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# Interactive effects of sex, social environment, dietary restriction, and methionine on survival and reproduction in fruit flies

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**Abstract** For the evolution of life histories, the trade-off between survival and reproduction is fundamental. Because sexes optimize fitness in different ways, this trade-off is expected to be resolved differently by males and females. Consequently, the sexes are predicted to respond differently to changes in resource availability. In fruit flies, research on dietary restriction has focused largely on females maintained in the absence of males, thereby neglecting sexual interactions that affect reproductive behavior of both sexes under more natural conditions. Here, we tested for the interactive effects of diet (40, 60, 100, and 300 % of standard yeast concentrations) and social environment (separate-sex vs. mixed-sex groups) on male and female *Drosophila melanogaster* life histories. Additionally, we evaluated the essential amino acid methionine as an agent that can uncouple the survival–reproduction trade-off. We show sex differences in the effect of social environment on survival patterns, but not on reproductive fitness. In females, yeast had a positive effect on reproduction and a negative effect on survival. In males, yeast had a negative effect on reproduction and the effect on survival depended on the social environment. Methionine reduced survival, but had no effect on reproduction. Our findings highlight the need to include both sexes and to vary social environments in research programs aimed at lifespan extension and call for further evaluation of the fecundity-restoring effect of methionine.

**Keywords** Sex differences · Cost of reproduction · Senescence · Mortality · Dietary restriction

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## Introduction

In the course of an organism's life, resources have to be acquired, processed, and allocated to a suite of different functions, which ultimately determine the number of descendants into the next generation—its Darwinian fitness. At the heart of this process are the two key fitness components, lifespan and reproductive

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output, which are thought to be mechanistically and genetically linked (Stearns 1992; Roff 2002). The possibility to gain the same fitness through a differently balanced product of age-dependent survival and reproduction theoretically allows for a wide range of possible solutions. Life-history theory posits that, under most circumstances, the relationship between these two main fitness components will be negative, i.e., survival and reproduction trade off against each other (Stearns 1992). The underlying principle for this assumption can be visualized as a so-called Y model of resource allocation (Roff 2002). At the stem of the Y, an organism has a pool of resources, which is usually limited. The quantity of resources available depends on an organism's ability to acquire them, which is determined by genetic factors and environmental conditions. Resources have to be allocated to different functions with the overall goal to optimize fitness, within the given constraints of limited resources. However, high variation in resource acquisition might mask negative correlations (i.e., trade-offs) between two traits (Van Noordwijk and Dejong 1986). In the two-trait Y model, resources that are allocated to mechanisms promoting cellular maintenance, and therefore, better survival, will not be available for reproduction. Direct cellular damage is part of the costs of reproduction. Comparative studies show ample variance in cellular damage between species (e.g., through oxidative stress; Shi et al. 2010), supporting the view that internal repair mechanisms are not universally fixed entities, but that they are under selection and can evolve within phylogenetic constraints. The disposable soma theory of senescence explicitly posits the trade-off between active maintenance of somatic cells and reproduction (Kirkwood 1977).

The effects of dietary restriction (DR), defined as a reduced acquisition of resources without malnutrition, on lifespan and reproduction generally support the disposable soma theory, as modeled by Shanley and Kirkwood (2000; Kirkwood and Shanley 2005). Fewer available resources under DR lead to elevated lifespan in most animal species tested so far (Partridge et al. 2005). This increase in lifespan is correlated with a decrease in reproduction and may have evolved to allow animals to survive periods of food shortage (for a discussion, see Kirkwood and Shanley 2005). Whereas it was formerly thought that calories are the key resource component responsible for the life-extending effect of a restricted diet, it has become clear in recent years that it is not the restriction of calories but of dietary protein that extends lifespan (Mair et al. 2005; Lee et al. 2008; Skorupa et al. 2008). More generally, it seems that the ratio between dietary components, especially proteins and carbohydrates, is the fundamental dietary characteristic that can explain patterns of survival and reproduction, including lifespan extension under DR, in *Drosophila melanogaster* (Lee et al. 2008; Skorupa et al. 2008) and other organisms (Maklakov et al. 2008). This puts the correctness of the term "dietary restriction" into question, but, due to the wide use of the term in the aging literature, we will keep using it when referring to a diet manipulation that restricts specific nutrients.

Recently, the tight link between survival and fecundity, posited by life-history theory and supported empirically (Rose and Charlesworth 1980; Stearns 1992), has been questioned, as interventions have been discovered that seem to prolong lifespan, without producing a negative effect on reproduction (Flatt 2011). These interventions range from single-gene alterations (Fontana et al. 2010; Magwire et al. 2010) to dietary compounds that are directly implicated in basic cellular processes (Bjedov et al. 2010) or compounds that potentially mimic the lifespan-extending effect of DR (Valenzano et al. 2006). Out of the second mentioned category, the effect of dietary methionine on lifespan has been examined in some detail (Troen et al. 2007; Grandison et al. 2009a; Dick et al. 2011; Kabil et al. 2011). Methionine is an essential amino acid that is involved in a very wide range of cellular processes. In previous studies, methionine was often experimentally restricted in relation to other amino acids, and a lifespan-extending effect was commonly observed in rodents (Miller et al. 2005), but not in fruit flies (Troen et al. 2007). More recently, methionine was found to be the only amino acid that, when added to a restricted *D. melanogaster* diet, could restore fecundity and simultaneously maintain lifespan at the level observed under DR (Grandison et al. 2009b). Given that this finding is correct, it implies that the trade-off between lifespan and fecundity can be decoupled by environmental manipulation.

The effects of dietary manipulations on survival and reproduction have been studied mostly in invertebrate laboratory model organisms like *D. melanogaster* and *Caenorhabditis elegans*. This is due to the ease of maintenance in the laboratory, their short generation time, and because of the vast accumulated knowledge of their biology. Many genetic pathways that control aging and the DR effects in these

invertebrate model species are evolutionary conserved (Greer and Brunet 2011). This means that findings about the genetic and environmental factors that affect aging in invertebrate model organisms can be used to guide biomedical research.

Commonly, only females are used in these studies (but see, e.g., Magwere et al. 2004) because it is much easier to estimate reproductive output in terms of female fecundity, compared to the estimation of male reproductive success. In addition, experimental females are normally kept without males for standard lifespan assays. This protocol facilitates the experimental setup to a large extent, but differs considerably from the social and reproductive environment in which the flies have evolved. However, this approach may not be warranted due to sex differences in life history strategies. Sexes can be defined by a dimorphism in sex cells, where males are the sex that produces large numbers of small gametes (sperm), whereas females produce small numbers of large, nutrient-rich gametes (eggs) (Parker et al. 1972). This translates into an increased reproductive investment per gamete in females, often leading to life history strategies that differ remarkably from those observed in males (Trivers 1972). Empirical studies have shown that the trade-off between survival and reproduction can be resolved differently between the sexes and can be condition-dependent (e.g. Maklakov et al. 2008). This emphasizes the need to investigate both sexes and to take the common social environment of the study species into account when making inferences about lifespan or fitness consequences of environmental or genetic manipulations carried out in the experiments.

DR studies in *D. melanogaster* on survival and reproduction in both sexes are scarce. Moreover, studies that explicitly examine the effects of DR, methionine availability, social environment, and sex on survival and reproductive fitness are currently lacking. Therefore, we conducted the present study to test for the interactive effects of these variables on survival and reproductive output. To this end, we assayed males and females in two different social environments: separate-sex (standard assay environment) or mixed-sex (recent evolutionary history of this population) groups. We used four diets that covered the dietary protein range from DR to very high protein levels (while keeping carbohydrate levels constant). Finally, methionine was either added to the diets or not. We used a full-factorial design, resulting in a total of 32 treatments.

## Methods

### Study animals

We used flies of the Dahomey laboratory population obtained from Prof. Tracey Chapman, University of East Anglia, Norwich, UK. This population has been maintained on overlapping generations at a size of ≥3,000 individuals, ever since the founders that initiated the population were brought in from the wild 40 years ago (Partridge and Fowler 1992). The Dahomey population has been kept on a standard diet, identical to our experimental 1.0 diet (see below in the "Diet preparation" section), except that we used Brewer's yeast, instead of Baker's yeast, in the present study. To reduce the potential parental effects in our experimental flies stemming from conditions in the population cages, we raised flies for two generations under standardized densities (180 eggs per vial) on standard (1.0) diet and used the third generation of flies in the present experiment. Experimental males and females were allowed to mate for 48 h after eclosing into adulthood.

### Experimental design

To test for the effects of sex and social environment, dietary protein and methionine in a full-factorial design, we set up 48 experimental vials (28.5×95 mm). We created three social treatment groups that consisted of either males or females kept in single-sex groups or mixed-sex groups. In all treatment groups, 40 flies were kept per vial. At the start of the experiment, these 40 animals consisted of either 40 males or 40 females per vial in the single-sex treatments or of 20 males with 20 females per vial in the mixed-sex treatment.

To test for the effects of dietary protein, we provided flies with one of four different diets that differed in their yeast concentration (40, 60, 100, or 300 g per 1 l of diet; see the "Diet preparation" section below). The methionine treatment consisted of diets that either contained no added methionine (M−) or that had methionine added (M+). For each treatment combination of social environment, yeast concentration, and methionine treatment we used two replicate vials, adding up to 48 experimental vials ($N=3_{\text{social}}\times4_{\text{yeast}}\times2_{\text{methionine}}\times2_{\text{replicate}}$) and 1,920 flies in total. The treatment effects included in this study spans many of the variables that currently are thought to have a strong implication for lifespan, and the full-factorial design employed makes it

possible to thoroughly analyze their effect as well as potential interactions between them. It is, however, important to note that longevity data are very sensitive even to small changes in experimental procedures, e.g., the type and manufacturer of yeast used in the diet (Bass et al. 2007).

### Mortality assays and maintenance

Mortality was checked each week on Monday, Wednesday, and Friday, when flies were flipped to new food vials. Once a week, fly density was adjusted using 3-day-old flies that were raised at a larval density of 180 per vial and allowed to mate for the first 2 days of adult life (from here on called *replacement* flies). To distinguish these flies from the experimental flies, we used flies homozygous for the recessive phenotypic marker *ebony* that gives them a darker body compared to wild-type flies. The *ebony* mutation had previously been backcrossed to the Dahomey population for four generations. During the whole experiment, if necessary, we used light $CO_2$ anesthesia and applied the same treatment to all experimental vials.

### Fitness assays

To measure reproductive fitness, we estimated female fecundity and male fertilization success every week. To estimate female fecundity, we flipped the experimental females into a fresh vial where they were allowed to lay eggs for 22 h. These eggs were subsequently counted. Counts for single-sex female treatments were divided by two in order to adjust for the fact that there was twice as many females in the vials with the single-sex treatments compared to the mixed-sex treatments. In the male fitness assay, we placed the focal males of a given vial (including experimental wild-type males and replacement *ebony* males) with the same number of *ebony* females for 5 h, after which they were separated again. We used 5-day-old *ebony* females that were raised on larval densities of 180 eggs per vial and that were allowed to mate on their first 2 days of adult life. We kept these ebony females on 1.0 diet for 24 h after we had separated them from the experimental males then put them on a new 1.0 diet vial for 22 h. This was done to give sperm transferred during the fitness assays enough time to distribute in the female reproductive tract, so that sperm stored from previous matings could not be used to a great extent any more (then P2>>P1; Boorman and Parker 1976). Eggs laid in these vials were trimmed to 180 eggs per vial and were allowed to develop and hatch. On day 11 after egg-laying, hatched offspring fathered by the experimental males were counted. We could separate these offspring by their body color, as offspring to experimental males had wild-type color and offspring to ebony males were dark. Offspring counts of single-sex male treatments were divided by two, following the same rationale as for single-sex female egg counts.

### Diet preparation

We manipulated diets by varying the yeast content. Diet components are given in Table 1. To prepare diets, we mixed agar, sugar, yeast, and water together and boiled the mixture in an autoclave at 121 °C for 30 min. When the solution had cooled down to 65 °C, we added propionic acid and nipagin solution to prevent fungal and bacterial growth and filled vials with 10 ml of food each. Methionine was added at a temperature of around 55 °C to assigned vials.

### Statistical analysis

#### *Survival*

We used Cox proportional hazard models to test for the effects of sex, social environment, protein, and

**Table 1** Diet composition

| Diet concentration (×SY) | 0.4 | 0.6 | 1.0 | 3.0 |
|---|---|---|---|---|
| Agar (g/L) | 15 | 15 | 15 | 15 |
| Sucrose (g/L) | 50 | 50 | 50 | 50 |
| Brewer's yeast (g/L) | 40 | 60 | 100 | 300 |
| Nipagin solution[a] (ml/L) | 30 | 30 | 30 | 30 |
| Propionic acid (ml/L) | 3 | 3 | 3 | 3 |

Diets for methionine treatment have 0.1 g/L methionine added (equivalent to 0.7 mM). For each diet, distilled water was added to 1 L. Agar was supplied by Bageriprodukter AB, sucrose by Nordic Sugar AB, Brewer's yeast by MP Biomedicals, methionine by Sigma-Aldrich, propionic acid by Acros Organics, and methyl 4-hydroxybenzoate for Nipagin solution from VWR

[a] 100 g/L methyl 4-hydroxybenzoate in 95 % ethanol

methionine treatment on survival patterns. A mixed model including vial as a random effect did not provide a better fit compared to a model excluding this effect (likelihood ratio test (LR): $p=0.623$; mixed model tested with function *coxme*, R package "coxme"; Therneau 2011a; model without random effect tested with function *coxph*, R package "survival"; Therneau 2011b). Therefore, we proceeded without random effects in subsequent Cox analyses, using function coxph. In the initial global model, we included all linear variables and all their two-, three-, and four-way interactions. We used backward model selection. Starting with the initial global model, we sequentially dropped one model term in each step, starting with the highest-order term (sex × yeast × social × methionine) and proceeding with the next lower-order term. To compare two models in each step (full vs. reduced), we used log-likelihood ratio tests, with twice the difference in log-likelihoods of the models taken as chi-square distributed, with degrees of freedom (*df*) equal to the number of reduced parameters (Bolker 2008). In cases with more than one model term of the same order (main effects and their two- and three-way interactions), we dropped the model term with the smallest effect first. Yeast was treated as a continuous variable. The assumption of proportional hazards was tested graphically (function *cox.zph*, R package "survival"; Therneau 2011b) and found to be met. Models on subsets of separate females are presented in their initial form, with all model parameters included. Given $p$ values qualitatively reflect the importance of model terms, i.e., terms with $p>0.05$ would be dropped during model selection. This is the same for all subset analyses, including lifespan and reproductive fitness.

We tested the effects of treatments and sex on average lifespan in a linear model with the same model terms and with the same model selection procedures as in the Cox proportional hazard analyses above. To test for the inclusion of a random effect, we tested the global model with a random effect of vial (using function *lme* in the R package "nlme"; Pinheiro et al. 2009) against the same model without the random effect (using function *gls* in the R package "nlme"; Pinheiro et al. 2009). The mixed model with the random effect provided a better fit (LR, $p=0.006$); therefore, we proceeded using the mixed models with function *lme* for further model selection. Multiple Tukey's HSD (Honestly Significant Difference) comparisons between treatment groups were accomplished using function *glht* in the "multcomp" R package (Hothorn et al. 2008). If not otherwise stated, reported $p$ values in the "Results" section refer to these analyses.

### *Fitness*

Female fitness was taken as the total number of eggs all females in a given vial laid across the weekly assays. Male fitness was defined as their net reproductive performance (mating and fertilization success) and measured as the total number of wild-type offspring sired during their lifetime. We chose to use lifetime reproductive fitness instead of age-dependent fitness measures in this analysis because it is the most meaningful measure of our reproductive fitness data that is based on populations of flies kept in vials, and not based on individuals, as is possible with time to death event data in survival analysis. Female fecundity was $\ln(x+1)$ transformed in order to make the data conform to a normal distribution. To be able to analyze and compare male and female fitness in the same models, we standardized fitness values within each sex by subtracting the mean and dividing by the standard deviation.

We used the same model to analyze fitness as we used to analyze lifespan. Including vial as a random effect did not improve model fit (LR, $p=0.557$), and the random effect vial was, therefore, dropped from all analyses of fitness.

### *Methionine*

Methionine was included in the global models of survival and fitness described above. To specifically test for the recently reported fecundity-restoring effect of methionine on dietary restricted females (fed 1.0 diet), we analyzed the effect of methionine, yeast, and methionine × yeast in a subset of separate females on diets 1.0 and 3.0 only.

### *Model tables*

All model tables are presented as effect tables with reference level "female" for sex and "mixed" for social environment.

## Results

### Age-dependent survival

All experimental variables (yeast, sex, social environment, and methionine) had an influence on survival on their own, but also interacted with the other variables to a high degree (Table 2).

The overall main effect of yeast on survival was negative (Fig. 1). Females always lived longer than males in separate-sex groups, whereas in mixed-sex groups, the difference was observed only in some treatments ($p_{0.4-}<0.0001$, $p_{0.4+}<0.0001$, $p_{0.6+}=0.057$). This resulted in two-way interactions between social environment and all other variables, as well as the three-way interactions social environment × sex × yeast and social environment × methionine × yeast (Table 2).

The effect of methionine depended on social environment and on yeast, but not on sex, and was relatively small compared to other treatment effects (Table 2). The overall main effect of methionine on survival was negative (Table 2). When only tested in females from separate-sex groups (which replicates the way fruit flies are maintained in most studies), methionine showed no effect on survival patterns, independent of whether only yeast ratios 1.0 and 3.0 were included (Table S1a; Grandison et al. 2009a used 1.0 diet as the restricted diet and 2.0 diet as the full feeding diet) or whether tested across all yeast ratios (Table S1b).

### Adult life expectancy

Adult life expectancy, defined as mean adult lifespan, was affected by all experimental variables (Table 3). Methionine had a weak negative direct effect on mean lifespan, independent of other treatments (Table 3; Fig. 2). Effects of sex and social environment on mean lifespan depended on the quadratic yeast term, i.e., they were curvilinear along the yeast gradient. The difference between social environments was generally more pronounced in females than in males, without any observed difference between separate-sex and mixed-sex males on diet 1.0, without methionine ($p>0.99$; Fig. 1). Individuals on diet 3.0 always had the lowest lifespan, independent of sex and social environment (Fig. 2). Lifespan of separate-sex and mixed-sex females declined with higher dietary yeast level, whereas separate-sex males on yeast diets lower than 3.0 did not differ in average lifespan and mixed-sex males had their highest average lifespan on yeast diet 1.0, with a similarly lower lifespan on diets 0.4 and 0.6 (Fig. 3). Analyses on subsets of separate-sex females showed no effect of methionine, independent of yeast level, similar to the results for survival curves (Table S2).

### Fitness

Reproductive fitness was affected by diet, sex, and social environment, but not by methionine (Tables 4 and S3). The relationship between yeast and fitness was curvilinear and differed between the sexes (Table 4; Fig. 4).

**Table 2** Final Cox proportional hazards model

| Source | Coefficient | Exp(coef) | SE(coef) | $z$ | $p$ value |
|---|---|---|---|---|---|
| Yeast | 0.728 | 2.071 | 0.049 | 14.988 | <0.0001 |
| Sex (male) | 0.615 | 1.849 | 0.089 | 6.917 | <0.0001 |
| Meth (plus) | 0.277 | 1.319 | 0.088 | 3.131 | 0.0017 |
| Social (sep) | −1.161 | 0.313 | 0.113 | −10.311 | <0.0001 |
| Yeast × sex (male) | −0.170 | 0.844 | 0.055 | −3.099 | 0.0019 |
| Yeast × meth (plus) | −0.096 | 0.909 | 0.055 | −1.755 | 0.0792 |
| Yeast × social (sep) | −0.334 | 0.716 | 0.067 | −5.010 | <0.0001 |
| Sex (male) × social (sep) | 0.645 | 1.906 | 0.128 | 5.031 | <0.0001 |
| Meth (plus) × social (sep) | −0.358 | 0.699 | 0.125 | −2.870 | 0.0041 |
| Yeast × sex (male) × social (sep) | 0.247 | 1.281 | 0.078 | 3.189 | 0.0014 |
| Yeast × meth (plus) × social (sep) | 0.172 | 1.187 | 0.077 | 2.236 | 0.0253 |

Exp(coef) gives the hazard ratio: Terms in brackets represent the factor levels against which the reference levels are compared to. “Plus” stands for “with methionine”, “sep” stands for “separate/single-sex”, “meth” stands for “methionine”

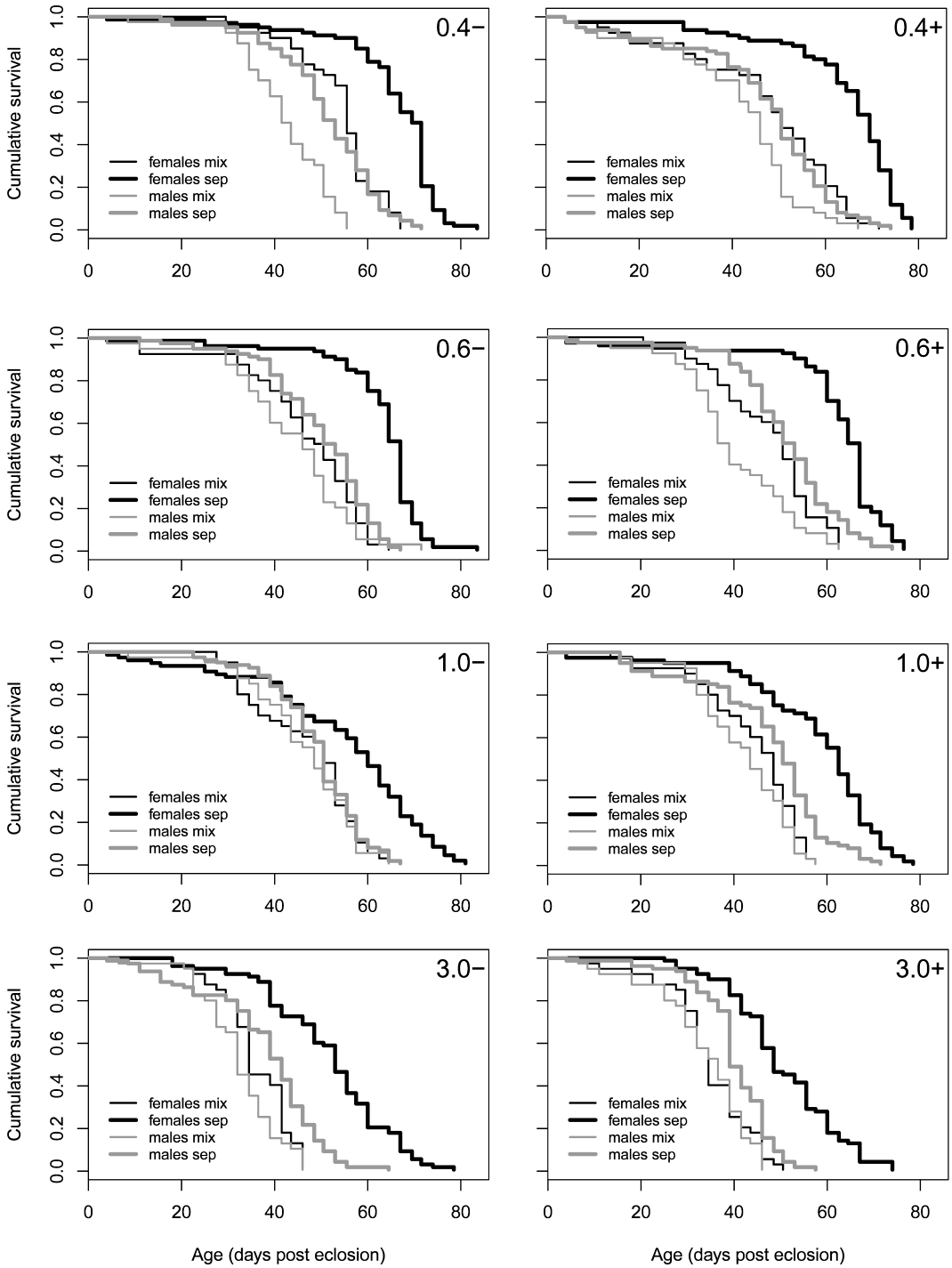


**Fig. 1** The effects of sex, social environment, diet and methionine on survival. Symbols – and + stand for without or with methionine, respectively. "Mix" stands for "mixed-sex", "sep" stands for "separate/single-sex"

Female reproductive fitness increased with yeast content. This relationship was strong from low to medium yeast content, and then leveled off towards the higher yeast concentrations. In contrast, there was a general negative relationship between male fitness and yeast concentration (Fig. 4).

**Table 3** Final mixed model for lifespan

| Source | Coefficient | SE | *t* | *p* value |
|---|---|---|---|---|
| (Intercept) | 54.245 | 2.147 | 25.261 | <0.0001 |
| Yeast | −10.460 | 3.889 | −2.689 | 0.0104 |
| Sex (male) | −15.371 | 2.182 | −7.043 | <0.0001 |
| Meth (plus) | −1.197 | 0.587 | −2.040 | 0.0480 |
| Social (sep) | 18.902 | 2.505 | 7.547 | <0.0001 |
| Yeast$^2$ | 1.492 | 1.089 | 1.371 | 0.1781 |
| Yeast×sex (male) | 19.968 | 3.928 | 5.083 | <0.0001 |
| Yeast×social (sep) | −9.153 | 4.507 | −2.031 | 0.0490 |
| Sex (male)×social (sep) | −7.768 | 1.040 | −7.468 | <0.0001 |
| Yeast$^2$×sex (male) | −5.299 | 1.100 | −4.818 | <0.0001 |
| Yeast$^2$×social (sep) | 2.597 | 1.262 | 2.058 | 0.0462 |

Abbreviations as in Table 2

## Discussion

We present evidence of strong interactive effects of social environment, sex, diet, and methionine on survival and reproduction in *D. melanogaster*. In terms of lifespan, diet affected females in a similar way, whether they were kept separately or in mixed-sex groups. This finding suggests that conclusions drawn from previous studies on females in separate-sex groups are also valid for females tested in a more natural setting. On the contrary, social environment had a strong effect on how male age-specific survival and lifespan responded to dietary manipulations. While predicted lifespan-extending DR effects were observed in females, males showed little increase in lifespan on restricted diets. Moreover, males that were allowed to interact with females (mixed-sex groups) showed a decrease in lifespan on restricted diets when

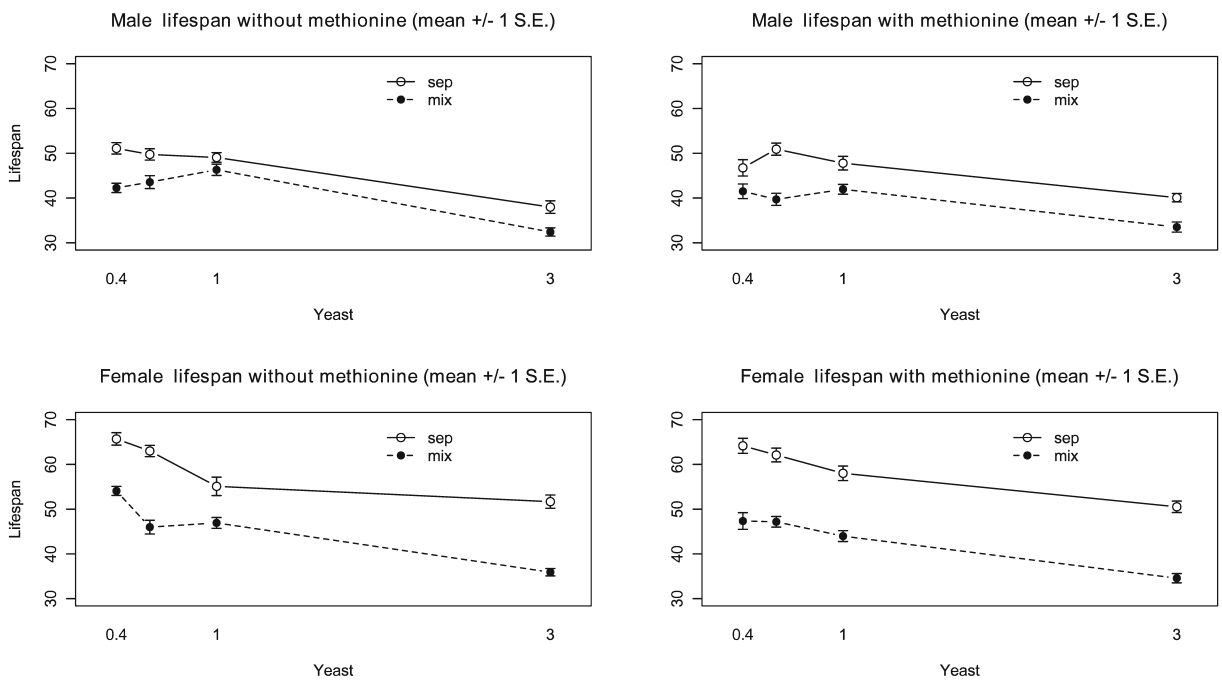


**Fig. 2** The effects of sex, social environment, diet and methionine on lifespan. Abbreviations as in Fig. 1

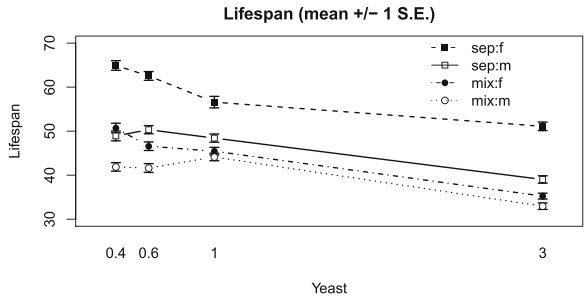


**Fig. 3** The effects of sex, social environment and diet on lifespan, independent of methionine. Depicted groups are: separate/single-sex females (sep:f), separate/single-sex males (sep:m), mixed-sex females (mix:f), mixed-sex males (mix:m)

no methionine was supplied. In general, methionine was not found to have the previously reported positive effect on reproductive fitness, but rather had an overall weak negative effect on survival. Strong sex × diet interactions for survival and reproduction suggest that the trade-off between survival and reproduction is sex-specific and that optimization of lifespan through diet should be explored separately for each sex.

The lifespan-extending effect of restricting specific dietary components, i.e., manipulating the available nutrient ratios, has been investigated in a large number of evolutionary, biomedical, and biodemographic studies (Everitt et al. 2010). *D. melanogaster* is one of the key invertebrate models in this research field (Grotewiel et al. 2005), but generalizing from experimental results that disregard fundamental biological relationships, as for example intersexual interactions, may not be warranted. In this study, we confirm the lifespan-extending effect of DR in female fruit flies and the concomitant resource-mediated trade-off between survival and fecundity, as predicted by life-history theory. This trade-off was largely unaffected by social environment in females. Female fruit flies in the two social environments differed in the mean of their survival and reproductive fitness response across all diets, with females in separate-sex groups having higher survival and reproductive fitness, compared to females in mixed-sex groups (corroborating previous findings; Kuijper et al. 2006), but there were no interactions between the effects of social environment and diet. Our data thus suggest that fecundity and lifespan patterns from studies of aging using similar experimental diets, conducted on once-mated female flies kept without males, would not differ fundamentally from the results gained from females in mixed-sex groups, when patterns of diet-dependent survival and fecundity are concerned. Of course we cannot rule out potential differences on a deeper level of biological organization that may nevertheless result in similar patterns of survival and fecundity in the two social contexts. There are many ways of how to manipulate social environment. One variable that often differs between studies is fly density, ranging from one fly per vial (Nuzhdin et al. 1997) to several hundred flies in demography cages (Min et al. 2007). Sex differences in demographic parameters may be density-dependent, requiring further examination of our results across a range of different densities.

**Table 4** Final model of reproductive fitness

| | Coefficient | SE | *t* | *p* value |
|---|---|---|---|---|
| (Intercept) | −2.545 | 0.372 | −6.843 | <0.0001 |
| Yeast | 2.755 | 0.674 | 4.089 | 0.0001 |
| Sex (male) | 2.302 | 0.519 | 4.439 | <0.0001 |
| Social (sep) | 1.272 | 0.125 | 10.181 | <0.0001 |
| Yeast$^2$ | −0.584 | 0.189 | −3.094 | 0.0031 |
| Yeast × sex (male) | −3.070 | 0.953 | −3.221 | 0.0021 |
| Yeast$^2$ × sex (male) | 0.584 | 0.267 | 2.188 | 0.0327 |

More generally, the fact that most studies differ to a certain extent in the implementation of the same treatments may complicate comparisons of results from different studies. However, Richter et al. (2010) also point to the benefit of introducing systematic variation in experimental designs, rather than trying to exactly repeat one specific treatment in order to test for the robustness of the results. Viewed in this context, we want to point out that our results are based on the data from a single study, and further research will no doubt reveal how robust our results are by testing the effects of dietary composition on lifespan across different environments and experimental setups, as well as in flies from different genetic backgrounds.

Males are rarely used in *Drosophila* aging studies but see (Magwere et al. 2004; Bross et al. 2005; Dick et al. 2011) and almost never in different social environments. In our study, males on restricted diets did not have higher average lifespan compared to the 1.0 diet. In fact, males in mixed-sex groups on restricted diets without methionine had lower average lifespan compared to the 1.0 diet. In contrast, other studies have found a positive effect of DR on male lifespan (Magwere et al. 2004; Bross et al. 2005; Dick et al. 2011). As it stands, we

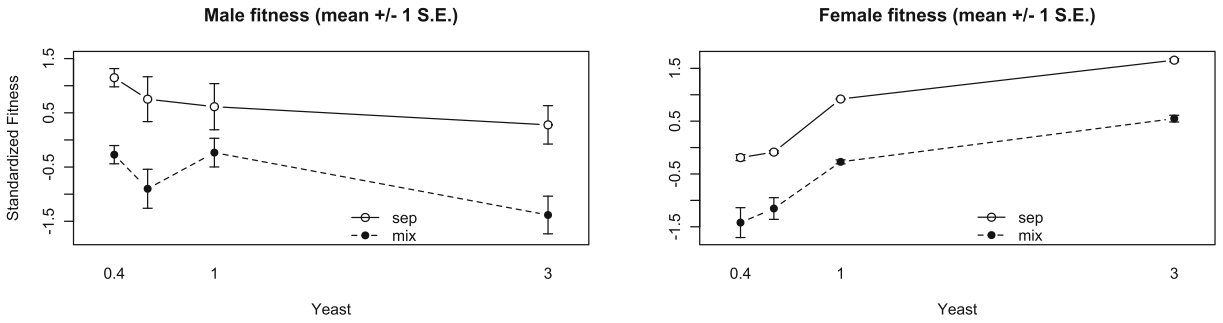


**Fig. 4** The effects of sex, social environment and diet on fitness. Abbreviations as in Fig. 1

cannot pinpoint the reason for this difference in the effect of restricted diet on male lifespan. Possible explanations include differences in fly maintenance (separate-sex vs. mixed-sex groups; density), diet (only yeast manipulated vs. yeast and sugar manipulated; different protein sources), and interactions thereof. Further studies are necessary to confirm this finding, but our results highlight that nutritional effects can be sex-specific and that it is possible for lifespan-extending genetic or environmental manipulations to be only successful in one sex, with a negative or no effect on the other sex. Therefore, it seems essential in most circumstances to include both sexes in aging studies that aim at screening for lifespan-extending interventions and to be aware of the social context-dependent nature of these effects. Diet effects on male reproductive fitness were also in stark contrast to the pattern observed in females: high dietary yeast concentration lowered male reproductive fitness, independent of social environment.

Methionine had a slight negative effect on survival and lifespan in our study, but it did not affect reproductive fitness in either sex. These results differ from previous studies that found methionine to have a fecundity-restoring effect when fed to once-mated females (Dick et al. 2011; Kabil et al. 2011). Grandison et al. (2009a) showed this with a concentration of methionine comparable to the one we used (0.7 mM), while the other studies got this result using a higher concentration (1.5 mM) of methionine (Dick et al. 2011; Kabil et al. 2011). Concentration of methionine could, therefore, potentially explain some of the discrepancy between results.

The fact that males show a much higher reproductive fitness on the lowest protein diet remains puzzling. Bodies of flies on a high-protein diet have been shown to have higher protein content, but not higher fat stores (Skorupa et al. 2008). More fat only accumulated in flies on carbohydrate-rich diets (Skorupa et al. 2008), excluding negative effects of high body fat as the cause of low reproductive fitness in males fed a high-yeast diet. A high-protein diet could have a sex-specific toxic effect on males through unknown dietary compounds contained in yeast or directly through higher protein body content, which male flies, in contrast to females, might not be able to tolerate. Fricke et al. (2008) found that diet affected the components of male reproductive success in a way that is very similar to our findings, at least for separate-sex males. The second-male paternity success in sperm competition (P2) was highest for males on low (0.5) and intermediate (1.0) protein diets, suggesting that P2 might be a good approximation of lifetime fertilization success.

In conclusion, we show that sex, diet, social environment, and methionine availability interact to affect survival and reproductive fitness in *D. melanogaster*, a classic model organism in aging research. There are four key messages that follow from our results. First, the effects of social environment were less pronounced in females, suggesting that the results obtained in many previous DR studies using once-mated females kept in isolation from males are likely to hold under more natural conditions. Second, this is unlikely to be the case for males, where the effects of diet on survival depended on social environment, with males that were allowed to reproduce normally showing reduced survival on DR diets. Third, we did document a significant effect of methionine in our experiment, but contrary to several recent studies, it was a weak negative effect on survival rather than a positive effect on reproductive performance, calling for further evaluation of the effect of essential amino acids balance on life history traits. Fourth, there were strong sex × diet interactions for survival and reproductive fitness, supporting the existence of sex-specific trade-offs between survival and reproduction and suggesting that

dietary optimization of lifespan should be conducted separately for each sex. Our results highlight the potential benefit of investigating the effect of DR and other lifespan-extending treatments for both sexes under different environmental conditions, in particular those reflecting the recent evolutionary history of the population.

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