

1 **FEMALE CHEMICAL WARFARE DRIVES FITNESS EFFECTS OF GROUP SEX RATIO**

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1 SUMMARY

2
3 In animals, skewed sex ratios can affect individual fitness either via sexual interactions (e.g. intersexual
4 conflict or intrasexual mate competition) or non-sexual interactions (e.g. sex-specific resource
5 competition). Because most analyses of sex ratio focus on sexual interactions, the relative importance of
6 these mechanisms remains unclear. We addressed this problem using the flour beetle *Tribolium*
7 *castaneum*, where male-biased sex ratios increase female fitness relative to unbiased or female-biased
8 groups. Although flour beetles show both sexual and non-sexual (resource) competition, we found that
9 sexual interactions did not explain female fitness. Instead, female fecundity was dramatically reduced
10 even after a brief exposure to flour conditioned by other females. Earlier studies suggested that quinones
11 (secreted toxins) might mediate density-dependent population growth in flour beetles. We identified
12 ethyl- and methyl- benzoquinone (EBQ and MBQ) as the primary components of adult stink glands that
13 regulate female fecundity. In female-biased groups (i.e. at high female density), females upregulated
14 quinones and suppressed each other's reproduction. In male-biased groups, low female density lead to
15 low quinone levels, allowing higher fecundity. Thus, quinones serve both as indicators and mediators of
16 female competition, resulting in the observed fitness decline in female-biased groups. Our results
17 underscore the importance of non-sexual interference competition that may often underlie the fitness
18 consequences of skewed sex ratios.

1 INTRODUCTION

2
3 Theoretical models predict that a balanced sex ratio is optimal for most diploid species [e.g. 1-4].
4 However, the adult sex ratio varies substantially both across species and populations, and a large body of
5 research has analyzed the evolutionary consequences of skewed sex ratios. In particular, the impact of sex
6 ratios on reproductive behavior and associated intra- or inter- sexual conflict has received a lot of
7 attention, since reproductive traits are expected to face strong natural as well as sexual selection. For
8 instance, male-biased sex ratios can be detrimental because of direct or indirect male harassment,
9 decreasing female longevity [e.g. 5], fecundity [e.g. 6], or offspring survival [e.g. 7]. More generally,
10 increased male-male competition can result in elevated sexual conflict, reducing female fitness [8]. On the
11 other hand, under a male-biased sex ratio, females may benefit from the opportunity to selectively mate
12 with higher-quality males [9], or benefit from polyandry by increasing offspring heterogeneity [10].

13 These hypotheses explaining the relationship between adult sex ratio and female fitness largely
14 depend on sexual interactions involving mating and associated behaviors. However, individual fitness is
15 also determined by non-sexual interactions that alter survival and life history [11]. Such non-sexual
16 interactions may be especially important for females, who often compete more strongly for resources
17 (such as food or space) rather than for mates [12]. A growing body of work on mammals suggests that
18 female competitive interactions may be important for many species [13]. For instance, in cooperatively
19 breeding meerkats, dominant females increase their reproductive success by suppressing subordinate
20 females' reproduction, reducing competition for resources [14]. Female parasitoid wasps face intense
21 intraspecific competition for suitable hosts, and use physiological and chemical mechanisms to determine
22 the outcome in their favour [15]. In such cases, resource rather than sexual competition may govern
23 female fitness. More generally, changes in sex ratio may be confounded with changes in the density of
24 one or both sexes. Hence it is important to quantify the contribution of sexual interactions vs. non-sexual
25 competition on individual fitness.

1 We tested the impact of adult sex ratio on female fitness in the red flour beetle *Tribolium*
2 *castaneum*, a global pest of stored grains. Typically, populations of *T. castaneum* exhibit a balanced sex
3 ratio [16]. Both sexes mate multiply and exhibit mate choice [16-20], but the degree and fitness
4 consequences of polyandry vary across populations [21]. Last-male sperm precedence is well known,
5 although there is large variation in male offense capability [22], which may potentially trade off with
6 sperm defense ability [23]. Hence, sexual selection, sexual conflict and intra-sexual competition are
7 important aspects of fitness in flour beetles. In addition, individuals compete strongly for resources,
8 which determines density-dependent population growth rate [24]. Together, these features make *T.*
9 *castaneum* an attractive model to analyze the impact of different forms of individual interactions on
10 fitness in the context of imbalanced sex ratios.

11 We tested the consequences of skewed adult sex ratio on multiple proxies of female fitness:
12 female reproductive fitness (egg production and total surviving offspring), lifespan, and immune function.
13 We found that for in each case, females from male-biased groups had higher fitness compared to females
14 from either unbiased or female-biased groups. Neither male intra-sexual competition nor opportunity for
15 female or male mate choice explained this fitness difference. Instead, we found that negative density-
16 dependent interactions between females were responsible for the fitness effects of group sex ratio. We
17 identified benzoquinones as key chemicals mediating these interactions, suggesting female interference
18 competition. Thus, we present a case where fitness consequences of group sex ratio arise from female-
19 driven non-sexual resource competition, rather than sexual interactions.

20

21 **METHODS**

22

23 **Generating experimental individuals and sex ratio groups**

24 We used an outbred laboratory population of *T. castaneum* [25] maintained on whole wheat flour
25 (henceforth “flour”) at 34°C ($\pm 1^\circ\text{C}$) on a 45-day discrete generation cycle. For all experiments, we
26 allowed ~2000 individuals to oviposit in 750 g of wheat flour for 48 h, and collected their offspring at the

1 pupal stage. We housed pupae of each sex separately in 1.5 ml micro-centrifuge tubes (3 pupae of the
2 same sex in 1g flour) for 10 days post-pupation. The pupal stage lasts for 3-4 days; we thus obtained ~7
3 day old (post-eclosion), sexually mature virgins. We grouped these adults into three sex ratio treatments
4 ($n = 8-9$ groups per sex ratio): (1) Male-biased groups (henceforth “MB”: 3 males + 1 female) (2)
5 Unbiased groups (henceforth “UB”: 3 males + 3 females) and (3) Female-biased groups (henceforth
6 “FB”: 1 male + 3 females). Each group was maintained in a plastic Petri plate (60 mm diameter, Tarsons)
7 at a density of 1 beetle/g flour (chosen to minimize resource limitation), and placed in a 34°C (± 1 °C)
8 incubator. Thus, MB and FB groups were given 4g of flour and UB groups received 6g of flour. We
9 chose the UB treatment such that we could determine the effect of skewed sex ratio given the same
10 number of males (UB vs. MB, 3 males each) or females (UB vs. FB, 3 females each); note that multiple
11 males or females were necessary to evaluate mate choice hypotheses. To test whether group size altered
12 the effects of group sex ratio, we set up additional groups with a total of 12 beetles/group ($n = 10$ groups
13 per sex ratio) but the same sex ratio (FB: 9 females + 3 males; MB: 3 females + 9 males) and density per
14 g flour.

15

16 **Measuring fitness effects of group sex ratio**

17 Females in each experimental group could oviposit continuously during the experiment, but adult
18 cannibalism would confound estimates of female fecundity and fertility. Hence, after constituting the
19 groups described above, we estimated the total offspring produced per female by periodically isolating
20 females: every 5 days for the first 30 days, every 7 days for the next 28 days, and then every 14 days for
21 another 42 days. Thus, we obtained estimates of female reproductive fitness as a function of sex ratio as
22 well as age, until females were 102 days old. During each fitness assay, females oviposited individually
23 for 24 hours in plastic Petri plates (60mm diameter, Tarsons) containing 5g flour, after which they were
24 placed again into their original group and provided fresh flour. Thus, offspring from each female
25 developed independently in the oviposition plates. After three weeks, we counted the total number of
26 offspring in each plate (including larvae, pupae and adults) as a proxy for the total reproductive fitness of

1 each female. To separate female fecundity (egg production) from fertility (egg survival), we counted the
2 number of eggs laid by 21-day-old females after the 24-hour oviposition period as a direct measure of
3 female fecundity. We also monitored mortality of individuals from each group, and tested proxies of
4 immune function (see SI methods for details).

5

6 **Quantifying mating behaviors**

7 To quantify mating-related behaviors as a function of group sex ratio, we set up additional experimental
8 groups using virgin adults ($n = 15-16$ groups per sex ratio). To identify females in FB and UB groups, we
9 used a paintbrush to mark the elytra with a small dot of non-toxic acrylic (model master) paint, using one
10 of three colours (yellow, green or red). To control for any effects of the paint on mating behavior, we
11 marked $1/3^{\text{rd}}$ of the MB females with each colour. Thus, all females in the experiment were marked
12 regardless of the sex ratio of their group. After marking the elytra, we placed females individually in
13 wells of a 96 well microplate and allowed the paint to dry for 6 hours. Since we wanted to quantify
14 mating behaviors directed towards females, we did not mark males. We distributed marked females into
15 sex ratio groups as described in the previous section. A week later, we separated individuals from the
16 flour, placing each group in a 60 mm Petridish whose bottom was covered with filter paper to allow
17 beetles traction for walking. We observed each group for 30 minutes, noting the number and timing of
18 matings received per female. A mating event was recorded when the male mounted the dorsum of the
19 female and attempted or achieved copulation. We quantified (1) total number of copulatory mounts
20 (matings) (2) total time spent in copula (3) average copulation time and (4) mating latency (time until the
21 first copulation). Immediately after the behavioral assay, we separated females and measured their
22 reproductive output (as described above) to test whether female fitness was correlated with mating
23 behaviour.

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1 **Testing the effect of conditioned flour on female fitness**

2 Flour used by *Tribolium* beetles gets “conditioned” by the accumulation of secreted quinones,
3 pheromones, and excreta. We tested whether flour conditioned by FB and MB groups differentially
4 affects the fitness of females exposed to the flour. We allowed FB and MB groups (constituted as
5 described earlier) to condition 4g of flour for 10 days. After this, we removed the adults, froze the flour at
6 -80 °C for 15 minutes and sifted it through a fine mesh (300 µm pore size, Daigger) to kill and remove
7 juvenile stages. We placed a virgin male and a virgin female (both 7 day old) in 2g of this conditioned
8 flour for 6 hours, 1 day or 4 days ($n = 12$ pairs per conditioning treatment per duration of exposure).
9 Following this, we isolated the female from each pair and allowed her to oviposit for 24 hours in fresh
10 flour. The next day, we counted the number of eggs laid per female as a measure of fecundity. Thus, we
11 obtained female fecundity after 6 hours, 1 day and 4 days of exposure to flour conditioned by either MB
12 or FB groups. In a second experiment, we conditioned the flour using either female-only or male-only
13 groups of beetles (three 7-day-old virgin beetles of the same sex). We then introduced a male and female
14 (7-day-old virgins) into 2g of this conditioned flour for either 6 hours or 24 hours ($n = 11-12$ pairs per
15 conditioning treatment per duration of exposure). Following this, we estimated the number of eggs laid by
16 each female as described above.

17

18 **Testing the impact of stink gland contents on female fecundity**

19 Flour beetles have paired abdominal and thoracic stink glands that secrete a mix of chemicals including
20 toxic quinones [26]. To test whether secreted products from beetles’ stink glands directly alter female
21 fitness, we measured female fecundity after a brief exposure to abdominal stink glands. We paired 7-day-
22 old virgins (1 male + 1 female) in 2 g flour for 2 days ($n = 10$ pairs per treatment). We separated the
23 females and transferred them individually into a 35mm Petridish whose bottom was covered with a filter
24 paper for traction, and a thin layer of flour to avoid starving the beetles during the assay. On the same day,
25 we separately dissected abdominal stink glands from 60 MB and 60 FB females (sex ratio groups
26 constituted as described earlier). For each set, we used a micro-pestle to pool and homogenize the glands

1 in 300 μ l hexane, and centrifuged the suspension (5000 rpm, 5 minutes). 10 μ l of the supernatant was thus
2 equivalent to the abdominal gland content of 2 females. We then performed serial dilution of the
3 supernatant to obtain abdominal gland content equivalents of 0.75, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.001
4 females in a total volume of 10 μ l ($n = 5-6$ / dilution/sex ratio). We soaked filter paper discs (10 mm
5 diameter) with 10 μ l of the supernatant (or hexane as control), kept them under laminar air flow for 10
6 minutes to evaporate the solvent, and then placed the discs at the center of a Petriplate containing the
7 experimental female. Twelve hours later, we tested the fecundity of each female as described above.

8 Next, we wanted to identify the chemical component of female stink glands responsible for
9 reduced female fecundity. Previous analyses show that ethylbenzoquinone (EBQ) and
10 methylbenzoquinone (MBQ) are two major components of *T. castaneum* stink glands [27]. Hence, we
11 quantified the amount of EBQ and MBQ produced by females in MB and FB groups. We constituted MB
12 and FB groups as earlier ($n = 10$ groups per sex ratio), and after 7 days we dissected abdominal and
13 thoracic stink glands of a randomly chosen female from each group. For each female, we combined
14 abdominal and thoracic gland samples and homogenized in 60 μ l cold methanol. Subsequently, we
15 quantified the amount of EBQ and MBQ in each sample using HPLC (see SI methods for details). For
16 calibration, we used pure MBQ (Sigma) and lab-synthesized EBQ (see SI Methods).

17 Finally, to directly test whether quinones regulate fecundity, we exposed 9 day-old test females
18 (reared as a single mating pair for 2 days before exposure, as described above) to abdominal stink gland
19 extracts of MB females containing varying concentrations of added EBQ and MBQ. We added 10 μ l of
20 the gland extract equivalent to 0.5 females, supplemented with 1, 5 or 10 μ g of either quinone to a filter
21 paper disc (10 mm diameter), and placed the disc in a 35 mm Petridish containing a female ($n = 7-10$
22 females per concentration per chemical). As controls, we exposed test females to discs spotted only with
23 solvent, or with the MB gland extract without quinones. After exposing females to each chemical mixture
24 for 12 hours, we measured fecundity as described earlier.

25

26

1 **RESULTS**

2

3 **Male-biased sex ratio increases female fitness**

4 We found that females from male biased (MB) groups outperformed females from unbiased (UB) and
5 female biased (FB) groups. Throughout their major reproductive lifespan, MB females consistently
6 produced more offspring compared to UB or FB females (Fig 1A; Table S1), resulting in over twice as
7 many total offspring per female (Fig 1B; Table S1). The differential reproductive success was driven by
8 offspring quantity: MB females laid ~three times more eggs per female per day, compared to UB or FB
9 females (Fig 1C; Table S1). We observed a similar pattern even when we used a larger group size (FB: 9
10 females + 3 males, and MB: 3 females + 9 females; Fig S1). Thus, a male-biased sex ratio increased
11 female reproductive fitness by increasing female fecundity. We also found significantly fewer deaths in
12 MB females, compared to both UB and FB females (Fig. S2A; Table S1; Fisher's exact test, two tailed p
13 value: MB vs. FB = 0.009; MB vs. UB = 0.001; UB vs. FB = 0.999). In FB replicates where a female died
14 earlier in the experiment, the sex ratio would become less skewed, leading us to underestimate the impact
15 of the skewed sex ratio. In contrast, female mortality in UB groups would increase the skew in sex ratio.
16 Despite these confounding factors, female fertility and fecundity in FB and UB groups was consistently
17 lower than MB groups, suggesting that early effects of sex ratio were very strong. MB females also had
18 better immune function (Fig S2B-D; Table S1). Together, these data indicate that low fecundity of FB and
19 UB females cannot be explained by tradeoffs with other fitness components. In subsequent experiments,
20 we focused on the mechanism underlying the observed impact of sex ratio on female reproductive fitness.

21

22 **Sexual interactions do not explain female fitness**

23 We evaluated the hypothesis that female fitness increases when females can selectively choose to mate
24 with superior males. This hypothesis predicts that FB females should have low fitness (no mate choice
25 possible), and UB and MB females should have equally higher fitness (choice between 3 males in each
26 case). Instead, we found that females in both FB and UB groups had lower fitness than females in MB

1 groups (Fig 1). Thus, increased opportunity for female mate choice was unlikely to explain the higher
2 fitness of MB females. An alternative hypothesis is that male mate choice drives female fitness. This
3 hypothesis predicts that within replicates of UB and FB groups, only one female would have high fitness.
4 However, when we only considered the female with the highest fitness in each group, we still found that
5 MB females outperformed FB and UB females (Fig S2E). Thus, it is unlikely that opportunity for male or
6 female mate choice was responsible for the increased fitness of MB females. Nonetheless, we specifically
7 tested the impact of sex ratio on mating behaviour and female fecundity. We found that although MB
8 females received more matings and tended to spend more time in copula (Fig S3, Table S2), female
9 fitness was not correlated with either of these behaviours (Fig 2). In other words, adding mating
10 behaviours to a model with group sex ratio as an explanatory variable did not improve the model fit
11 (Table S2). Group sex ratio did not affect other aspects of mating behaviour (Fig S3). Thus, sexual
12 interactions between individuals could not explain the higher fitness of MB females.

13

14 **Female-secreted benzoquinones explain the fitness impact of sex ratio**

15 Flour beetles secrete toxic compounds in the flour, which are suggested to decrease female fecundity at
16 high concentrations [28,29]. Thus, it is possible that greater accumulation of toxins in flour used by FB
17 groups was responsible for the decreased fecundity of FB females. To test this, we measured the
18 reproductive output of mated females exposed to flour previously “conditioned” by either FB or MB
19 groups. We found that females responded rapidly to cues in conditioned flour, with a large reduction in
20 fecundity after only 6 hours of exposure to flour conditioned by FB groups (Fig 3A, Fig S4A; Table S3).
21 Critically, flour conditioned by females also produced a similar decline in fecundity (Fig 3B, Fig S4B;
22 Table S3). These results indicated that the reduced fitness of FB females was mediated by female-
23 secreted chemicals in the flour, rather than via direct inter- or intra- sexual interactions.

24 Previous work shows that female flour beetles secrete more quinones (produced by stink glands)
25 than males [25,27]. Hence, we tested whether stink gland secretions (rather than other secretions or
26 excreta in the flour) were responsible for the observed effects of female-conditioned flour on female

1 fitness. We found that fecundity of previously mated test females decreased after exposure to abdominal
2 stink gland contents of FB females, whereas gland contents of MB females did not alter fecundity (Fig
3 3C; Table S3). These results confirmed that the contents of female stink glands directly affected female
4 fitness. Next, we measured the concentration of benzoquinones (ethyl benzoquinone, EBQ and methyl
5 benzoquinone, MBQ) in abdominal and thoracic stink glands dissected from females from MB vs. FB
6 groups. We found that stink glands of FB females had higher amounts of both EBQ and MBQ (Fig 3D;
7 Table S3), suggesting that these quinones may be responsible for lower fecundity of FB females. Finally,
8 we tested whether adding quinones to gland extracts of MB females could mimic the effects of glands
9 from FB females. Indeed, we found that adding EBQ or MBQ to stink gland extracts of MB females
10 reduced the fecundity of test females (Fig 3E; Table S3).

11 Together, our results show that at high female density (i.e. in UB and FB groups), females
12 upregulate EBQ and MBQ production, suppressing oviposition by all females in the group and resulting
13 in low female fitness. In contrast, MB females secrete less MBQ and EBQ, allowing MB females to
14 maximize oviposition and fitness. We infer that the observed female response to high female density
15 reflects female interference competition for shared dietary and oviposition resources.

16

17 **DISCUSSION**

18

19 A rich body of theoretical and empirical work documents the varied causes and consequences of adult sex
20 ratio for individual fitness. Perhaps due to the obvious implications for sexual conflict and sexual
21 selection, the large majority of this work has been interpreted in light of female mate choice and male-
22 male intrasexual competition. However, we found that neither male mate competition nor increased
23 opportunity for female mate choice explained higher female fitness in male-biased groups of flour beetles.
24 Instead, female competitive interactions were responsible for the observed fitness difference between
25 male-biased and female-biased groups, regardless of group size. Similarly, in houseflies a male-biased sex
26 ratio increased female fecundity and offspring survival [30]. Although male houseflies courted more

1 frequently in male biased groups, female fitness was not associated with male courtship and was not
2 affected by the opportunity for female mate choice. As discussed by the authors, these results suggest
3 cryptic effects of social, non-sexual interactions between individuals as drivers of increased female fitness
4 under a male biased sex ratio. Such non-sexual competitive interactions between females may be
5 widespread, with multiple examples in mammals [reviewed in 13] as well as in social insects (discussed
6 in [31]) and mosquitofish [32,33]. Thus, non-sexual interactions between females may frequently mediate
7 the outcome of altered sex ratio in multiple taxa.

8 Importantly, we identified female-secreted benzoquinones as mediators of female competitive
9 interactions in flour beetles. Studies on *Tribolium* have long implicated quinones as a mechanism of
10 negative density-dependent population growth [34-36], though this was never explicitly tested. Quinones
11 are secreted from abdominal and thoracic glands [26], and accumulate in flour in a density-dependent
12 manner [16]. At high concentrations, quinones are toxic, causing developmental abnormalities in
13 juveniles [16], and may thus represent a form of interference competition at high density. Indeed, beetles
14 typically avoid flour containing high amounts of quinones [37]. The individual response to major quinone
15 components also increases as a function of population density [38]. For instance, exposing a pair of focal
16 beetles to olfactory cues (primarily quinones) from crowded beetles for three days decreased female
17 oviposition rate by up to 25% [36]. Therefore, we hypothesized that quinones might be responsible for the
18 observed differences in female fitness as a function of sex ratio. If true, the quinone hypothesis would
19 predict that MB groups should produce less quinones than FB or UB groups, and exposure to more
20 quinones should decrease fecundity. We found support for both these predictions in independent
21 experiments: MB females had less quinones in their stink glands, and adding quinones to gland extracts of
22 MB females mimicked the fitness impact of gland extracts of FB females. Our results also suggest that
23 females are particularly sensitive to conditioned flour, and respond by modulating their fecundity.
24 Previous experiments support this finding: under a balanced sex ratio, net female fecundity decreased
25 rapidly with increasing population density, but the decline was slower in highly male biased groups
26 containing a single female [39]. Previous work also shows that sensitivity to conditioned flour is a

1 heritable trait that can rapidly evolve under selection in laboratory populations, and is controlled by a
2 single (or few) genes [40]. Thus, density dependence itself may evolve via sensitivity to quinones. Higher
3 quinone sensitivity in females may also explain our observation that male mortality was not affected by
4 group sex ratio, even though males were exposed to high quinone levels in FB and UB groups.

5 Could quinones also account for the observed impact on other fitness proxies such as lifespan and
6 immune traits? Quinones are toxic for *T. castaneum* juveniles [16], and cause high adult mortality in
7 related species such as *T. destructor* [41]. A recent study showed that beetles grew faster and larger in
8 male-biased groups [42], possibly due to greater amounts of toxic quinones in female-biased groups. Thus,
9 it is fairly straightforward to explain the lower lifespans of UB and FB females. However, it is more
10 difficult to explain the mechanism responsible for poor immune function in these females. One possibility
11 is that similar to the impact on lifespan, quinone toxicity may decrease overall body condition, resulting
12 in poor immune responses. Alternatively, quinone production may trade off with innate immunity.
13 However, these possibilities need to be explicitly tested. Although our results suggest that quinones
14 accumulate in the flour in proportion to their concentration in stink glands, further work is necessary to
15 confirm this and to test whether females may also control the secretion rate of stink gland contents in a
16 density-dependent manner. The precise mechanism through which benzoquinones suppress fecundity also
17 remains to be elucidated; possibilities include direct effects on egg maturation or oviposition behaviour,
18 or indirect effects via physiological costs of producing quinones.

19 Our finding that female fitness increases as a function of male biased sex ratio contradicts some
20 previous work with *T. castaneum* showing inter-sexual conflict. Male competitive ability and mating
21 success increased significantly in experimentally evolved male-biased populations [43]. Increasing the
22 number of males in mating trials decreased the fitness of evolved FB females, but not of evolved MB
23 females. As the authors discuss, these data suggest that females became more sensitive to (as yet
24 unknown) deleterious effects of multiple mating, indicating underlying sexual conflict. However, unlike
25 *Drosophila* where sexual conflict is mediated via mating-related harm to females (discussed in [43]),
26 there is no direct evidence for such harm in *T. castaneum*. Furthermore, the impact of female-female

1 interactions in the evolved FB lines is unclear. Testing quinone production and sensitivity in the evolved
2 male-biased and female-biased lines may help to understand the conflicting results. In contrast, two other
3 studies with *T. castaneum* corroborate our results, showing increasing female fitness with increasing
4 male-biased sex ratios. First, populations selected for female-biased sex ratios showed a reduction in
5 fitness [44]. Second, Wade measured the total number of offspring produced by groups with sex ratios
6 ranging from 0.2 to 0.8 after 50 days [45]. Re-analyzing these data, we found that per capita female
7 fitness decreased as a function of the proportion of females in the group (Fig S5A). Similarly, in another
8 set of experiments (Wade, unpublished data), per capita female productivity declined as a function of the
9 number of females in groups with different sex ratios maintained at a constant density (Fig S5B). Despite
10 the many differences in experimental design, these results support our findings and suggest that female
11 resource competition may be the most important determinant of female fitness in *T. castaneum*.

12 In closing, we suggest that the role of females in the evolution and consequences of group sex
13 ratio has generally been underappreciated so far [13]. This is unfortunate because sex ratios provide a
14 context where both sexual selection and resource competition may be altered. Our work demonstrates that
15 fitness consequences of male biased sex ratios in flour beetles are driven by chemically mediated female
16 non-sexual competitive interactions. Specifically, we show that females use benzoquinones as a major
17 weapon in this warfare, although the mechanism responsible for the resulting suppression of fecundity
18 remains unclear. We suggest that such effects of intrasexual female interference competition may not be
19 uncommon, and may frequently confound the effects of sex ratio on female fitness.

20

21 **AUTHOR CONTRIBUTIONS**

22 IK and DA conceived and designed experiments with input from other authors; IK, AP, SI, MU and PL
23 conducted beetle experiments; RS, NJ and RV designed and conducted chemical synthesis and analyses;
24 DA and IK analyzed data and wrote the manuscript with input from other authors.

25

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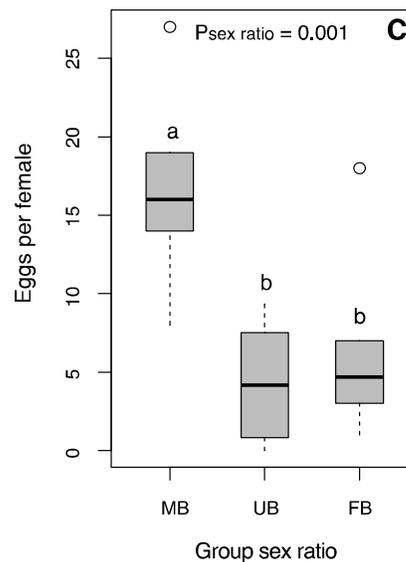
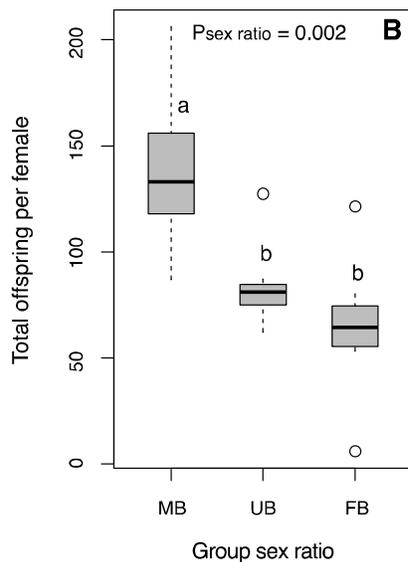
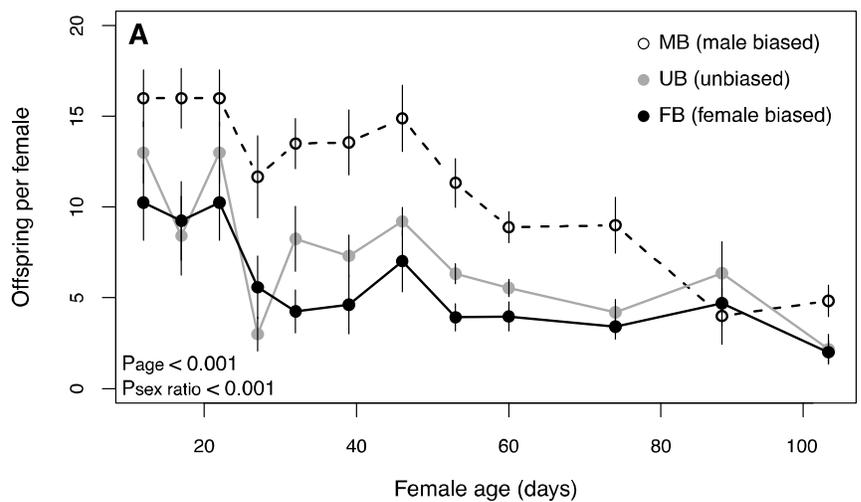
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1 FIGURES

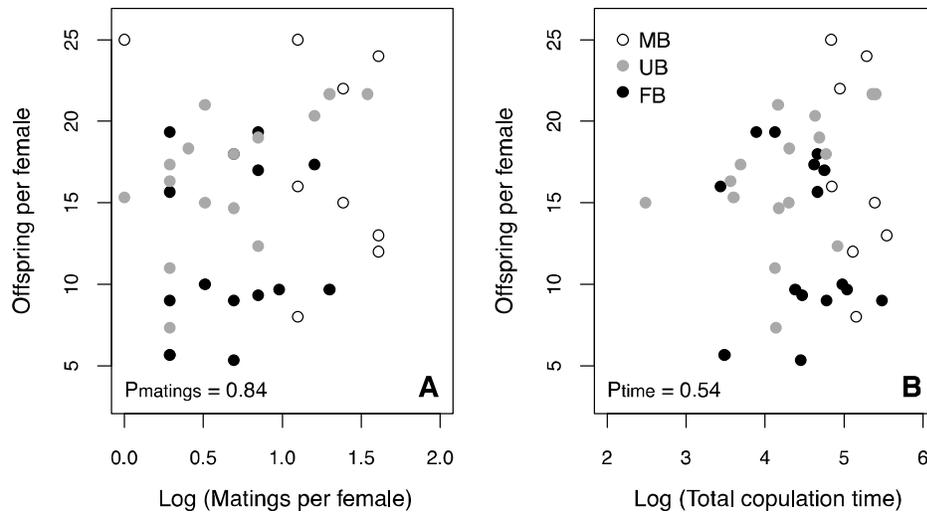
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3 **Figure 1: Female fitness as a function of group sex ratio.** (A) Average offspring (\pm se) produced per
4 female as a function of age (days post eclosion) (B) Total offspring produced per female during the
5 experiment and (C) Fecundity of 28-day-old females as a function of sex ratio. MB = male-biased groups
6 (3 males + 1 female); UB = unbiased groups (3 males + 3 females); FB = female-biased groups (1 male +
7 3 females). Box plots show median and quartiles, and lowercase alphabets indicate significantly different
8 groups inferred from pairwise comparisons.



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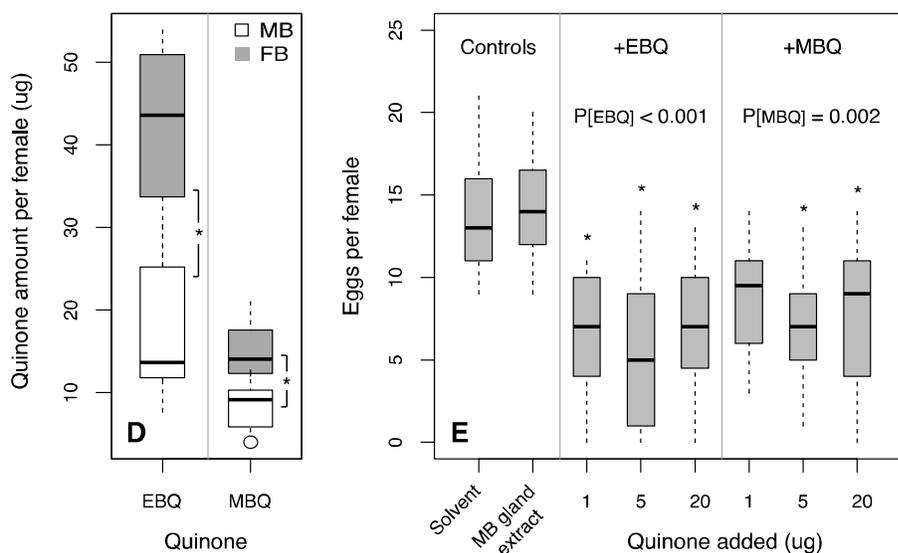
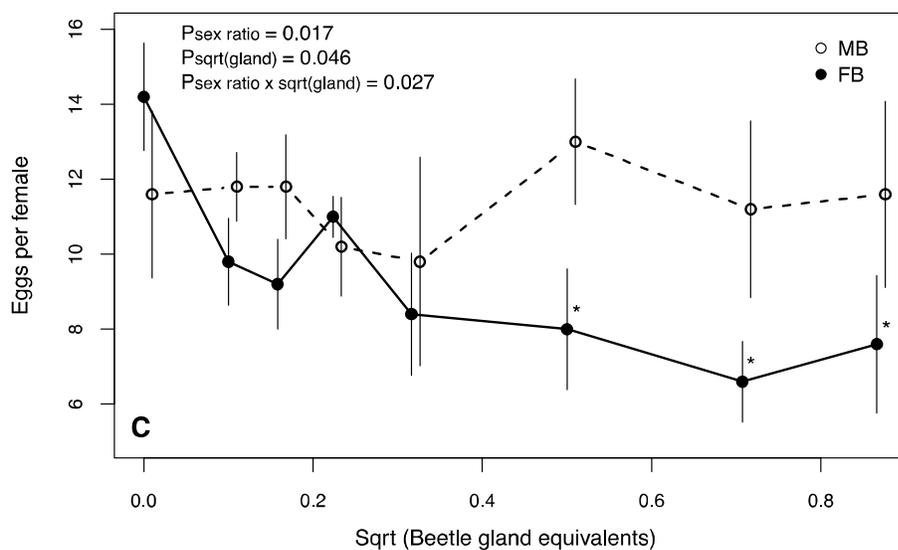
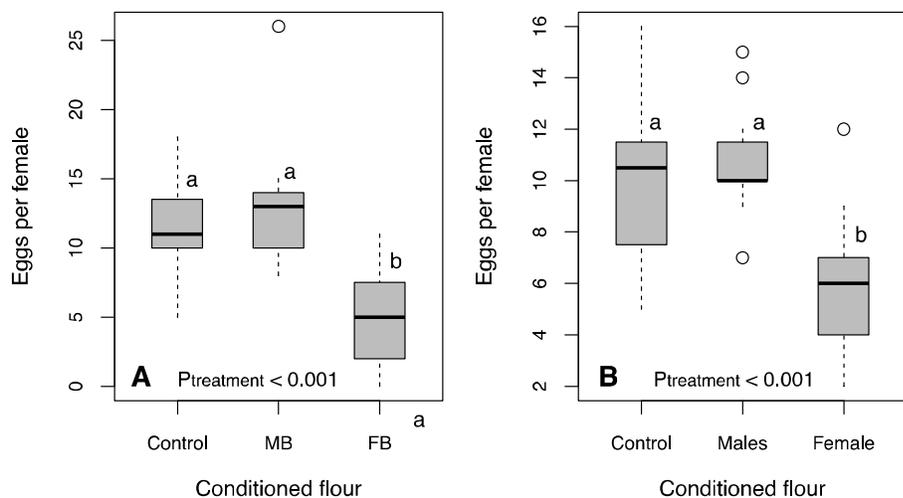
1 **Figure 2: Female reproductive fitness as a function of mating behaviour.** Average offspring per
2 female, as a function of (A) Total number of matings per female in a 30-min observation period (B) Total
3 time spent in copula per female. The x-axis was log-transformed in both panels. MB = male-biased
4 groups (3 males + 1 female); UB = unbiased groups (3 males + 3 females); FB = female-biased groups (1
5 male + 3 females).

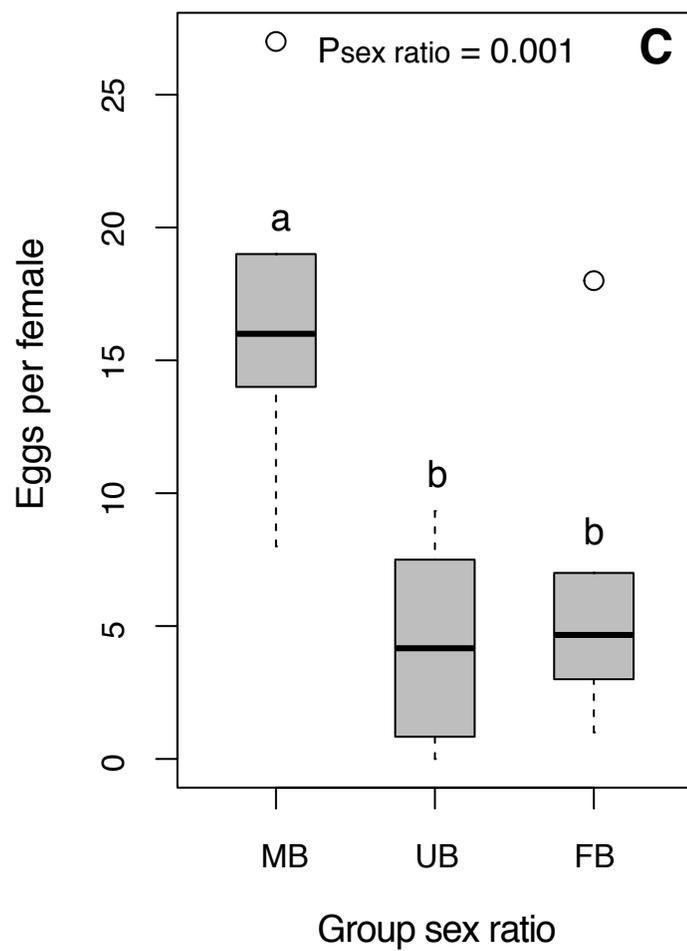
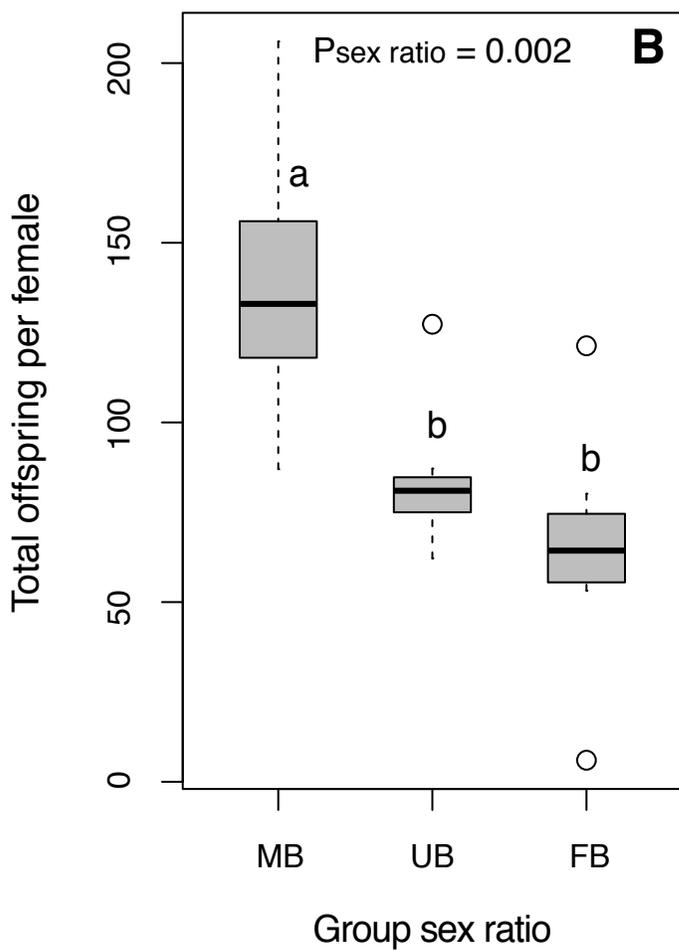
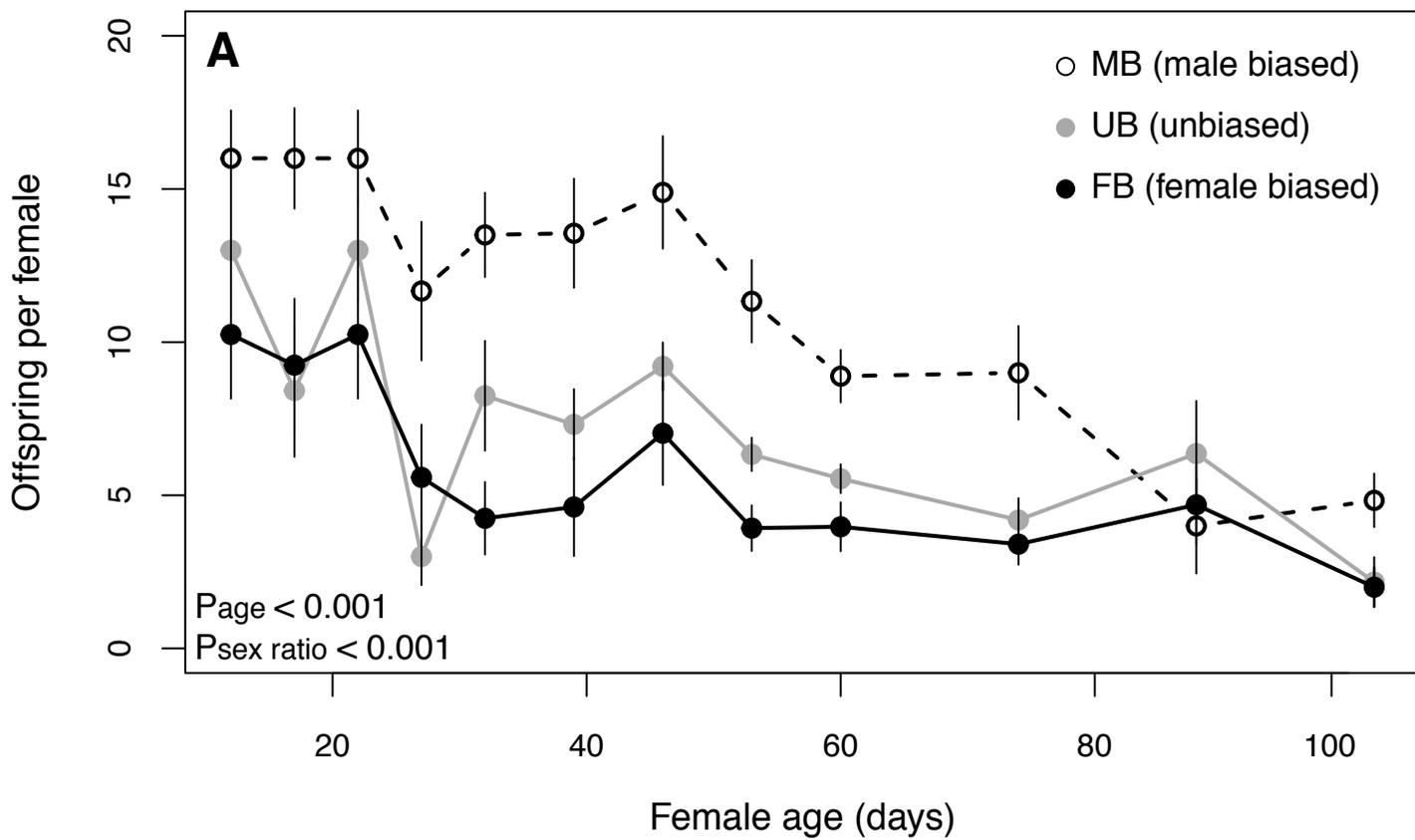


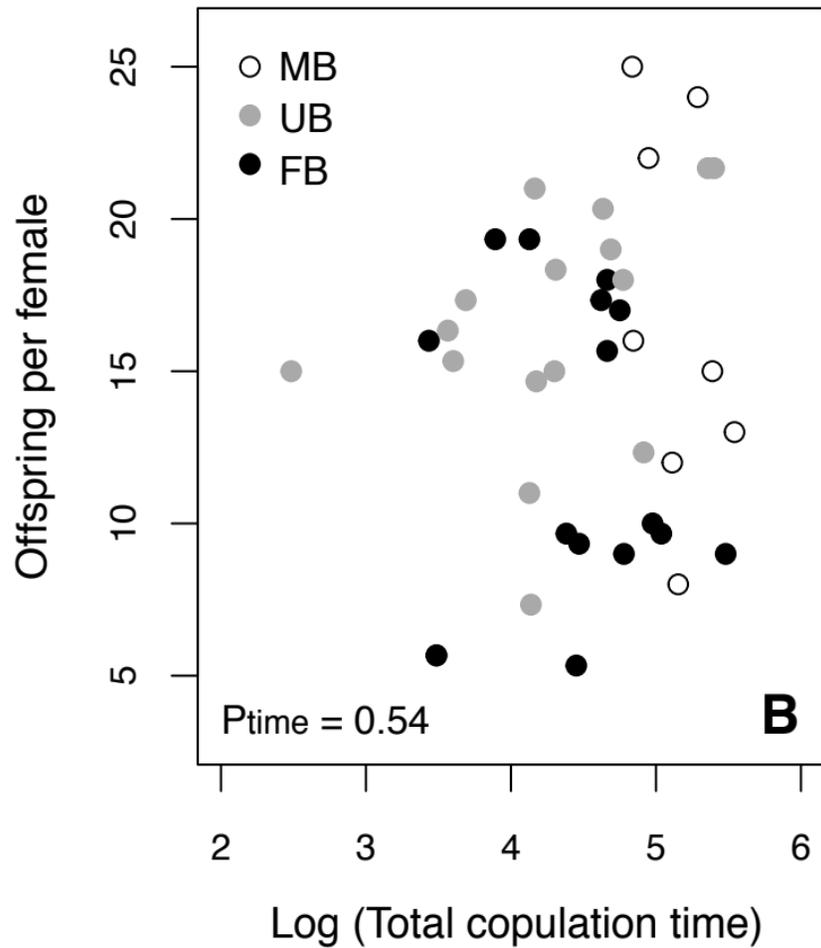
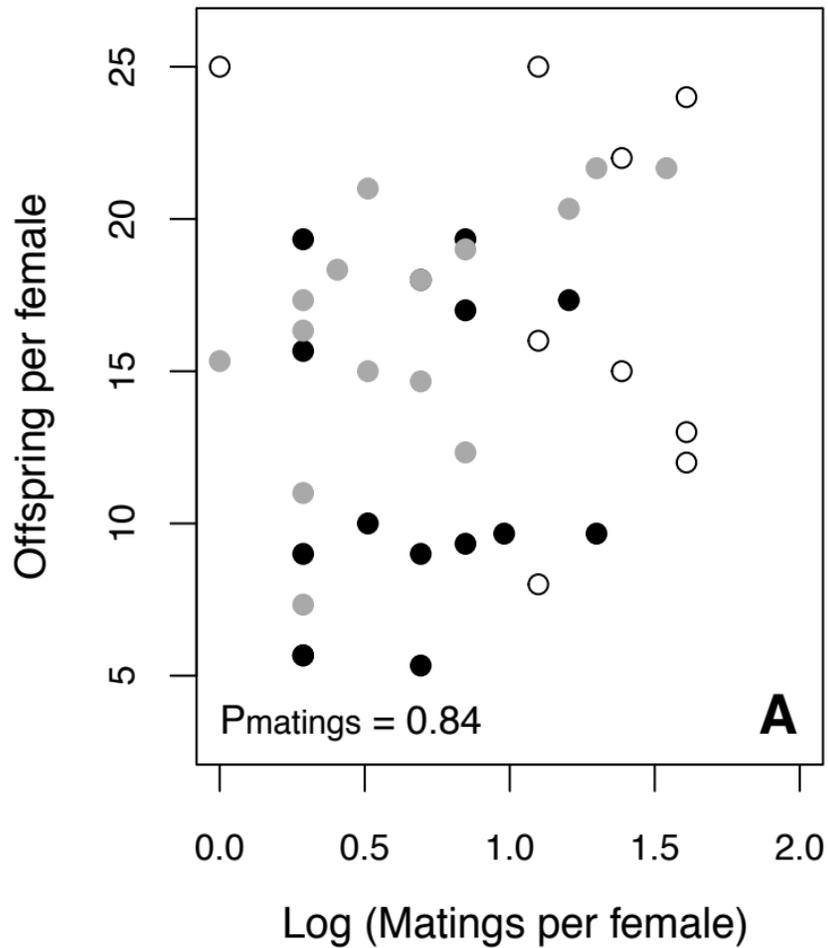
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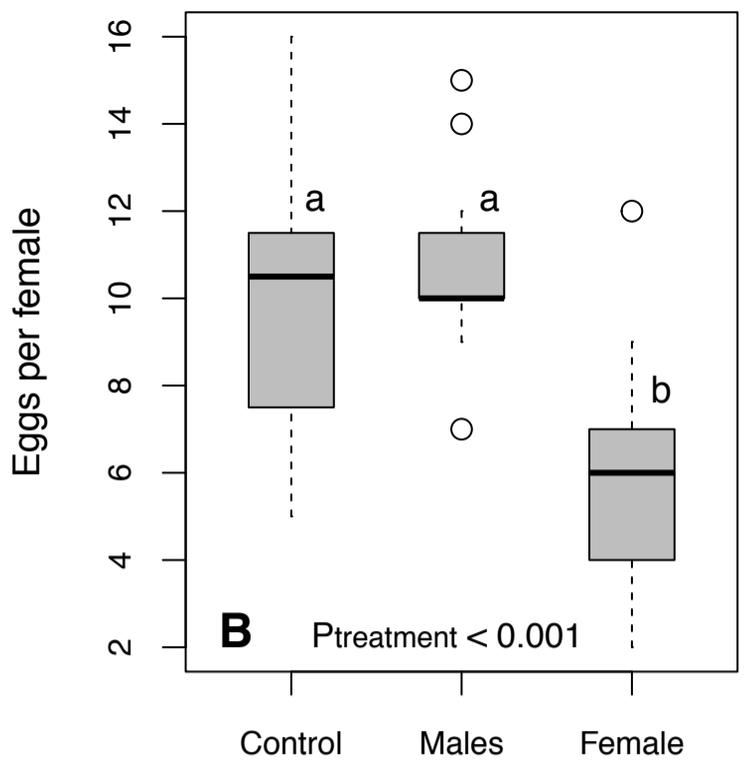
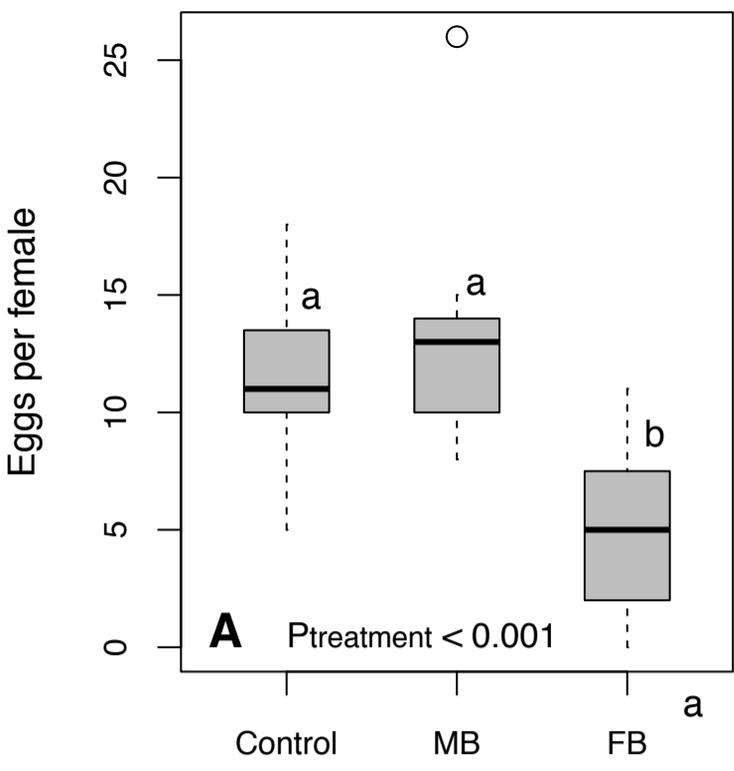
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1 **Figure 3: Quinones in female stink glands mediate fitness effects of group sex ratio.** Fecundity of test
2 females exposed to flour conditioned by (A) MB vs. FB groups, or (B) virgin females vs. males, for 6
3 hours (controls: unconditioned flour). Lowercase alphabets indicate significantly different groups inferred
4 from pairwise comparisons. (C) Mean fecundity (\pm se) of test females exposed to increasing
5 concentrations of stink gland extracts of females from MB vs. FB groups. MB data are slightly displaced
6 along the x-axis for clarity. Asterisks indicate treatments that are significantly different from the
7 respective control for each treatment (concentration 0). (D) Amount of ethyl- and methyl- benzoquinones
8 (EBQ and MBQ) in stink glands of females from MB vs. FB groups. (E) Fecundity of test females
9 exposed to MB stink gland extracts containing varying amounts of either EBQ or MBQ (controls: solvent
10 used for gland extraction, and MB gland extract without added quinones). Box plots show median and
11 quartiles; asterisks indicate groups that are significantly different from the control (MB gland extract).









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