Sexual selection and the evolution of condition-dependence: an experimental test at two resource levels

Eleanor Bath[1], **Wayne Rostant[2], Harrison J. Ostridge[3], Sophie Smith[4], Janet S. Mason[2],**
Timm Rafaluk-Mohr[1], Judith E. Mank[5,6,7], Tracey Chapman[2], Jennifer C. Perry[2,8],

[1]Department of Biology, University of Oxford, Oxford, United Kingdom
[2]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom
[3]Department of Genetics, Evolution and Environment, University College London, London, United Kingdom
[4]Department of Life Sciences, Imperial College London, London, United Kingdom
[5]Department of Zoology, University of British Columbia, Vancouver, BC, Canada
[6]Biodiversity Research Centre, University of British Columbia, Vancouver, BC, Canada
[7]Centre for Ecology and Conservation, University of Exeter, Exeter, United Kingdom
[8]Department of Biology, St Francis Xavier University, Antigonish, NS, Canada

Corresponding author: Department of Biology, St Francis Xavier University, Antigonish, NS, Canada. Email: jperry@stfx.ca

Abstract

Stronger condition-dependence in sexually selected traits is well-documented, but how this relationship is established remains unknown. Moreover, resource availability can shape responses to sexual selection, but resource effects on the relationship between sexual selection and condition-dependence are also unknown. In this study, we directly test the hypotheses that sexual selection drives the evolution of stronger-condition-dependence and that resource availability affects the outcome, by evolving fruit flies (*Drosophila melanogaster*) under relatively strong or weak sexual selection (through varied sex ratios) and at resource-poor or resource-rich adult diets. We then experimentally manipulated condition via developmental diet and assessed condition-dependence in adult morphology, behavior, and reproduction. We observed stronger condition-dependence in female size in male-biased populations and in female ovariole production in resource-limited populations. However, we found no evidence that male condition-dependence increased in response to sexual selection, or that responses depended on resource levels. These results offer no support for the hypotheses that sexual selection increases male condition-dependence or that sexual selection's influence on condition-dependence is influenced by resource availability. Our study is, to our knowledge, the first experimental test of these hypotheses. If the results we report are general, then sexual selection's influence on the evolution of condition-dependence may be less important than predicted.

Keywords: condition-dependence, experimental evolution, nutrition, plasticity, sex ratio, sexual conflict, sexual selection

Life history theory predicts that sexually selected traits, and more generally any traits under directional selection, should evolve stronger condition-dependence (Andersson, 1982; Andersson, 1986; Biernaskie et al., 2018; Garland & Kelly, 2006; Hoglund & Sheldon, 1998; Houle, 1998; Nur & Hasson, 1984; Rowe & Houle, 1996; but see Johnstone et al., 2009), where condition is defined as the pool of metabolic resources an individual has available to allocate to trait expression (Andersson, 1982, 1986; Cotton et al., 2004; Nur & Hasson, 1984; Rowe & Houle, 1996). Consistent with this prediction, sexually selected traits are often found to exhibit heightened condition-dependence (Bonduriansky, 2007; Bonduriansky & Rowe, 2005; Cotton et al., 2004; Delcourt & Rundle, 2011; Emlen et al., 2012; Gosden & Chenoweth, 2011; Johnstone, 1995; Knell & Simmons, 2010; Sentinella et al., 2013). For example, in the carrion fly *Prochyliza xanthostoma*, male sexually selected traits exhibit stronger condition-dependence compared with non-sexually selected traits (Bonduriansky & Rowe, 2005). In the horned dung beetle *Onthophagus taurus*, in which males develop into "fighter" or

"sneaker" morphs, body size and testes size are under stronger sexual selection in fighters and sneakers, respectively; correspondingly, body size exhibits stronger condition-dependence in fighters and testes size exhibits stronger condition-dependence in sneakers (Knell & Simmons, 2010). The ample evidence for the co-occurrence of sexual selection and increased condition-dependence appears to provide strong support for the predicted causal relationship between them.

However, the prediction that sexual selection drives the evolution of stronger condition-dependence lacks direct experimental support. The evidence to date comes from studies that compare the extent of condition-dependence in sexually selected traits with that of traits not under sexual selection (i.e., other traits within the same sex or homologous traits in females or in males of other species; Bonduriansky & Rowe, 2005; Cotton et al., 2004; Delcourt & Rundle, 2011; Johnstone, 1995; Knell & Simmons, 2010). Although these comparisons are informative, they do not test causality in the relationship between sexual selection and condition-dependence. This is important because there is a plausible alternative

hypothesis: instead of sexual selection driving the evolution of increased condition-dependence, condition-dependent traits might more often become targets of sexual selection, because they better reveal the bearers' condition or quality to choosy receivers (Johnstone et al., 2009). This mechanism would generate the same pattern of co-occurrence between condition-dependence and sexual selection as that predicted by life history theory (and widely documented). Resolving these hypotheses requires the experimental manipulation of sexual selection and testing for any associated evolution of condition-dependence.

Any effect of sexual selection on the evolution of condition-dependence is likely to vary across environments. It is clear that the ecological context shapes evolutionary responses to sexual selection (Andersson, 1994; Emlen & Oring, 1977) and that nutritional resources can be particularly influential (e.g., Chapman & Partridge, 1996; Janicke et al., 2015; Rostant et al., 2020). One possibility is that a food-restricted environment dampens responses to sexual selection, through reducing an individual's resources available for investment in costly sexually selected traits (Figure 1). This mechanism may explain the evolution of reduced female resistance to male harm under food restriction, compared with food abundance, in fruit flies (Rostant et al., 2020). Alternatively, food restriction might strengthen the effect of sexual selection on condition-dependence. If food restriction leads to the evolution of increased efficiency in nutrient processing and reduced metabolic demand (e.g., as observed in fruit flies (Bochdanovits & de Jong, 2003; Cavigliasso et al., 2020) and *Plutella* moths (Warbrick-Smith et al., 2006; reviewed by Ahmad et al., 2018)), then the increased efficiency might allow more investment in sexually selected traits when food becomes available. By this mechanism, food-restricted environments should allow both the evolution of increased condition-dependence and the potential for condition-dependence to respond more strongly to sexual selection (Figure 1). Overall, it remains unclear whether sexual selection drives the

evolution of stronger condition-dependence, and whether the effect of sexual selection on condition-dependence depends on the nutritional context.

We investigate here the hypotheses that stronger sexual selection drives the evolution of increased condition-dependence, and that responses to sexual selection vary with the nutritional environment, by using the experimental evolution of the fruit fly *Drosophila melanogaster*. We evolved replicated populations of fruit flies under three strengths of sexual selection, manipulated through the population sex ratio, and two nutritional regimes, in a fully factorial design. The sex ratio treatment involved male-biased, female-biased, or equal sex ratio treatments, representing relatively strong, weak, or baseline sexual selection on males, respectively (Edmunds, 2020; Pavković-Lučić et al., 2009; Zikovitz & Agrawal, 2013; see also Aronsen et al., 2013). The nutritional regime involved a resource-poor or resource-rich adult diet. After ≥ 23 generations of experimental evolution, we tested for treatment effects on condition-dependence in morphological, behavioral, and reproductive traits (Table 1). We assayed male phenotypes that are known to be targets of sexual selection (Table 1) and female phenotypes, to explore female responses to the sexual and nutritional environment and to test whether evolutionary responses in condition-dependence are limited to males. We generated individuals in good or poor condition by assigning experimentally evolved flies to a high- or low-quality developmental diet (respectively). This is a widely used approach for manipulating condition (e.g., Amitin & Pitnick, 2007; Bonduriansky & Rowe, 2005; Bonduriansky et al., 2015; Gosden & Chenoweth, 2011; Hill, 2011; Izzo & Tibbetts, 2015; Katsuki et al., 2012; Kemp & Rutowski, 2007; Łopuch & Radwan, 2009; McGraw et al., 2007; Miller et al., 2016; Zikovitz & Agrawal, 2013) that can yield similar patterns to genetic manipulations of condition in *D. melanogaster* (Bonduriansky et al., 2015).

If sexual selection causes the evolution of increased condition-dependence, then we expect the response to sexual selection to be strongest in males, with a stronger predicted response after evolution in male-biased populations and weaker response in female-biased populations (i.e., interacting effects of sex ratio and developmental diet). However, because males and females share a genome, we might also expect female condition-dependence to evolve through a positive intersex genetic correlation with male condition-dependence. Alternatively, female condition-dependence might evolve because female traits themselves are under directional selection (e.g., through fecundity selection or resistance to male harm).

Material and methods

Fly stocks and experimental evolution

The experimental evolution regime has been previously described (Dore et al., 2021; Rostant et al., 2020). Briefly, the experimental populations derived from an outbred, lab-adapted Dahomey stock and evolved at either male-biased (70 males:30 females), female-biased (25:75), or equal sex ratios (50:50), and at either a high-yeast adult diet (a standard sugar-agar-yeast (SYA) medium; 100 g brewer's yeast, 50 g sucrose, 15 g agar, 30 ml Nipagin (10% solution), 3 ml propionic acid, and 0.97 L water per liter of medium) or a low-yeast adult diet (SYA medium as before, but with only 20% yeast content (20 g/L)). Yeast is the main source of protein

Predictions:
- Increased condition-dependence evolves under stronger sexual selection (SS)
- Resource limitation during evolution might mute (dashed line) or amplify (dotted line) the effect of sexual selection
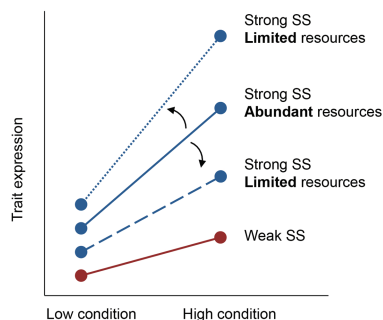
Figure 1. Theory predicts that elevated sexual selection (SS; blue lines) drives the evolution of stronger condition-dependence, resulting in a steeper slope for the relationship between trait expression and condition than under weaker sexual selection (red line). In resource-poor conditions, sexual selection's effect on the evolution of condition-dependence might be muted, if individuals have limited resources for costly trait expression after meeting physiological needs (dashed line). Alternatively, the effect of sexual selection might be stronger in resource-poor conditions, if more efficient nutrient processing evolves under resource limitation, allowing disproportionately higher investment in trait expression for individuals in good condition (dotted line).

Table 1. Traits for which condition-dependence was assayed in this study, after experimental evolution at a male-biased, female-biased, or equal sex ratio and on a resource-poor or resource-rich adult diet.

Sex	Trait category	Trait [†]	Result: Evidence for evolution in condition-dependence? [‡]
Male	Morphology	Mass	No
		Size [§]	No
		Wing shape	No
	Behavior	Courtship frequency	No
		Courtship intensity	No
		Mating success	No
	Reproductive success	Siring success in competitive conditions	No
Female	Morphology	Mass	No
		Size [§]	Increased condition-dependence in male-biased populations
		Wing shape	No
	Reproductive success	Number of ovarioles	Increased condition-dependence in resource-poor populations
		Offspring production	No

[†] Previous studies suggest that each male trait is under sexual selection in *D. melanogaster* or congeners (body size: Bangham et al., 2002, Jagadeeshan et al., 2015, Partridge & Farquhar, 1983, Partridge et al., 1987, Turiegano et al., 2013, Wigby et al., 2016; post-copulatory fertilization success and wing shape and courtship behavior: Abbott et al., 2010, Debelle et al., 2017, Markow & Ricker, 1992, McGuigan, 2009).
[‡] A significant interaction between developmental diet and sex ratio, evolutionary diet, or both.
[§] Scores on the first principal component axis summarizing variation in midfemur length and mean wing centroid size.

in *D. melanogaster* diets and also contains micronutrients (e.g., vitamins, nucleic acids, and cholesterol; Sang, 1978). We, therefore, refer to low- and high-yeast diets as "resource-poor" and "resource-rich", respectively. Three replicate populations were established for each combination of sex ratio and diet treatments, for a total of 18 populations. During experimental evolution, adult flies interacted in ventilated plastic chambers for 10 days in non-overlapping generations. On the 10th day, eggs were collected for the propagation of the next generation and the resulting larvae were grown at a standardized density of 100 larvae per 7 ml of standard SYA medium. Previous studies of these populations have reported the experimental evolution of mating behavior (Sepil et al. 2022; sampled after 35 generations), ejaculate properties (Hotzy et al., 2022; 38 generations), and lifespan (Rostant et al., 2020; 30 generations) at time points similar to those we sampled (generation 23 for morphology and 36 for behavior and reproduction).

To generate white-eyed females and competitor males for the behavioral experiments (white eyes were used to score paternity; see *Behavior and reproduction*), we backcrossed a loss of function allele (w^{1118}) for the white gene into a Dahomey wild-type background and reared flies in vials containing 100 larvae and 7 ml of SYA. All fly husbandry and experiments were conducted at 25°C on a 12:12 h light:dark cycle.

Manipulation of condition through variation of developmental diet

Supplementary Figure S1 illustrates our experimental protocol. Before manipulating condition, we cultured flies from each replicate population on standard SYA media for two generations to minimize parental carry-over effects. We then manipulated condition by culturing the offspring of these flies on a low (20% yeast (20 g/L) or high (120% yeast (120 g/L)) resource diet (relative to SYA media), in

vials containing 100 larvae and 7.5 ml of food media, for each replicate population. To apply the diet treatment, we transferred newly emerged larvae from each population to the assigned diet. We collected the resulting virgin adults for the assays of morphology, behavior, and reproduction (described below) within 6 h of eclosion, using ice anesthesia, and housed them at a density of 15–20 flies in same-sex vials containing standard SYA food medium. We chose 120% yeast for the high-resource diet for two reasons. First, we chose 120% yeast so that both developmental diet treatments differed from the developmental diet experienced during experimental evolution (100% yeast), to avoid the confound of the low-resource treatment being distinct and the high-resource treatment being similar (had we chosen a 100% yeast diet as the high-resource treatment). Second, 120% yeast represents a high-resource developmental diet but not "overfeeding," and results in flies that are phenotypically similar to those that developed on a 100% yeast diet (Duxbury & Chapman, 2020; Fricke et al., 2010). Because our goal was to generate individuals in good or poor condition, we did not aim to recapitulate the developmental nutrition levels that the populations experienced during experimental evolution.

Larvae reared on a low-resource diet develop more slowly and development time is more variable, compared with larvae reared on a higher-resource diet (Duxbury & Chapman, 2020). To account for this difference, we set up larvae on the low-resource diet on days 7 and 8, and 9 days before setting up larvae on the high-resource diet (following Duxbury & Chapman, 2020), so that peak emergence for both treatments occurred simultaneously.

Our goal was to assess the experimental evolution of condition-dependence in male sexually selected traits and, for comparison, in female traits. Previous studies suggest that all male traits assayed in our study are subject to sexual selection (Table 1). These, or similar traits, have shown evidence of

evolved responses in previous experimental evolution studies in *D. melanogaster* or congeners (e.g., Abbott et al., 2010; Bacigalupe et al., 2008; Crudgington et al., 2010; Holland & Rice, 1999; Innocenti et al., 2014; Linklater et al., 2007; Nandy et al., 2013, 2014; Pavković-Lučić & Kekić, 2011; Perry et al., 2016; Snook et al., 2005; Trajković et al., 2021; Wigby & Chapman, 2004). We generated one set of flies for measurement of morphology (at generation 23 of experimental evolution) and another set for behavioral assays (at generation 36). These time points reflected the team's availability throughout the project.

Measurements of morphology

After 23 generations of experimental evolution, we grew flies under the developmental diet treatments described above and then assayed adult mass, midfemur length, wing size, and wing shape (Table 1). Adult flies were preserved in 70% ethanol 2 days post-eclosion. We removed the left midleg and both wings from 15 specimens of each sex, developmental diet treatment, and replicate population and digitally photographed them on a hemocytometer. We used ImageJ software (Abramoff et al., 2004) to measure midfemur length. To measure wing size and shape, we used tpsDig software (version 2.12, Rohlf, 2006) to place 11 landmarks on wing venation (Supplementary Figure S2A). Following leg and wing removal, we placed specimens in a drying oven at 60°C for 48 hr and measured the dry mass of individual specimens (UMX2 ultra-microbalance; Mettler-Toledo, Leicester, U.K.).

Measurements of behavior and reproduction

After 36 generations of experimental evolution, we assayed behavioral and reproductive traits in separate experiments for males and females (Table 1) that were cultured on one of the developmental diet treatments as described above. The measurements for both experiments were initiated with 1-day-old adult flies.

In the male experiment, we measured the courtship behavior (frequency and intensity), mating rate, and siring success of focal males under competitive conditions. To do this, we set up 1,338 replicate vials housing 1 focal male and 2 Dah[w^{1118}] competitor males, along with 3 Dah[w^{1118}] females. We transferred groups to fresh vials on day 5 or 6 after the vials were set up using light CO_2 anesthesia. We inspected vials daily and replaced females and rival males when deaths occurred (191 and 64 replacements, respectively). We terminated vials in which focal males died. We conducted behavioral observations on day 8, when three observers (blind to treatment) conducted spot check observations and recorded whether the focal male was courting or mating, and if courting, whether the male was chasing or orienting towards a female (less intense courtship), performing the wing extension associated with courtship song (moderate courtship), or attempting copulation (more intense courtship). Each vial was observed a total of 6 times beginning at 0h Zeitgeber time. Observations were made at least 20 min apart to avoid double-counting matings. We assayed male reproductive success over day 10—the day on which eggs were collected for propagation during experimental evolution—to capture the selective window for reproductive success. On day 9, groups were transferred to fresh vials containing live yeast granules atop the food media. On day 11, adults were discarded. Vials were frozen 14 days later after adult offspring emergence. We counted the number of offspring of each sex and eye color for each vial.

In the female experiment, we evaluated offspring production for 988 experimental females, each housed in a vial with a single Dah[w^{1118}] male. The experiment was conducted in two blocks offset by one day. We inspected vials daily and replaced males when deaths occurred. We discarded vials in which the female died. Large, high-quality females can attract more courtship from males, which depresses fecundity (Lin et al., 2018; Long et al., 2009). To assess whether offspring production was affected by male courtship effort or mating rate, we conducted behavioral observations on day nine. A single observer (blind to treatment) conducted six spot-check observations beginning at 0 hr Zeitgeber time and recorded whether the female was being courted (with any of the courtship behaviors noted above) or was mating. Observations were at least 20 min apart. We assayed female offspring production over day 10 to capture the selective window for reproductive success. On day 8, each male–female pair was transferred to a fresh vial containing live yeast granules atop the food media. On day 11, adults were discarded and vials were frozen 14 days later. We counted the number of offspring produced.

As a proxy for reproductive capacity (Klepsatel et al., 2013), we measured ovariole number for five females per treatment combination and replicate population.

Analyses

For all linear models, we tested whether model residuals approximated a normal distribution and applied transformations where appropriate (noted in Tables 2 and 3). Models were conducted in JMP 14.2.0 except where noted. Least squares means are given with standard errors throughout.

Morphology

We measured mass along with two univariate traits related to body size (midfemur length and wing size) and multivariate wing shape. As a measure of wing size, we calculated the centroid size for the left and right wings based on the landmarks placed on wing venation and took the mean centroid wing size for each specimen. We then used principal component (PC) analysis to summarize variation in body size. To compare wing shape among treatment groups independently from size, we converted the landmark data to shape variables and calculated a Procrustes superimposition using IMP CoordGen (version 8; Sheets, 2001a), scaled to the size standard (Zelditch et al., 2012).

To test for the effects of the experimental evolution treatments on condition-dependence in mass, body size (PC1 scores), and wing shape, we tested for interactions between developmental diet and either sex ratio or evolutionary diet (or three-way interactions between all three treatments) using linear models. Models included the fixed effects of developmental diet, sex ratio, and evolutionary diet and all interactions, along with the random effects of replicate population and the interaction between replicate population and developmental diet, such that all hypotheses were tested at the population level to avoid pseudoreplication (Arnqvist, 2020). We ran separate models for males and females. For wing shape, we used a univariate linear model of shape variables (i.e., a single column containing all 18 shape variables) that included the above factors, a random factor to account for individual male ID, and the random factor "shape variable" (18 levels). We adopted this approach instead of a multivariate analysis of variance to allow the inclusion of the random factors

Table 2. Models of male traits in relation to sex ratio (SR), evolutionary diet (ED), and developmental diet (DD). Significant interactions involving DD indicate a change in condition-dependence in response to an experimental evolution treatment.

Term (numerator df)	F-statistics (error df) and p-values							
	Mass	Size (PC1) [*]	Wing shape [†]	Courtship frequency	Courtship intensity	Mating success	Likelihood of siring offspring	Proportion of offspring sired [‡]
SR (2)	2.5 (12.1), $p = .13$	0.4 (12.0), $p = .70$	0.8 (12.0), $p = .49$	8.8 (9.1), $p = .008$	2.6 (12.0), $p = .11$	0.3 (12.0), $p = .75$	0.0 (10.6), $p = .96$	2.8 (13.0), $p = .10$
ED (1)	0.8 (12.1), $p = .39$	1.9 (12.0), $p = .19$	0.2 (12.0), $p = .69$	0.0 (9.3), $p = .92$	1.1 (12.0), $p = .32$	0.7 (12.0), $p = .41$	0.2 (10.6), $p = .67$	2.4 (13.1), $p = .15$
DD (1)	11.0 (10.9), $p = .007$	322.8 (12.0), $p < .0001$	172.1 (12.1), $p < .0001$	2.1 (13.8), $p = .17$	0.0 (8.7), $p = .96$	2.3 (12.1), $p = .16$	5.6 (11.0), $p = .04$	0.3 (13.4), $p = .60$
SR × ED (2)	0.6 (12.1), $p = .55$	0.0 (12.0), $p = .99$	0.4 (12), $p = .65$	4.0 (9.1), $p = .06$	1.9 (12.0), $p = .19$	0.3 (12.0), $p = .76$	0.5 (10.6), $p = .64$	0.5 (13.0), $p = .62$
SR × DD (2)	0.5 (10.9), $p = .61$	0.0 (12.0), $p = .99$	0.4 (12.1), $p = .69$	0.4 (13.7), $p = .70$	0.0 (8.7), $p = .99$	0.9 (12.1), $p = .43$	2.7 (11.0), $p = .11$	2.4 (13.2), $p = .12$
ED × DD (1)	0.1 (10.9), $p = .73$	0.0 (12.0), $p = .90$	2.3 (12.1), $p = .15$	2.8 (13.8), $p = .12$	2.0 (8.7), $p = .19$	2.6 (12.1), $p = .13$	0.2 (11.0), $p = .67$	1.2 (13.4), $p = .30$
SR × ED × DD (2)	3.6 (10.9), $p = .06$	2.1 (12.0), $p = .16$	1.9 (12.1), $p = .19$	0.9 (13.7), $p = .41$	0.3 (8.7), $p = .76$	2.0 (12.1), $p = .18$	2.0 (11.0), $p = .18$	0.6 (13.2), $p = .57$

[*] Scores on the first principal component axis summarizing 91.4% of variation in midfemur length and mean wing centroid size. Both midfemur length and wing size loaded positively (eigenvector values of 0.71).

[†] Results for right wings are given here; similar results for left wings are given in Supplementary Table S1.

[‡] Arcsin-square root transformed.

specified above so that we could avoid pseudoreplication (following Snijders & Bosker, 2012). An assumption of this approach is that the shape variables are uncorrelated with each other within and among groups (Pitchers et al., 2013). We examined patterns of shape variation by canonical variates analysis (CVA) and by thin-plate spline visualizations of shape variation, using IMP CVAGen (Sheets, 2001b).

Behavior and reproduction

We tested for the evolution of condition-dependence in male courtship behavior (frequency and intensity), mating frequency, and siring success. Models for courtship frequency and intensity included the same factors as described above for mass and body size. We weighted courtship frequency by the number of observation periods (of 6) in which courtship could have been observed (i.e., discounting periods in which focal males were mating and could not have been courting). We calculated a weighted courtship intensity score (modified from Manning, 1960), with orienting and chasing behavior scored 1, wing extension scored 2, and attempted mating scored 3. Scores were averaged across all observation periods in which courtship was observed, excluding vials when the focal male died or was lost before behavioral observations (12 and 17, respectively). We observed few males mating twice (5/1,309) and no male was observed mating more than twice, so we analyzed the population frequency of males that achieved at least one mating in a model that included the fixed factors given above and the random factor of replicate population.

We assessed male siring success by the number of daughters sired by focal males. Because the white gene is located on the X chromosome, daughters sired by red-eyed focal males had red eyes and daughters sired by white-eyed rival males had white eyes (all sons had white eyes). Approximately 30% of males failed to sire offspring (365 of 1,204 for which daughter counts were available). We therefore first modeled the likelihood of siring offspring in a model that included the fixed factors given above and the random factor of the replicate population. We then modeled the proportion of offspring sired (within the subset of males that sired any offspring), weighted by the total number of daughters, using a linear model that included the same factors as for mass and body size.

Finally, we tested how condition-dependence evolved for female offspring production, measured in the female experiment. The model included factors as given above for mass and body size, along with the fixed factor block. We excluded females that died before the experiment was complete (97/988) and females that produced no offspring (54/891; there were too few females that produced no offspring for a meaningful test of treatment effects on whether females produced any offspring). To evaluate differences in the frequency with which females received courtship, which might influence their fecundity (Lin et al., 2018; Long et al., 2009), we analyzed courtship frequency weighted by the number of observation periods (of 6) in which courtship could have been observed (discounting periods in which mating was observed). The model included the same factors as for female offspring production. We observed only 13 females mating (of 924 females alive on the day behavior was observed) and we were, therefore, unable to evaluate treatment effects on mating rate. The low female re-mating rate we observed is consistent with previous research (Chapman et al., 1994;

Table 3. Model outputs for female traits in relation to sex ratio (SR), evolutionary diet (ED), and developmental diet (DD). Significant interactions involving DD indicate a change in condition-dependence in response to an experimental evolution treatment.

Term (numerator df)	F-statistics (error df) and *p*-values				
	Mass	Size (PC1)[*]	Wing shape [†]	Number of ovarioles	Offspring production[‡]
SR (2)	1.1 (12.1), *p* = .35	0.1 (12.0), *p* = .94	2.1 (12), *p* = .17	1.5 (12.3), *p* = .25	0.9 (11.9), *p* = .43
ED (1)	0.3 (12.1), *p* = .60	0.5 (12.0), *p* = .48	0.1 (12), *p* = .78	0.1 (12.3), *p* = .78	0.0 (11.9), *p* = .96
DD (1)	322.1 (12.3), ***p* < .0001**	514.8 (12.0), ***p* < .0001**	227.6 (12), ***p* < .0001**	137.4 (11.8), ***p* < .0001**	2.3 (12.5), *p* = .15
SR × ED (2)	1.5 (12.1), *p* = .26	0.2 (12.0), *p* = .84	1.2 (12), *p* = .33	1.1 (12.3), *p* = .37	0.8 (11.9), *p* = .49
SR × DD (2)	1.3 (12.3), *p* = .32	4.3 (12.0), ***p* = .04**	0.7 (12), *p* = .53	3.1 (11.8), *p* = .08	1.3 (12.5), *p* = .30
ED × DD (1)	4.2 (12.3), *p* = .06	0.4 (12.0), *p* = .54	0.9 (12), *p* = .35	5.5 (11.8), ***p* = .04**	0.2 (12.5), *p* = .68
SR × ED × DD (2)	1.7 (12.3), *p* = .22	2.6 (12.0), *p* = .12	1.1 (12), *p* = .36	0.7 (11.8), *p* = .50	1.8 (12.5), *p* = .20

[*] Scores on the first principal component axis summarizing 91.4% of variation in midfemur length and mean wing centroid size. Both midfemur length and wing size loaded positively (eigenvector values of 0.71).
[†] Results for right wings are given here; similar results for left wings are given in Supplementary Table S1.
[‡] Square-transformed.

Lawniczak & Begun, 2005; Rostant et al., 2020). We analyzed the number of female ovarioles in a model that included the factors given for mass and body size.

Results

No evidence that male condition-dependence evolved in response to sex ratio

The prediction that stronger sexual selection would drive the evolution of stronger condition-dependence in male traits, resulting in a stronger response to developmental diet in male-biased populations and a weaker response in female-biased populations, was not supported. We found no strong evidence that male responses to developmental diet depended on sex ratio (SR × DD row in Table 2; slope of sex ratio lines in Figure 2).

The developmental diet itself had strong effects on morphology, modest effects on reproductive traits, and no effects on behavior (Table 2; difference between resource-poor and resource-rich developmental diets in Figure 2). A resource-rich developmental diet led to increased mass and size, modified wing shape (Supplementary Figure S2B), and increased likelihood of siring offspring (from 66% (382/580) to 73% (457/624)).

Male courtship frequency differed among sex ratio treatment groups, independent of developmental diet (Table 2). Males from female-biased populations evolved more frequent courtship compared with males from male-biased or equal sex ratio populations (weighted courtship frequency: female-biased, 0.303 ± 0.006; male-biased, 0.270 ± 0.006; and equal sex ratio, 0.279 ± 0.007; Figure 2C). There was no strong evidence that other male traits evolved in response to sex ratio (Table 2; Figure 2).

Condition-dependence in female size evolved in response to sex ratio

Females that evolved in male-biased populations showed a stronger response to developmental diet in body size, compared with females that evolved in female-biased or equal sex ratio populations (a significant sex ratio × developmental diet interaction, Table 3; slope of sex ratio lines in Figure 3B; mean difference between resource-rich and resource-poor developmental diets in PC scores for size: male-biased, 1.63 ± 0.11; female-biased, 1.20 ± 0.11; and equal sex ratio, 1.35 ± 0.11; Figure 3).

Most female traits responded strongly to developmental diet (Table 3; difference between resource-poor and resource-rich developmental diets in Figure 3). A resource-rich developmental diet led to increased mass and size, modified wing shape (Supplementary Figure S2C), and the production of more ovarioles (from 24.2 ± 0.7 to 32.9 ± 0.7; Table 3; Figure 3C). However, offspring production showed no strong response to developmental diet (Figure 3D). We found no strong evidence that female traits had evolved in response to sex ratio (Table 3; Figure 3), apart from the interaction between sex ratio and developmental diet for female size noted above.

Male courtship is known to suppress female fecundity, and we, therefore, wondered whether females from different sex ratio groups might attract different levels of courtship, which might have obscured an effect of sex ratio on offspring production. However, we observed no differences in female receipt of courtship among treatment groups (Supplementary Table S2).

No evidence for interactions between sex ratio and evolutionary diet

We found no evidence that the effect of sex ratio on condition-dependence varied with resource levels experienced during experimental evolution in either male or female traits (no two- or three-way interactions involving sex ratio and evolutionary diet; Tables 2 and 3).

Condition-dependence in female ovariole number evolved in response to evolutionary diet

Females that evolved on a resource-poor adult diet showed a stronger response to developmental diet in their production of ovarioles, compared with females that evolved on a
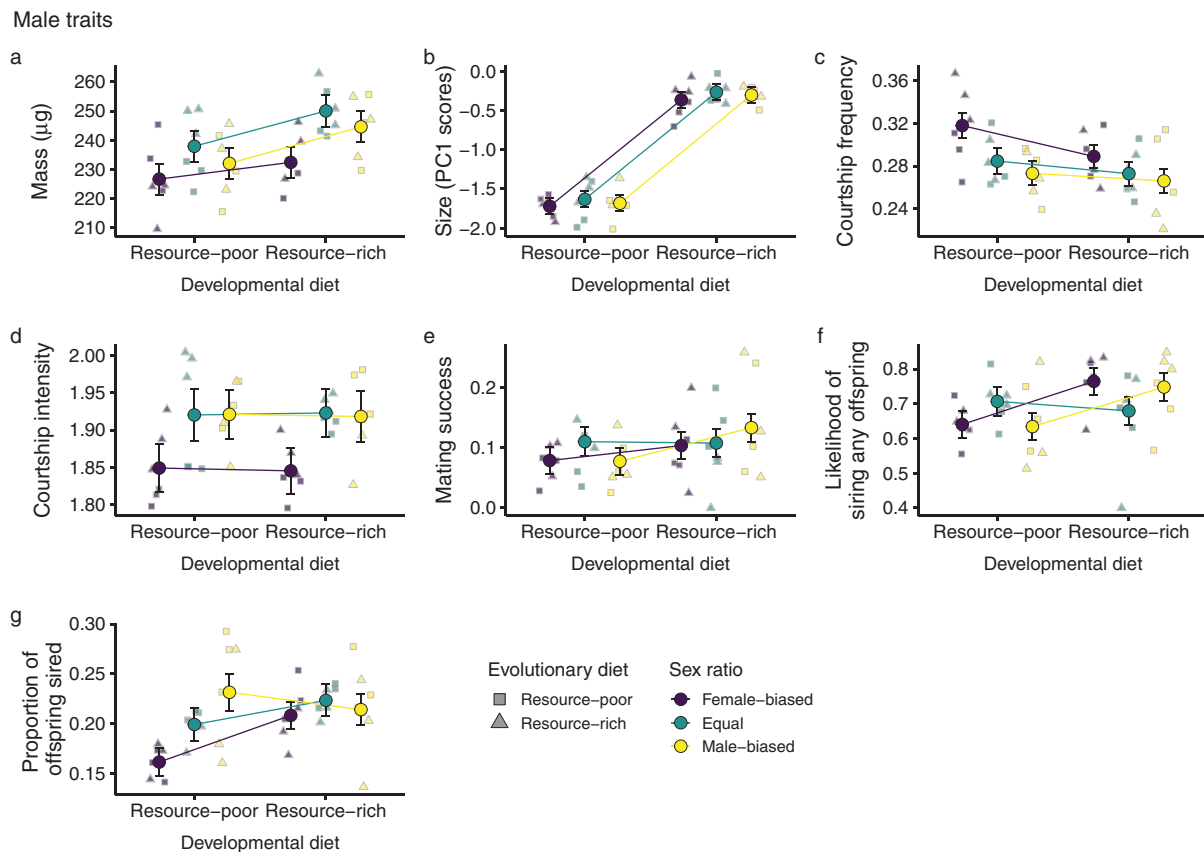
Male traits

Figure 2. Condition-dependence in male traits, after experimental evolution in male-biased, female-biased or equal sex ratio populations and at a resource-poor or resource-rich adult diet, for mass (A), body size (B); PC scores summarizing variation in wing size and leg length), courtship frequency (C) and intensity (D), mating success (E); the likelihood of being observed to mate), the likelihood of siring offspring (F), and the proportion of offspring sired (G). Condition-dependence is reflected by the slope of the relationship between trait expression in experimentally evolved male fruit flies randomly assigned to a resource-poor or resource-rich developmental diet (i.e., in poor or good condition, respectively). Small symbols represent replicate populations, squares represent the resource-poor treatment and triangles represent the resource-rich treatment. Large circles represent model means for each sex ratio treatment, including both resource-rich and resource-poor populations. Least squares means are given with standard errors.

resource-rich adult diet (a significant evolutionary diet × developmental diet interaction; Table 3 and Figure 4; mean difference between resource-rich and resource-poor developmental diets: resource-poor evolutionary diet, 10.4 ovarioles ± 1.1; resource-rich evolutionary diet, 6.9 ovarioles ± 1.0).

Apart from this interaction, there was no evidence that other female or male traits evolved in response to the evolutionary diet (no main effects and no other interactions with evolutionary diet; Tables 2 and 3; Figures 2 and 3).

Discussion

In the well-documented positive association between sexual selection and condition-dependence, the direction of causality has remained obscure, because it is not clear whether (a) sexual selection increases condition-dependence or (b) condition-dependent traits are more likely to come under sexual selection (Johnstone et al., 2009). In this study, we have explored the first of these hypotheses. We observed the experimental evolution of condition-dependence in response to skewed population sex ratios and resource levels. However, we observed evolutionary change in condition-dependence only in female body size and ovariole number. In contrast, we found no evidence that condition-dependence in a suite of male sexually selected traits evolved in response to the

strength of sexual selection, manipulated via the sex ratio. These results show that there is some potential for condition-dependence to evolve rapidly in response to the social environment, but the evolvability of condition-dependence was limited to females in the conditions of our study. The results offer no support for the hypothesis that sexual selection drives the evolution of increased condition-dependence in males. If these results reflect general patterns, then the role of sexual selection in influencing condition-dependence may be less important than predicted.

Our results also give insight into the unresolved issue of whether sexual selection's effects on condition-dependence are influenced by food resource abundance. We found that the resource abundance that adults experienced during experimental evolution did not influence how condition-dependence responded to the sex ratio for any male or female trait.

No increase in male condition-dependence in male-biased populations

We predicted that male traits would evolve increased condition-dependence under the stronger sexual selection of male-biased populations. The prediction was not supported: no male trait showed differences in condition-dependence among treatments. Hence, the results offer no support for the hypothesis that sexual selection has a causal role in increasing
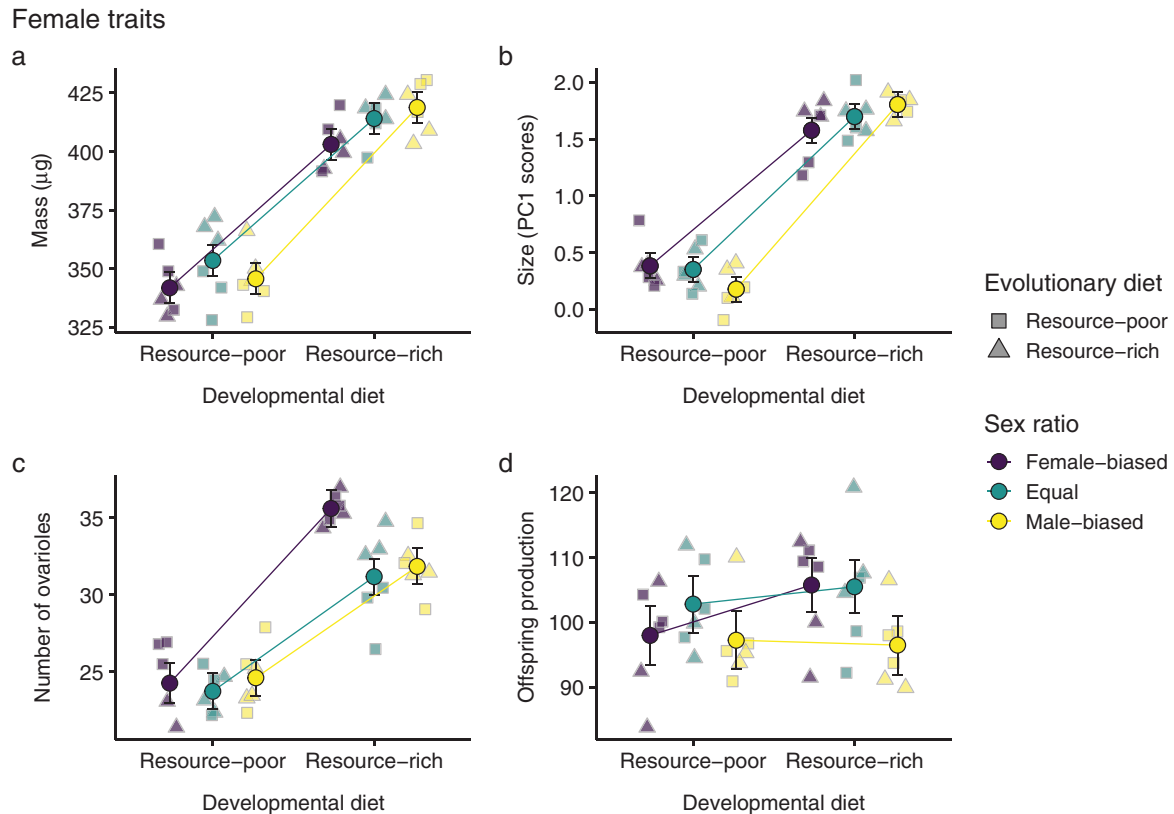
Female traits

Figure 3. Condition-dependence in female traits, after experimental evolution in male-biased, female-biased or equal sex ratio populations and at a resource-poor or resource-rich adult diet, for mass (A), body size (B); PC scores summarizing variation in wing size and leg length), number of ovarioles (C), and offspring production (D). Condition-dependence is reflected by the slope of the relationship between trait expression in experimentally evolved female fruit flies randomly assigned to a resource-poor or resource-rich developmental diet (i.e., in poor or good condition, respectively). Least squares means are given with standard errors.

the degree of condition-dependence in sexually selected traits. One possible explanation is sexual selection generally does drive the evolution of stronger condition-dependence but that our study could not detect it. A false negative could have arisen because one of the ingredients of evolution—genetic variation, selection, and time—was insufficient. However, this explanation is not fully satisfying for a few reasons. One is that we observed the experimental evolution of condition-dependence in two female traits (Table 1), and previous studies of these populations have also reported the evolution of phenotypes at time points similar to those we sampled (Hotzy et al., 2022; Rostant et al., 2020; Sepil et al., 2022). This suggests that there was sufficient genetic variation, differences in selection among treatments, and time for the evolution of at least some traits in this study, although it remains possible that one or more of these factors was lacking for male condition-dependence. Other reasons come from previous studies. First, stronger sexual selection in male-biased groups is supported by both theory (e.g., Kokko & Monaghan, 2001; Kvarnemo & Ahnesjo, 1996) and by measurably stronger sexual selection on male mating success in male-biased groups of *D. melanogaster* in a laboratory setting (Edmunds, 2020). Second, previous studies of our experimentally evolving populations report evolution in several behavioral and life history traits in response to sex ratio (Bath et al., 2021; Dore et al., 2021; Rostant et al., 2020). Finally, there is evidence for standing genetic variation in sensitivity to developmental

diet in laboratory populations of *D. melanogaster* and congeners (Hillesheim & Stearns, 1991; Delcourt & Rundle, 2011; Bhumika, 2019; but see Gosden & Chenoweth, 2011). Nonetheless, because surprising (null) results warrant extra scrutiny, it would be worthwhile to explore the possibility that, given more time, condition-dependence would evolve in response to sex ratio, by assaying these populations after more prolonged experimental evolution.

An alternative explanation is that stronger sexual selection did not, in fact, favor increased male condition-dependence in male-biased sex ratios. This possibility suggests the need for additional experimental studies of the evolution of condition-dependence in response to sexual selection, in *D. melanogaster* and other organisms. If the pattern we found is general, then caution is warranted in inferring causality from the association between sexually selected traits and heightened condition-dependence (e.g., Cotton et al., 2004). Such a pattern would be consistent with the theory that predicts no relationship between the strength of sexual selection and condition-dependence (Johnstone et al., 2009), despite other theories predicting that sexual selection should drive the evolution of increased male condition-dependence (Andersson, 1982, 1986; Biernaskie et al., 2018; Hoglund & Sheldon, 1998; Houle 1998; Nur & Hasson, 1984; Rowe & Houle, 1996). We note that our study was not designed to evaluate the alternative hypothesis that male condition-dependent traits are more likely to come under sexual selection because
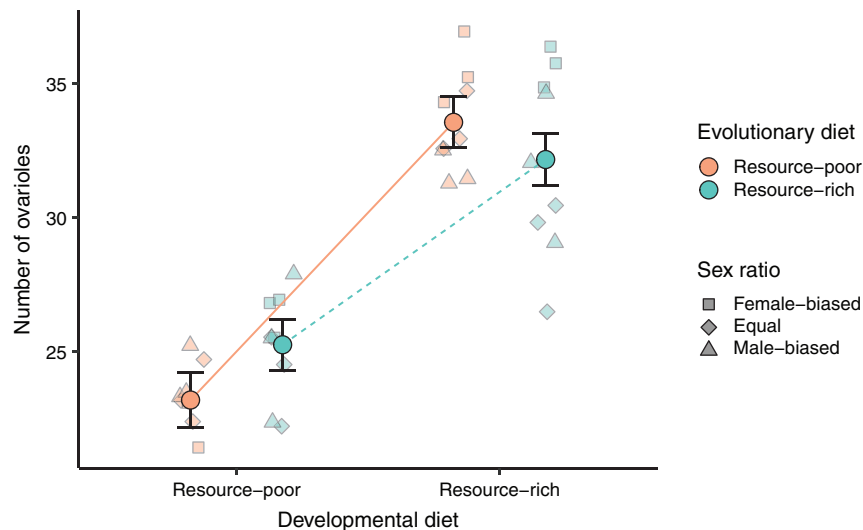
Figure 4. Condition-dependence in female ovariole production, after experimental evolution at a resource-poor or resource-rich adult diet and in male-biased, female-biased, or equal sex ratio populations. Condition-dependence is reflected by the slope of the relationship between trait expression in experimentally evolved female fruit flies randomly assigned to a resource-poor or resource-rich developmental diet (i.e., in poor or good condition, respectively). Least squares means are given with standard errors.

females prefer them for the information they convey about males. Such a study would need to introduce novel and artificial sources of sexual selection, because if the hypothesis is correct, then it is likely that past and ongoing sexual selection will have already found all condition-dependent traits that can impart information about male quality.

Increased condition-dependence in female size in female-biased populations

We found that female body size evolved increased condition-dependence in male-biased populations. Female traits can evolve as a byproduct of selection on males coupled with a positive intersex genetic correlation, but we observed no changes in male condition-dependence with a sex ratio that could explain the result in females. It is therefore likely that female condition-dependence evolved in response to differences in the directional selection on females among sex ratio groups. Directional selection is expected to lead to increased plasticity (e.g., increased condition-dependence in response to diet) because selection will favor individuals that are able to enact a stronger plastic response in the direction favored by selection (Garland & Kelly, 2006). Although there was no strong evidence that female body size itself evolved in response to sex ratio, it is possible that the reaction norm for body size was evolvable while body size itself was not (see Pigliucci, 2005; Scheiner, 1993; Via, 1993). One might expect a stronger selection for larger female size in female-biased populations, because females from these populations have evolved heightened aggression toward other females (Bath et al., 2021) and larger females are more likely to win contests (Bath et al., 2017). However, this possibility is inconsistent with our finding of increased female condition-dependence in male-biased populations. Instead, selection might have favored larger females in male-biased populations to buffer the harmful effects of excess male courtship. Indeed, larger female *D. melanogaster* appear better able to persist in egg-laying despite male harassment (Turiegano et al., 2013). It is interesting that we observed a trend for females from female-biased

populations to produce more ovarioles despite being similar in size, or smaller, than females from male-biased and equal sex ratio populations, after development on a resource-rich diet. In female-biased populations, females face less harassment from males, but stronger competition for oviposition sites, which might lead to selection for more efficient packaging of ovarioles rather than increased size. Future work that directly establishes sources of variation in selection on females in relation to sex ratio will be helpful.

No evidence that effect of sex ratio depended on adult resource levels during experimental evolution

We found no evidence that the effect of sex ratio on condition-dependence differed depending on adult resource levels experienced during experimental evolution, even though there are several reasons to expect an interaction. One expectation is that responses to sex ratio will be dampened when populations evolve in resource-limited environments because the greater investment would be more costly in environments with limited adult resources (Figure 1). A previous study of our populations found that females evolved reduced resistance to male harm when they evolved in adult food-limited populations (Rostant et al., 2020), consistent with this expectation. It is also possible that the strength of sexual selection is reduced in resource-limited populations; for example, because poor-quality adult food depresses egg production (e.g., McCracken et al., 2020), which results in a lower opportunity for sexual selection in some species (Janicke et al., 2015; see also De Lisle & Rowe, 2014). Alternatively, it is possible that resource-limited environments will strengthen the effect of sexual selection on condition-dependence, if resource limitation drives the evolution of a general increase in nutrient processing efficiency and reduction in metabolic demand. In fact, these responses have been observed in response to resource limitation in experimental evolution studies of *D. melanogaster* (Bochdanovits & de Jong, 2003; Cavigliasso et al., 2020; but see Kristensen et al., 2011) and other insects (Warbrick-Smith et al., 2006; reviewed by Ahmad et al., 2018). It is

unclear from our results whether neither of these processes had much impact or whether they tended to counterbalance each other, such that no interaction between sex ratio and evolutionary diet was detectable. The potential for resource limitation to influence sexual selection deserves further study.

Increased condition-dependence of female ovariole production in resource-limited populations

We found that the number of ovarioles females produced was more condition-dependent following evolution under adult resource limitation. This result is consistent with the hypothesis that resource limitation selects for more efficient nutrient acquisition, extraction, and processing, such that individuals can maximally exploit abundant resources when they become available. Previous studies reported more efficient nutrient processing after evolution on a limited diet during development (Bochdanovits & de Jong, 2003; Cavigliasso et al., 2020; Warbrick-Smith et al., 2006). Our result is consistent with the hypothesis that more efficient nutrient processing might also evolve in response to adult resource restriction and that the increased efficiency might extend to the larval stage, permitting the greater female ability to convert nutrients into reproductive potential. If this hypothesis is correct, some puzzles remain. First, if limited adult resources cause the evolution of increased efficiency, then one might expect females that evolved under resource limitation to exhibit increased ovariole production under both low and high developmental resources, compared with females that evolved under abundant resources. This would result in the main effect of the evolutionary diet. However, we observed no significant differences between evolutionary diet treatments at either developmental diet level, and if anything, a trend towards increased ovariole production by females that evolved under abundant resources on a limited developmental diet. However, it is not clear that females that evolved under limited resources should produce more ovarioles on a limited developmental diet even if they had evolved increased efficiency. Instead, evolving in a limited resource environment might select females that reduce ovariole production when resources are limited in order to better match reproductive potential to the nutritional environment. Further study of the fitness consequences of plasticity in ovariole numbers in varying nutritional environments will help to clarify these predictions. A second puzzle is that the effect was limited to females and to ovariole numbers. One possible explanation is that female reproductive potential might be a target of especially strong directional selection. This hypothesis is consistent with the stronger selection on female fecundity observed in low-food environments (compared with high-food environments) in the snail *Physa acuta* (Janicke et al., 2015).

We did not find that high-condition females produced more offspring, despite having more ovarioles. In previous studies of *D. melanogaster*, ovariole number was positively related to offspring production when females were housed singly (e.g., Cohet & David, 1978), but not when females were housed in groups (e.g., Markow et al., 2012; Wayne et al., 2006), as in our study. One possibility is that high-condition females might have attracted more courtship, limiting their fecundity (e.g., Lin et al., 2018; Long et al., 2009), but we found no differences in courtship depending on a female developmental diet under the conditions of our experiments. It would be interesting for future work on these populations to investigate condition-dependence in egg size, as a proxy for offspring quality,

following recent findings of reduced egg size in response to experimental evolution under larval crowding (which should generate adult flies of poor condition; Venkitachalam et al., 2022).

Overall, we observed evolved changes in condition-dependence for two female traits and no male traits. This pattern is consistent with other experimental evolution studies that have found a stronger response to sex ratio in females (Bath et al., 2021; Stångberg et al., 2020; Wigby & Chapman, 2004), and the observation of stronger condition-dependence in response to developmental diet for female *D. melanogaster* than for males (Millington et al., 2021; Shingleton et al., 2017). Stronger responses in females might reflect stronger selection on females or more standing genetic variation or evolvability in females, and the generality of this pattern represents an exciting avenue for further research.

More frequent male courtship in female-biased populations

Our focus was on the experimental evolution of condition-dependence, but our study's design allowed us to evaluate how traits evolved in response to sex ratio and evolutionary diet, independent of condition. We found that males evolved more frequent courtship in female-biased populations, compared with male-biased and equal sex ratio populations. This result is consistent with a previous study of these populations that found less frequent courtship song by males from male-biased populations, compared with equal sex ratio populations (Dore et al., 2021). It is also consistent with a report of more prolonged courtship song in polyandrous populations of *Drosophila pseudoobscura* (compared with monogamous; Debelle et al., 2017; see also Wensing et al., 2017). However, another study in an independent set of populations found the opposite: males from male-biased populations displayed more frequent courtship (Nandy et al., 2013), highlighting the possibility that trait evolution might strongly depend on idiosyncratic differences in experimental conditions among studies. We found no evidence for experimental evolution in other traits in response to evolutionary diet or sex ratio, largely consistent with previous experimental evolution studies that manipulated sex ratio (Linklater et al., 2007; Reuter et al., 2008; Wigby & Chapman, 2004; but see Nandy et al., 2013).

Conclusion

Our study is, to our knowledge, the first to directly test the hypothesis that sexual selection drives the evolution of stronger condition-dependence. Overall, support for the long-standing hypothesis that sexual selection causes traits to evolve stronger condition-dependence awaits further tests. Given the strong observational support for stronger condition-dependence in sexually selected traits, and the centrality of condition-dependence and sexual selection in evolutionary biology, causality is important to resolve. A robust answer will require input from multiple systems and conditions. Our results highlight the importance of testing the widely assumed causal relationship between sexual selection and condition-dependence.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpac066)

Data availability

Author contributions

Acknowledgments

References

Abbott, J. K., Bedhomme, S., & Chippindale, A. K. (2010). Sexual conflict in wing size and shape in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 23(9), 1989–1997. https://doi.org/10.1111/j.1420-9101.2010.02064.x

Abramoff, M. D., Magalhaes, P. J., & Ram, S. J. (2004). Image processing with image. *Biophotonics International*, 11, 36–42.

Ahmad, M., Keebaugh, E. S., Tariq, M., & Ja, W. W. (2018). Evolutionary responses of *Drosophila melanogaster* under chronic malnutrition. *Frontiers in Ecology and Evolution*, 6, 47.

Amitin, E. G., & Pitnick, S. (2007). Influence of developmental environment on male- and female-mediated sperm precedence in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 20(1), 381–391. https://doi.org/10.1111/j.1420-9101.2006.01184.x

Andersson, M. (1982). Sexual selection, natural selection and quality advertisement. *Biological Journal of the Linnean Society*, 17(4), 375–393. https://doi.org/10.1111/j.1095-8312.1982.tb02028.x

Andersson, M. (1986). Evolution of condition-dependent sex ornaments and mating preferences: Sexual selection based on viability differences. *Evolution*, 40(4), 804–816. https://doi.org/10.2307/2408465

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

Arnqvist, G. (2020). Mixed models offer no freedom from degrees of freedom. *Trends in Ecology and Evolution*, 35(4), 329–335. https://doi.org/10.1016/j.tree.2019.12.004

Aronsen, T., Berglund, A., Mobley, K. B., Ratikainen, I. I., & Rosenqvist, G. (2013). Sex ratio and density affect sexual selection in a sex-role reversed fish. *Evolution*, 67(11), 3243–3257. https://doi.org/10.1111/evo.12201

Bacigalupe, L., Crudgington, H. S., Slate, J., Moore, A. J., & Snook, R. R. (2008). Sexual selection and interacting phenotypes in experimental evolution: A study of *Drosophila pseudoobscura* mating behavior. *Evolution*, 62, 1804–1812.

Bangham, J., Chapman, T., & Partridge, L. (2002) Effects of body size, accessory gland and testis size on pre- and postcopulatory success in *Drosophila melanogaster*. *Animal Behaviour* 64, 915–921.

Bath, E., Bowden, S., Peters, C., Reddy, A., Tobias, J. A., Easton-Calabria, E., Seddon, N., Goodwin, S. F., & Wigby, S. (2017). Sperm and sex peptide stimulate aggression in female Drosophila. *Nature Ecology & Evolution*, 1, 0154.

Bath, E., Edmunds, D., Norman, J., Atkins, C., Harper, L., Rostant, W. G., Chapman, T., Wigby, S., & Perry, J. C. (2021). Sex ratio and

the evolution of aggression in fruit flies. *Proceedings of the Royal Society B: Biological Sciences*, 288, 20203053.

Bhumika, A. K. S. (2019). Patterns of morphological divergence in fruit fly: Response to nutritional variations through changes in allometric relationships and trait sizes. *Journal of Zoology*, 309, 22–34.

Biernaskie, J. M., Perry, J. C., & Grafen, A. (2018). A general model of biological signals, from cues to handicaps. *Evolution Letters*, 2(3), 201–209. https://doi.org/10.1002/evl3.57

Bochdanovits, Z., & Jong, G. de (2003). Experimental evolution in *Drosophila melanogaster*: Interaction of temperature and food quality selection regimes. *Evolution*, 57, 1829–1836.

Bonduriansky, R. (2007). The evolution of condition-dependent sexual dimorphism. *American Naturalist*, 169(1), 9–19. https://doi.org/10.1086/510214

Bonduriansky, R., Mallet, M. A., Arbuthnott, D., Pawlowsky-Glahn, V., Egozcue, J. J., & Rundle, H. D. (2015). Differential effects of genetic vs. environmental quality in *Drosophila melanogaster* suggest multiple forms of condition dependence. *Ecology Letters*, 18(4), 317–326. https://doi.org/10.1111/ele.12412

Bonduriansky, R., & Rowe, L. (2005). Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution*, 59(1), 138–151. https://doi.org/10.1554/04-445

Cavigliasso, F., Dupuis, C., Savary, L., Spangenberg, J. E., & Kawecki, T. J. (2020). Experimental evolution of post-ingestive nutritional compensation in response to a nutrient-poor diet. *Proceedings of the Royal Society B: Biological Sciences*, 287, 20202684.

Chapman, T., & Partridge, L. (1996). Female fitness in *Drosophila melanogaster*: An interaction between the effect of nutrition and of encounter rate with males. *Proceedings of the Royal Society B: Biological Sciences*, 263, 755–759.

Chapman, T., Trevitt, S., & Partridge, L. (1994). Remating and male-derived nutrients in *Drosophila melanogaster* J. *Evolutionary Biology*, 7, 51–69.

Cohet, Y., & David, J. R. (1978). Control of the adult reproductive potential by preimaginal thermal conditions: A study in *Drosophila melanogaster*. *Oecologia*, 36, 295–306.

Cotton, S., Fowler, K., & Pomiankowski, A. (2004). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society B: Biological Sciences*, 271, 771–783.

Crudgington, H. S., Fellows, S., & Snook, R. R. (2010). Increased opportunity for sexual conflict promotes harmful males with elevated courtship frequencies. *Journal of Evolutionary Biology*, 23(2), 440–446. https://doi.org/10.1111/j.1420-9101.2009.01907.x

De Lisle, S. P., & Rowe, L. (2014). Interactive effects of competition and social environment on the expression of sexual dimorphism. *Journal of Evolutionary Biology*, 27(6), 1069–1077. https://doi.org/10.1111/jeb.12381

Debelle, A., Courtiol, A., Ritchie, M. G., & Snook, R. R. (2017). Mate choice intensifies motor signalling in *Drosophila*. *Animal Behaviour*, 133, 169–187. https://doi.org/10.1016/j.anbehav.2017.09.014

Delcourt, M., & Rundle, H. D. (2011). Condition dependence of a multicomponent sexual display trait in *Drosophila serrata*. *American Naturalist*, 177(6), 812–823. https://doi.org/10.1086/659949

Dore, A. A., Rostant, W. G., Bretman, A., & Chapman, T. (2021). Plastic male mating behavior evolves in response to the competitive environment. *Evolution*, 75, 101–115.

Duxbury, E. M. L., & Chapman, T. (2020). Sex-specific responses of life span and fitness to variation in developmental versus adult diets in *Drosophila melanogaster*. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 75, 1431–1438.

Edmunds, D. 2020. The function and evolutionary ecology of intra-sexual aggression in *Drosophila melanogaster*. DPhil thesis, *Zoology*, University of Oxford.

Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I., & Lavine, L. C. (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science*, 337(6096), 860–864. https://doi.org/10.1126/science.1224286

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science*, 197(4300), 215–223. https://doi.org/10.1126/science.327542

Fricke, C., Bretman, A., & Chapman, T. (2010). Female nutritional status determines the magnitude and sign of responses to a male ejaculate signal in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 23, 157–165.

Garland, T., & Kelly, S. A. (2006). Phenotypic plasticity and experimental evolution. *Journal of Experimental Biology*, 209(12), 2344–2361. https://doi.org/10.1242/jeb.02244

Gosden, T. P., & Chenoweth, S. F. (2011). On the evolution of heightened condition dependence of male sexual displays. *Journal of Evolutionary Biology*, 24(3), 685–692. https://doi.org/10.1111/j.1420-9101.2010.02205.x

Hill, G. E. (2011). Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecology Letters*, 14, 625–634.

Hillesheim, E., & Stearns, S. C. (1991). The responses of *Drosophila melanogaster* to artificial selection on body weight and its phenotypic plasticity in two larval food environments. *Evolution*, 45(8), 1909–1923. https://doi.org/10.2307/2409839

Hoglund, J., Sheldon, B. C., & Hoglund, J. (1998). The cost of reproduction and sexual selection. *Oikos*, 83(3), 478–483. https://doi.org/10.2307/3546675

Holland, B., & Rice, W. R. (1999). Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences*, 96(9), 5083–5088. https://doi.org/10.1073/pnas.96.9.5083

Hotzy, C., Fowler, E., Kiehl, B., Francis, R., Mason, J., Moxon, S., Rostant, W., Chapman, T., & Immler, S. (2022). Evolutionary history of sexual selection affects microRNA profiles in *Drosophila* sperm. *Evolution*, 76(2), 310–319. https://doi.org/10.1111/evo.14411

Houle, D. (1998). How should we explain variation in the genetic variance of traits? *Genetica*, 102, 241–253.

Innocenti, P., Flis, I., & Morrow, E. H. (2014). Female responses to experimental removal of sexual selection components in *Drosophila melanogaster*. *BMC Evolutionary Biology*, 14, 239.

Izzo, A., & Tibbetts, E. A. (2015). Heightened condition dependence of a sexually selected signal in male *Polistes dominulus* paper wasps. *Ethology*, 121(6), 586–592. https://doi.org/10.1111/eth.12371

Jagadeeshan, S., Haerty, W., Moglinicka, M., Ahuja, A., De Vito, S., & Singh, R. S. (2015). Evolutionary consequences of male driven sexual selection and sex-biased fitness modifications in *Drosophila melanogaster* and members of the *simulans* clade. *International Journal of Evolutionary Biology* 2015, 756269.

Janicke, T., David, P., & Chapuis, E. (2015). Environment-dependent sexual selection: Bateman's parameters under varying levels of food availability. *American Naturalist*, 185(6), 756–768. https://doi.org/10.1086/681128

Johnstone, R. A. (1995). Sexual selection, honest advertisement and the handicap principle: Reviewing the evidence. *Biological Review*, 70(1), 1–65. https://doi.org/10.1111/j.1469-185x.1995.tb01439.x

Johnstone, R. A., Rands, S. A., & Evans, M. R. (2009). Sexual selection and condition-dependence. *Journal of Evolutionary Biology*, 22(12), 2387–2394. https://doi.org/10.1111/j.1420-9101.2009.01822.x

Katsuki, M., Okada, Y., & Okada, K. (2012). Impacts of diet quality on life-history and reproductive traits in male and female armed beetle, *Gnatocerus cornutus*. *Ecological Entomology*, 37(6), 463–470. https://doi.org/10.1111/j.1365-2311.2012.01390.x

Kemp, D. J., & Rutowski, R. L. (2007). Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution*, 61(1), 168–183. https://doi.org/10.1111/j.1558-5646.2007.00014.x

Klepsatel, P., Gáliková, M., De Maio, N., Ricci, S., Schlötterer, C., & Flatt, T. (2013). Reproductive and post-reproductive life history of wild-caught *Drosophila melanogaster* under laboratory conditions. *Journal of Evolutionary Biology*, 26(7), 1508–1520. https://doi.org/10.1111/jeb.12155

Knell, R. J., & Simmons, L. W. (2010). Mating tactics determine patterns of condition dependence in a dimorphic horned beetle. *Proceedings of the Royal Society B: Biological Sciences*, 277, 2347–2353.

Kokko, H., & Monaghan, P. (2001). Predicting the direction of sexual selection. *Ecology Letters*, s, 159–165.

Kristensen, T. N., Overgaard, J., Loeschcke, V., & Mayntz, D. (2011). Dietary protein content affects evolution for body size, body fat and viability in *Drosophila melanogaster*. *Biology Letters*, 7, 269–272.

Kvarnemo, C., & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology and Evolution*, 11(10), 404–408. https://doi.org/10.1016/0169-5347(96)10056-2

Lawniczak, M. K., & Begun, D. J. (2005). A QTL analysis of female variation contributing to refractoriness and sperm competition in *Drosophila melanogaster*. *Genetical Research*, 86, 107–114.

Lin, W. S., Yeh, S. R., Fan, S. Z., Chen, L. Y., Yen, J. H., Fu, T. F., Wu, M. S., & Wang, P. Y. (2018). Insulin signaling in female *Drosophila* links diet and sexual attractiveness. *The FASEB Journal*, 32, 3870–3877.

Linklater, J. R., Wertheim, B., Wigby, S., & Chapman, T. (2007). Ejaculate depletion patterns evolve in response to experimental manipulation of sex ratio in *Drosophila melanogaster*. *Evolution*, 61(8), 2027–2034. https://doi.org/10.1111/j.1558-5646.2007.00157.x

Long, T. A. F., Pischedda, A., Stewart, A. D., & Rice, W. R. (2009). A cost of sexual attractiveness to high-fitness females. *PLoS Biology*, 7(12), e1000254. https://doi.org/10.1371/journal.pbio.1000254

Łopuch, S., & Radwan, J. (2009). Condition dependence of sexual attractiveness in the bank vole. *Behavioral Ecology and Sociobiology*, 63, 339–344.

Manning, A. (1960). The sexual behaviour of two sibling *Drosophila* species. *Behaviour*, 15(1-2), 123–145. https://doi.org/10.1163/156853960x00133

Markow, T. A., Beall, S., & Castrezana, S. (2012). The wild side of life: Drosophila reproduction in nature. *Fly*, 6, 981–101. https://doi.org/10.4161/fly.19552

Markow, T. A., & Ricker, J. P. (1992). Male size, developmental stability, and mating success in natural populations of three *Drosophila* species. *Heredity*, 69, 122–127.

McCracken, A. W., Adams, G., Hartshorne, L., Tatar, M., & Simons, M. J. P. (2020). The hidden costs of dietary restriction: Implications for its evolutionary and mechanistic origins. *Science Advances*, 6, eaay3047.

McGuigan, K. (2009). Condition dependence varies with mating success in male *Drosophila bunnanda*. *Journal of Evolutionary Biology*, 22, 1813–1825.

McGraw, L. A., Fiumera, A. C., Ramakrishnan, M., Madhavarapu, S., Clark, A. G., & Wolfner, M. F. (2007). Larval rearing environment affects several post-copulatory traits in *Drosophila melanogaster*. *Biology Letters*, 3(6), 607–610. https://doi.org/10.1098/rsbl.2007.0334

Miller, C. W., McDonald, G. C., & Moore, A. J. (2016). The tale of the shrinking weapon: Seasonal changes in nutrition affect weapon size and sexual dimorphism, but not contemporary evolution. *Journal of Evolutionary Biology*, 29(11), 2266–2275. https://doi.org/10.1111/jeb.12954

Millington, J. W., G. P. Brownrigg, C. Chao, Z. Sun, P. J. Basner-Collins, L. W. Wat, B. Hudry, I. Miguel-Aliaga, & E. J. Rideout. (2021). Female-biased upregulation of insulin pathway activity mediates the sex difference in *Drosophila* body size plasticity. *eLife Sciences*, 10, e58341.

Nandy, B., Gupta, V., Sen, S., Udaykumar, N., Samant, M. A., Ali, S. Z., & Prasad, N. G. (2013). Evolution of mate-harm, longevity and behaviour in male fruit flies subjected to different levels of interlocus conflict. *BMC Evolutionary Biology*, 13, 212.

Nandy, B., Gupta, V., Udaykumar, N., Samant, M. A., Sen, S., & Prasad, N. G. (2014). Experimental evolution of female traits under different levels of intersexual conflict in *Drosophila melanogaster*. *Evolution*, 68, 412–425.

Nur, N., & Hasson, O. (1984). Phenotypic plasticity and the handicap principle. *Journal of Theoretical Biology*, 110(2), 275–297. https://doi.org/10.1016/s0022-5193(84)80059-4

Partridge, L., & Farquhar, M. (1983). Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. *Animal Behaviour*, 31, 871–877.

Partridge, L., Hoffmann, A., & Jones, J. S. (1987). Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. *Animal Behaviour*, 35, 468–476.

Pavković-Lučić, S., & Kekić, V. (2011). Are larger and/or more symmetrical *Drosophila melanogaster* (Diptera, Drosophilidae) males more successful in matings in nature? *Revista Brasileira de Entomologia*, 55, 583–588.

Pavković-Lučić, S., Kekić, V., & Čvoro, A. (2009). Larger male mating advantage depends on the sex ratio in *Drosophila melanogaster*. *Ethology Ecology and Evolution*, 21(2), 155–160. https://doi.org/10.1080/08927014.2009.9522504

Perry, J. C., Joag, R., Hosken, D. J., Wedell, N., Radwan, J., & Wigby, S. (2016). Experimental evolution under hyper-promiscuity in *Drosophila melanogaster*. *BMC Evolutionary Biology*, 16, 131.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology and Evolution*, 20(9), 481–486. https://doi.org/10.1016/j.tree.2005.06.001

Pitchers, W., Pool, J. E., & Dworkin, I. (2013). Altitudinal clinal variation in wing size and shape in African *Drosophila melanogaster*: One cline or many? *Evolution*, 67, 438–452.

Reuter, M., Linklater, J. R., Lehmann, L., Chapman, T., Fowler, K., & Hurst, G. D. D. (2008). Adaptation to experimental alterations of the operational sex ratio in populations of *Drosophila melanogaster*. *Evolution*, 62, 401–412.

Rohlf, F. J. (2006). tpsDig, http://life.bio.sunysb.edu/morph/.

Rostant, W. G., Mason, J. S., Coriolis, J.-C. de, & Chapman, T. (2020). Resource-dependent evolution of female resistance responses to sexual conflict. *Evolution Letters*, 4, 54–64.

Rowe, L., & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society B: Biological Sciences*, 263, 1415–1421.

Sang, J. H. 1978. The nutritional requirements of *Drosophila*. In M. Ashburner, & T. R. F. Wright (Eds.), *The genetics and biology of Drosophila* (pp. 159–192). Academic Press.

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, 24, 35–68.

Sentinella, A. T., Crean, A. J., & Bonduriansky, R. (2013). Dietary protein mediates a trade-off between larval survival and the development of male secondary sexual traits. *Functional Ecology*, 27, 1134–1144.

Sepil, I., Perry, J. C., Dore, A., Chapman, T., & Wigby, S. (2022). Experimental evolution under varying sex ratio and nutrient availability modulates male mating success in *Drosophila melanogaster*. *Biology Letters*, 18, 20210652.

Sheets, H. D. (2001a). IMP CoordGen. http://www3.canisius.edu/~sheets/morphsoft.html.

Sheets, H. D. (2001b) IMP CVAGen. http://www3.canisius.edu/~sheets/morphsoft.html.

Shingleton, A. W., Masandika, J. R., Thorsen, L. S., Zhu, Y., & Mirth, C. K. (2017). The sex-specific effects of diet quality versus quantity on morphology in *Drosophila melanogaster*. *Royal Society Open Science*, 4(9), 170375. https://doi.org/10.1098/rsos.170375

Snijders, T. A. B. and R. J. Bosker. (2012). *Multilevel analysis: An introduction to basic and advanced multilevel modeling*. Sage Publishers.

Snook, R. R., Robertson, A., Crudgington, H. S., & Ritchie, M. G. (2005). Experimental manipulation of sexual selection and the evolution of courtship song in *Drosophila pseudoobscura*. *Behavior Genetics*, 35(3), 245–255. https://doi.org/10.1007/s10519-005-3217-0

Stångberg, J., Immonen, E., Moreno, P. P., & Bolund, E. (2020). Experimentally induced intrasexual mating competition and sex-specific evolution in female and male nematodes. *Journal of Evolutionary Biology*, 33(12), 1677–1688. https://doi.org/10.1111/jeb.13706

Trajković, J., Pavković-Lučić, S., Miličić, D., & Savić, T. (2021). Different diets can affect attractiveness of *Drosophila melanogaster* males via changes in wing morphology. *Animal Behaviour*, 171, 51–62.

Turiegano, E., Monedero, I., Pita, M., Torroja, L., & Canal, I. (2013). Effect of *Drosophila melanogaster* female size on male mating success. *Journal of Insect Behavior*, 26, 89–100.

Venkitachalam, S., Das, S., Deep, A., & Joshi, A. (2022). Density-dependent selection in *Drosophila*: Evolution of egg size and hatching time. *Journal of Genetics*, 101, 13.

Via, S. (1993). Adaptive phenotypic plasticity: Target or by-product of selection in a variable environment? *American Naturalist*, 142(2), 352–365. https://doi.org/10.1086/285542

Warbrick-Smith, J., Behmer, S. T., Lee, K. P., Raubenheimer, D., & Simpson, S. J. (2006). Evolving resistance to obesity in an insect. *Proceedings of the National Academy of Sciences*, 103(38), 14045–14049. https://doi.org/10.1073/pnas.0605225103

Wayne, M. L., Soundararajan, U., & Harshman, L. (2006). Environmental stress and reproduction in *Drosophila melanogaster*: Starvation resistance, ovariole numbers and early age egg production. *BMC Evolutionary Biology*, 6, 57.

Wensing, K. U., Koppik, M., & Fricke, C. (2017). Precopulatory but not postcopulatory male reproductive traits diverge in response to mating system manipulation in *Drosophila melanogaster*. *Ecology and Evolution*, 7(23), 10361–10378. https://doi.org/10.1002/ece3.3542

Wigby, S., & Chapman, T. W. (2004). Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution*, 58, 1028–1037.

Wigby, S., Perry, J. C., Kim, Y. -H., & Sirot, L. K. (2016). Developmental environment mediates male seminal protein investment in *Drosophila melanogaster*. *Functional Ecology*, 30, 410–419.

Zelditch, M. L., Swiderski, D. L., & Sheets, H. D. (2012). *Geometric morphometrics for biologists*. Elsevier Academic Press.

Zikovitz, A. E., & Agrawal, A. F. (2013). The condition dependency of fitness in males and females: The fitness consequences of juvenile diet assessed in environments differing in key adult resources. *Evolution*, 67, 2849–2860.