alternatively, males might avoid mating with infected females due to the risk of infection during copulation. A recent study with *D. melanogaster* showed transmission of *S. marcescence* infection from males to females during mating (Miest and Bloch-Qazi, 2008). However, this was achieved by experimentally dipping the male genitalia into bacterial slurry before mating. Therefore, there is still no evidence that "infected" males can transfer bacteria during mating. Similarly, as yet, there is no evidence that "infected" *Drosophila* females can transfer bacteria to males during copulation (Knell and Webberley, 2004; Miest and Bloch-Qazi, 2008). However, since in our experiment, infected females also have significantly reduced reproductive output, the mating bias of males towards sham infected females does benefit males and is, hence, adaptive. It is important to point out that our experimental design to assess possible male mate choice was very similar to those of other studies which addressed male mate choice explicitly (Byrne and Rice, 2006; Nandy et al., 2012). We would also like to point out that this result comes from the same experimental system (LH laboratory population) that was used by Byrne and Rice (2006) and Nandy et al. (2012). Further, we have used a single pathogen (*S. marcescens*). Hence, we would consider the adaptive male mate choice with

respect to female infection status observed in our study as an indication of an exciting possibility that needs further strengthening.

Our results about mating bias are in contrast to those of Sullivan and Jaenike (2006) who found that the number of infected and uninfected females of *Drosophila innubila* that received mating was not different in a male mate choice experiment. However, this study used maternally inherited male-killing pathogen *Wolbachia* which differs from *Serratia marcescens* in its transmission mode and effect on host life-history (Bandi et al., 2001; Champion de Crespigny et al., 2006).

Males can exhibit mate choice not only in terms of mating bias but also in the form of variation in ejaculate investment. Copulation duration (CD) is often used as an indicator of male reproductive investment and is often positively correlated with components of male reproductive success (Bretman et al., 2009; Friberg, 2006; Nandy and Prasad, 2011). Additionally, variation in CD is also an indirect measure of cryptic male mate choice (Friberg, 2006; Martin and Hosken, 2002; Siva-Jothy and Stutt, 2003). In the present study, we did not find any significant effect of male or female infection status on CD, indicating that there was no difference in male investment across different females. However, a very recent study by Lüpold et al. (2011) reported that male might adjust their ejaculate investment or amount of sperm transferred without any alteration in CD. Since we did not measure the amount of ejaculate or count sperms directly, we cannot completely rule out the possibility of post-copulatory, cryptic male mate choice.

Our result about CD is similar to those of Pai and Yan (2003) who found that in flour beetles, *Tribolium castaneum*, CD was not affected upon rat tapeworm infection (Pai and Yan, 2003). However, our result is in contrast to a study in crickets which found a negative correlation between immune activation and copulation duration (Fedorka and Mousseau, 2006). This

difference can be attributed to (among other things) the difference between mating strategies of *Drosophila* and crickets. Unlike *Drosophila*, nuptial gifts are transferred during copulation in crickets. In an earlier report, Fedorka et al. (2005) showed that immune-compromised male crickets have smaller gift sizes and initiate early termination of copulation (Fedorka et al., 2005).

Our results add to the small but growing body of evidence for adaptive male mate choice (Byrne and Rice, 2006; Long et al., 2009; Edward and Chapman, 2011; Nandy et al., 2012). While several previous studies have suggested that infection or activation of immune system can adversely affect male sexual signals (Rantala and Kortet, 2003; Fedorka and Mousseau, 2006) and can therefore potentially affect female choice, ours is the first study to document pre-copulatory male mate choice in response to female infection status in *D. melanogaster*. However, the cues that the males use to ascertain infection status of females in our study are not clear. In some of the previous studies (Byrne and Rice, 2006; Long et al., 2009; Edward and Chapman, 2011) males could potentially depend on female body size as an indicator of female quality. However in our study, body sizes of infected and sham infected females are not expected to be different. Alternatively, pathogenic bacteria might subtly affect a suit of traits including behaviour which might be used as a cue by the males. One interesting possibility is that males could use changes in cuticular hydrocarbon profile as a cue of female quality (Nandy et al., 2012). In *D. melanogaster*, cuticular hydrocarbon profile can be affected by the commensal microbes present in the organism (Sharon et al., 2010). This can in turn drive rapid divergence of mating preferences (Sharon et al., 2010). Therefore, while it is not clear whether pathogenic bacteria can drive changes in cuticular hydrocarbons, a potential for such change does exist and can possibly be used as a cue by the males for biasing their mating efforts.

The broader question of how common and important is adaptive male mate choice, is still open. It is interesting to note that all the studies on male mate choice in fruit flies have used only two populations of *D. melanogaster* (LH and Dahomey). While male mate choice in Dahomey populations seems to be highly variable and dependent upon the exact assay conditions (Edward and Chapman, 2013), males from the LH population show adaptive male mate choice when assayed in a wide range of experimental conditions (Byrne and Rice, 2006; Long et al., 2009; Nandy et al., 2012). Our results obtained under different conditions compared to previous studies (Byrne and Rice, 2006; Long et al., 2009; Nandy et al., 2012) clearly show the consistency of adaptive male mate choice in LH populations. It has been suggested that the differences in male mate choice between the LH and Dahomey populations might be due to the different selection pressures acting on these populations (Edward and Chapman, 2013). Clearly, further studies would be required to understand the extent of occurrence and variation in adaptive male mate choice.

To summarise, the present study documents the effect of bacterial infection on male mating bias in *D. melanogaster*. We found evidence that female infection status can potentially affect adaptive male mate choice with males preferring to mate with sham infected females of higher reproductive potential over infected females. The bias in mating was not due to higher receptivity of sham infected females. Further, we did not find any evidence of post-copulatory male mate choice in terms of altered copulation duration across different female types.

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References

Andersson, M., 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Bandi, C., Dunn, A.M., Hurst, G.D.D., Rigaud, T., 2001. Inherited microorganisms, sex-specific virulence and reproductive parasitism. Trends in Parasitology, 17, 88-94.

Bateman, A.J., 1948. Intra-sexual selection in *Drosophila*. Heredity, 2, 349-368.

Bonduriansky, R., 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biological Reviews of Cambridge Philosophical Society, 76, 305–339.

Bonduriansky, R., Brooks, R. J., 1998. Male antler flies (*Protopiophila litigata* ; Diptera: Piophilidae) are more selective than females in mate choice. Canadian Journal of Zoology, 76, 1277–1285.

411 Bretman, A., Fricke, C., Chapman, T., 2009. Plastic responses of male *Drosophila*
412 *melanogaster* to the level of sperm competition increase male reproductive fitness.
413 Proceedings of the Royal Society B: Biological Sciences, 276, 1705-1711.

414 Burton-Chellew, M.N., Sykes, E.M., Patterson, S., Shuker, D.M., West, S.A., 2007. The cost
415 of mating and the relationship between body size and fitness in males of the parasitoid wasp
416 *Nasonia vitripennis*. Evolutionary Ecology Research, 9, 921–934.

417 Byrne, P.G., Rice, W.R., 2006. Evidence for adaptive male mate choice in the fruit fly
418 *Drosophila melanogaster*. Proceedings of the Royal Society B: Biological Sciences, 273,
419 917-922.

420 Champion de Crespigny, F.E., Wedell, N., 2006. *Wolbachia* infection reduces sperm
421 competitive ability in an insect. Proceedings of the Royal Society B: Biological Sciences,
422 273, 1455–1458.

423 Chippindale, A.K., Rice, W.R., 2001. Y chromosome polymorphism is a strong determinant
424 of male fitness in *Drosophila melanogaster*. Proceedings of the National Academy of
425 Sciences of the United States of America, 98, 5677-5682.

426 Cordts, R., Partridge, L., 1996. Courtship reduces longevity of male *Drosophila*
427 *melanogaster*. Animal Behaviour, 52, 269–278.

428 Cox, C.R., Gilmore, M.S., 2007. Native microbial colonization of *Drosophila melanogaster*
429 and its use as a model of *Enterococcus faecalis* pathogenesis. Infection and Immunity, 75,
430 1565–1576.

431 Dewsbury, D.A., 1982. Ejaculate cost and mate choice. American Naturalist, 119, 601–610.

Edward, D. A., Chapman, T., 2011. The evolution and significance of male mate choice. TREE, 26, 647– 654.

Edward, D.A., Chapman, T., 2013. Variation in Male Mate Choice in *Drosophila melanogaster*. PLoS ONE, 8(2), e56299.

Fedorka, K.M., Mousseau, T.A., 2006. Immune system activation affects the male sexual signal and reproductive potential in crickets. Behavioral Ecology, 18, 231-235.

Fedorka, K.M., Zuk, M., Mousseau, T.A., 2005. Natural selection drives the link between male immune function and reproductive potential. Canadian Journal of Zoology, 83, 1012–1014.

Flyg, C., Kenne, K., Boman, H.G., 1980. Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants with a decreased resistance to Cecropia immunity and a decreased virulence to *Drosophila*. Journal of General Microbiology, 120, 173-81.

Flyg, C., Xanthopoulos, K. 1983. Insect pathogenic properties of *Serratia marcescens*. Passive and active resistance to insect immunity studied with protease-deficient and phage-resistant mutants. Journal of General Microbiology, 129, 453–464.

Friberg, U., 2006. Male perception of female mating status: its effect on copulation duration, sperm defence and female fitness. Animal Behaviour, 72, 1259–1268.

Gage, A.R., Barnard, C.J. 1996. Male crickets increase sperm number in relation to competition and female size. Behavioral Ecology and Sociobiology, 38, 349–353.

Galvani, A., Johnstone, R.A., 1998. Sperm allocation in an uncertain world. Behavioral Ecology and Sociobiology, 44, 161–168.

453 Gems, D., Riddle, D.L., 1996. Longevity in *Caenorhabditis elegans* reduced by mating but
454 not gamete production. Nature, 379, 723–725.

455 Gromko, M. H., Newport, M. E. A., Kortier, M. G., 1984. Sperm dependence of female
456 receptivity to remating in *Drosophila melanogaster*. Evolution, 38, 1273–1282.

457 Hamilton, W.D., M. Zuk., 1982. Heritable true fitness and bright birds: a role for parasites?
458 Science, 218, 384-387.

459 Imroze, K., Prasad, N.G., 2011. Sex-specific effect of bacterial infection on components of
460 adult fitness in *Drosophila melanogaster*. Journal of Evolutionary Biology Research, 3, 79-
461 86.

462 Judge, K.A., Brooks, R., 2001. Chorus participation by male bull-frogs, *Rana catesbeiana*: a
463 test of the energetic constraint hypothesis. Animal Behaviour, 62, 849–861.

464 Katvala, M., Kaitala, A., 2001. Male choice for current egg fecundity in a polyandrous egg-
465 carrying bug. Animal Behaviour, 62, 133–137.

466 Khan, I., Prasad, N.G., 2013. The aging of the immune response in *Drosophila melanogaster*.
467 The Journals of Gerontology: Series A, 68, 129-35.

468 Knell, R.J., Webberley, K.M., 2004. Sexually transmitted diseases of insects: distribution,
469 evolution, ecology and host behaviour. Biological reviews, 79, 557-581.

470 Kotiaho, J.S., Simmons, L.W., 2003. Longevity cost of reproduction for males but no
471 longevity cost of mating or courtship for females in the male-dimorphic dung beetle
472 *Onthophagus binodis*. Journal of Insect Physiology, 49, 817–822.

473 Lazzaro, B.P., Sackton, T.B., Clark, A.G., 2006. Genetic variation in *Drosophila*
474 *melanogaster* resistance to infection: a comparison across bacteria. Genetics, 174, 1539–
475 1554.

476 Lazzaro B.P., Sceurman B.K., Clark A.G., 2004. Genetic basis of natural variation in *D.*
477 *melanogaster* antibacterial immunity. Science, 303, 1873–1876.

478 Lefevre, G., Johnson, U. B., 1962. Sperm transfer, storage, displacement, and utilization in
479 *Drosophila melanogaster*. Genetics, 47, 1719–1736.

480 Long, T. A. F., Pischedda, A., Stewart, A. D., Rice, W. R., 2009. A cost of sexual
481 attractiveness to high-fitness females. PloS Biology, 7(12), e1000254.

482 Lupold, S., Manier, M. K., Ala-Honkola, O., Belote, J. M., Pitnick, S., 2011. Male
483 *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity and
484 age. Behavioral Ecolology, 22, 184–191.

485 Martin, O.Y., Hosken, D.J., 2002. Strategic ejaculation in the common dung fly *Sepsis*
486 *cynipsea*. Animal Behaviour, 63, 541–546.

487 McKean, K.A., Nunney, L., 2001. Increased sexual activity reduces male immune function in
488 *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United
489 States of America, 98, 904-909.

490 McKean, K.A., Yourth, C.P., Lazzaro, B.P., Clark, A.G., 2008. The evolutionary costs of
491 immunological maintenance and deployment. BMC Evolutionary Biology, 8, 76–95.

492 Miest, T.S., Bloch-Qazi, M.C., 2008 Sick of mating: Sexual transmission of a pathogenic
493 bacterium in *Drosophila melanogaster*. Fly (Austin), 2(4), 215-219.

494 Nandy, B., Prasad, N.G., 2011. Reproductive behavior and fitness components in male
495 *Drosophila melanogaster* are nonlinearly affected by the number of male co-inhabitants early
496 in adult life. Journal of Insect Science, 11, 67.

497 Nandy, B., Joshi, A., Ali, Z.S., Sen, S., Prasad, N.G., 2012. Degree of adaptive male mate
498 choice is positively correlated with female quality variance. Scientific Reports, 2, 447; DOI:
499 10.1038/srep00447.

500 Pai, A., Yan, G., 2003. Effects of tapeworm infection on male reproductive success and
501 mating vigor in the red flour beetle, *Tribolium castaneum*. Journal of Parasitology, 89, 516–
502 521.

503 Papadopoulos, N. T., Liedo, P., Müller, H., Wang, J., Carey, J. R., 2011. Cost of reproduction
504 in male medflies: The primacy of sexual courting in extreme longevity reduction. Journal of
505 Insect Physiology, 56, 283–287.

506 Partridge, L., Farquhar, M., 1981. Sexual activity reduces lifespan of male fruitflies. Nature,
507 294, 580–582.

508 Perez-Staples, D., Aluja, M., 2006. Sperm allocation and cost of mating in a tropical tephritid
509 fruit fly. Journal of Insect Physiology, 52, 839–845.

510 Pitafi, K.D., Simpson, R., Day, T. 1995. Male mate choice for fecund females in seaweed
511 flies. Pakistan Journal of Zoology, 27, 233–240.

512 Prasad, N.G., Bedhomme, S., Day, T., Chippindale, A.K., 2007. An evolutionary cost of
513 separate genders revealed by male-limited evolution. American Naturalist, 169, 29–37.

514 Rantala, M.J., Kortet, R., 2003. Courtship song and immune function in the field cricket
515 *Gryllus bimaculatus*? Biological Journal of Linnean Society, 79, 503-510.

516 Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I., 2010. Commensal
517 bacteria play a role in mating preference of *Drosophila melanogaster*. Proceedings of the
518 National Academy of Sciences of the United States of America, 107, 20051–20056.
519
520 Siva-Jothy, M.T., Stutt, A.D., 2003. A matter of taste: direct detection of female mating
521 status in the bedbug. Proceedings of the Royal Society B: Biological Sciences, 270, 649–652.
522
523 Sullivan, J., Jaenike, J., 2006. Male-killing *Wolbachia* and male mate choice: a test with
524 *Drosophila innubila*. Evolutionary Ecology Research, 8, 91–102.
525
526 Trivers, R.L., 1972. Parental investment and sexual selection. In: Sexual selection and the
527 descent of man: 1871-1971 (Campbell B, ed). Chicago: Aldine-Atherton; 136 -179.
528
529 Wedell, N., Cook, PA., 1999. Strategic sperm allocation in the small white butterfly *Pieris*
530 *rapae* (Lepidoptera: Pieridae). Functional Ecology, 13, 85–93.
531 Wegener, B.J., Stuart-Fox, D.M., Norman, M.D., Wong, B.B.M., 2013. Strategic male mate
532 choice minimizes ejaculate consumption, 24(3), 668-671.
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Tables

Table 1. **Effect of male and female infection status on mating latency (ML).** Summary of three way mixed model ANOVA on ML with treatment as fixed factor crossed with replicate experiments as random factor.

Factor	df	SS	*F*	*P*
Female infection status (F)	2	5.965	0.389	0.678
Male infection status (M)	2	8.802	0.574	0.564
Replicate experiments (R)	1	0.804	0.105	0.747
F*M	4	20.762	0.678	0.609
F*R	2	1.032	0.068	0.935
M*R	2	3.011	0.196	0.822
F*M*R	4	23.625	0.771	0.546
Error	104	796.42		

Table 2. Effect of male and female infection status on copulation duration (CD). Summary of three way mixed model ANOVA on CD with treatment as fixed factor crossed with replicate experiments as random factor.

Factor	df	SS	*F*	*P*
Female infection status (F)	2	9.274	0.238	0.788
Male infection status (M)	2	34.917	0.899	0.41
Replicate experiments (R)	1	0.163	0.008	0.927
F*M	4	49.225	0.633	0.639
F*R	2	8.536	0.219	0.8031
M*R	2	2.598	0.0669	0.935
F*M*R	4	41.854	0.538	0.707
Error	104	2019.682		

Table 3. Effect of male and female infection status on reproductive output. Summary of three way mixed model ANOVA on reproductive output with treatment as fixed factor crossed with replicate experiment as random factor. Significant value is shown in bold.

Factor	df	SS	*F*	*P*
Female infection status (F)	2	2254.1	28.027	**<0.0001**
Male infection status (M)	2	70.378	0.875	0.42
Replicate experiments (R)	1	2.77	0.069	0.793
F*M	4	21.578	0.134	0.969
F*R	2	92.947	1.155	0.319
M*R	2	40.894	0.508	0.603
F*M*R	4	54.103	0.336	0.852
Error	93	3739.757		

567 **Table 4.** Summary of Wilcoxon rank sum test of the mating success of the three types of
568 females.

Replicate Experiments	Infection status	Mean	SE	Chi-square	df	*P*
1	I	0.904	0.023	0.3758	2	0.83
	NI	0.908	0.022			
	SI	0.927	0.023			
2	I	0.938	0.032	2.7764	2	0.25
	NI	0.867	0.03			
	SI	0.88	0.032			

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Table 5. Mean mating bias score from each of the four replicate experiments. The 95% CI around the mean did not overlap the expected mean value of 0.5 in any of the replicate experiments.

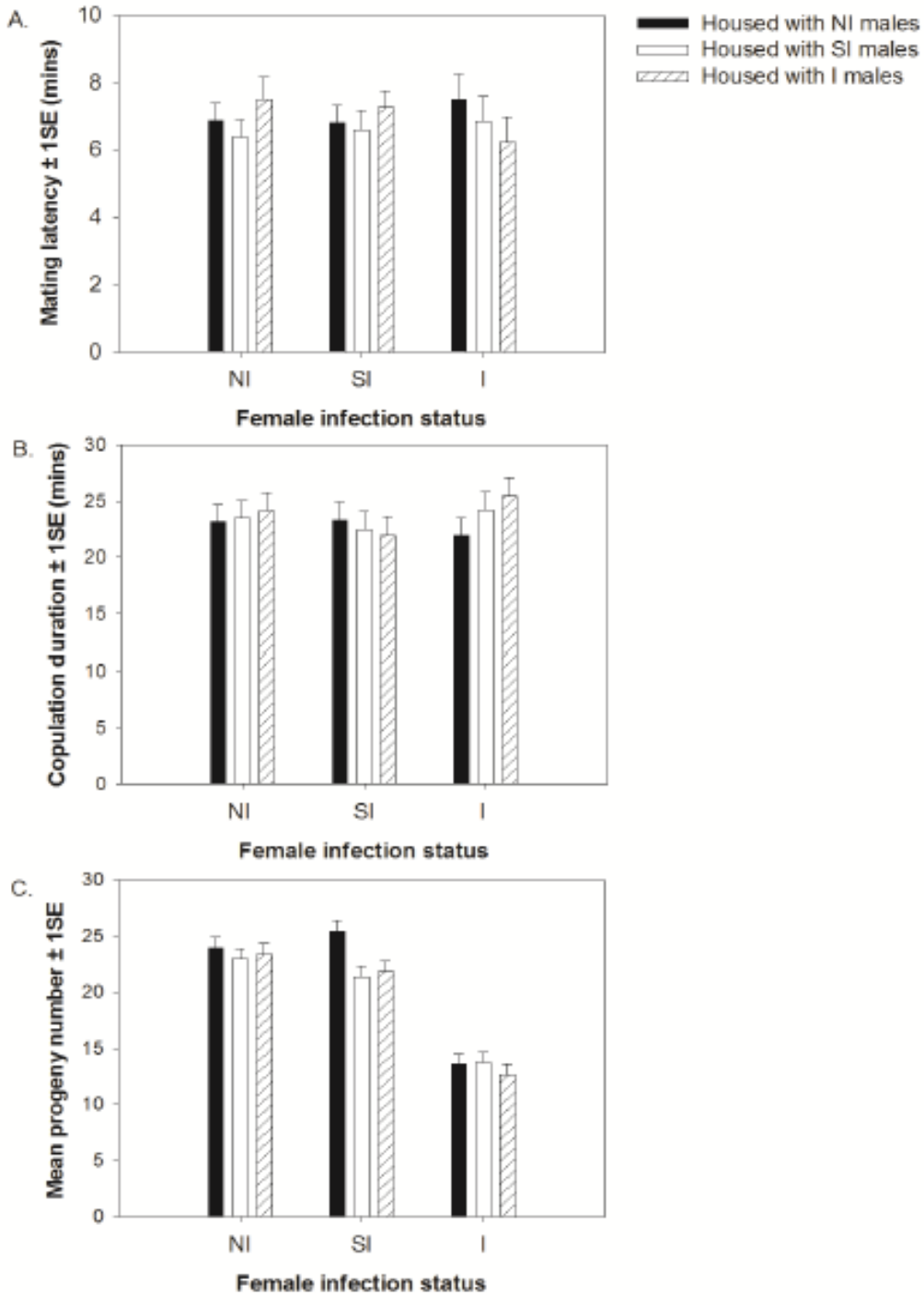
Replicate Experiment	Mean Bias Score	- 95% CI	+95% CI
1	0.447	0.412	0.482
2	0.478	0.465	0.491
3	0.469	0.419	0.496
4	0.459	0.424	0.494

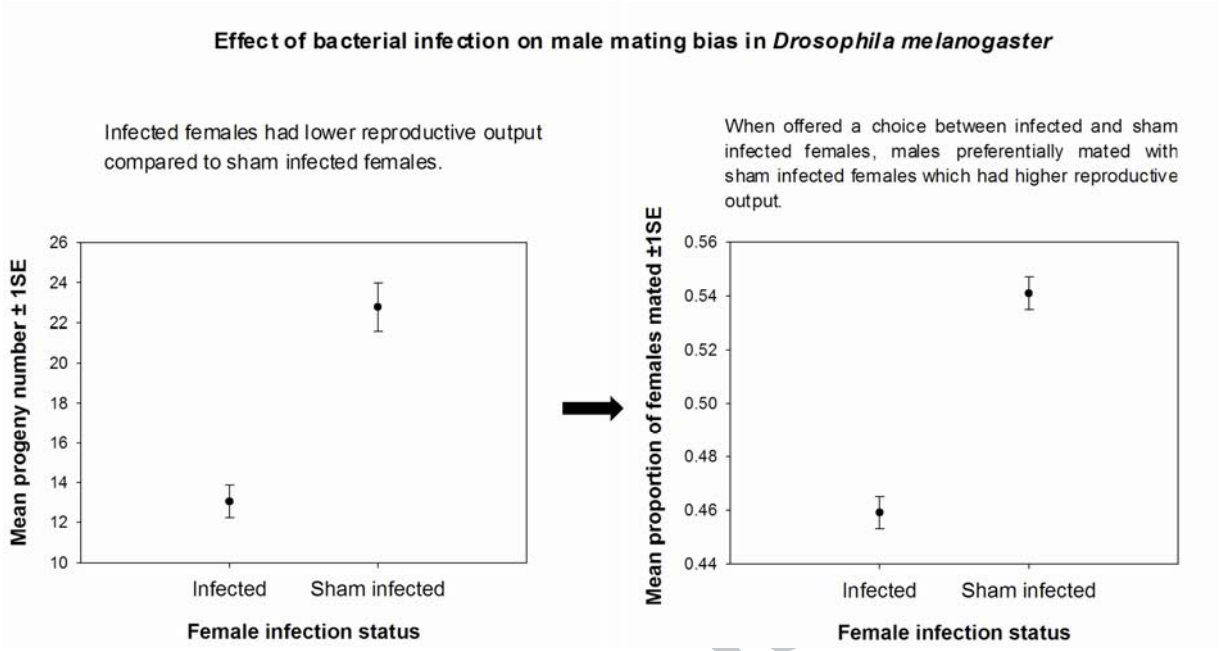
Figure captions

Figure 1. Effect of male and female infection status on (a) ML (b) CD and (c) Reproductive output. Values represent means (±1SE) calculated across the two replicate experiments. There was no significant effect of male or female infection status on ML and CD. Reproductive output was significantly affected by female infection status but not male infection status. Infected females had significantly lower reproductive output compared to sham infected and non-infected/ unhandled control females.

Figures

Figure 1





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Highlights

- We examined the effect of female infection status on male mate choice in *Drosophila melanogaster*.
- Males preferentially mated with sham infected females.
- Sham-infected females had higher reproductive output compared to infected females.
- We found potential evidence of pre-copulatory male mate choice
- We did not find any evidence of post-copulatory male mate choice measured in terms of variation in copulation duration.