Socially plastic responses in females are robust to evolutionary manipulations of adult sex ratio and adult nutrition

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Author's contributions

T.C., W.H., M.J.G.G and N.M conceptualised the study. N.M. conducted the labwork and the data analyses and wrote the first draft of the manuscript. T.C., W.H. and N.M contributed to writing and revising the manuscript and approved the final version.

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Data availability

Supplementary figures and tables are presented in the Supplementary Information (including statistical analysis code). Raw data are available at https://doi.org/10.5061/dryad.qbzkh18s6.

Ethics approval

Protocols were approved by the Animal Welfare and Ethical Review Board of the UEA. Formal ethical approval was not required. All methods were incompliance with relevant guidelines.

Competing interest

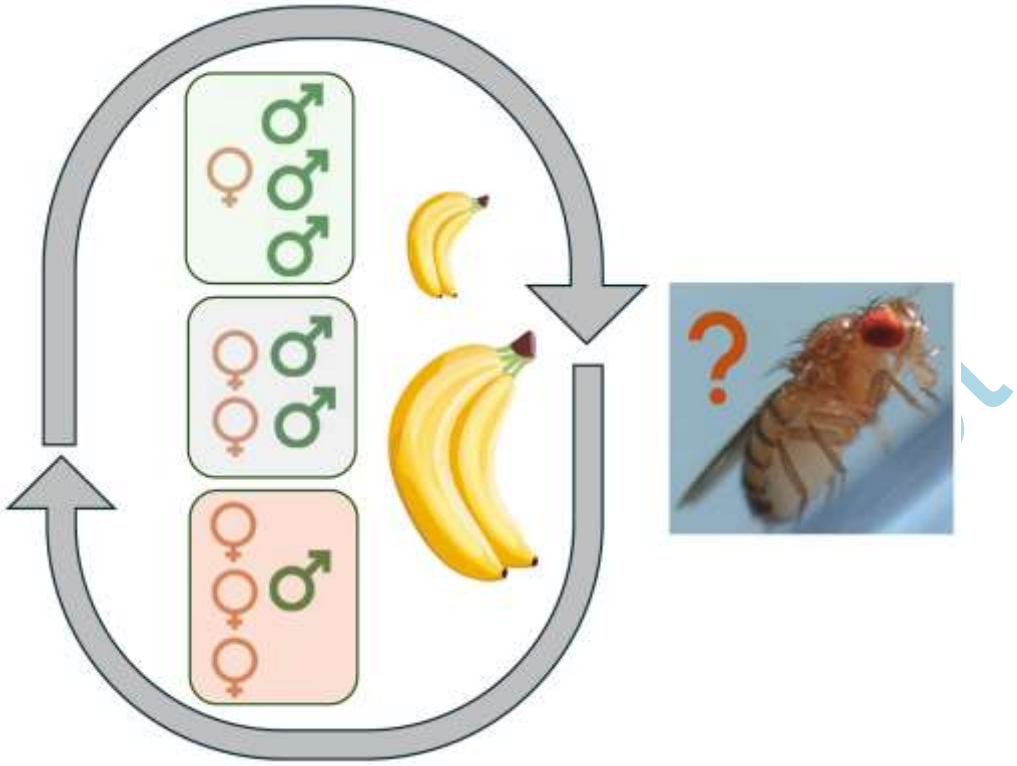
The authors declare that they have no competing interests.

Abstract

Socially plastic behaviours are widespread among animals and can have a significant impact on fitness. Here we investigated whether the socially plastic responses of female *Drosophila melanogaster* can evolve in predictable ways following long term manipulation of adult sex ratio and adult nutrient availability. Previous reports show that female *D. melanogaster* respond plastically to their same-sex social environment, and lay significantly fewer eggs after mating when previously exposed to other females. In this study, we tested two hypotheses, using females drawn from lines with an evolutionary history of exposure to variation in adult sex ratio (male biased, female biased or equal sex ratio) and adult nutritional environment (high or low quality). The first was that a history of elevated competition in female-biased regimes would select for increased plastic fecundity responses in comparison to females from other lines. The second was that these responses would also be magnified under poor nutritional resource regimes. Neither hypothesis was supported. Instead, we found that plastic fecundity responses were retained in females from all lines, and did not differ significantly across any of them. The lack of differences does not appear to be due to insufficient selection, as we did observe significant evolutionary responses in virgin egg laying patterns according to sex ratio and nutritional regime. The lack of variation in the magnitude of predicted plasticity is consistent with the idea that the costs of maintaining plasticity are low, benefits high, and that plasticity itself can be relatively hard wired.

Keywords sexual selection, plasticity, mating behaviour, experimental evolution

Graphical abstract



Background

Plastic responses expressed by individuals in response to environmental cues can be vital components of fitness (Wedell et al. 2002, Bretman et al. 2011a, Kasumovic and Brooks 2011, Moczek et al. 2011, Dingemanse and Wolf 2013, Snell-Rood 2013, Pfennig 2021, Sheehy and Laskowski 2023). Such responses allow organisms to match their reproductive effort or tailor their life history to the expected or prevailing environment (Price et al. 2003, Van Buskirk 2012). Plastic responses can be influenced by conditions experienced by parents, in anticipation of the environment likely to be experienced by offspring (Kasumovic and Brooks 2011, Snell-Rood et al. 2013). They may also be set during development in anticipation for the expected adult environment (Lange et al. 2023, Yoon et al. 2023). Behavioural plasticity or allocation of resouces to reproduction may also vary in response to the immediate conditions experienced during adulthood (Bretman et al. 2011a). Plastic responses to environmental conditions such as diet can also balance lifespan and reproductive success according to the level of resources available (Zajitschek et al. 2013, Zajitschek et al. 2016, Bretman et al. 2023) and can even change the direction of trade offs between lifespan and reproductive effort (Collins et al. 2023).

The capacity for individuals to express plasticity will be affected by the potential fitness gains balanced against costs of being plastic (Dingemanse and Wolf 2013, Snell-Rood 2013, Pfennig 2021, Sheehy and Laskowski 2023). The extent, tempo and predictability of variation in expected or prevailing environments is expected to be a crucial determinant of the potential fitness benefits of plasticity. Variation is a pre-requisite for plasticity. However, if environments change too rapidly, plasticity responses and any associated fitness effects, may be mismatched to them (Bretman et al. 2012). If environments are generally stable, fitness benefits of plasticity will also be minimal (Scheiner and Levis 2021). Much about the evolutionary drivers, pace and extent of plasticity evolution remains unclear (Lange et al. 2021). This is the topic we address here by testing key hypotheses for the evolution of reproductive plasticity in females drawn from populations subjected to long term variation in two key factors: socio-sexual and nutritional environments. How plasticity is expected to respond to variation in those two factors is described below.

The socio-sexual environment has emerged as one of the key drivers favouring the evolution of plastic behaviours. Evidence for this has been gained by using phenotypic engineering of the immediate number, density or sex of potential competitors, of the sensory cues available (Bretman et al. 2010, Bretman et al. 2011b) or longer-term manipulations of the adult sex ratio over evolutionary time (Emlen and Oring 1977, Clutton-Brock 1988, 2007). A key reason predicting strong effects of the socio-sexual environment on plasticity is that alterations to the density or composition of same or opposite sex individuals in an individual's immediate environment can be highly dynamic. This can result in rapid variation in the intensity of sexual and / or ecological competition for resources, creating potential for benefits to individuals that can respond and match their behaviour to the environment (Wedell et al. 2002, Bretman et al. 2011a, Kasumovic and Brooks 2011, Moczek et al. 2011, Dingemanse and Wolf 2013, Snell-Rood 2013, Pfennig 2021, Sheehy and Laskowski 2023). For example, an increase in the frequency or intensity of contacts between conspecific males is expected to signal an increased likelihood of competition for matings or fertilisations (Bretman et al. 2011a). Similarly, elevated contacts between females should indicate higher competition for resources such as food, mates or, other key reproductive resources (e.g. nest and oviposition sites) (Zajitschek et al. 2013, Zajitschek et al. 2016, Bretman et al. 2023). Many experimental studies that have manipulated the socio-sexual environment over the short term are consistent with these predictions. For example, male Mediterranean fruit flies that perceive elevated sexual competition due to the presence of a conspecific male in the mating arena, transfer significantly more sperm to females during mating (Gage 1991). Similarly, male *D. melanogaster* respond to elevated sexual competition by mating for longer, transferring more of key seminal fluid proteins (Wigby et al. 2009) and sperm (Garbaczewska et al. 2013, Moatt et al. 2014, Hopkins et al. 2019), thus achieving higher reproductive success (Bretman et al. 2009). Females also show related plastic responses to the presence of conspecifics (Sarin and Dukas 2009, Bailly et al. 2023). In *D. melanogaster*, mated females are more aggressive towards rival females than are virgins (Nilsen et al. 2004, Bath et al. 2017). Female fecundity is also surprisingly plastic, and varies according to the pre- and post-mating social environment (Fowler et al. 2022a, Bailly et al. 2023). One example, is seen in *D. melanogaster*, in which females maintained in same sex conspecific groups prior to

mating lay significantly fewer eggs after mating than those kept alone (Fowler et al. 2022a).

The strength of plastic behaviours expressed in response to socio-sexual environmental variation can itself also evolve. Individuals experiencing high and / or highly dynamic levels of competition over many generations are expected to retain strong plasticity. However, predicting exactly how the magnitude or sensitivity of plasticity should evolve in response to variation in the socio-sexual environment (e.g. to the degree of adult sex ratio bias) is not yet clear. The finding that that plasticity in male courtship repertoire evolves under long term exposure to male- (but not female-) biased regimes in *D. melanogaster* (Dore et al. 2020b) suggests that high same-sex competition could be an important driver. There are far fewer experimental studies to date that address this type of question, and this is an omission we tackle here.

A second key factor expected to drive selection for plastic behaviours, and in particular the ability of individuals to express them, is an individual's condition, which can vary with factors such as temperature, nutrition and population density. The general prediction, based on findings and theory from non plastic traits, is that individuals in good condition should retain the capacity to express plastic traits (Zajitschek et al. 2013, Zajitschek et al. 2016, Rostant et al. 2020, Bretman et al. 2023). However, the fitness gains from expressing plasticity may be higher for the poor quality individuals that can express them, as even small gains in relative fitness may have greater value when resources are limited. Many studies provide evidence to support the idea that condition underpins the expression of plastic, sexually-selected or life history traits (Bath et al. 2021). For example, in the moth *Plodia interpunctella*, males raised under high population density take longer to mature but have larger testes (Gage 1995). In the Yellow dung fly (*Scathophaga stercoraria*) an increase in temperature is strongly correlated with a reduction in testes size (Bernasconi et al. 2002). Consistent with the prediction outlined above, previous research using the lines employed in this study, has shown females evolving within resource-rich regimes were able to evolve resistance to the deleterious effects of continual exposure to males, whereas females evolving under resource poor regimes were not (Rostant et al. 2020). Some studies also show the effects of short or long term manipulations of condition on the expression of plastic behaviours

specifically. For example, in *D. melanogaster,* increased availability of nutrition under lower larval density results in the production of larger females that are more aggressive (Bath et al. 2018) and increased aggression was stronger in populations maintained over time under strong female bias (Bath et al. 2017).

Collectively, these studies show that phenotypic and evolutionary manipulations of the socio-sexual environment and condition can result in changes to plastic behaviours. However, there are so far very few studies that have tested the effect of simultaneous long term manipulations of condition and the socio-sexual environment, on the extent of plasticity, particularly in females. This is the specific omission we address in this study. Here we tested whether plastic responses in female fecundity (Fowler et al. 2022a) could evolve in experimental evolution regimes selected under male-biased (MB), equal sex (ES) and female-biased (FB) adult sex ratios maintained under high or low quality adult diets. The specific predictions were that a history of elevated competition among females (in FB regimes) would:

(i) select for increasingly sensitive plastic fecundity responses to the presence of conspecifics, to counter the effects of increased competition

(ii) be magnified in individuals drawn from poor adult nutritional resource regimes, to increase relative fitness when resources are poor, by limiting competition.

Methods

Base stock maintenance and non focal fly collection

All non-focal flies were reared from the wild-type Dahomey population, which was maintained at 25°C in a humidified room (50-60% RH) held on a 12 h light: 12 h dark cycle. Flies were reared on a sugar-yeast-agar (SYA) medium (100g brewer's yeast, 50g sucrose, 15g agar, 30mL Nipagin (10% solution), 3mL propionic acid, 0.97L water). Flies for use in experiments were grown by allowing females to first oviposit for 24 h on agar-grape juice plate (50g agar, 600mL red grape juice, 42mL Nipagin (10% solution), 1.1L water) to acclimatise, and then on a fresh agar-grape juice plate for 4 h. Larvae were collected from the 4 h egg collection plates and reared under a

controlled density of 100 larvae per vial (24 x 75 mm) each containing 7ml SYA medium. At eclosion, adults were separated by sex within 6 h of eclosion to ensure virginity and stored 10 per vial in single sex groups in vials on standard SYA medium.

Experimental evolution line maintenance

Females from the experimental evolution lines were derived from those used in previous studies (Dore et al. 2020b, Rostant et al. 2020, Bath et al. 2021, Sepil et al. 2022). These lines comprise 3 independent replicates each of of equal sex (EQ (50M: 50F)), female biased (FB (25M: 75F)) and male biased (MB (70M: 30F)) lines, maintained as adults on either high or low SYA diets (100% versus 20% of the standard amount of yeast; 3 sex ratio regimes x 2 nutritional regimes x 3 replicates = 18 populations). It was only the adult nutritional environment that was varied, as larvae from all regimes were always reared each generation on the same standard SYA medium. Regimes were maintained as adults within plastic boxes (12cmW x 18cmL x 8.5cmD, with gauze lid) under the same culturing conditions as the Dahomey wild type. Adults in the high yeast lines were given access to two fresh, standard SYA medium every 2-3d, whilst the low yeast lines were similarly supplied with 20% SYA medium. 9d after setting up the adults in the boxes, each line was supplied with an agar-grape juice egg collection plate, which was replaced on day 10. Egg collection plates were maintained at 25°C following their removal from the boxes and kept within in cotton bags for 2d. 400 larvae were then picked from the egg collection plates and placed 100 per vial for each line. After eclosion of the adults from these vials, flies were anaesthetised using CO_2, counted into the appropriate sex ratios, and placed again in plastic boxes. The lines have been maintained under these conditions since 23/12/2013. The flies used in the experiments were derived from generation 102 for block 1,& generation 109 for blocks 2 & 3.

Collection of experimental evolution line females

To minimise parental effects, and more easily allow the detection of evolved responses, experimental flies were reared under two generations of common garden conditions (equal sex ratio and nutrition) prior to the experimental set up. To initiate these cultures, additional flies from the standard maintenance of the lines were

transferred to 70ml bottles of standard SYA for 24h, the adults then removed, and the deposited eggs allowed to mature to adulthood. Upon emergence the populations were transferred to an egg laying chamber (12cm diameter x 18cm high) and provided with an agar-grape juice egg collection plate for 24h to acclimatise, which was then replaced with a fresh agar-grape juice egg collection plate for 4h. Larvae were then picked at standard densities of 100 per vial into standard SYA vials. Upon eclosion adults were separated by sex to ensure virginity and stored 10 per vial on standard SYA medium.

Effect of evolutionary manipulation of adult sex ratio and nutrition on socially plastic fecundity responses in females

Following the 2 generations of common garden rearing described above, focal virgin females from all experimental evolution lines were exposed to two social treatments: 'alone' or 'grouped' with 3 non-focal wild type virgin females, for 3d prior to mating (n=30 focal females per replicate per treatment). Non-focal females in the grouped treatments were made identifiable by wing clipping under CO_2 anaesthesia 1d prior to introduction to focal females. The experiment was carried out in two blocks (replicate populations 1 first and replicate populations 2 & 3 simultaneously).

After the 3 days of alone and grouped social treatments, matings were performed. Mating assays were started at 30 mins after lights on and ran for up to 1.5h. Experimental females were each transferred into vials containing 1 wild type male (placed in each vial 24h previously). The vacated social exposure vials were retained to count virgin eggs (note that in the alone treatment all virgin eggs counted were from the focal females, in the grouped treatment virgin eggs of focal and non focal females were mixed). In the mating assay, time of entry, mating latency and duration were all recorded. Any individuals for which matings lasted less than 5min were discarded, as these matings were likely to have been incomplete with no sperm transfer (Gilchrist and Partridge 2000). After mating, males were removed, and focal females retained for 24h before also being removed. Eggs in each of the mating vials were then counted and the vials incubated for 12d for freezing and subsequent counting of all emerging progeny.

Statistical analysis

All statistical analysis was performed using R Core team V-4.0.2 (2020 2020). All three replicates were analysed simultaneously, with the replicates ('Population') designated as a random factor. The Shapiro-Wilk test, Q-Q plots and histograms were used to check data were normally distributed and the Levene's test to check the homogeneity of variances across treatments. Analysis of egg number and progeny were analysed using linear mixed effects models from the lme4 package (Bates et al. 2015) and Chi-squared test were used to drop non-significant terms (supplementary material). Mating latency and duration were also analysed using the same method after being log10 transformed. To analyse differences between group treatments, a Tukey post hoc analysis was conducted using the 'emmeans' package (Lenth 2022). Additionally, a Generalized Linear Mixed Effects Model (GLMER) with a Poisson error distribution, and a negative binomial GLMER were used to check model fit versus the LMER. The GLMER with poisson structure did not fit the data well, whilst the negative binomial reported similar results to the LMER. Models were compared using Log-likelihood, Akaike's information criterion (AIC) and residual plots. The data were initially analysed using the whole dataset. In subsequent analyses, zero egg counts were removed to allow the data for the egg laying and non egg laying females to be analysed separately, using a binomial generalised linear mixed model. For virgin egg data, it was not distinguish between the eggs laid by focal and non-focal females in the grouped treatments. Therefore, we analysed the virgin egg count data separately for alone and grouped treatments.

Results

Evolution of plasticity in females was robust and did not change following evolutionary manipulations of adult sex ratio and nutrition

The main finding was that females from the sex ratio regimes retained plastic fecundity responses across all sex ratio and nutritional evolutionary treatments. Counter to the predictions, this effect was not magnified in FB or low nutrition adult

diet regimes, with the extent of fecundity plasticity remaining similar in magnitude across all treatments. These results are presented in more detail, below.

(i) Mating latency, duration and post-mating fecundity plasticity

We first analysed latency to mate, which was not significantly different across evolutionary sex ratio or diet regimes. However, latency did differ according to the social environment, being significantly longer overall in grouped compared to alone females (t = 2.418, residual df = 877.72, *p=0.0158*; Fig. 1A; Fig. S1A-C). No such effect was seen in mating duration, which also did not differ aross sex ratio or diet regimes (t = -0.550, residual df = 878.054 *p = 0.582*, Fig. 1B; Fig. S2A-C). In terms of the main hypotheses tested, we found that fecundity plasticity was retained in all treatments, with grouped treatment females consistently laying significantly fewer eggs after mating in comparison to the alone females (t = -2.728, residual df = 871.1465, *p=0.00649*; Fig. 2A; Fig. S3A-C). However, the magnitude of this plasticity was not significantly different across any of the sex ratio or diet regimes (table S1). Consistent with the fecundity data, grouped females also produced significantly fewer offspring than alone females in the 24h after mating (t = -2.274, residual DF = 870.232, *p=0.0232*), again with no significant difference between sex ratio or diet regimes (Fig. 2B; Fig. S4A-C). There was no effect of social treatment, sex ratio or diet regime on egg-adult viability (percentage of post-mating eggs developing to adulthood after 12d; Fig. 2C; Fig. S5A-C; table S2).

The results show that fecundity plasticity was retained in all of the experimental evolution regimes – thus all females exhibited similar fecundity plasticity to that previously reported in wild type females (Fowler et al. 2022a). Counter to the prediction, fecundity plasticity was not increased in females from FB regimes, or in females drawn from the low nutrition adult diet regimes.

(ii) Virgin egg laying patterns prior to mating

It was not possible to distinguish focal from non focal virgin eggs, thus the number of virgin eggs laid was analysed separately for each of the two social treatments. This showed that low adult nutrition regime females held in social isolation (alone treatment) laid significantly more virgin eggs than did high food regime females (t = 2.319, p = 0.0204; Fig. S6A). There was no detectable effect of sex ratio or adult diet

regime on the number of virgin eggs laid among grouped treatment females (Fig. S6B), though note here that we cannot distinguish the eggs of focal and non focal females and thus any patterns present might have been obscured. An analysis of the frequency of egg laying versus non egg laying females showed that, in the alone social treatment, MB females from the poor food regimes were significantly more likely to lay eggs in comparison to their high food counterparts ($t = -1.538$, $p = 0.0004$; Figure S7A) an effect that was not observed for the other sex ratio regimes. Among the grouped treatment females, there was no significant difference in the frequency of egg laying between females from the different sex ratios or dietary regimes (Figure S7B), though again note that we cannot distinguish egg laying here by focal versus non focal females. Overall, these results suggested there were significant responses of virgin egg laying behaviour to both nutritional and adult sex ratio regimes, which was evident in the focal females held alone prior to mating.

Discussion

Our main aim was to investigate whether plastic fecundity responses to the presence of conspecific females had evolved in lines with an evolutionary history of variation in sexual selection and adult diet availability. To test this, we compared the mating behaviour and fecundity plasticity of females drawn adult sex ratio regimes maintained on high and low adult diets, raised through 2 generations of common garden rearing, and then housed alone or with three conspecific females prior to mating. We found that female plastic fecundity responses were retained in all regimes, but that they did not differ in response to long term variation in the adult sex ratio or adult diet regimes. The results revealed consistent plasticity in mating latency, which was a new, unexpected finding, with females from all regimes mating sooner when socially isolated prior to mating. Among females socially isolated prior to mating, we also detected evidence of evolutionary responses of virgin egg laying patterns according to adult diet and sex ratio regimes.

Effect of long term manipulation of socio-sexual environment (adult sex ratio) and adult nutrition on mating latency, duration and fecundity plasticity

An unexpected finding of this study was of consistent plasticity in mating latency, with females from all regimes mating significantly faster when they were held alone prior to mating rather than with other females. Neither adult diet nor sex ratio regime had any effect on this plasticity, which suggests that the response of female mating latency to the pre-mating social environment was robust to long term perturbations of the socio-sexual and nutritional environment. A plastic response in mating latency has not been consistently observed in previous related studies (Fowler et al. 2022a, Fowler et al. 2022b). It is possible that the culturing procedures used to maintain the experimental evolution regimes also conferred consistent benefits of plasticity in mating latency – potentially due to greater predictability of conditions (specified densities, timings of culturing stages and non overlapping generations) in comparison to previous studies. Females experiencing social isolation may perceive mating opportunities and competition for egg laying sites as low against a normal expectation of multiple matings (Imhof et al. 1998). This could increase their willingness to mate rapidly with the first prospective partner encountered. We observed no differences in mating duration in females held alone or in groups prior to mating across any of the sexual selection or nutrition regimes. Hence, although females have the potential to influence mating duration (Lefranc and Bundgaard 2000), the results are consistent with previous reports that plasticity in extended mating duration is primarily under male control (Bretman et al. 2009, Bretman et al. 2013, Fowler et al. 2022b).

Our main aim was to test whether fecundity plasticity evolved following long term variation in adult sex ratio and nutritional regimes. However, the results showed that this plasticity was retained across all evolutionary regimes, with females housed alone prior to mating producing significantly more eggs after mating than did females kept in groups, consistent with previous results (Fowler et al. 2022a). Egg to adult viability did not differ across any regimes and hence, owing to their higher fecundity, females that were socially isolated prior to mating also produced significantly more offspring after mating than grouped females. Females held in groups prior to mating are expected to perceive higher levels of resource competition, and thus lay fewer eggs after mating to reduce it. This informed the prediction, that the increased level

of female-female competition in FB regimes would lead to enhanced plasticity in response to the same sex environment (Rosvall 2011). However, this was not observed, as the plastic fecundity responses of females when exposed to conspecific females were retained and did not evolve differences across any of the regimes.

Fecundity plasticity was also predicted to respond differentially to the nutritional regimes. Our hypothesis was that it would be costly for females to oviposit eggs in an area in which other females were also likely to do so, and that these costs would be magnified under food limitation. Protein restrictive diets can reduce protein content in eggs (Kutzer and Armitage 2016) and low protein in adult diets generally significantly reduces female fecundity (Dick et al. 2011, Zajitschek et al. 2019). Therefore, we expected that females from lines maintained under an evolutionary history of restrictive adult diets (20% of standard yeast levels) might produce fewer eggs or become more efficient in nutrient acquisition under competition. However, there was no evidence for this, with no significant differences in post-mating fecundity attributable to the different adult dietary regimes. This could indicate that the females gain sufficient protein from the carry over effects of resources gained during larval development (Aguila et al. 2013) at least for an initial batch of eggs (Aguila et al. 2013, Bowman and Tatar 2016) or that the restricted adult diet lines have evolved to cope with limited protein availability in adulthood without reducing egg quality (Kutzer and Armitage 2016). Additional experiments will be required to test these ideas.

The lack of response to sex ratio regime in reproductive output was surprising, given reported plasticity effects in males (Bretman et al. 2009, Dore et al. 2020a, Dore et al. 2020b) and evolved changes in other traits in females (Holland and Rice 1999, Wigby and Chapman 2004, House et al. 2019, Rostant et al. 2020) in response to similar variations in adult sex ratio in other studies. Sexual selection is predicted to become more intense in populations in which the adult sex ratio becomes unbalanced (Emlen and Oring 1977, Clutton-Brock 1988, Pitnick 1993, Clutton-Brock 2007, Hollis et al. 2019). Under a male biased regime, competition between males should increase as males seek to maximise their reproductive success in the light of reduced mating opportunities (Godwin et al. 2017, Hollis et al. 2019, Sepil et al. 2022). Female biased regimes are expected to have opposing effects, potentially

increasing competition among females for access to males or resouces (Simmons and Kvarnemo 2006, Pomfret and Knell 2008, Holveck et al. 2015). The lack of response of variation in adult sex ratio is consistent with the idea that the maintenance of this type of plasticity may carry low fitness costs, or that any such cost is accumulated across lifespan (Chapman et al. 1995, Holland and Rice 1999, Wigby and Chapman 2004, Tilszer et al. 2006, Nandy et al. 2013), which would not have been captured here. Additional studies of the benefits of fecundity plasticity would be very useful.

Evolutionary responses in virgin egg laying patterns in response to long term manipulation of socio-sexual environment and adult nutrition

The lack of predicted responses in fecundity plasticity could be explained by lack of sufficient selection pressure. However, potentially arguing against this were the evolutionary responses to nutritional and sex ratio regimes that were observed in virgin egg laying patterns evident in the socially isolated females prior to mating (note we could not test for such effects in grouped females because we could.not distinguish eggs laid by focal or non focal females that were held in groups prior to mating). The number of virgin eggs laid by focal females kept alone was significantly higher for females drawn from the low adult food regimes, but was unaffected by adult sex ratio regime. This effect could result from elevated selection for food utilisation efficiency (Bowman and Tatar 2016). MB females from the low food regimes were also more likely to lay eggs in comparison to MB females from high food regimes. This could indicate that virgin egg laying for these females is potentially costly. However, the significance of virgin egg production overall is not well understood and costs of virgin egg production are generally assumed to be low (Tatar and Promislow 1997).

Response to selection and effective population size

There is little evidence to suggest that fecundity plasticity did not respond due to a lack of selection pressure or limits on effective population size. For example, responses were observed in virgin egg laying patterns in socially isolated females, and in addition, reproductive plasticity in males drawn from these same lines has previously been described (Dore et al. 2020b). Effective population size does vary across the regimes, and though this must be carefully considered (Snook et al.

2009), there is limited evidence that the differences were sufficient to confound the results of studies such as this one.

Conclusion

Overall, consistent with previous works, we show that females adjust their post-mating fecundity according to the social environment.This plasticity was unexpectedly robust to long term evolutionary manipulations of sexual selection and adult resource availability. The results suggest that this type of plasticity can be fairly hard wired to evolutionary perturbations.

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Author contributions

T.C., W.H., M.J.G.G and N.M conceptualised the study. N.M. conducted the labwork and the data analyses and wrote the first draft of the manuscript. T.C., W.H. and N.M contributed to writing and revising the manuscript and approved the final version.

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Data availability

Supplementary figures and tables are presented in the Supplementary Information (including statistical analysis code). Raw data are available at https://doi.org/10.5061/dryad.qbzkh18s6.

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Competing interest

The authors declare that they have no competing interests.

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Figures

Fig 1. (A) Latency to mate ('loglat' in log_{10} minutes), and (B) mating duration ('logdur' in log_{10} minutes) of females from the sex ratio and diet regimes exposed for 3 days prior to mating to conspecifics or left alone. Females from the Female-biased (FB), Equal sex (EQ) or Male-biased (MB) sex ratios and standard 100% protein (High) or 20% protein (Low) diet regimes were tested. Focal test females were either housed alone (A) or grouped with three conspecific non focals (G) for 3 days prior to mating. All conspecific non focal females and males were standard wild type. Boxplots show median line, with boxes representing upper and lower 25% quartile and whiskers representing the range, and points representing individual records, outliers highlighted in yellow.

Fig.2. (A) Post-mating fecundity ('Eggs', number of eggs per female per 24h following mating), (B) Progeny production ('progeny', number of progeny emerging from the eggs laid in (A) per female per 24h following mating), (C) Egg to adult viability ('Egg viability', number of progeny / eggs per female per 24h following mating) of females from the sex ratio and diet regimes exposed for 3 days prior to mating to non focal conspecifics or left alone. Females from the Female-biased (FB), Equal sex (EQ) or Male –biased (MB) sex ratios and standard protein (High) or 20% protein (Low) diet regimes were tested. Experimental females were either housed alone (A) or grouped with three non focal conspecifics (G) prior to mating assay. All conspecific non focal females and males were standard wild type. Boxplots as per Fig.1.

Figure 1

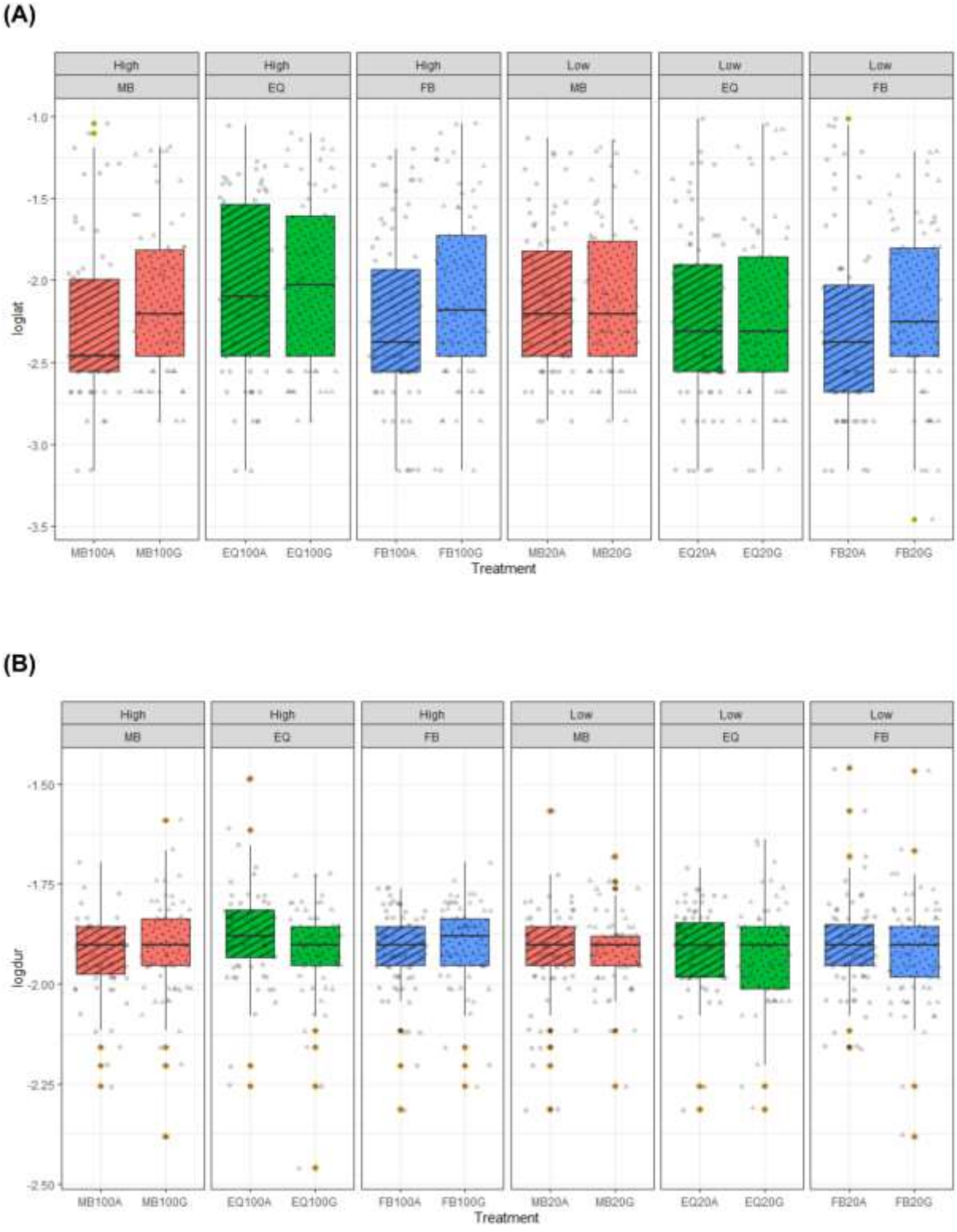

Figure 2A-B

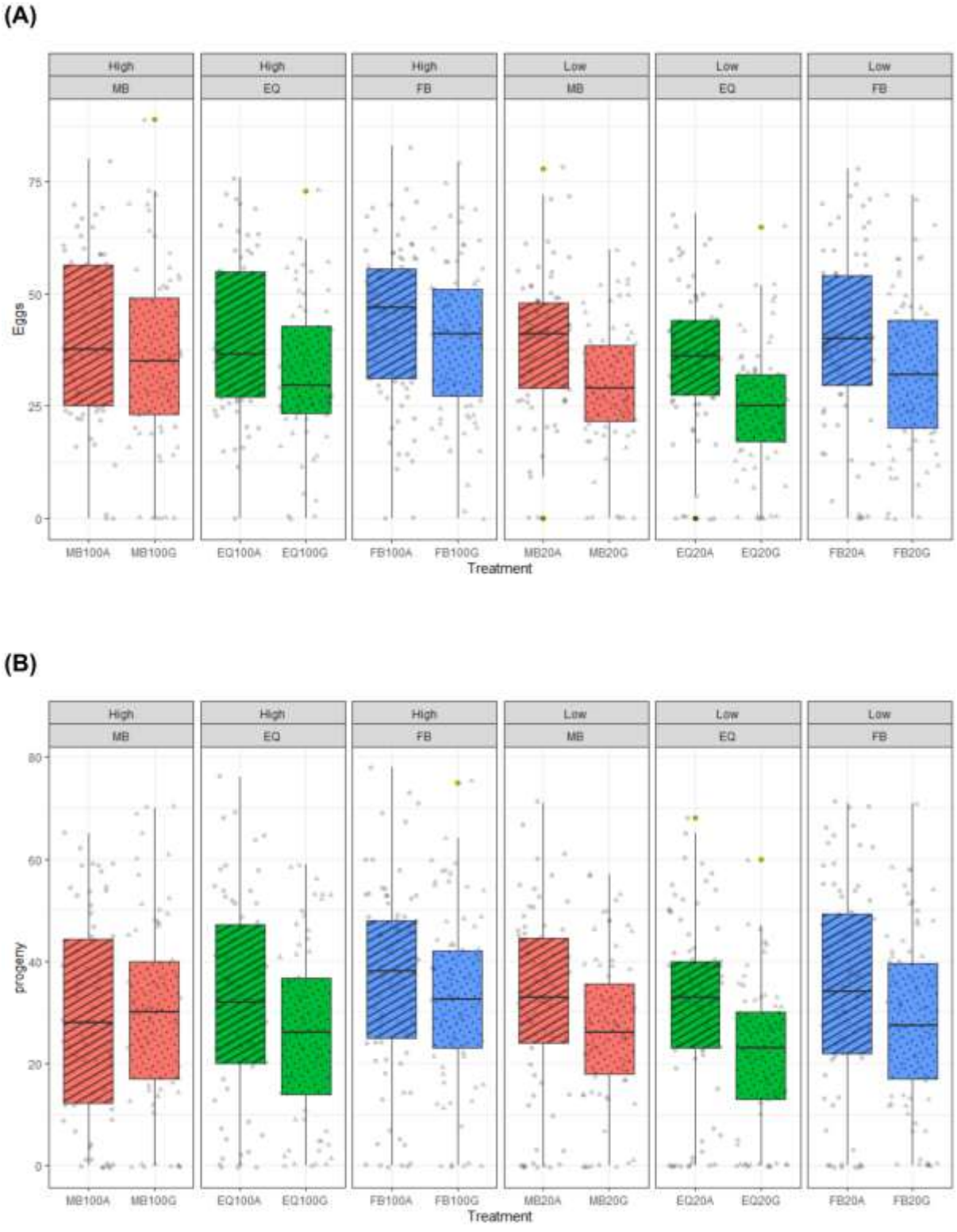

Figure 2C