Diet quality impairs male and female reproductive performance and affects the opportunity for selection in an insect model

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Abstract

Environmental factors can have profound effects on the strength and direction of selection and recent studies suggest that such environment-dependent selection can be sex-specific. Sexual selection theory predicts that male fitness is more condition dependent compared to female fitness, suggesting that male fitness is more sensitive to environmental stress. However, our knowledge about the effect of environmental factors on sex-specific reproductive performance and on sex differences in the opportunity for selection is still very limited. In the present study, we investigated the sex-specific effects of diet quality (yeast deprivation and flour type) in the red flour beetle Tribolium castaneum. Specifically, we manipulated yeast supplementation in wheat and whole-wheat flour in competition assays allowing us to test for sex-specific effects of food quality (i) on reproductive success and (ii) on the opportunity for selection. Our data show that yeast deprivation in wheat flour had significantly negative effects on body mass and reproductive success of both sexes, while high-quality flour (whole-wheat flour) was able to buffer the negative impact to a large extent. Importantly, our data suggest no sex-specific effect of dietary stress on reproductive success because the magnitude of the negative effect of yeast deprivation was similar for males and females. Moreover, our study demonstrates that low food quality inflated the opportunity for selection and did not differ between sexes neither under benign nor stressful dietary conditions. We discuss the implications of our findings for the adaptation to stressful environments.

KEYWORDS
condition dependence, dietary restriction, environmental stress, opportunity for selection, sex-specific selection, sexual selection

TAXONOMY CLASSIFICATION
Applied ecology, Evolutionary ecology
1 | INTRODUCTION

Environmental factors have a profound impact on the demography of populations. They affect reproductive performance and can alter the strength and direction of selection (Riegel et al., 2017; Willi & Hoffmann, 2009). In general, stressful conditions are predicted to lower a population’s growth (Riegel et al., 2017; Sommer et al., 2016; Willi & Hoffmann, 2009) and may impose stronger selection on deleterious alleles (Cally et al., 2019; Martinossi-Allibert et al., 2019; Whitlock & Agrawal, 2009). Importantly, these effects can differ between males and females as a consequence of sex-specific life-history strategies (Berger et al., 2016; Janicke et al., 2015; Martinossi-Allibert et al., 2017, 2019; Moiron et al., 2022). The postulated ‘live-fast-die-young’ strategy of males implies that males invest more in current reproduction whereas females allocate more resources into future reproduction (Bonduriansky et al., 2008; Vinogradov, 1998; but see: Travers et al., 2015). Hence, females are expected to live longer (Viña et al., 2005), have a better immune competence (Kelly et al., 2018) and may also be more resilient when facing environmental stress. Moreover, environmental conditions often change the pool of resources that can be allocated to different fitness routes, also called conditions (Rowe & Houle, 1996). Sexual selection theory predicts that male reproductive performance is particularly condition-dependent so that an unfavorable environment may have a stronger negative impact on males (Whitlock & Agrawal, 2009; Winkler et al., 2021). Despite the outlined theoretical framework predicting pervasive sex differences in stress response, the empirical evidence for sex-specific effects of environmental factors is limited (but see e.g., Duxbury & Chapman, 2020; Reddiex et al., 2013).

Diet quality can severely influence an individual’s reproductive success (Brooker et al., 2013; Duxbury & Chapman, 2020; Eldridge & Krapu, 1988; García-González et al., 2016; Geister et al., 2008; Katsuki et al., 2012; Naya et al., 2007). However, evidence for sex-specific effects of diet quality is scarce. In the beetle Gnatocerus cornutus, diet quality has been shown to have sex-specific fitness effects, with a stronger impact on males through larval nutritional environment and on females by adult diet (Katsuki et al., 2012). Moreover, in Drosophila melanogaster females benefited more than males from a high-quality diet (Duxbury & Chapman, 2020), and also the black field cricket (Teleogryllus commodus) was found to show sex-specific diet optima (Maklakov et al., 2008).

In the present study, we explore the effect of diet quality on the sex-specific strength of selection in the red flour beetle Tribolium castaneum. To this end, we manipulated the diet by removing the baker’s yeast (Saccharomyces cerevisiae) supplement from the flour mixture that forms the habitat of T. castaneum. Yeast supplementation could be important for red-flour beetles, as it adds proteins (Schmidt et al., 1956) and nutritive supplements (James et al., 1987) to their diet. In addition, baker’s yeast contains immunostimulating compounds (e.g., β-glucans and nucleic acids) (Abdel-Tawwab et al., 2008; Siwicki et al., 1994) that have been shown to increase fitness in several fish species (Abdel-Tawwab et al., 2008; Ortuño et al., 2002; Sakai et al., 2001; Siwicki et al., 1994). Furthermore, lipids contained in a yeast-enriched diet, have been found to influence membrane lipid composition and fitness in D. melanogaster (Brankatschk et al., 2014, 2018; Guo & Reinhardt, 2020). This is in line with previous studies in T. castaneum indicating that baker’s yeast supplementation leads to shorter development time and increased productivity of populations (Sokoloff et al., 1966). Nevertheless, the effect of yeast availability on individual fitness and more importantly sex-specific selection is unknown in T. castaneum. A previous study manipulated yeast availability for T. castaneum males and found that dietary restriction reduced spermatogenesis and testes size investment (Godwin et al., 2017). These findings suggest that yeast deprivation likely has a strong effect on male reproductive success.

Using two identical fitness assays, we studied the effect of yeast availability in both wheat flour and whole-wheat flour on male and female reproductive success. While wheat flour is typically the standard laboratory food, whole-wheat seems to be beneficial for development time (Chapman, 1924; Sokoloff et al., 1966; Wong & Lee, 2011) and overall fitness of Tribolium (Good, 1933; Park, 1934; Sokoloff et al., 1966; Wong & Lee, 2011), although these early studies focused on T. confusum and not T. castaneum (but see: Sokoloff et al., 1966; Wong & Lee, 2011). This could be due to the additional vitamins (i.e. vitamin E and folate), minerals and higher sugar content in whole-wheat flour compared to wheat flour (Kumar et al., 2011; Likes et al., 2007). In the present study, we tested if the benefits of whole-wheat flour can mitigate the negative impact of the removal of yeast from the diet.

We first evaluated the sex-specific effects of diet quality on body mass as an indicator of condition. Secondly, we assessed the effect of diet on reproductive success using an assay in which stressed individuals are challenged by unstressed competitors. And finally, we estimated the sex-specific opportunity for selection (measured as the variance in reproductive success [Crow, 1958; Wade & Shuster, 2005]) in populations of stressed and unstressed individuals. We hypothesize that diet has a stronger effect on reproductive success and on the opportunity for selection in males, due to the predicted higher condition dependence of male fitness (Janicke et al., 2016; Whitlock & Agrawal, 2009).

2 | MATERIALS AND METHODS

Stock cultures and experimental cultures were kept in incubators at 30°C (±1°C) at 50% humidity (±5%) without light. Experimental procedures were performed at room temperature. All flour had been frozen for sterilization prior to experiments at −30°C for at least 24 h. The Ga1 (wildtype line) and Rd (‘reindeer’ mutant line) used in this experiment had been originally supplied by the U.S. Department of Agriculture and kept in the laboratory for over a year in standard culturing conditions. Rd is a dominant mutation that affects antenna morphology and was used as a paternity marker. All stock beetles were kept in a flour mixture that consisted of organic wheat flour (type 405, Alnatura, Darmstadt, Germany) and 5% dry baker’s yeast.
In this study we manipulated the diet in two ways: First, we manipulated yeast availability in standard, organic wheat flour (type 405, Alnatura, Darmstadt, Germany), with either control (5% yeast) or no yeast. Secondly, we manipulated yeast availability in organic whole-wheat flour (Alnatura, Darmstadt, Germany), with either control (5% yeast), low yeast availability (1% yeast) or no yeast. The low yeast availability treatment was with 1% yeast similar to a previous study (Godwin et al., 2017) but can still be considered arbitrary and results might be sensitive to the chosen range.

2.1 | Impact of food quality on reproductive performance

In the first assay, we manipulated the diet quality (i.e. yeast availability) of all focal individuals to investigate the effect of dietary stress on reproductive success of males and females in a setup allowing for intra-sexual competition.

We set up base cultures of 80 individuals of Ga1 (wildtype) and Rd adults in each of the treatments. Stock adults were left to mate and oviposit for 3 days in 60g of flour according to treatment, afterwards the adults were discarded and the eggs were left to hatch. 31 days after the base cultures had been set up, we collected and sexed pupae and established sex-separated groups of 40 individuals. 8 days after the last pupae had been sexed, all pupae had emerged and were fully adult. We then set up the mating groups for the experiment.

Groups contained the focal individual (female or male) and a competitor (Rd), as well as two mating partners. All mating group beetles were synchronized in age (±3 days) and competitors as well as mating partners were always raised on the control diet. All wildtype mating partners were marked with a dot of Revell emaillle paint, to differentiate them from the focal. We allowed the treatment groups to mate undisturbed for 3 days in empty arenas (plastic Petri dishes, diameter 3.3 cm). All mating trial arenas were scratched at the bottom to increase traction for the beetles and contained no flour to prevent oviposition. After the mating trial, we froze the males at −30°C for preservation to take phenotypic measurements (see below), while we separated the females to oviposit for 2 weeks in the control environment. In addition, to investigate if females recovered from the treatment when returned to the control diet (i.e. 5% yeast) to lay eggs, we transferred all yeast-deprived females from the whole-wheat flour treatment after 1 week to a new egg-laying vial (Appendix 1: Figure A1). After 2 weeks, females were removed and frozen at −30°C until further processing. Once all offspring reached the adult stage after 50 days, they were frozen and scored for genotype (wildtype or Rd; with Rd phenotype clearly discernible from the wildtype due to shorter and thicker antennae).

All frozen individuals from the mating groups were weighed to the nearest 0.01 mg on a Sartorius R200D balance (Göttingen, Germany) within 2 months after the main experiment. Body mass was used as a proxy for condition (Rowe & Houle, 1996). The repeatability of body mass, estimated by measuring twice a subset of the samples was high (Intra-class correlation coefficient, ICC = 0.937, p < .001, N = 96).

2.2 | Impact of food quality on the opportunity for selection

In the second assay, we manipulated the diet quality (i.e. yeast availability) of all individuals (i.e. focal, competitors and mating partners) during development. Hence we mimicked population level dietary manipulation, aiming to measure the opportunity for selection in males and females separately. After the mating trials, all females were given the opportunity to lay eggs on their respective rearing diet (control or yeast deprived). Apart from this, the experimental design followed the procedures described above and was performed in parallel with the first assay.

2.3 | Statistical analyses

We performed all analyses in R version 4.0.4 (R Core Team, 2018) and used ‘ggplot2’ for creating all figures (Wickham, 2016). In a first series of analyses, we tested for an effect of yeast availability on body mass and reproductive performance. Specifically, we used general linear models (GLMs) with yeast availability defined as the only fixed effect and the body mass (family: Gaussian) or the number of offspring (family: quasi-Poisson) as response variables. These models were done separately for males and females. For males, we also tested the effect of yeast availability on the genetic mating success (i.e. the number of fertilized females) using GLMs (family: quasi-Poisson). To test for sex differences, we computed selection coefficients (s) as:

\[ s = \left( \frac{W_{\text{control}} - W_{\text{stressed}}}{W_{\text{control}}} \right) = 1 - \left( \frac{W_{\text{stressed}}}{W_{\text{control}}} \right) \]

With W representing the reproductive success (i.e. offspring number) of control or stressed individuals. Thus, s represents a standardized measure for the strength of selection against stressed individuals (Janicke & Chapuis, 2016; Zikovitz & Agrawal, 2013). We used bootstrapping and permutation tests (10,000 permutations) to compare s between sexes using the boot package in R (Canty & Ripley, 2019).

In a second series of analyses, we tested the effect of yeast availability on the opportunity for selection, which is defined as the variance in relativized reproductive success (i.e. observed values divided by the group mean) and serves as a proxy for the total net selection (Winkler et al., 2021) in a population (Crow, 1958; Moorad & Wade, 2013; Wade & Shuster, 2005). Similar to the analysis of s, we applied bootstrapping to estimate I and its 95% confidence intervals (10,000 bootstrap samples) using the boot package in R (Canty & Ripley, 2019). Finally, we decomposed the opportunity for selection of male reproductive success into the variance of the mating success...
MS; number of females that produced offspring by the focal male), partner’s fecundity (Fec; average number of offspring produced by female partners), paternity share (PS; the proportion of offspring sired per female partner) and each of their covariances. Specifically, we modeled male reproductive success as the product of the genetic mating success, the partner’s fecundity, and the paternity share of the focal male (Janicke et al., 2015). To estimate the variance components (with 95% CI) we used bootstrapping (Canty & Ripley, 2019) with 10,000 bootstrap replicates.

3 | RESULTS

Overall, we observed that development time was considerably longer if yeast was removed from the diet. We did not follow developmental time systematically for all treatments but we collected pupae after 31 and 27 days in the control diet (wheat and whole-wheat, respectively) and this shifted to 48 and 31 days when yeast was removed from the diet. There were no large differences in development time between 5% and 1% yeast in whole-wheat flour, with the collection of pupae starting after about 29 days in both treatments. In addition, we tracked the survival of females during 2 weeks after eclosion and observed strongly increased mortality when yeast was constantly removed from the diet. In particular, 19% of females died before the end of the egg-laying period when no yeast was available in wheat flour, which represents a significantly higher mortality compared to 4% of control females that died at the same time (Pearson’s Chi-squared test with Yates’ continuity correction: df = 127, χ² = 12.77, p-value < .001). In contrast, in whole-wheat flour only 0% (control), 0.5% (1% yeast) and 1.1% (no yeast) of females died during the egg laying period (Pearson’s Chi-squared test: df = 281, χ² = 1.80, p-value = .407).

3.1 | Yeast availability influenced body mass only in wheat flour

Since our food quality treatment might have affected net energy intake and/or vitamin availability, we measured body mass as a proxy for the condition of focal individuals. For these analyses, we combined data from both assays of the experiment (see Section 2) because the treatment of the focal individual was identical. There was a decrease in body mass in wheat flour when yeast was removed (Figure 1a and Table 1). This effect was significantly larger in females compared to males (GLM on relativized data with sex by treatment interaction: df = 181, t = 2.05, p-value = .041). Specifically, in females the mean, proportional reduction in body mass was 8.98%, while in males body mass was only reduced by 4.08% compared to the control. By contrast, yeast availability in whole-wheat did not influence body mass (Figure 1b and Table 1).

3.2 | Yeast deprivation impacts reproductive success only in wheat flour

In the first assay, we only subjected the focal individual to the treatment while potential competitors and partners were exposed to control food. This enabled us to measure the effect of the treatment on the reproductive success of focal individuals under intra-sexual competition. There was a significant reduction in the absolute number of offspring produced by females and males in wheat flour if the yeast was removed from their diet (Figure 2a and Table 2). This effect was not significantly different between the sexes (GLM with sex by treatment interaction: df = 88, t < 0.01, p-value = .998). In contrast, there was no significant reduction in the offspring number of both sexes in whole-wheat flour if yeast was reduced or removed from the diet (Figure 2b and Table 2).
In agreement with the finding that the treatment had a limited influence on the body mass of focal individuals, we also found that body mass did not explain the observed fitness effects of the treatment (Table A1).

### 3.3 | No change in egg laying over time

In order to provide a more detailed test on the effect of food quality on female reproductive performance, we examined if egg laying changed over the course of 2 weeks after the mating trials. For this, we transferred females to a fresh vial after the first week of egg laying in the yeast availability experiment to whole-wheat. A decrease in female reproductive success in the second week of egg laying would indicate that sperm or egg limitation plays a role in female reproductive success. In contrast, an increase in egg laying in week two would indicate that females recovered gradually from negative effects of the previous low-quality diet. There were no differences in female reproductive success between the first and the second week (Figure A1 and Table A2).

### 3.4 | No sex-specific selection against yeast-deprived individuals

When examining the selection coefficients (a standardized measure for the strength of selection against stressed individuals), there we observed significant selection against yeast-deprived individuals in wheat flour (Figure 3 and Table 3) but no differences in the strength of selection between males and females (Table 3).
For males, we were able to estimate the genetic mating success (number of fertilized females) as a fitness component contributing to the measured reproductive success. In the assay in which only the focal was treated, yeast deprivation had a negative effect on the genetic mating success of males in wheat flour (Figure A2a,b and Table 4). Nevertheless, there was no difference in the genetic mating success of males in whole-wheat (Figure A2c–e and Table 4).

### 3.6 | Sex-specific effects of diet on the opportunity for selection

One main objective of our study was to test if a low-quality diet (i.e. no yeast supplementation) affected the opportunity for selection (I) in *T. castaneum* in a sex-specific manner. Therefore, we manipulated the diet quality of all individuals (i.e. focal, potential mating partners and potential competitors) of the mating groups in a second experimental assay to mimic selection in a population under dietary stress.
We measured $I$ as the variance in relative reproductive success of the focal. We found that in this assay, there was a significant effect of the diet treatment on reproductive success of both sexes in wheat as well as whole-wheat flour (Figure A3 and Table A3). Importantly, we found that $I_{\text{Females}}$ was higher when yeast was removed from the diet (Figure 4a and Table 5). We also detected an increase in $I_{\text{Males}}$ but this effect was less pronounced and statistically non-significant (Figure 4a and Table 5). Likewise, in whole-wheat flour the absence of yeast increased $I_{\text{Females}}$ compared to the control, but $I_{\text{Males}}$ only increased slightly (Figure 4b and Table 5). The reduction of yeast to 1% did not increase the opportunity for selection in either of the sexes compared to the control. Finally, there was only a non-significant trend towards larger $I_{\text{Females}}$ compared to $I_{\text{Males}}$ in wheat and whole-wheat when yeast was removed from the diet (Table A4).

### 3.7 Variance decomposition for focal males

To better understand the observed effects of food quality on the opportunity for selection in males, we partitioned male reproductive success into three main fitness components: genetic mating success, paternity share and partner’s fecundity. We found that the variance in mating success was higher in the no-yeast treatment in wheat-flour compared to the control (Figure 5a and Table A5), though the

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**TABLE 4** Results of general linear models (quasi-Poisson) testing for an effect of food quality on the genetic mating success of focal males including ANOVA results and post-hoc tests.

| Flour type   | Treatment effect | Post-hoc comparisons |
|--------------|------------------|----------------------|
|              | df   | f     | p-Value | Contrast          | Estimate | SE  | t value | Adj. p-Value |
| Wheat        | 41   | 6.79  | .013    | Control - No yeast| -0.35    | .14 | -2.52   | .016          |
| Whole-wheat  | 102  | 2.90  | .059    | Control - 1% yeast| -0.10    | .10 | -0.95   | .342          |
|              |      |       |         | Control - No yeast| -0.25    | .11 | -2.34   | .064          |
|              |      |       |         | 1% yeast - No yeast| -0.15    | .09 | -1.58   | .174          |

*Note: Assay in which only the focal subjected to treatment. p-values adjusted for false discovery rate (Benjamini & Hochberg, 1995).*

**FIGURE 4** Effect of yeast availability on the opportunity for selection ($I$) estimated as the variance of relativized number of offspring by the focal males and females in wheat flour (a) and in whole-wheat flour (b). Assay for measuring the opportunity for selection, with all individuals in the group subject to treatment. Bars show means and 95% CI of $I$ obtained from bootstrapping.

**TABLE 5** Opportunity for selection ($I$) estimated as the variance of relativized number of offspring estimated via bootstrapping.

| Flour type   | Sex | Treatment | $I$ (95%CI)  |
|--------------|-----|-----------|--------------|
| Wheat        | Male| Control   | 0.197 (0.124, 0.277) |
|              |     | No yeast  | 0.517 (0.215, 0.869) |
|              | Female| Control | 0.207 (0.113, 0.296) |
|              |     | No yeast  | 0.961 (0.542, 1.353) |
| Whole-wheat  | Male| Control   | 0.308 (0.208, 0.421) |
|              |     | 1% yeast  | 0.317 (0.223, 0.418) |
|              |     | No yeast  | 0.451 (0.298, 0.623) |
|              | Female| Control | 0.200 (0.103, 0.296) |
|              |     | 1% yeast  | 0.310 (0.193, 0.417) |
|              |     | No yeast  | 0.671 (0.481, 0.883) |

*Note: Assay for measuring the opportunity for selection, with all individuals subject to treatment.*
95% CI overlaps slightly. There was also a trend for a larger variance in partner’s fecundity, while there was no difference in the variance in paternity share. In whole-wheat, the effects of yeast availability on the variances in mating success and partner’s fecundity were smaller but showed the same direction as observed in wheat flour (Figure Sb and Table A5).

3.8 | Lower body mass and poor fitness of Rd males

Finally, we aimed to evaluate the competitiveness of the used Rd mutant strain in both assays of the experiment by comparing the body mass and reproductive success of Rd competitors with the wildtype focal. Overall, we found Rd of both sexes to have lower body mass compared to wildtype (Figure A4 and Tables A6 and A7). Furthermore, Rd males had significantly fewer offspring compared to wildtype males (65% less; Figure A4 and Table A6). In contrast, there was no difference in offspring numbers of Rd females compared to wildtype females (Figure A4 and Table A7).

4 | DISCUSSION

This study demonstrates that low food quality impairs the reproductive performance of both sexes and affected the opportunity for selection in the red flour beetle Tribolium castaneum. More specifically, our data reveal four main findings: (i) body mass, female survival, male mating success and offspring production were negatively impacted by removing yeast from a wheat diet, (ii) the magnitude of the effect of poor diet on offspring production was similar for males and females, (iii) the negative effects of yeast deprivation observed on wheat diet were buffered or undetectable on a whole-wheat flour diet, and (iv) a poor diet quality inflated the opportunity for selection primarily for females.

4.1 | Negative impact of yeast deprivation only in wheat flour

Yeast deprivation reduced body mass significantly on a wheat but not on a whole-wheat diet. This effect was greater in females compared to males suggesting that yeast had a stronger effect on female condition. The absence of an effect of yeast deprivation in whole-wheat indicates that whole-wheat mitigates the negative impact of yeast reduction in both sexes. Interestingly, the reduction in body mass did not explain the observed effect of diet on reproductive success in both sexes (see below), which is in line with a previous study reporting no effect of lowered body mass on fitness in T. castaneum cultured on a low-quality diet using a non-nutritional filler (Plesnar-Bielak et al., 2017). We note that the present study might underestimate the influence of yeast deprivation on body mass because rearing density was presumably not balanced across treatments. In particular, we expect that density during development was lower for the yeast-deprived focal individuals due to a reduced hatching success and/or survival of larvae and pupae. This could have led to an underestimation of the effect of the yeast deprivation, as high density might negatively impact body mass in insects (Morimoto et al., 2016; Than et al., 2020).

Furthermore, we observed that yeast availability affected development time and had a strong impact on the mortality of focal females during the experiment, but only in wheat and not in whole-wheat flour. However, we only estimated mortality for females over a two-week period, which is relatively short given an average reproductive lifespan of 0.5–1 year (Fedina & Lewis, 2008) and a total lifespan of up to 4 years (Pointer et al., 2021). In addition, our findings on development time are rather exploratory since we only measured the time needed for cultures to yield a sufficient number of pupae for sexing rather than tracking individual development. Nevertheless, previous studies on effects of yeast-deprived diet in Tribolium also reported a significant increase in development time (Sokoloff et al., 1966). These findings on development, survival and body mass suggest that our experimental manipulation of yeast availability affected the pool of resources (i.e. condition) of focal males and females in the wheat diet treatment but not necessarily in the whole-wheat diet. However, condition itself is difficult to measure and certainly not fully captured by body mass (Barnett et al., 2015; Wilder et al., 2016). Moreover, diet manipulation may have sex-specific effects that are independent of condition. For these reasons, we stress that our findings on the effect of diet on reproductive performance should not be interpreted solely in the context of condition-dependence. The main focus of our study was to explore the causal link between dietary stress and reproductive performance, which can, but does not have to be mediated by the effects of diet on condition.

Most importantly, we found a negative impact of yeast deprivation on male and female reproductive success in wheat flour. For males, this is in line with the previous finding that investment into spermatogenesis and testes mass was reduced under yeast deprivation in wheat flour (Godwin et al., 2017). In contrast, we did not detect statistically significant effects of yeast availability in whole-wheat flour indicating that the higher nutritional quality of whole-wheat flour (Kumar et al., 2011; Likes et al., 2007) buffered the negative effects of removing baker’s yeast from the diet of T. castaneum. Opposite to our prediction of higher condition-dependence of male reproductive success as a consequence of male-biased sexual selection, our results suggest that condition had a similar effect on reproductive success in both sexes. Remarkably, male genetic mating success (i.e., number of successfully inseminated females) was lower in yeast-deprived individuals that grew on wheat flour. Therefore, the observed effect of yeast availability on male reproductive success might, at least partially, be driven by a lowered competitiveness at pre-copulatory episodes of sexual selection.

In the context of what is known from other model systems, our findings support the idea that sex-specific effects of dietary stress on reproductive success can vary across species. In the fruit fly Drosophila melanogaster, low food quality provided during development had a
Figures 5 Effects of yeast availability on the variance of relativized male mating success (MS), partner's fecundity (Fec), paternity share (PS) and the respective covariances in wheat flour (a) and in whole-wheat flour (b). Analysis based on experimental assay in which all individuals have been subjected to the food treatment. Means of bootstrapped variances and 95% CIs are shown.

Stronger negative effect on reproductive success in males compared to females (Zikovitz & Agrawal, 2013). By contrast, a study on the freshwater snail Physa acuta suggests that dietary stress impairs primarily female reproductive success (Janicke & Chapuis, 2016), though this finding might be a consequence of condition-dependent sex allocation, which is restricted to simultaneous hermaphrodites. The observed absence of a sex-specific-effect of food quality on reproductive success in T. castaneum may have at least three reasons. First, the alleged sex difference in the strength of sexual selection might actually be too small to generate sex-specific stress responses. Several lines of empirical work suggest that sexual selection is stronger in males and operates primarily along post-copulatory episodes (Fedina & Lewis, 2008). However, a formal comparison of the standardized metrics (e.g., the Bateman gradient or Jones index (Jones, 2009)) for the strength of sexual selection between males and females is still lacking for T. castaneum. Second, yeast availability may not only affect the individual's condition in males and females but may also contain compounds/elements that are more critical for egg production rather than for sperm production or any other sexually selected trait of males. Hence, female reproduction might be more sensitive to yeast deprivation despite higher condition-dependence of male reproductive success. Nevertheless, a previous study manipulating yeast availability in wheat flour in T. castaneum males found an effect on spermatogenesis and testes investment, suggesting a marked effect on male reproductive performance (Godwin et al., 2017). Third, the absence of sex-specific stress response may also reflect the methodological limitations of our experimental setup. Specifically, body mass of both sexes and male reproductive success of the Rd mutant strain (which served as competitors during mating trials) was considerably lower compared to wildtype individuals, which has already been found in previous studies (Godwin et al., 2018; Sbilordo & Martin, 2014). Hence, competition for mates and/or their gametes might have been low for males, which may translate into an underestimation of condition-dependence of male reproductive performance. In addition, we only measured reproductive success over an egg-laying period of 2 weeks, therefore we did not capture possible effects of diet quality on reproductive senescence. Further work on the effect of food availability is clearly needed to fully understand the effect of dietary stress on male and female reproductive success in Tribolium beetles. This may include experimental setups that apply alternative methods to manipulate conditions (e.g., food quantity rather than quality) and studies testing explicitly the effect of pre- and post-copulatory competition on diet-induced stress responses in males and females. Moreover, the apparent inter-specific variation in sex-specific stress responses and its evolutionary link to differences in the strength of sexual selection on males and/or females across species constitutes a promising avenue to follow using comparative approaches.

4.2 Yeast availability inflates the opportunity for selection

The main objective of the second mating assay (i.e. all individuals where subjected to the treatment) was to test for an effect of food quality on the opportunity of selection (I) and whether this effect differs between males and females. As predicted, a reduction in food quality increased I in both sexes though this effect was statistically significant only in females under both wheat flour and whole-wheat flour. This result is in line with the prediction that environmental stress may unmask cryptic genetic variation and therefore inflate I (Paaby & Rockman, 2014). Similar to our findings, food stress has been found to increase I of both sex functions in the freshwater snail P. acuta (Janicke et al., 2022). Interestingly, our variance decomposition of male reproductive success suggests that the increase of I in yeast-deprived populations can partly be attributed to a higher variance in mating success (also called the ‘opportunity for sexual selection’). This suggests that pre-copulatory sexual selection in terms of male–male competition is higher in yeast-deprived populations of T. castaneum.
Sexual selection theory predicts that stronger sexual selection on males manifests in a higher $I_{\text{Males}}$ (Bateman, 1948), which is well supported across a broad range of animal taxa (Janicke et al., 2016; Winkler et al., 2021). Our data do not support this hypothesis as we did not detect consistent sex differences in $I$ across the tested food treatments. On the assumption that phenotypic variance in reproductive success translates into genetic variance in fitness, this finding may have implications for the adaptation to stressful food conditions. This is because populations are expected to purge deleterious alleles at a low demographic cost only if net selection (measured in terms of genetic variance in fitness but often approximated by estimating $I$) is stronger on males (Whitlock & Agrawal, 2009). Our data do not indicate stronger net selection on males and hence, under the tested experimental conditions, sexual selection would not be expected to purge deleterious alleles at low demographic costs. However, as mentioned before, male–male competition was presumably limited in our experimental setup, which may have restricted $I_{\text{Males}}$ to be low and the sex difference in net selection might be more male-biased under conditions allowing for more intense male–male competition.

5 | CONCLUSION

Overall, our study demonstrates that yeast restriction can impose major fitness costs on male and female T. castaneum beetles, which can be partially buffered by higher nutritional quality of whole-wheat compared to wheat flour. Moreover, a lowered diet quality leads to an increased opportunity for selection especially in females, suggesting high demographic costs for the adaptation to environments of poor diet quality.

AUTHOR CONTRIBUTIONS

Lennart Winkler: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); resources (equal); software (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). Tim Janicke: Conceptualization (equal); formal analysis (supporting); funding acquisition (lead); methodology (equal); project administration (lead); resources (equal); supervision (lead); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data analyzed in this study are uploaded to the Dryad data repository (https://doi.org/10.5061/dryad.05qftf6h; Winkler & Janicke, 2022).

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APPENDIX 1

Influence of body mass on the effect of treatment on fitness

**TABLE A1** ANOVA of general linear models (GLMs) of quasi-Poisson family modeling the influence of yeast availability in wheat and whole-wheat flour on offspring number of focal when including body mass.

| Design                        | Flour type     | Sex | Coefficient                  | df  | F-Value | p-Value |
|-------------------------------|----------------|-----|------------------------------|-----|---------|---------|
| Only focal subject to treatment | Wheat flour    | Male | Treatment                    | 39  | 76.57   | <.001   |
|                               |                |     | Focal body mass [mg]         | 0.01| .917    |         |
|                               |                |     | Treatment × body mass        | 0.36| .552    |         |
|                               |                | Female | Treatment                    | 44  | 63.78   | <.001   |
|                               |                |     | Focal body mass [mg]         | 0.19| .667    |         |
|                               |                |     | Treatment × body mass        | <0.01| .984    |         |
| All animals subject to treatment | Wheat flour    | Male | Treatment                    | 38  | 68.20   | <.001   |
|                               |                |     | Focal body mass [mg]         | 0.01| .917    |         |
|                               |                |     | Treatment × body mass        | 0.32| .574    |         |
|                               |                | Female | Treatment                    | 48  | 93.46   | <.001   |
|                               |                |     | Focal body mass [mg]         | 0.22| .638    |         |
|                               |                |     | Treatment × body mass        | <0.01| .959    |         |
| Only focal subject to treatment | Whole-wheat flour | Male | Treatment                    | 98  | 1.19    | .310    |
|                               |                |     | Focal body mass [mg]         | 0.71| .403    |         |
|                               |                |     | Treatment × body mass        | 1.04| .355    |         |
|                               |                | Female | Treatment                    | 103 | 1.07    | .347    |
|                               |                |     | Focal body mass [mg]         | 2.56| .112    |         |
|                               |                |     | Treatment × body mass        | 4.92| .009    |         |
| All animals subject to treatment | Whole-wheat flour | Male | Treatment                    | 125 | 44.90   | <.001   |
|                               |                |     | Focal body mass [mg]         | 1.84| .177    |         |
|                               |                |     | Treatment × body mass        | 0.42| .660    |         |
|                               |                | Female | Treatment                    | 135 | 62.28   | <.001   |
|                               |                |     | Focal body mass [mg]         | 1.65| .201    |         |
|                               |                |     | Treatment × body mass        | 2.88| .060    |         |
EGG LAYING SUCCESS OVER TIME

**FIGURE A1** Total wildtype offspring after week one (a) and week two (b) of focal females in whole wheat with varying yeast concentration. Assay with just the focal subjected to treatment.

**TABLE A2** ANOVA of general linear model of quasi-Poisson family comparing the total number of offspring sired by the focal female in week one and two of the whole-wheat experiment separately for each treatment.

| Flour type | Contrast                  | df  | Estimate | SE   | F-Value | p-Value |
|------------|---------------------------|-----|----------|------|---------|---------|
| Control    | Week 1 vs. 2              | 44  | <~0.01   | <0.12| <0.01   | .966    |
| Low yeast  | Week 1 vs. 2              | 82  | 0.14     | 0.11 | 1.46    | .230    |
| No yeast   | Week 1 vs. 2              | 86  | 0.15     | 0.12 | 1.57    | .214    |

Note: Assay with just the focal subjected to treatment.

GENETIC MATING SUCCESS OF MALES

**FIGURE A2** Histograms of genetic mating success of focal males in wheat (a, b) and whole-wheat flour (c–e) with yeast supplementation (a, c), without yeast supplementation (b, e) or low yeast supplementation (d). Only focal males subject to treatment.
ADDITIONAL ANALYSES FOR THE ASSAY FOR MEASURING THE OPPORTUNITY FOR SELECTION

**Figure A3** Effect of food availability on (a) number of offspring produced by the focal male or female with or without yeast supplementation in wheat flour and (b) number of offspring produced by the focal male or female with full, 1% or no yeast supplementation in whole-wheat flour. For males, the total number of offspring was divided by the number of females in the assayed groups (i.e. two) to enable comparison with female values. Assay for measuring the opportunity for selection, with all animals subject to treatment.

**Table A3** Results of general linear models (quasi-Poisson) modeling the effect of food quality on the total number of offspring produced by the focal including ANOVA results and post-hoc tests.

| Flour type | Sex | Treatment effect | Post-hoc comparisons |
|------------|-----|------------------|----------------------|
|            |     | df   | F-Value | p-Value | Contrast   | Estimate | SE  | t-value | Adj. p-Value |
| Wheat      | Male | 40   | 72.84   | <.001   | Control - No yeast | -2.54    | .47  | -5.44   | <.001        |
|            |     | 51   | 124.35  | <.001   | Control - No yeast | -2.77    | .40  | -6.92   | <.001        |
| Whole-wheat| Male | 131  | 48.88   | <.001   | Control - 1% yeast | -0.24    | .11  | -2.25   | .026         |
|            |     |      |         |         | Control - No yeast | -1.37    | .16  | -8.64   | <.001        |
|            |     |      |         |         | 1% yeast - No yeast | -1.13    | .16  | -6.98   | <.001        |
|            |     |      |         |         | Control - 1% yeast | -0.43    | .10  | -4.21   | <.001        |
|            |     |      |         |         | Control - No yeast | -1.43    | .14  | -9.98   | <.001        |
|            |     |      |         |         | 1% yeast - No yeast | -1.00    | .15  | -6.71   | <.001        |

Note: Assay for measuring the opportunity for selection, with all animals subject to treatment. p-values adjusted for false discovery rate.

**Sex bias in the opportunity for selection**

**Table A4** Sex bias in variance on number of offspring by the focal estimated via bootstrapping. Experiment with all animals subject to treatment.

| Flour type | Treatment | Sex bias | Lower 95% CI | Upper 95% CI | p-Value |
|------------|-----------|----------|--------------|--------------|---------|
| Wheat      | Control   | -0.010   | -0.125       | 0.112        | .887    |
|            | No yeast  | -0.443   | -0.959       | 0.098        | .057    |
| Whole-wheat| Control   | 0.108    | -0.034       | 0.255        | .649    |
|            | 1% yeast  | 0.007    | -0.137       | 0.159        | .306    |
|            | No yeast  | -0.219   | -0.479       | 0.035        | .094    |

Note: Positive sex bias values indicate male bias. p-values estimated via permutation test.
Variance decomposition for focal males

**Table A5** Variance of standardized relative reproductive success estimated via bootstrapping.

| Flour type | Variance component | Control estimate (95%CI) | Low yeast (95%CI) | No yeast (95%CI) |
|------------|--------------------|--------------------------|-------------------|-----------------|
| Wheat      | MS                 | 0.046 (0.018, 0.071)     | NA                | 0.327 (0.051, 0.504) |
|            | Fec                | 0.196 (0.109, 0.309)     | NA                | 0.467 (0.193, 0.763) |
|            | PS                 | 0.087 (0.056, 0.121)     | NA                | 0.045 (<0.001, 0.111) |
|            | cov(MS, Fec)       | 0.005 (~0.047, 0.053)    | NA                | 0.246 (~0.054, 0.501) |
|            | cov(MS, PS)        | -0.004 (~0.028, 0.023)   | NA                | 0.011 (~0.026, 0.072) |
|            | cov(Fec, PS)       | 0.085 (0.040, 0.133)     | NA                | -0.073 (~0.189, ~0.001) |
| Whole-wheat| MS                 | 0.134 (0.082, 0.197)     | 0.148 (0.086, 0.213) | 0.225 (0.143, 0.308) |
|            | Fec                | 0.241 (0.162, 0.328)     | 0.220 (0.146, 0.304) | 0.391 (0.271, 0.512) |
|            | PS                 | 0.145 (0.095, 0.192)     | 0.112 (0.071, 0.154) | 0.165 (0.088, 0.240) |
|            | cov(MS, Fec)       | 0.058 (~0.013, 0.137)    | 0.102 (0.028, 0.182) | 0.100 (~0.006, 0.210) |
|            | cov(MS, PS)        | 0.006 (~0.034, 0.047)    | 0.020 (~0.021, 0.068) | -0.053 (~0.102, ~0.004) |
|            | cov(Fec, PS)       | 0.075 (0.014, 0.136)     | 0.061 (0.012, 0.111) | -0.001 (~0.092, 0.079) |

Note: Assay for measuring the opportunity for selection, with all animals subject to treatment.

**RD MASS AND COMPETITIVENESS**

**Figure A4** Body mass (a) and number of offspring (b) of wildtype (Ga1) focals and Rd competitors. Shown data only includes control treatment. Assay 1 & 2 combined.

**Table A6** ANOVA of general linear model of gaussian family modeling the differences in body mass between competitor Rd and wildtype individuals.

| Sex  | Mean wildtype | Mean Rd | df  | F-Value | p-Value |
|------|---------------|---------|-----|---------|---------|
| Male | 2.01          | 1.62    | 127 | 360.25  | <.001   |
| Female | 2.55       | 2.13    | 136 | 301.75  | <.001   |

Note: Data only including control treatment. Assay 1 & 2 combined.

**Table A7** ANOVA of general linear model of quasi-Poisson family modeling the differences in offspring number between competitor Rd and wildtype individuals.

| Sex  | Mean wildtype | Mean Rd | df  | F-Value | p-Value |
|------|---------------|---------|-----|---------|---------|
| Male | 146.33        | 51.75   | 128 | 143.51  | <.001   |
| Female | 92.59       | 91.08   | 138 | 0.14    | .711    |

Note: Data only including control treatment. Assay 1 & 2 combined.