**Title**

The relationship between longevity and diet is genotype dependent and sensitive to desiccation

**Running title**

Dietary reaction norms are genotype dependent

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## Summary

**Dietary restriction (DR) is a key focus in ageing research. Specific conditions and genotypes were recently found to negate lifespan extension by DR, questioning the universal relevance of DR. However, the conceptual framework of dietary reaction norms explains why DR's effects might not be apparent in some situations. When dietary reaction norms shift with genetic or environmental effects, a specific dyad of diets tested can result in a null effect. Only if a full reaction norm is tested can lifespan (or any trait) be shown to be refractory to diet. We tested comprehensively, for the first time, the importance of dietary reaction norms by measuring longevity and fecundity on five diets in five genotypes, with and without water supplementation, using high sample sizes in the fly (N>25,000). We detected substantial genetic variation in the reaction norm between diet and lifespan. Environments supplemented with water rescued putative desiccation stress but only at the richest diets. Fecundity declined at these richest diets, but contrary to effects on lifespan, was unaffected by water and is thus most likely caused by nutritional toxicity. Our results demonstrate empirically that any conclusion on the absence of DR is only justified when a range of diets is considered in a reaction norm framework.**

## Introduction and Results

Dietary restriction (DR), the limitation of food intake but avoiding malnutrition, extends lifespan. The generality of the DR response has been questioned, however, by reports that DR does not extend lifespan under certain experimental conditions (Austad, 2012; Ja et al., 2009; Piper et al., 2010) or in some genotypes (Liao et al., 2010; Tatar, 2011). These conclusions are routinely based on experiments using two diets (dietary dyad) alone, whereas it is recognised that a change in the continuous relationship between diet and lifespan (reaction norm) can obscure lifespan extension by DR (Flatt, 2014; Tatar, 2011). The bell-shaped nature of the dietary reaction norm dictates that one particular diet concentration, in one genotype or environment, will result in the longest lifespan; with lower or higher diet concentrations inducing a shortened lifespan due to overfeeding or malnutrition. Where a particular dietary dyad falls on this reaction norm will determine the magnitude of the DR effect and can even lead to the erroneous conclusion that DR shortens lifespan (Fig.1).

Given this, it is currently unclear to what extent genetic variation in dietary reaction norms confounds DR research. When specific environmental effects interact or interfere with the DR reaction norm, the use of dietary dyads could similarly lead to misleading conclusions. For flies specifically, water supplementation has been suggested to diminish the effect of DR on lifespan (Dick et al., 2011; Ja et al., 2009). The conclusion that water completely explains DR has been discredited (Piper et al., 2010), but flies nonetheless consider water a nutrient and consume 1-2µl per day, with higher consumption at higher dietary yeast concentrations

(Fanson et al., 2012). Hence, erroneous conclusions could be drawn from diet responses if desiccation presents a genotype- or diet-specific hazard.

Here, we present DR reaction norms for fecundity and longevity across five genotypes in the fly with and without water supplementation using high sample sizes. Longer lifespans were observed at intermediate dietary yeast concentrations, consistent with DR, with a reduction in survival at the lowest and highest yeast concentrations (Fig.2A,S4; Table S1-6). We detected strong genetic variation in the response to diet ($\chi2=162$, df=16, $P<0.001$) with the diet of maximum longevity differing between genotypes. To test the effect of desiccation, we compared longevity under control conditions to water-supplemented. Water reduced mortality particularly at higher yeast concentrations, with genetic variance for this reduction ($\chi2=160$, df=16, $P<0.001$; Fig.2B; Table S2-6), ranging from a two- to twenty-fold reduction in hazard rate. To assess statistically whether water supplementation abolished DR-induced life extension (Ja et al., 2009) we ran our models within the water treatment only, but found no evidence for this suggestion (Fig.S1, Table S7-12). Given that water supplementation ameliorated, but did not eliminate, elevated mortality under these high yeast concentrations, we conclude desiccation can play an experimentally confounding role in DR, but is not causal.

DR is known to reduce reproductive output and is interpreted as a response to decreased energy availability (Moatt et al., 2016). The effect of overfeeding on reproduction, although appreciated in humans (Broughton & Moley, 2017), has received little attention (McCracken et al., 2020). These two responses were evident in egg laying: an increase with yeast concentration, and a stabilisation, or decline at the highest yeast concentrations (Fig.S2,3; Table S13,14). As with mortality, genetic lines also differed in the reproductive response to diet ($F=6.3$, df=16, $P< 0.001$). Reduced egg laying together with a reduction in survival, lowered predicted lifetime reproductive at the richest diet (Fig.S3). Egg laying was not affected by water (Fig.S2; Table S13,14; $P>0.15$), even when water rescued desiccation stress at the high yeast concentrations (Fig.2,S2) implicating nutritional toxicity in the egg laying, and part of the mortality, responses at the richest diets.

## Conclusions

These data now directly demonstrate that specific care is needed when studying DR across genotypes, experimental conditions or environments. We acknowledge that carrying out full reaction norms in all DR experiments would be laborious. We suggest selecting dietary dyads that differ only minimally when genetic variance in DR is the object of study. Such a strategy reduces the chance that tested diets diverge considerably from maximal lifespans, leading to starvation or nutritional toxicity (Fig.1). Furthermore, we suggest when environmental conditions, such as water (Ja et al., 2009), sex (Regan et al., 2016) and microbiome (Wong et al., 2014) are presumed to negate the DR response, that a post-hoc reaction norm is performed.

Similar considerations hold for mechanistic research: e.g. when a genetic manipulation removes the DR response, only a full dietary reaction norm can demonstrate how such an effect arises: by either a shift in, or compression of, the reaction norm. The importance of reaction norms when studying DR has been stressed before, but this is the first high sample size data across multiple wild-type genotypes and diets that demonstrates this empirically.

Support by NERC ACCE & Wellcome Royal Society SHDF. AM and MS conceived and planned the experiment. AM and EB carried out the experiment. AM performed the analysis and wrote the manuscript with supervision from MS. MS supervised the project. The data that support the findings of this study are openly available in Dryad. The authors declare no conflicts of interest.

## Main Figures

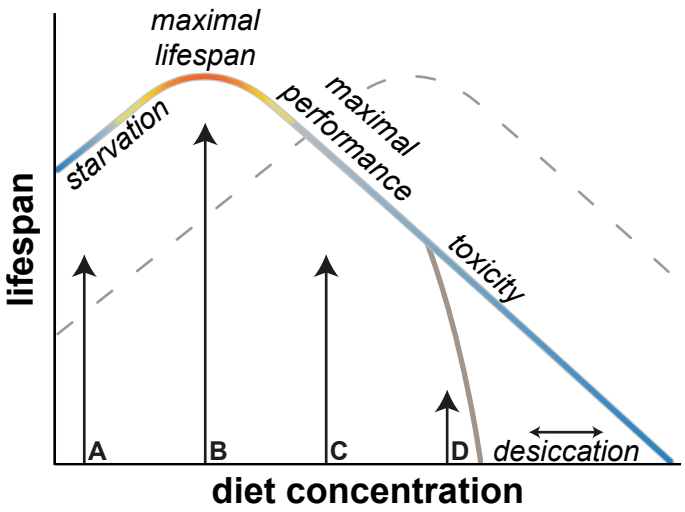


**Fig.1** Diet concentration has a bell-shaped relationship with lifespan, ranging from malnutrition (A), DR (B), maximal performance or highest Darwinian fitness at a relatively rich diet (C), to overfeeding leading to nutritional toxicity (D). As a detailed reaction norm is rarely known, a dietary dyad although often used can lead to misleading conclusions. A dietary dyad (A & C) can show no response at all due to the symmetry in the shape of the reaction norm. Furthermore, genetic or environmental effects can alter the shape or shift the reaction norm (dashed line), or lead to effects at only specific parts of the reaction norm (solid gray, e.g. desiccation). For example, diets B & C result in a DR response on the focal curve, but malnutrition on the dashed curve.

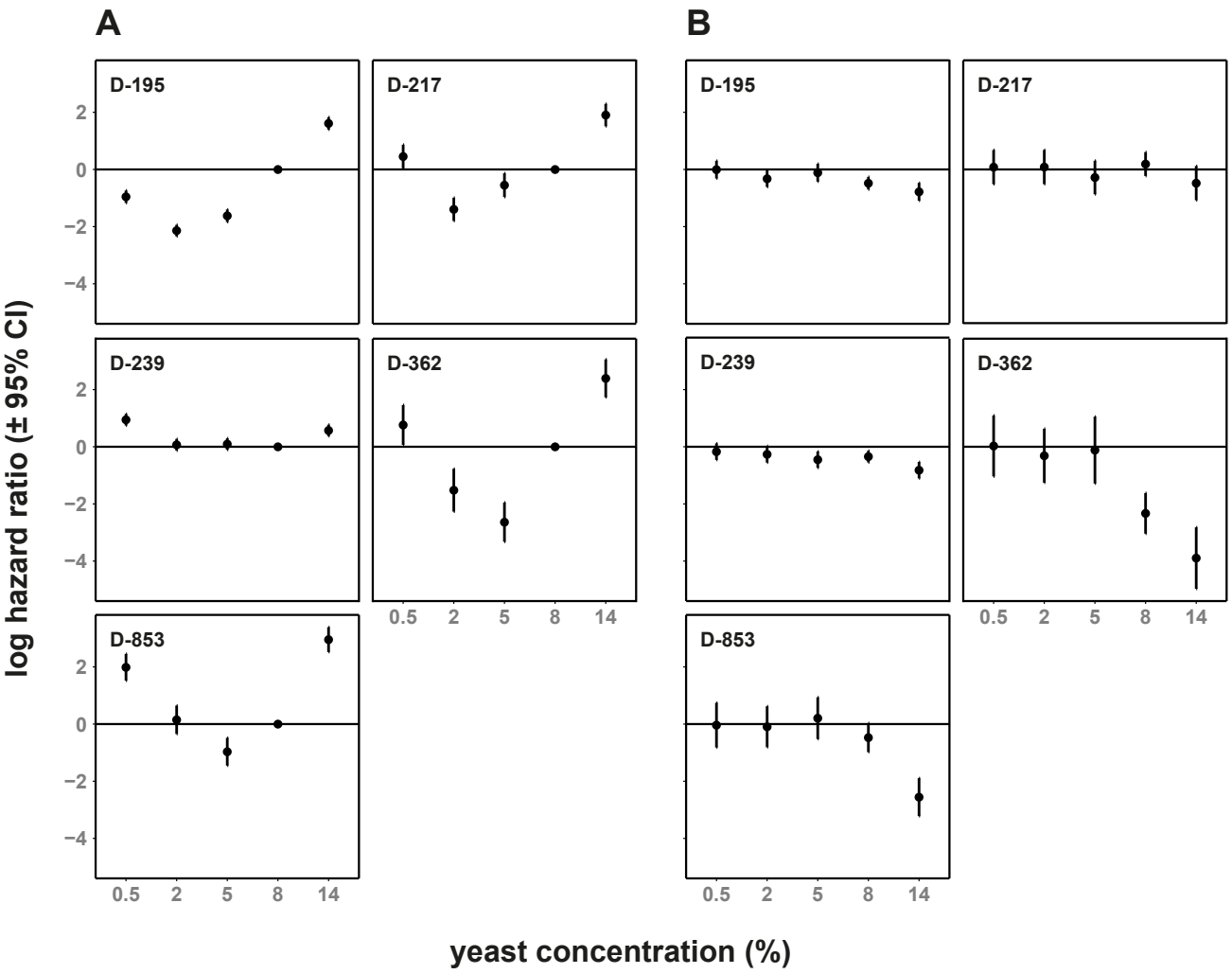


**Fig.2 A** Dietary reaction norms vary by genotype. **B** Water-supplementation rescues desiccation stress at high yeast concentrations, and the extent of this is genotype dependent. Note, hazard ratios represent risk to die, therefore higher values indicate shorter lifespans and are relative, in this case to the 8% diet. Effects in B are plotted as the additional effect of water supplementation as determined by Cox mixed-effect hazard models.

# Supplementary Figures

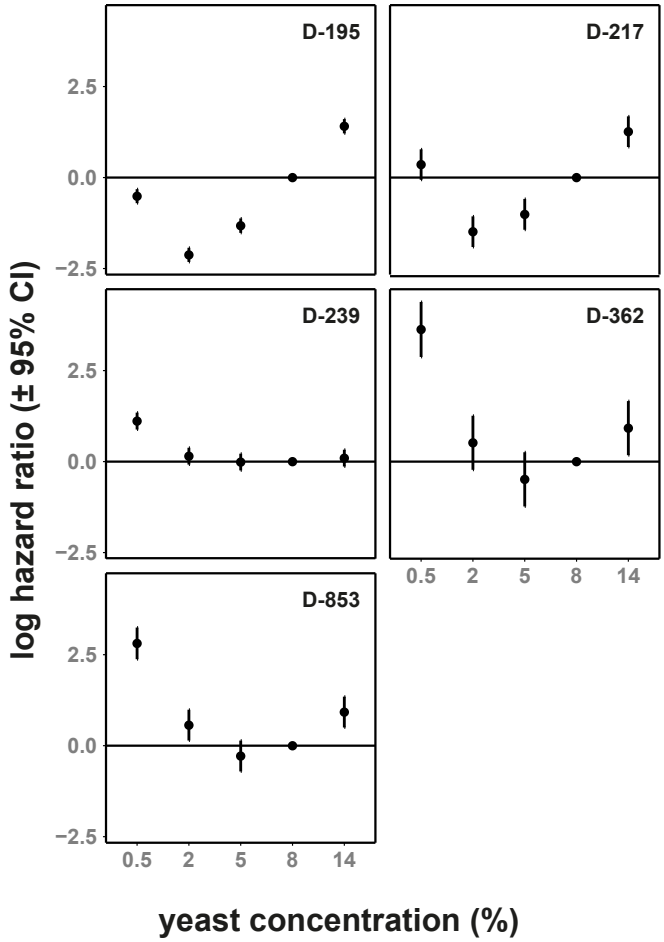


**Fig. S1. Log hazard ratios of diet within water-treated cages in a panel of DGRP genotypes.** Reaction norms to diet still differ in water-treated circumstances. Hazard ratios represent the inverse of typical survival reaction norms to diet. 8% yeast treatment was treated as a reference and as such, no CIs are available. Rates here are relative to 8% yeast diet, and lines represent this standard. N = 12,737 females total; 2,396-2629 per genotype. Hazard ratios have the benefit over median lifespan in that they are directly related to the appropriate statistics used for time-to-event data. In addition, they are directly comparable in a quantitative fashion across genotypes of different lifespans, as they express a relative risk.

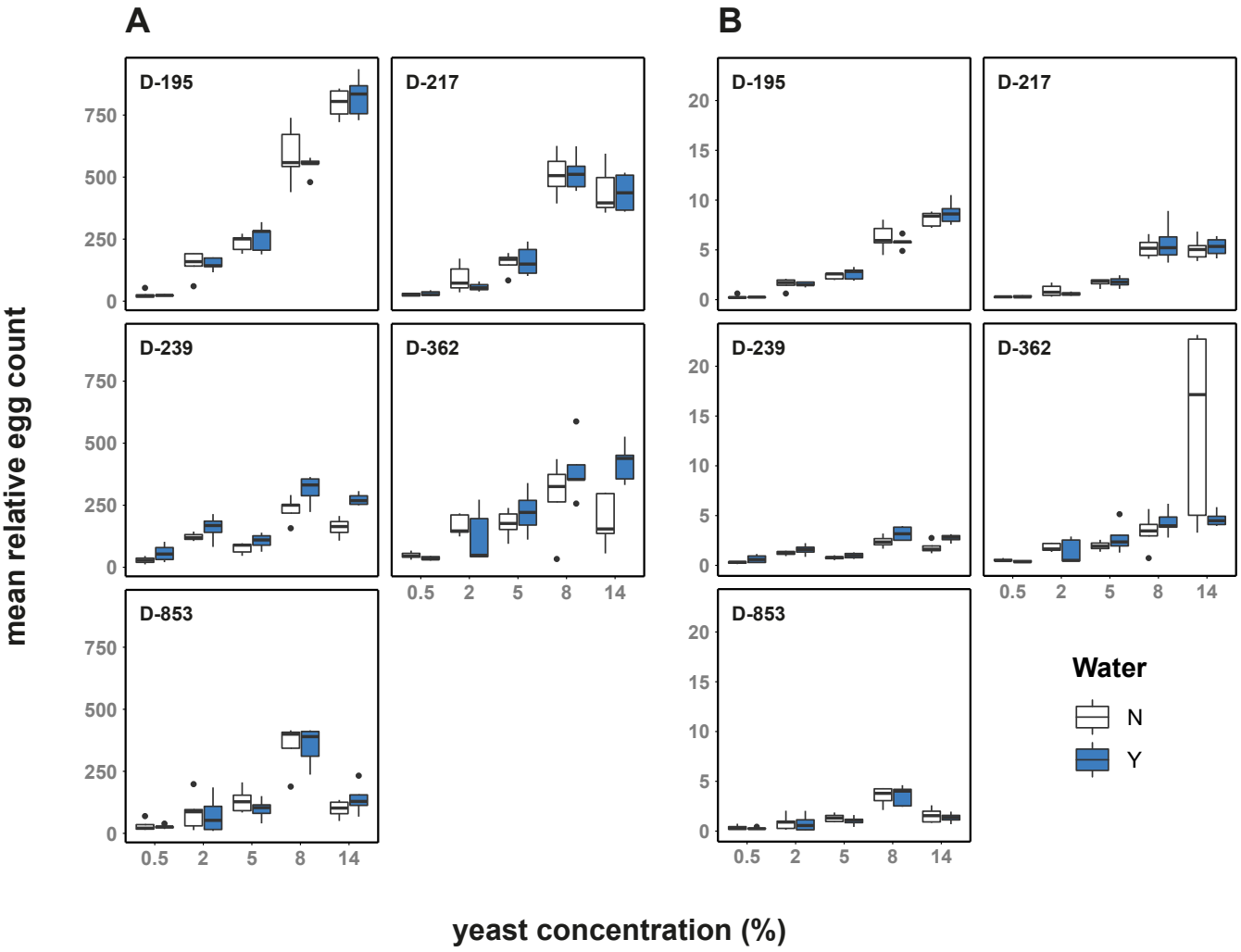


**Fig. S2. Fecundity analysis of panel under all conditions.** Fecundity has a positive relationship with dietary yeast concentration, except at the highest yeast concentration assayed (14%) for most genotypes. **A** - raw egg counts. **B** - mortality-corrected counts. Counts generated using QuantiFly software. Counts are relative, but directly comparable. Flies assayed at age 11-12 days, with boxplots aggregating totals (median, with the box depicting a quartile each way, and whiskers showing the range; outliers plotted as dots). Each cage was assayed on 1 scoring day at this age. Mortality corrected counts (**B**) generated by dividing raw counts, by N flies remaining in cage at the time of assaying. N = 25,519 females total; 4,800-5,282 per genotype. Note that DGRP-362 experienced significant mortality at this age under 14% yeast dietary treatment. This is the cause of the discrepancy in significance between raw, and age-adjusted fecundity counts.Note, egg-laying was not assessed throughout life and in natural circumstances lifespan of the fly is truncated by extrinsic factors, e.g. predation. We nonetheless, tentatively conclude that the enhanced mortality and reduced egg laying on very rich diets is caused by nutritional toxicity.

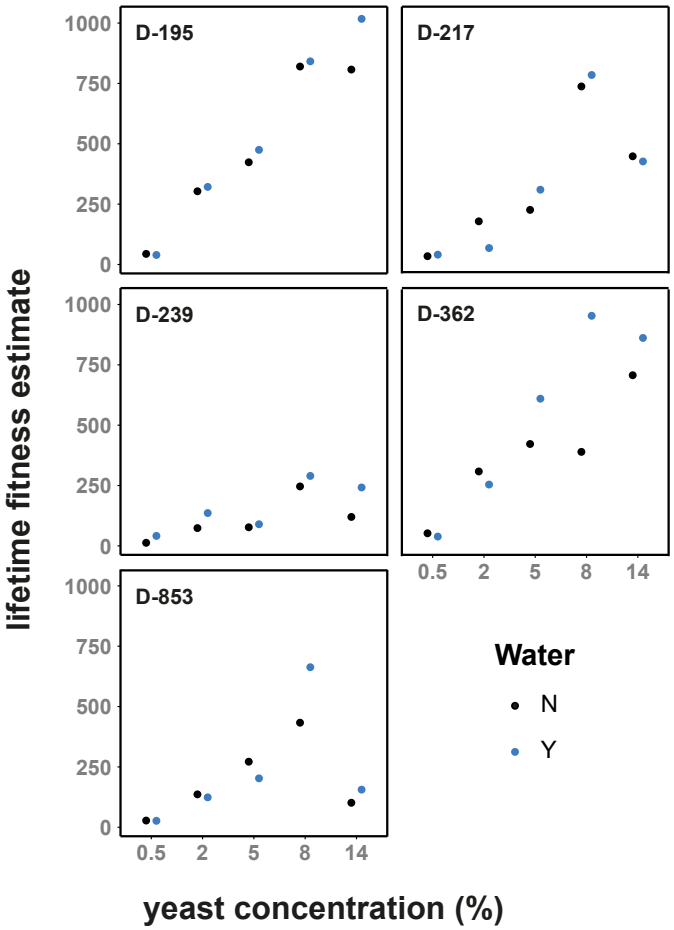


**Fig. S3. Lifetime reproductive fitness estimates of panel under all conditions.** Lifetime fitness has a positive relationship with dietary yeast concentration, except at the highest yeast concentration assayed (14%) for most genotypes. Mortality-adjusted egg counts from Fig. S2 were multiplied by the area under the relevant survival curve (restricted mean) to generate lifetime estimates. N = 25,519 females total; 4,800-5,282 per genotype.

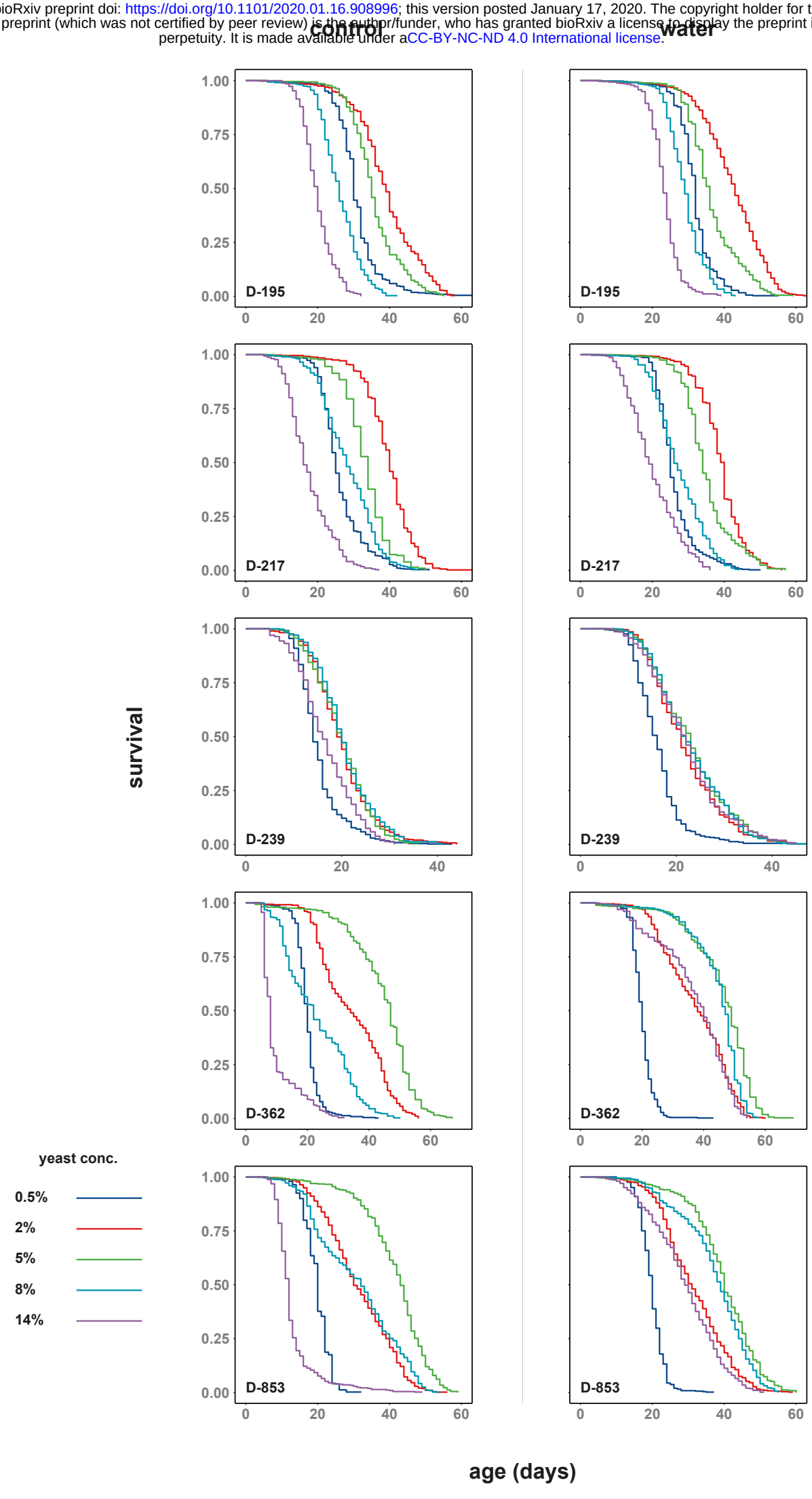

**Fig. S4. Survival curves of panel in response to diet.** Dietary reaction norms vary in a genotype-specific manner. Survival curves are separated by genotype, and water-supplementation status. N = 25,519 females total; 4,800-5,282 per genotype.

# Materials and Methods

## Fly husbandry, experimental protocol and demography cages

For lifespan experiments adults were provided with either 0.5%, 2%, 5%, 8% or 14% autolysed yeast media. All other media components (13% table sugar, 6% cornmeal, 1% agar and 0.225% [w/v] nipagin) remained the same, given the dietary protein axis is the main lifespan determinant in flies (Lee et al. 2008; Jensen et al. 2015). Purpose-built demography cages included two grommets, for the supplementation of food, and water-agar (2% agar) or empty vial. Cages contained between 70-125 females each (mode of ~ 100 females), with 5 cages per treatment, per genotype (N = 50 cages per genotype) with the exception of DGRP-195. This genotype consisted of an additional 2 cages of water-supplemented, and control cages at 2% which served as controls for a separate experiment. All experimental flies were mated on 8% media for 48 hours, and further grown on 8% media until age 3-4 days, when they were divided between dietary treatments. For a more detailed description, see McCracken et al., 2020.

## Fecundity

Feeding vials were imaged and analysed using QuantiFly (Waithe et al. 2015) to determine the relative quantity of egg laying. Feeding vials were removed, during normal scoring periods, from all cages containing eggs from flies aged 11 or 12; water agar vials were checked, but not assayed, given negligible egg counts.

## Treatment groups

Ten dietary regimes were imposed on several genotypes of female flies using 5 dietary yeast concentrations detailed above, with the addition, or absence of water-agar supplementation. To test the effect of water supplementation on longevity, we provided an additional vial of water-agar (‘water supplementation’), or an empty vial (‘control’), to each cage. Separation of food and water sources allowed flies to choose their source of nourishment, and eliminated the need for hydration to be coupled with food intake. Dietary treatments were balanced for age, and date of eclosion. All flies presented were grown within one batch. The experiment was carried out on a small panel of DGRP lines (Mackay et al. 2012); DGRP-195; 217; 239; 362; 853), at high sample size (N = 25, 519).

**Data analysis**

Mixed cox-proportional hazard models were used that included 'cage' as random term to correct for uncertainty of pseudo-replicated effects within demography cages (Ripatti and Palmgren 2000; Therneau, Grambsch, and Shane Pankratz 2003). Additional specific tests of coefficients are provided that combine the single and interaction term (in a z-test, using the maximum s.e. of the factors compared) to test how survival was changing in water-treated flies, compared to respective control treatments. For survival comparisons, we report the full model, and models fitted within each genotype separately. The latter corrects for deviations in proportionality of hazards between the genotypes. Qualitative conclusions remain similar, and formal tests for proportionality of hazards are not available for mixed effects cox regressions. Coefficients are reported as logged hazards with significance based on z-tests. Right-censoring was included, and dietary treatments were considered ordinal factors.

Egg laying was analysed via linear model of log-transformed fecundity count data. Age was not treated a variable given the one day disparity between assay ages. Experimentally, treatments and genotypes were split evenly over these ages. BIC with backward elimination of terms was used for model optimisation, and detailed a multiplicative interaction between genotype and diet alone. Water was added to our models to demonstrate its negligible effect on fecundity. Estimates from models are presented as the effect of diet; these were estimated from the same model. Additional specific tests of coefficients are also provided here. We specifically test the effect of 14% yeast, compared to 8% in the same combining coefficient manner as with survival.

For hazard ratio figures, ratios are plotted as coefficients derived from within-line Cox mixed-effect models, with error bars representing 95% confidence intervals.

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Table S1. Effect of diet and water supplementation on mortality across 5 DGRP lines (DGRP-195 is reference).

| | Full Model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| water | -0.390 | 0.677 | 0.228 | 0.086 |
| 0.5% yeast | -0.796 | 0.451 | 0.228 | <0.001 |
| 2% yeast | -1.856 | 0.156 | 0.206 | <0.001 |
| 5% yeast | -1.296 | 0.274 | 0.225 | <0.001 |
| 14% yeast | 1.043 | 2.837 | 0.226 | <0.001 |
| 217 | -0.444 | 0.641 | 0.226 | 0.05 |
| 239 | 0.763 | 2.145 | 0.227 | 0.001 |
| 362 | 0.104 | 1.110 | 0.227 | 0.645 |
| 853 | -1.090 | 0.336 | 0.223 | <0.001 |
| 0.5% yeast * water | 0.470 | 1.601 | 0.322 | 0.144 |
| 2% yeast * water | 0.030 | 1.031 | 0.300 | 0.92 |
| 5% yeast * water | 0.277 | 1.319 | 0.322 | 0.39 |
| 14% yeast * water | -0.156 | 0.856 | 0.322 | 0.629 |
| 217 * 0.5% yeast | 1.195 | 3.302 | 0.320 | <0.001 |
| 217 * 2% yeast | 0.632 | 1.881 | 0.310 | 0.042 |
| 217 * 5% yeast | 0.786 | 2.195 | 0.321 | 0.014 |
| 217 * 14% yeast | 0.643 | 1.903 | 0.322 | 0.046 |
| 239 * 0.5% yeast | 1.793 | 6.008 | 0.321 | <0.001 |
| 239 * 2% yeast | 1.887 | 6.599 | 0.308 | <0.001 |
| 239 * 5% yeast | 1.429 | 4.174 | 0.321 | <0.001 |
| 239 * 14% yeast | -0.382 | 0.683 | 0.324 | 0.238 |
| 362 * 0.5% yeast | 1.754 | 5.775 | 0.319 | <0.001 |
| 362 * 2% yeast | 0.262 | 1.300 | 0.311 | 0.399 |
| 362 * 5% yeast | -1.322 | 0.267 | 0.315 | <0.001 |
| 362 * 14% yeast | 1.880 | 6.551 | 0.316 | <0.001 |
| 853 * 0.5% yeast | 3.087 | 21.901 | 0.318 | <0.001 |
| 853 * 2% yeast | 2.029 | 7.604 | 0.307 | <0.001 |
| 853 * 5% yeast | 0.287 | 1.332 | 0.320 | 0.37 |
| 853 * 14% yeast | 2.100 | 8.168 | 0.320 | <0.001 |
| 217 * water | 0.551 | 1.736 | 0.322 | 0.087 |
| 239 * water | -0.101 | 0.904 | 0.322 | 0.754 |
| 362 * water | -2.015 | 0.133 | 0.319 | <0.001 |
| 853 * water | -0.101 | 0.904 | 0.323 | 0.753 |
| 217 * 0.5% yeast * water | -0.568 | 0.567 | 0.455 | 0.212 |
| 217 * 2% yeast * water | -0.114 | 0.893 | 0.441 | 0.797 |
| 217 * 5% yeast * water | -0.702 | 0.496 | 0.456 | 0.123 |
| 217 * 14% yeast * water | -0.448 | 0.639 | 0.456 | 0.327 |
| 239 * 0.5% yeast * water | -0.145 | 0.865 | 0.457 | 0.751 |
| 239 * 2% yeast * water | 0.126 | 1.134 | 0.440 | 0.775 |
| 239 * 5% yeast * water | -0.427 | 0.652 | 0.455 | 0.348 |
| 239 * 14% yeast * water | -0.383 | 0.682 | 0.457 | 0.402 |
| 362 * 0.5% yeast * water | 1.990 | 7.319 | 0.456 | <0.001 |
| 362 * 2% yeast * water | 2.068 | 7.912 | 0.439 | <0.001 |
| 362 * 5% yeast * water | 2.007 | 7.444 | 0.457 | <0.001 |
| 362 * 14% yeast * water | -1.942 | 0.143 | 0.451 | <0.001 |
| 853 * 0.5% yeast * water | -0.027 | 0.974 | 0.458 | 0.954 |
| 853 * 2% yeast * water | 0.351 | 1.420 | 0.441 | 0.427 |
| 853 * 5% yeast * water | 0.433 | 1.541 | 0.457 | 0.344 |
| 853 * 14% yeast * water | -2.056 | 0.128 | 0.455 | <0.001 |

Table S2. Effect of diet and water supplementation on mortality within DGRP-195.

| | Estimates from individual model | | | | Effect of water, compared to no water | | |
|---|---|---|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p | estimate | exp | p |
| water supplementation | -0.481 | 0.618 | 0.113 | <0.001 | | | |
| 0.5% yeast | -0.954 | 0.385 | 0.112 | <0.001 | | | |
| 2% yeast | -2.139 | 0.118 | 0.103 | <0.001 | | | |
| 5% yeast | -1.620 | 0.198 | 0.112 | <0.001 | | | |
| 14% yeast | 1.612 | 5.012 | 0.112 | <0.001 | | | |
| 0.5% yeast * water | 0.475 | 1.608 | 0.158 | 0.003 | -0.006 | 0.994 | 0.971 |
| 2% yeast * water | 0.156 | 1.169 | 0.147 | 0.289 | -0.324 | 0.723 | 0.028 |
| 5% yeast * water | 0.369 | 1.446 | 0.160 | 0.021 | -0.112 | 0.894 | 0.483 |
| 14% yeast * water | -0.301 | 0.740 | 0.159 | 0.059 | -0.781 | 0.458 | <0.001 |

Table S3. Effect of diet and water supplementation on mortality within DGRP-217.

| | Estimates from individual model | | | | Effect of water, compared to no water | | |
|---|---|---|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p | estimate | exp | p |
| water supplementation | 0.190 | 1.209 | 0.213 | 0.373 | | | |
| 0.5% yeast | 0.454 | 1.574 | 0.211 | 0.032 | | | |
| 2% yeast | -1.395 | 0.248 | 0.208 | <0.001 | | | |
| 5% yeast | -0.549 | 0.578 | 0.212 | 0.01 | | | |
| 14% yeast | 1.905 | 6.716 | 0.200 | <0.001 | | | |
| 0.5% yeast * water | -0.108 | 0.898 | 0.306 | 0.724 | 0.082 | 1.085 | 0.789 |
| 2% yeast * water | -0.105 | 0.901 | 0.306 | 0.732 | 0.085 | 1.089 | 0.781 |
| 5% yeast * water | -0.470 | 0.625 | 0.300 | 0.117 | -0.280 | 0.755 | 0.350 |
| 14% yeast * water | -0.668 | 0.513 | 0.306 | 0.029 | -0.479 | 0.620 | 0.118 |

Table S4. Effect of diet and water supplementation on mortality within DGRP-239.

| | Estimates from individual model | | | | Effect of water, compared to no water | | |
|---|---|---|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p | estimate | exp | p |
| water supplementation | -0.343 | 0.710 | 0.105 | 0.001 | | | |
| 0.5% yeast | 0.946 | 2.575 | 0.104 | <0.001 | | | |
| 2% yeast | 0.070 | 1.072 | 0.104 | 0.502 | | | |
| 5% yeast | 0.094 | 1.098 | 0.103 | 0.363 | | | |
| 14% yeast | 0.572 | 1.771 | 0.103 | <0.001 | | | |
| 0.5% yeast * water | 0.173 | 1.188 | 0.148 | 0.243 | -0.170 | 0.843 | 0.249 |
| 2% yeast * water | 0.079 | 1.082 | 0.148 | 0.593 | -0.264 | 0.768 | 0.075 |
| 5% yeast * water | -0.108 | 0.898 | 0.147 | 0.464 | -0.451 | 0.637 | 0.002 |
| 14% yeast * water | -0.476 | 0.621 | 0.147 | 0.001 | -0.819 | 0.441 | <0.001 |

Table S5. Effect of diet and water supplementation on mortality within DGRP-362.

| | Estimates from individual model | | | | Effect of water, compared to no water | | |
|---|---|---|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p | estimate | exp | p |
| water supplementation | -2.330 | 0.097 | 0.363 | <0.001 | | | |
| 0.5% yeast | 0.763 | 2.144 | 0.356 | 0.032 | | | |
| 2% yeast | -1.520 | 0.219 | 0.383 | <0.001 | | | |
| 5% yeast | -2.638 | 0.072 | 0.351 | <0.001 | | | |
| 14% yeast | 2.393 | 10.950 | 0.339 | <0.001 | | | |
| 0.5% yeast * water | 2.358 | 10.571 | 0.545 | <0.001 | 0.028 | 1.029 | 0.958 |
| 2% yeast * water | 2.016 | 7.509 | 0.482 | <0.001 | -0.314 | 0.731 | 0.515 |
| 5% yeast * water | 2.213 | 9.145 | 0.597 | <0.001 | -0.116 | 0.890 | 0.845 |
| 14% yeast * water | -1.564 | 0.209 | 0.549 | 0.004 | -3.894 | 0.020 | <0.001 |

Table S6. Effect of diet and water supplementation on mortality within DGRP-853.

| | Estimates from individual model | | | | Effect of water, compared to no water | | |
|---|---|---|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p | estimate | exp | p |
| water supplementation | -0.473 | 0.623 | 0.259 | 0.067 | | | |
| 0.5% yeast | 1.987 | 7.293 | 0.239 | <0.001 | | | |
| 2% yeast | 0.150 | 1.162 | 0.254 | 0.554 | | | |
| 5% yeast | -0.967 | 0.380 | 0.248 | <0.001 | | | |
| 14% yeast | 2.955 | 19.204 | 0.223 | <0.001 | | | |
| 0.5% yeast * water | 0.436 | 1.547 | 0.399 | 0.274 | -0.037 | 0.964 | 0.926 |
| 2% yeast * water | 0.379 | 1.461 | 0.365 | 0.299 | -0.094 | 0.910 | 0.797 |
| 5% yeast * water | 0.680 | 1.973 | 0.373 | 0.068 | 0.206 | 1.229 | 0.58 |
| 14% yeast * water | -2.083 | 0.125 | 0.339 | <0.001 | -2.556 | 0.078 | <0.001 |

Table S7. Effect of diet on mortality across 5 water-supplemented DGRP lines (DGRP-195 is reference).

| | Full Model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| 0.5% yeast | -0.345 | 0.709 | 0.228 | 0.131 |
| 2% yeast | -1.921 | 0.146 | 0.202 | <0.001 |
| 5% yeast | -1.060 | 0.347 | 0.224 | <0.001 |
| 14% yeast | 0.930 | 2.535 | 0.225 | <0.001 |
| 217 | 0.107 | 1.113 | 0.228 | 0.638 |
| 239 | 0.683 | 1.980 | 0.228 | 0.003 |
| 362 | -1.991 | 0.137 | 0.218 | <0.001 |
| 853 | -1.242 | 0.289 | 0.221 | <0.001 |
| 217 * 0.5% yeast | 0.658 | 1.931 | 0.320 | 0.04 |
| 217 * 2% yeast | 0.567 | 1.764 | 0.311 | 0.068 |
| 217 * 5% yeast | 0.096 | 1.101 | 0.321 | 0.764 |
| 217 * 14% yeast | 0.206 | 1.229 | 0.324 | 0.524 |
| 239 * 0.5% yeast | 1.771 | 5.877 | 0.318 | <0.001 |
| 239 * 2% yeast | 2.119 | 8.326 | 0.305 | <0.001 |
| 239 * 5% yeast | 1.042 | 2.834 | 0.321 | 0.001 |
| 239 * 14% yeast | -0.799 | 0.450 | 0.322 | 0.013 |
| 362 * 0.5% yeast | 3.937 | 51.246 | 0.315 | <0.001 |
| 362 * 2% yeast | 2.434 | 11.404 | 0.307 | <0.001 |
| 362 * 5% yeast | 0.691 | 1.995 | 0.318 | 0.03 |
| 362 * 14% yeast | -0.071 | 0.932 | 0.323 | 0.826 |
| 853 * 0.5% yeast | 3.228 | 25.234 | 0.312 | <0.001 |
| 853 * 2% yeast | 2.482 | 11.960 | 0.303 | <0.001 |
| 853 * 5% yeast | 0.739 | 2.094 | 0.322 | 0.022 |
| 853 * 14% yeast | 0.033 | 1.033 | 0.319 | 0.918 |

Table S8. Effect of diet on mortality within water-supplemented DGRP-195.

| | Estimates from individual model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| 0.5% yeast | -0.512 | 0.599 | 0.098 | <0.001 |
| 2% yeast | -2.124 | 0.120 | 0.100 | <0.001 |
| 5% yeast | -1.322 | 0.267 | 0.102 | <0.001 |
| 14% yeast | 1.410 | 4.095 | 0.100 | <0.001 |

Table S9. Effect of diet on mortality within water-supplemented DGRP-217.

| | Estimates from individual model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| 0.5% yeast | 0.357 | 1.429 | 0.214 | 0.095 |
| 2% yeast | -1.487 | 0.226 | 0.215 | <0.001 |
| 5% yeast | -1.011 | 0.364 | 0.216 | <0.001 |
| 14% yeast | 1.260 | 3.527 | 0.215 | <0.001 |

Table S10. Effect of diet on mortality within water-supplemented DGRP-239.

| | Estimates from individual model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| 0.5% yeast | 1.114 | 3.046 | 0.119 | <0.001 |
| 2% yeast | 0.150 | 1.162 | 0.118 | 0.204 |
| 5% yeast | -0.012 | 0.988 | 0.117 | 0.919 |
| 14% yeast | 0.096 | 1.101 | 0.118 | 0.413 |

Table S11. Effect of diet on mortality within water-supplemented DGRP-362.

| | Estimates from individual model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| 0.5% yeast | 3.631 | 37.764 | 0.386 | <0.001 |
| 2% yeast | 0.518 | 1.678 | 0.379 | 0.172 |
| 5% yeast | -0.487 | 0.614 | 0.380 | 0.2 |
| 14% yeast | 0.920 | 2.509 | 0.379 | 0.015 |

Table S12. Effect of diet on mortality within water-supplemented DGRP-853.

| | Estimates from individual model | | | |
|---|---|---|---|---|
| coefficient | estimate | exp | s.e. | p |
| 0.5% yeast | 2.811 | 16.623 | 0.222 | <0.001 |
| 2% yeast | 0.564 | 1.759 | 0.216 | 0.009 |
| 5% yeast | -0.284 | 0.753 | 0.216 | 0.19 |
| 14% yeast | 0.923 | 2.518 | 0.216 | <0.001 |

Table S13. Effect of diet and water supplementation on fecundity across 5 DGRP lines, derived from linear model estimates oflog-transformed raw fecundity counts (DGRP-195 is reference). Counts generated using QuantiFly software. Counts are relative, but directly comparable.

| | Full Model | | | Compared to 8% | |
|---|---|---|---|---|---|
| coefficient | estimate | s.e. | p | estimate | p |
| intercept | 2.731 | 0.067 | <0.001 | | |
| water | 0.039 | 0.027 | 0.15 | | |
| 0.5% yeast | -1.374 | 0.092 | <0.001 | | |
| 2% yeast | -0.595 | 0.092 | <0.001 | | |
| 5% yeast | -0.367 | 0.092 | <0.001 | | |
| 14% yeast | 0.157 | 0.092 | 0.09 | 2.888 | <0.001 |
| 217 | -0.044 | 0.092 | 0.634 | | |
| 239 | -0.331 | 0.095 | 0.001 | | |
| 362 | -0.294 | 0.092 | 0.002 | | |
| 853 | -0.221 | 0.095 | 0.021 | | |
| 217 * 0.5% yeast | 0.113 | 0.130 | 0.387 | | |
| 217 * 2% yeast | -0.268 | 0.134 | 0.048 | | |
| 217 * 5% yeast | -0.164 | 0.132 | 0.216 | | |
| 217 * 14% yeast | -0.224 | 0.132 | 0.092 | -0.110 | 0.404 |
| 239 * 0.5% yeast | 0.509 | 0.139 | <0.001 | | |
| 239 * 2% yeast | 0.310 | 0.139 | 0.027 | | |
| 239 * 5% yeast | -0.119 | 0.134 | 0.373 | | |
| 239 * 14% yeast | -0.262 | 0.136 | 0.056 | -0.436 | 0.001 |
| 362 * 0.5% yeast | 0.524 | 0.130 | <0.001 | | |
| 362 * 2% yeast | 0.212 | 0.130 | 0.106 | | |
| 362 * 5% yeast | 0.180 | 0.130 | 0.168 | | |
| 362 * 14% yeast | -0.202 | 0.130 | 0.122 | -0.339 | 0.009 |
| 853 * 0.5% yeast | 0.249 | 0.134 | 0.065 | | |
| 853 * 2% yeast | -0.233 | 0.132 | 0.079 | | |
| 853 * 5% yeast | -0.137 | 0.132 | 0.3 | | |
| 853 * 14% yeast | -0.657 | 0.134 | <0.001 | -0.721 | <0.001 |

Table S14. Effect of diet and water supplementation on fecundity across 5 DGRP lines, derived from linear model estimates oflog-transformed age-adjusted fecundity counts (DGRP-195 is reference). Counts generated using QuantiFly software. Counts are relative, but directly comparable.

| | Full Model | | | Compared to 8% | |
|---|---|---|---|---|---|
| coefficient | estimate | s.e. | p | estimate | p |
| intercept | 0.776 | 0.067 | <0.001 | | |
| water | -0.004 | 0.027 | 0.873 | | |
| 0.5% yeast | -1.379 | 0.093 | <0.001 | | |
| 2% yeast | -0.602 | 0.093 | <0.001 | | |
| 5% yeast | -0.384 | 0.093 | <0.001 | | |
| 14% yeast | 0.148 | 0.093 | 0.114 | 0.925 | <0.001 |
| 217 | -0.050 | 0.093 | 0.594 | | |
| 239 | -0.350 | 0.096 | <0.001 | | |
| 362 | -0.239 | 0.093 | 0.011 | | |
| 853 | -0.241 | 0.096 | 0.013 | | |
| 217 * 0.5% yeast | 0.101 | 0.132 | 0.444 | | |
| 217 * 2% yeast | -0.301 | 0.136 | 0.028 | | |
| 217 * 5% yeast | -0.113 | 0.134 | 0.398 | | |
| 217 * 14% yeast | -0.165 | 0.134 | 0.218 | -0.067 | 0.617 |
| 239 * 0.5% yeast | 0.542 | 0.141 | <0.001 | | |
| 239 * 2% yeast | 0.311 | 0.141 | 0.028 | | |
| 239 * 5% yeast | -0.120 | 0.136 | 0.378 | | |
| 239 * 14% yeast | -0.237 | 0.138 | 0.087 | -0.439 | 0.001 |
| 362 * 0.5% yeast | 0.485 | 0.132 | <0.001 | | |
| 362 * 2% yeast | 0.177 | 0.132 | 0.182 | | |
| 362 * 5% yeast | 0.186 | 0.132 | 0.16 | | |
| 362 * 14% yeast | 0.166 | 0.132 | 0.209 | 0.075 | 0.568 |
| 853 * 0.5% yeast | 0.284 | 0.136 | 0.038 | | |
| 853 * 2% yeast | -0.227 | 0.134 | 0.091 | | |
| 853 * 5% yeast | -0.111 | 0.134 | 0.406 | | |
| 853 * 14% yeast | -0.550 | 0.136 | <0.001 | -0.643 | <0.001 |