The evolutionary potential of diet-dependent effects on lifespan and fecundity in a multi-parental population of *Drosophila melanogaster*

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Abstract

The nutritional conditions experienced by a population play a major role in shaping trait evolution in many taxa. Constraints exerted by nutrient limitation or nutrient imbalance can influence the maximal value that fitness components such as reproduction and lifespan attains, and organisms may shift how resources are allocated to different structures and functions in response to changes in nutrition. Whether the phenotypic changes associated with changes in nutrition represent an adaptive response is largely unknown, and whether the response of fitness components to diet even has the potential to evolve in response to selection is not generally known. In this study, we use an admixed multiparental population of *Drosophila melanogaster* reared in three different diet conditions to estimate quantitative genetic parameters for lifespan and fecundity. We develop a new image analysis technique for estimating fecundity, removing the need to hand count eggs and use this method to show there is significant genetic variation for fecundity in our population. Our results also show that lifespan has moderate to high heritabilities within diets, and genetic correlations for lifespan between diets that are less than one demonstrate a genotype by diet interaction. These findings demonstrate substantial standing genetic variation in our population that is comparable to natural populations and highlights the potential for adaptation to changing nutritional environments.

Key words: life history, heritability, genotype by environment interaction, *Drosophila melanogaster,* multiparental population

Introduction

An organism's diet has a direct influence on nearly all phenotypes by determining the amount of nutrients and energy available to build structures and perform functions. In addition to this unavoidable direct effect, organisms may also alter how resources are allocated to different traits when experiencing different diets. In an environment where resource availability varies across space and/or time, and the optimal allocation of resources changes with diet, we expect to see the evolution of such phenotypically plastic resource allocation strategies.

In many non-human populations including nematode, fruit fly (Chippindale *et al.*, 1993b), killifish (Vrtílek and Reichard, 2014), waterstrider (Kaitala, 1991) and mouse (Sprott, 1997), nutrient limitation without starvation results in lifespan extension, and is often but not always accompanied by a reduction in reproductive output (Hsin and Kenyon, 1999; Barnes *et al.*, 2006; Flatt *et al.*, 2008). These diet-dependent changes have been hypothesized to result from an increased allocation of a limited nutritional resource to somatic maintenance (van Noordwijk and de Jong, 1986; Sohal and Weindruch, 1996; Simmons and Bradley, 1997; Hughes and Reynolds, 2005), and a decreased allocation to reproduction. Previous models predict this pattern is adaptive in environments with fluctuating resource availability (Neel, 1962; Wells, 2009; Fisher *et al.*, 2010; Warren *et al.*, 2013). However, these models have not been formally tested, nor has the evolutionary potential of the focal traits been established in most systems (Ng'oma *et al.*, 2017), which is an important first step.

The capacity for a given trait to evolve in a population depends on the proportion of trait variation that is attributable to genetic variation and the intensity of selection

61 acting on it (Roff 2006; Brčić-Kostić, 2010). Selection can have a direct effect on trait
62 evolution if the trait directly affects fitness, or indirectly if the trait is correlated with a trait
63 under selection (Lande and Arnold, 1983; Hunt *et al.*, 2004). However, even when a
64 selective pressure is observed to act on the trait, the amount of evolutionary change
65 depends on the amount of genetic variation expressed for that trait (Fisher, 1930).
66 Quantitative genetic parameters, such as the heritability of a given trait, remain some of
67 the more informative parameters we can estimate in order to learn about the genetic
68 basis of a trait, to predict trait values in a population, and to predict the selection
69 response, even with the advances in genomics and molecular genetics in recent years.
70 Studies in many species have frequently demonstrated genotype by diet variation for
71 complex traits, such as gene expression and fitness in yeast (Gagneur *et al.*, 2013),
72 cuticular hydrocarbons in *D. simulans* (Ingleby *et al.*, 2013), and metabolic phenotypes
73 in *D. melanogaster* (Reed *et al.*, 2010, 2014; Qi *et al.*, 2012). These studies suggest
74 that genotype by environment interactions (GEI) are common and could be important in
75 the nutrition-dependent response of life history traits such as fecundity and lifespan.
76 There has been a significant effort to understand the genetic basis for the dietary
77 restriction (DR) response with both molecular genetic approaches (Mair and Dillin,
78 2008; Grandison *et al.*, 2009; Teleman, 2010; Nässel *et al.*, 2015) and mapping
79 approaches (Rikke *et al.*, 2010; Stanley *et al.*, 2017). As is the case for most complex
80 traits, mapping approaches to identify the genetic loci determining how lifespan changes
81 with diet have failed to identify the specific contributors. Studies focused on mapping in
82 natural populations often do not identify the regions of the genome as QTL that harbor
83 candidate genes identified by molecular approaches (Remolina *et al.*, 2012; Burke,

King, *et al.*, 2014; Reed *et al.*, 2014; Megan Ulmer Carnes *et al.*, 2015) and there are several studies in which gene expression patterns do not change in the direction one would expect from these loss-of-function studies (Min *et al.*, 2008; Giannakou *et al.*, 2008; Stanley *et al.*, 2017). In light of these studies, a possible hypothesis is that the DR response is highly polygenic and best understood by quantifying holistic measures of genetic variation, such as the heritability and GEI. Additionally, it is possible that the limited genetic variation included in QTL mapping approaches limits the ability to identify causative variants that are segregating in natural populations. The majority of QTL studies in the past have used only two genomes to create the mapping population. More recently, multiparent populations (MPPs) have been gaining in popularity, where multiple (typically at least 4, and often many more) genomes are used to seed the population, thereby including increased genetic variability (Kover *et al.*, 2009; McMullen *et al.*, 2009; Huang *et al.*, 2011; Aylor *et al.*, 2011; Threadgill and Churchill, 2012; King, Macdonald, *et al.*, 2012; King, Merkes, *et al.*, 2012; Cubillos *et al.*, 2013).

In this paper, we employ a half-sibling, split environment design using an admixed multiparent population derived from an established mapping resource, the *Drosophila* Synthetic Population Resource (DSPR), to estimate quantitative genetic parameters for lifespan and fecundity in three different diets. We are able to estimate the evolutionary potential of how lifespan and fecundity respond to diet in order to establish, 1) the ability of these traits to evolve in general, 2) demonstrate levels of genetic variation that are comparable with wild derived populations, and 3) establish the validity of the evolve and resequence approach using a synthetic population as a base population.

Methods

Experimental setup

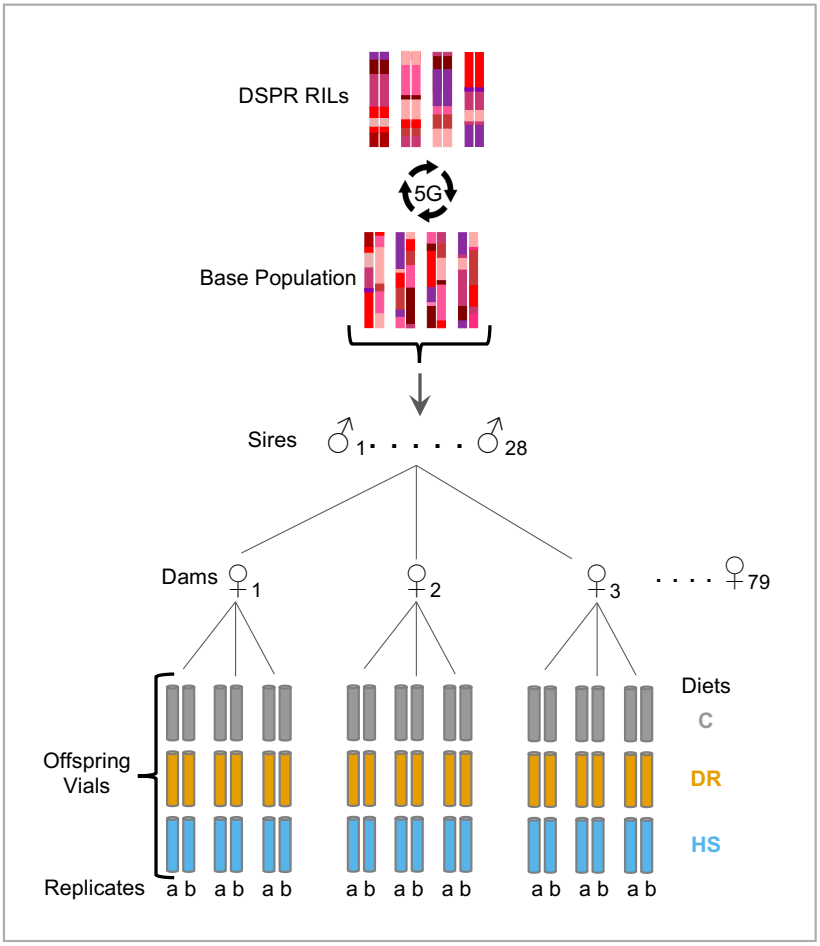
Generation of experimental population. We used an eight-way multi-parent population, the DSPR, to characterize the phenotypic response of lifespan and fecundity of mated female *D. melanogaster* to three nutritional environments and to estimate the heritabilities of these traits. The development, and genetic properties of the DSPR are described in detail in (King, Macdonald, *et al.*, 2012; King, Merkes, *et al.*, 2012). Briefly, the DSPR is a set of recombinant inbred lines created from 8 inbred founder lines that were crossed for 50 generations and then inbred via full sibling mating for 25 generations. To generate our starting population, for each of 835 recombinant inbred lines (RILs) of the *B* sub-population of the DSPR, five young females that had mated intra-line in vials, were randomly selected and distributed to each of six cages (20.3 cm x 20.3 cm x 20.3 cm). Eggs were collected in milk bottles (250 mL) twice on the next two successive days, each after a 22-hour oviposition period on maintenance diet (Table S1). Eclosed flies were released to six cages in populations >2,000 per cage and allowed to mate *en-masse* for five generations. A polystyrene plate, 100 mm x 15 mm (cat. No. FB0875713 Fisher Scientific, USA) containing maintenance food was supplied to each cage three times a week (Monday, Wednesday, Friday). A separate micro-plate (60 mm x 15 mm, cat. no. FB0875713A, Fisher Scientific, USA) with moist cotton wool was supplied to each cage to serve as a supplemental drinking source. Very thin slices of food with visually estimated 50-90 eggs were transferred to 30 vials (25 mm x 95 mm, Polystyrene Reload, cat. no. 32-109RL, Genesee Scientific, USA) for each cage for each successive generation. Egg vials from each cage for each next

130 generation were distributed across all cages to ensure a genetically homogenous
131 experimental population. We maintained all populations on a three week cycle (21 days
132 from oviposition to egg collection) on a cornmeal-dextrose-yeast diet for 5 generations
133 before the half-sibling experiment was started.

134 *Experimental design.* We employed a split-environment, half-sibling breeding
135 design to determine the phenotypic effects of three dietary conditions and estimate
136 quantitative genetic parameters. We created a set of paternal half-sibling families by
137 sequentially mating each of 28 males to three females (here on, sires and dams,
138 respectively) on a common cornmeal-dextrose-yeast (maintenance) diet (Table S1).
139 Each dam was housed in its own vial and each sire rotated among these three vials. In
140 round one, to ensure each dam had a mating opportunity early, the sire spent 2 days
141 with each dam. Each sire was then rotated among the three dams for two additional
142 rounds lasting 3.5 days each encounter. Two dams were kept waiting in media vials
143 when the sire was matched with the third dam. Whole families were restarted with new
144 flies when a sire died during crossing. In case of death of a waiting unmated dam, only
145 that dam was replaced, whereas if a mated dam died, a new dam family was restarted.
146 Starting with 61 sires and 183 dams, 28 half-sibling families and 77 maternal full-sibling
147 families successfully produced enough offspring to be included in the experiment (Fig.
148 1).

149 From each of the 77 dams, in most cases, we set up two replicate vials of 24
150 female and 6 male offspring in each of three diet treatments (described below). We
151 included males to provide mating opportunities to females throughout their lifetime but
152 chose a smaller number of males to reduce harassment of females within the vials. As

153 expected, even successful sire and dam pairs did not always produce enough offspring
154 for a full complement. Thus, some vials contained fewer than 24 females though never
155 below 15 females, and the dataset is not fully balanced with all sire families having all
156 three dams, split in all three diets, and with two replicates per diet. The realized totals of
157 sire half-sibling families and dam full-sibling families available for analysis in each diet
158 are presented in Table S2.
159



160

161 Figure 1: Experimental design. A set of 835 DSPR RILs were crossed for 5 generations
162 to create an outbred synthetic population. From this population, 28 randomly chosen

163 sires were each mated to 3 dams (actual N = 77 ♀). The resulting offspring from each
164 dam were split to each of C, DR, and HS diet treatments with two replicate vials in each
165 diet.
166 *Diet treatments.* Offspring of each sire were split across three experimental diets:
167 control (C), dietary restriction (DR), and high sugar (HS). These diets were also used in
168 our previous mapping study using the DSPR RILs by Stanley *et al*. (2017) and the
169 composition of each is detailed in Table S1. To preserve quality, diets were stored at 4
170 °C and used within two weeks of preparation. To permit phenotype measurement and
171 ensure that food did not degrade, individuals were moved to vials with fresh food three
172 times per week. All flies in all experiments described here were reared in a growth
173 chamber at 23˚C, ≥ 50% relative humidity, and a 24:0 light:dark cycle.
174 *Phenotype measurement*
175 We measured two phenotypes: 1) lifespan - the total number of days each fly
176 lived from the date of eclosion, and 2) weekly fecundity - the total number of eggs a set
177 of females in each vial produced within a 24-hour period measured once per week. Both
178 phenotypes were measured from the same set of experimental vials over a full lifespan.
179 For lifespan, the number and sex of dead, and number of censored (i.e., escapees,
180 accidental deaths) events were recorded three times each week during transfer of flies
181 to new food until all flies in each vial had died. To estimate fecundity, females were
182 provided with fresh media each Monday and following a 24 hour egg laying period,
183 these egg vials were collected on Tuesday and stored at -20 ºC until processing. We
184 continued this process each week until all females within a vial had died. We modified a
185 protocol developed in Michael Rose's laboratory by Larry Gonzaga Cabral at UC Irvine

186 based on personal communication and video tutorials (Rose Lab Data 2012a,b). A
187 vacuum apparatus was custom prepared following recommendations in the links above.
188 We added 2.5 mL of 50% bleach to each vial (frozen or thawed) and gently swirled for
189 about 2 min 50 s on an ORBITRON Rotator 1 (Boekel Scientific, USA) to separate eggs
190 from the media. Eggs were flushed from each vial onto a black filter disc fit in a vacuum
191 apparatus with a brief spray of embryo solution (12% Triton X-100, SIGMA-ALDRICH,
192 USA) followed by a spray of water to rinse. As a quality check, flask filtrate was re-
193 filtered to a new disc periodically, and processed vials inspected under a dissecting
194 microscope. The filter discs were prepared from black landscape fabric (DEWITT Weed
195 Barrier Pro, www.dewittcompany.com) to a radius of 3.65 cm. Discs bearing eggs were
196 photographed using a Canon EOS Rebel TS*i* (Canon Inc, Japan) camera vertically
197 attached to a steel clamp using the following settings: exposure time 1/25 seconds,
198 aperture F5.6, and ISO 100. Egg-bearing discs were mounted on a black stage placed
199 within a cubical photo studio 35cm x 35cm x 35cm constructed with PVC frame and
200 enclosed by a white fabric (Fig. S1). The entire setup was placed in a dark room lit only
201 by two facing white light lamps, 23W, 1200 lumens (model # LBP23PAR2/5K,
202 UTILITECH, China) clamped to the stage outside the studio box to allow for soft uniform
203 lighting across the imaging platform. Image files were uploaded to a computing system
204 and immediately inspected with ImageJ (Abramoff *et al.*, 2004) for quality.
205 *Estimation of egg counts*
206 We developed a predictive model to automate the counting of eggs from the
207 images we generated as described above. To train the model and assess its
208 performance, eggs on 345 discs were manually counted using the *Multi-point* tool in

ImageJ (Abramoff *et al.*, 2004). To ensure that all possible imaging conditions were available for model training, manually counted discs were selected across the whole lifespan series and across all three diet treatments. Some background reflectivity of the discs and small debris present meant that some non-zero white area was present in almost all images, so our training set includes an over-representation of images with relatively few eggs, to prevent upward bias in the fecundity predictions.

Each image was manually cropped to be square at the edges of the disc, and the background outside the disc was set to black to eliminate any reflectivity or debris occurring outside of the disc using GNU Image Manipulation Program (GIMP v.2.8; The GIMP team 2015; http://www.gimp.org). Subsequently, the image was binarized by thresholding 8 bit grayscale values using the opencv (Bradski, 2000) package in python (ver 3.6; https://github.com/EGKingLab/h2lifespan). From this thresholded image, the area of white pixels was summed (https://github.com/EGKingLab/h2lifespan). Because images were not all the same size due to slight variations in camera position, we included size of the image as a covariate in the linear model predicting egg count from white pixel area and image dimension:

$$Egg\ Count = \beta_0 + \beta_1 \sqrt{Area} + \beta_2\ ImageSize$$

where *Area* is the sum of the white pixels and *Image Size* is the linear dimension of one edge of the image in pixels. We use $\sqrt{Area}$ because pixel area is predicted to scale as a square of egg count.

The optimal grayscale threshold value was determined by cross-validation using R (v. 3.5; https://github.com/EGKingLab/h2lifespan). We tested threshold values between 30 and 150 (for 8-bit images with 256 levels of gray), across a range of

training/testing data splits (0.5 to 0.9) and a range of proportions of data (0.5 to 1.0). The latter was used to test the adequacy of our data. Each model was fit 1,000 times at each combination of threshold, train/test split, and data proportion, with the root mean squared error (RMSe) retained for each iteration (https://github.com/EGKingLab/h2lifespan). The optimal threshold value was determined from the mean RMSe for the 1,000 iterations at each combination (Fig. 2a, b). That threshold was 53 for the training set, using 90% of the data each iteration for training, and only required using 50% of the total data in any one iteration. The resulting predictive model performed well for our training set (see Results). The trained model was then used to predict egg counts for all 3,768 images (Fig. 2c). Images with negative predicted egg counts were set to 0. All images were also visually inspected, and those determined to be of poor quality, with excessive background reflectivity, were counted manually and were not included in the training or prediction set.

Statistical analysis

All following statistical analyses were performed in R (ver. 3.5; R Core Team 2018), and all code is available online (https://github.com/EGKingLab/h2lifespan).

Survival estimates

We converted daily counts of dead and censored individuals to individual events at a given age to perform survival analysis. Using the R package survival (ver. 2.38; (Therneau and Grambsch, 2000; Therneau, 2015), we used the Kaplan-Meier estimator (Kaplan and Meier, 1958) to estimate survival in each diet:

```
survfit(Surv(time, status) ~ diet)
```

where time is time (days), status is alive/dead, diet is diet treatment (C, DR, HS) and to visualize survivorship curves in each diet.

To assess statistical differences between diets accounting for family membership, we used hierarchical Bayesian inference to test for the effects of sire, dam nested in sire, and treatment using a set of seven nested models, which tested variously for effects of diet treatment, sire and dam, identical to the models used to test survival estimates (Table 1). We used the brms interface (Bürkner, 2017) to the statistical modeling language stan (Carpenter *et al.*, 2017), which samples posterior estimates of modeled parameters using Hamiltonian Monte Carlo. Lifespan was modeled with a Weibull distribution, which is commonly used to analyze time to failure (in this case, fly death). We used broad priors for the Weibull shape parameter ($\Gamma(0.01, 0.01)$), Student's $t(3, 4, 10)$ for the intercept, and Student's $t(3, 0, 10)$ for the standard deviation, which were quickly overwhelmed by the large amount of data (7486 observations). Models were compared using leave-one-out (loo) cross-validation and loo model weights (Vehtari *et al.*, 2017; Yao *et al.*, 2018), the latter which assigns relative out of sample predictive ability to each model compared. Posterior estimates of DR and HS diet treatments were compared to the STD diet treatment via credible intervals. We sampled each model for 2000 iterations with 50% discarded for warm-up and 4 replicate chains, to yield approximately 4000 total samples. We found that due to the large amount of data, this sampling approach was adequate. Adequate sampling was determined by $\hat{R}$ values of 1 (Gelman and Rubin, 1992; Brooks and Gelman, 1998).

Fecundity estimates

We focused on two fecundity measurements from our weekly fecundity measures. We calculated the number of eggs per female for each of our weekly measures by dividing by the number of females alive in each vial. We then obtained an estimate of total fecundity per female by simply summing across weeks (hereafter referred to as total fecundity). We note that this is not strictly a measure of lifetime fecundity as we measured fecundity only once per week, though we would expect this estimate to be highly correlated with lifetime fecundity. Second, we considered a snapshot of early life fecundity by choosing the time point closest to 5 days post-eclosion (hereafter referred to as early fecundity). The actual age of the females varies slightly as the vials were set up over several days, but our fecundity measurements always took place on a 24 hour period beginning on Monday.

We tested for the effects of sire, dam nested in sire, and treatment using a set of seven nested hierarchical (mixed) linear models (Gelman and Hill, 2007), which tested variously for effects of diet treatment, sire and dam, identical to the models used to test survival estimates (Table 1). Sampling was done on zero-centered values for early life and total fecundity using Hamiltonian Monte Carlo using the statistical modeling language stan (Carpenter *et al.*, 2017), via the rstanarm interface (ver. 2.13.1; (Stan Development Team, 2016). We used mildly regularizing priors: Normal(0, 10) for intercepts, Normal(0, 10) for diet treatment parameters, and Cauchy(0, 1) for variances. Priors for covariance matrices were set to 1 for regularization, concentration, shape, and scale (i.e., decov(1, 1, 1, 1)). Models were sampled for 20,000 iterations, with 10,000 discarded for burnin. Adequate sampling was assessed by $\hat{R}$, and models were

compared using leave-one-out (loo) cross-validation and loo model weights as described above.

Heritability in single diets

To estimate variance components, heritability, and phenotypic correlations, we used MCMCglmm (v. 2.24; Hadfield, 2010), a Bayesian inference package for R (v. 3.5; R Core Team, 2016) to fit variations of the 'animal model' (Kruuk, 2004; Wilson *et al.*, 2010; Ingleby *et al.*, 2013). Using the animal model we first inferred, in each diet separately, the additive genetic effect (V_A) from pedigree information where the identities of sire and dam are included in the random effect

$$\mathbf{y} \sim \beta + \mathbf{Zu}$$

where **y** is the vector of phenotypes (lifespan, early fecundity, or total fecundity), β is the mean phenotype, **Z** includes information about sire and dam identity (the "animal" term), and **u** is the parameter vector that is estimated for the random effect, distributed as Normal(0, σ^2). We used weakly informative priors: $V = 1$, $v = 0.002$, although analyses were robust when more informative priors were tested. The models were sampled for 2 x 10^6 iterations, discarding the first 1.5 x 10^4 iterations as burnin, with a thinning interval of 50 iterations. These sampling parameters yielded effective sample size >10,000. We evaluated the models for convergence and autocorrelation of the samples, ensuring autocorrelation of <0.1 for the first lag, and absence of trend in the trace plots of the mean β (i.e., intercept term), additive variance V_A, and residual V_R (the later two denoted in the output as 'animal' and 'units', respectively).

319 *Genetic correlations between diets*

320 A single phenotype that is measured in multiple environments (here diets) may
321 be regarded as different phenotypes in those environments (Falconer, 1952; Lynch and
322 Walsh, 1998)(Falconer 1952, Lynch and Walsh 1998). Based on this premise, we
323 computed genetic correlations for phenotype-diet combinations by fitting a Bayesian
324 multivariate animal model:

325 $$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu}$$

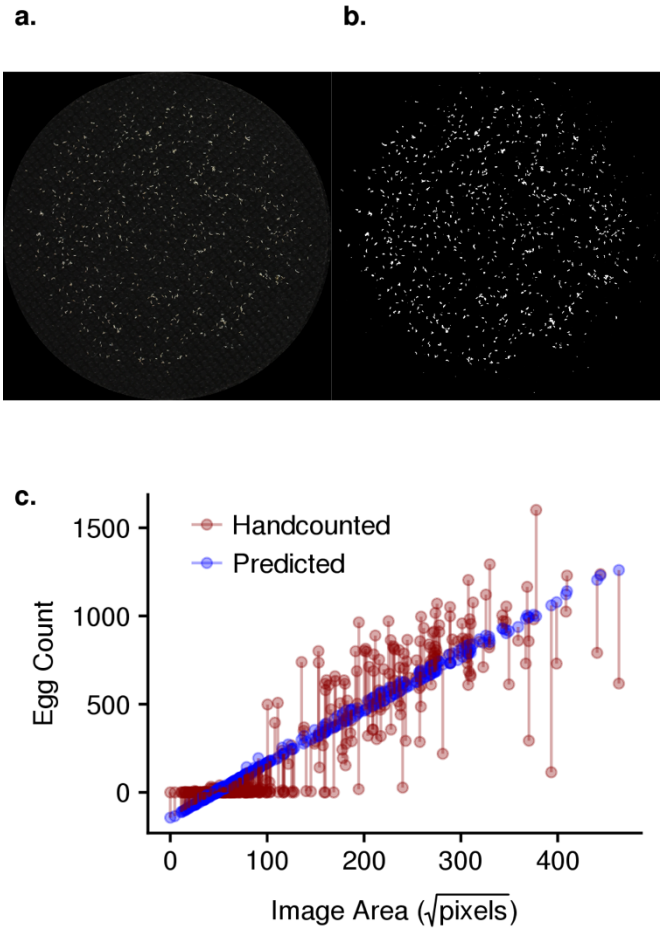
326 where **y** is a matrix of phenotypes (C, DR, HS), **β** is a vector of trait (**X**) means (with no
327 intercept term), **Z** includes information about sire and dam identity (the "animal" term),
328 and **u** is the parameter vector that is estimated for the random effect. Among the
329 random effects, covariance was free to vary (i.e., 'random = ~us(trait):animal') with one
330 restricting the variance to zero (i.e., 'random = ~idh(trait):animal'). If family genetic
331 effects remain the same or change in proportion between diets, then the genetic
332 correlation of family members between diets is 1. Genetic correlations that are credibly
333 smaller than 1 indicate a significant genotype x diet interaction. We therefore compared
334 the model above with a model in which the correlation between diets is constrained to
335 be 1 to test if our estimated genetic correlations were significantly different from 1. In
336 bivariate models, we tested several slightly stronger variations of priors (V = diag(3), v =
337 1.002). Model were run for 6.5 x 10^6 iterations, with 5 x 10^4 discarded as burning, with
338 thinning every 500 iterations. Twelve chains were run in parallel, yielding >10,000
339 samples total.

Results

Performance of image analysis to estimate the number of eggs

Studies measuring fecundity in *D. melanogaster* typically count the number of eggs laid by females by counting eggs on the food surface visually using a dissecting microscope, a method that requires a significant time investment. (Nouhaud *et al.*, 2018) has also developed an image-based method for counting *D. melanogaster* eggs but it relies on imaging the surface of the food and our egg densities would be very high with 24 females per vial, making it infeasible for this experiment. We employed a high throughput method for obtaining egg counts by developing a predictive model to obtain estimated egg counts from images of eggs that were separated from the food surface and filtered onto a dark fabric disc by taking advantage of the simple relationship between the number of eggs present and the amount of white area on a thresholded image of a disc (Fig. 2). We used a set of hand counted images to optimize the model and show that it performs well.

Figure 2: Performance of the model predicting egg counts from images. **a.** A representative raw image of eggs separated from fly media and filtered onto black filter fabric. **b.** The same image thresholded at the optimal threshold level identified by the model. **c.** The performance of the predictive model. The square root of image area is on the x-axis and the egg count is on the y-axis. True, hand-counted values are shown in red and the predicted values from the model are shown in blue. Pairs of values (hand-counted and predicted) for individual images are connected by a red line.



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We tested several parameters to produce an optimal model. First, by using different proportions of our total dataset of hand counted images, we showed our hand counted set was sufficiently large to train the predictive model and achieve a high predictive ability. Using only 50% of the hand counted data, we achieved similar error rates in the resulting model, demonstrating our dataset was sufficiently large to saturate the model. We also tested a range of threshold values to identify the value that would remove the most background noise without obscuring actual eggs. To assess the performance of our model across different parameters, we employed cross validation to ensure we tested the ability of the model to predict egg counts for new data (data not used to fit the model). For each iteration, 90% of the data was used to train the model and we assessed the ability of this model to predict the values of the remaining 10% of the data (see Methods). Our best performing model was able to predict new data well, with a mean absolute error of ~177 eggs and a high correlation (r = 0.88) between the predicted egg count and the manual egg counts (Fig. 2c). We note that with a 24 hour egg laying period for a vial with several females, this error rate represents a relatively small number of eggs.

After identifying the optimal threshold value, we fit a model using our full dataset and found similar error rates when comparing the predicted values from this model to the hand counted images (r = 0.88, mean absolute error = 128 eggs; Fig. 2c). We were then able to use this model to predict egg counts for our entire dataset of 3,768 images.

390 *Phenotypic response to dietary treatment*

391 *Lifespan*

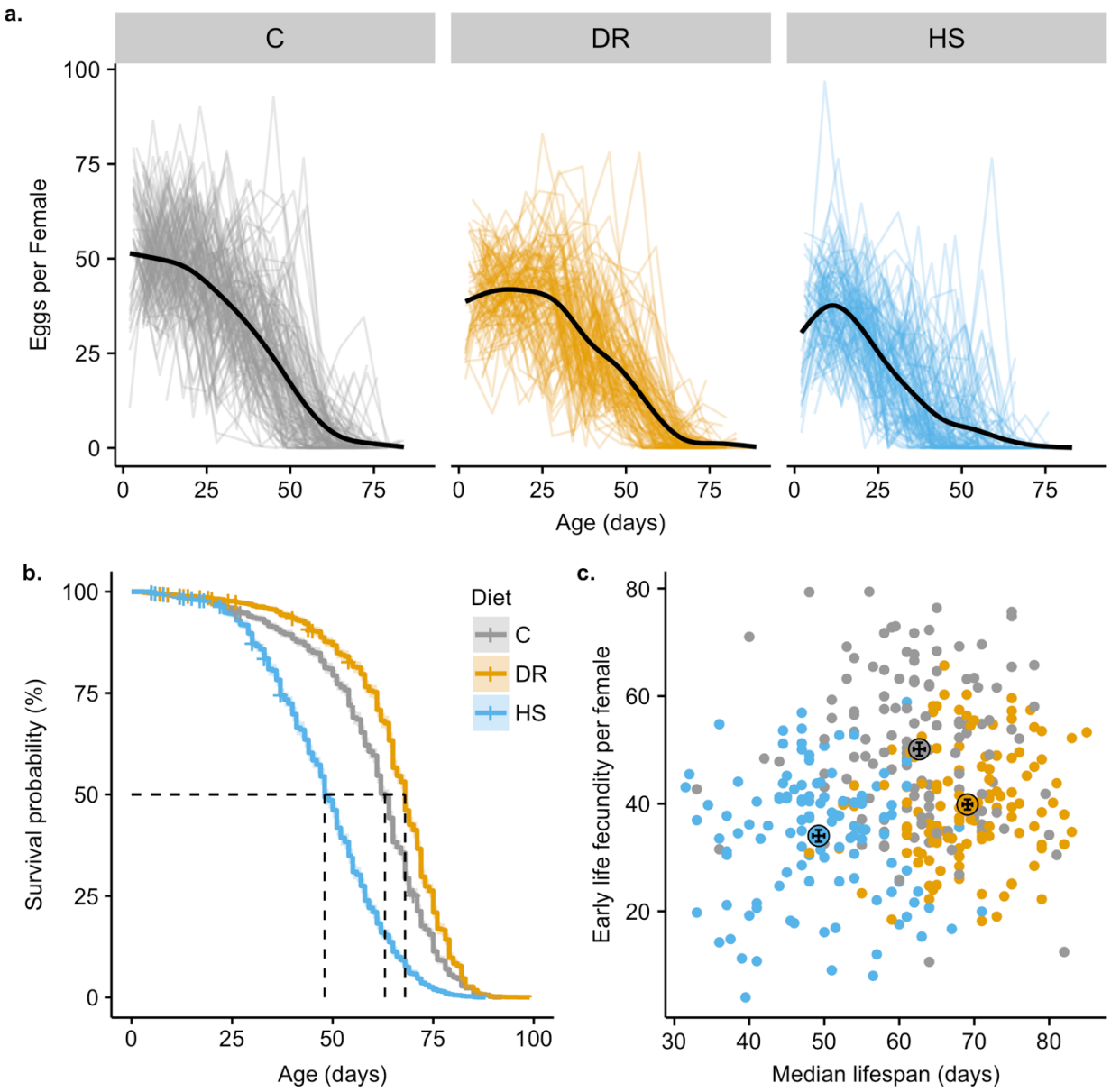
392 We used a split family design, splitting offspring from families into three different
393 diets. Relative to the control diet, median survival was 24% lower on high sugar (HS: 48
394 days vs. C: 63 days) and 8% higher on DR diet (68 days) (Fig. 3). Lifespan trajectories
395 began to diverge early, from about 25 days post oviposition and remained diverged until
396 under 10% survivorship (Fig. 3). Individual sire family responses to diet are presented in
397 Fig. S2.

398 We compared six nested models of survival, incorporating diet treatment and
399 various aspects of relatedness and compared these models using leave-one-out model
400 weighting (Table 1). The model including sire, dam nested in sire, diet, and a sire by diet
401 interaction has the overwhelming model support (>95% of model weight), indicating a
402 significant effect of diet, the presence of significant genetic variance for survival within
403 the diet treatments, and significant genetic variation for the response to diet.
404 Specifically, the results of this favored model show that, compared to the C diet group,
405 individuals in the DR group were credibly less likely to die (99% credible interval = 0.01 -
406 0.11) and individuals in the HS treatment group were credibly more likely to die (99%
407 credible interval = -0.22 - -0.13).

408 Table 1. Comparison of models of lifespan, early fecundity, and total fecundity across
409 diet treatments. Models 1 and 2 do not include any aspects of pedigree, and models 3-6
410 use a hierarchical model. Terms in parentheses are random effects. All random effects
411 were "intercept" terms, allowing separate intercepts for each level of sire ID, dam ID
412 nested in sire ID, or sire ID nested in diet treatment. Model 1 includes only an intercept

term, allowing comparison to model 2. In all cases, model 2 was vastly superior to model 1.

	Model	**Lifespan**	**Early Fecundity**	**Total Fecundity**
1	~ Intercept	3.5%	3.1%	1.1%
2	~ Diet	0%	10.3%	0.0%
3	~ Diet + (Sire ID)	0%	0.0%	2.1%
4	~ Diet + (Dam ID by Sire ID)	1.1%	86.6%	19.4%
5	~ Diet + (Sire ID) + (Dam ID by Sire ID)	0.2%	0.0%	0.0%
6	~ Diet + (Sire ID) + (Dam ID by Sire ID) + (Sire ID by Diet)	95.1%	0.0%	77.4%



415

416 Figure 3: Phenotypic response to diet treatment. The different colors represent different

417 diet treatments (grey = C, orange = DR, blue = HS). **a.** Fecundity per female sampled

418 over a 24 hour period once per week over lifespan. A smooth (loess) fit is shown for

419 each treatment. Individual lines represent each replicate vial. **b.** Survival probability

420 versus age in each of the three diet treatments. Median survival (50% survival

probability) is shown with the dotted line for each diet. Censored events are marked with a + sign on each survivorship curve. **c.** The relationship between early life fecundity (24 hour sample period closest to 5 days post-eclosion) and median lifespan for each vial. The average values for both within each treatment are shown by the large point outlined in black. Error bars are the mean +/- 1 standard error. Note that the error bars all fit within the size of the point.

Fecundity

Fecundity was estimated once per week in the same treatment setups from which lifespan was recorded in order to compare the two phenotypes in the same set of flies. As expected, in all treatments, fecundity declined with age. We used a Bayesian model comparison approach to analyze the effects of diet and family on total fecundity and early fecundity (Table 1). This analysis revealed a strong effect of diet for both total fecundity and early fecundity, as models including diet were strongly preferred over a model without diet. For both measures of fecundity, the highest values were in the C diet and the lowest in the HS diet. There was a credible effect of family for both fecundity measures, indicating significant genetic variation for fecundity. For early fecundity, the model including only dam nested within sire was favored heavily. For total fecundity, the model that included sire, dam nested in sire, and the interaction between sire and diet was favored over the other models, indicating genetic variation for the response to diet in addition to genetic variation for fecundity.

442 *Estimates of Quantitative Genetic Parameters*

443 We have a high sample size for lifespan with data for up to 48 individuals spread
444 across two vials for each dam family in each diet. While these same individuals
445 contributed to our measurements of weekly fecundity, we have a single measurement
446 for each vial of females at any given time point and do not have measurements per
447 each individual, as measuring fecundity for each individual female was not feasible for
448 this study. Thus, for estimating quantitative genetic parameters, our replication for
449 fecundity is low (2 per family per diet). The results of the above models reflect the
450 expected uncertainty from this low replication with wide posterior distributions. Thus,
451 while the models we fit above demonstrate the presence of significant genetic variation,
452 our estimates of heritability values have high uncertainty and wide credible intervals.
453 Therefore, we focus on discussing our results for lifespan below and results for
454 fecundity can be found in Fig. S3.

455 *Heritability of lifespan within diets*

456 We estimated the heritabilities of our phenotypes separately in each diet
457 treatment using a Bayesian approach (see Methods). Our heritability estimates for
458 lifespan are moderately high, ranging from 0.31 to 0.47 and do not differ substantially
459 across diets with all three credible intervals overlapping (Figure 4a; heritability (h^2) and
460 HPDI (highest posterior density interval) = C: h^2 0.47 HPDI 0.34 – 0.61; HS: h^2 0.37,
461 HPDI 0.25 – 0.50; DR: h^2 0.31, HPDI 0.21 – 0.43).

462 *Correlation of lifespan across dietary treatments*

463 To determine if there is substantial genetic variation for the response to the
464 environment (GEI), we fit a multivariate form of the animal model, which considers

465 lifespan within each diet as a separate phenotype (Falconer, 1952; Lynch and Walsh,
466 1998; Wilson *et al.*, 2010). When the genetic correlation between environments is less
467 than one, it indicates significant genetically based differences in the response to the
468 environment, or a significant GEI. In all pairwise diet comparisons, our estimates of the
469 genetic correlations are credibly less than one, indicating substantial genetically based
470 variation in the response to diet (genetic correlation (r_g) and HPDI = DR vs. C: r_g 0.48
471 HPDI 0.34 – 0.61; HS vs. C: r_g 0.29 HPDI 0.21 – 0.43; HS vs. DR: r_g 0.34 HPDI 0.25 –
472 0.50 (Fig. 4b). This variability is also apparent when examining the variation among sire
473 families in their response to diet for both lifespan and total fecundity (Fig. 4c & d). In
474 addition, the model comparisons we employed for survival and total fecundity both
475 favored models including a sire by diet interaction, which is consistent with the presence
476 of a significant GEI.

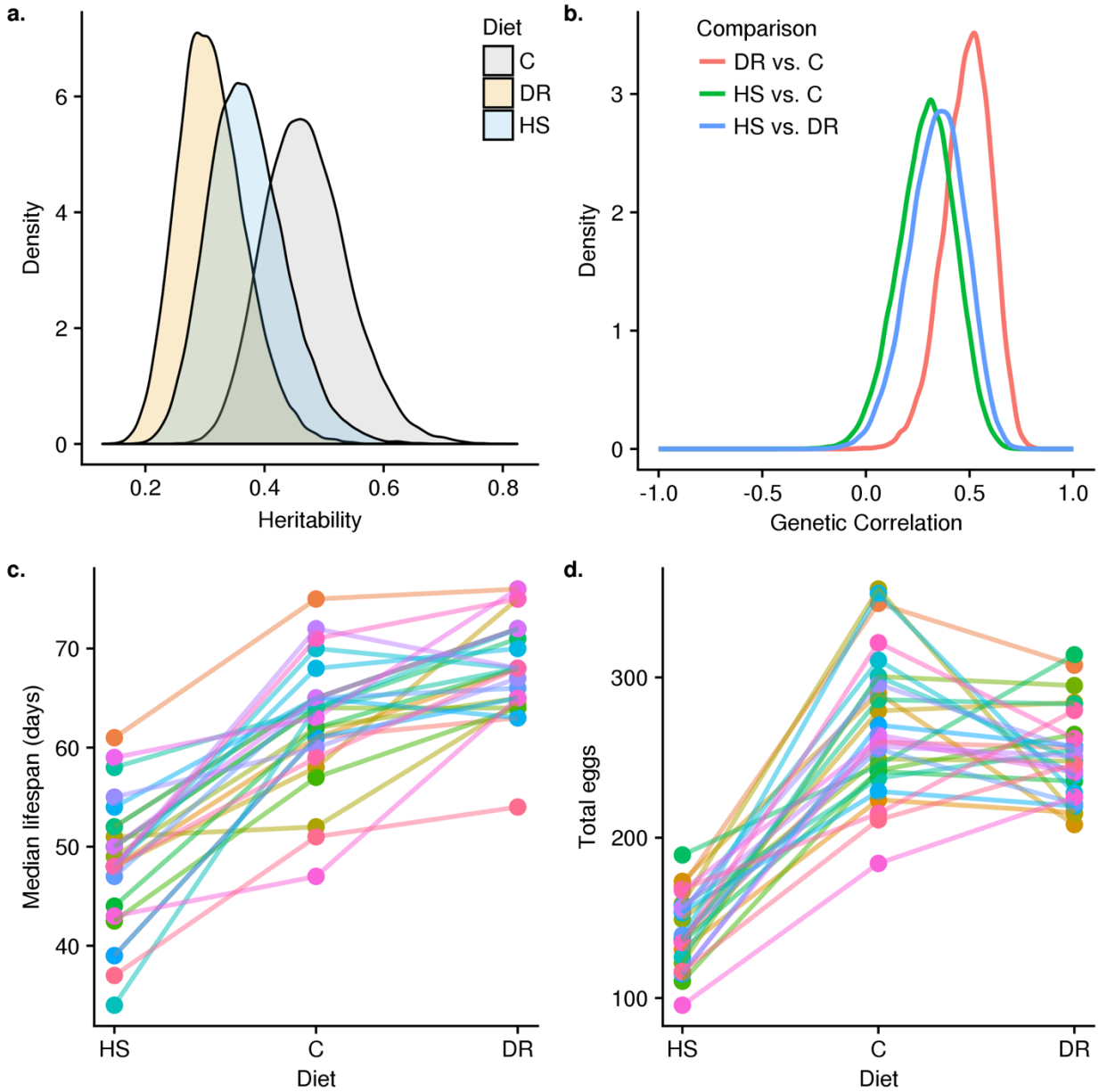


Figure 4: **a.** Density plots of the estimated posterior probabilities for lifespan heritability within each diet. **b.** Density plots of the estimated posterior probabilities for the genetic correlation between lifespan in pairs of diets. **c.** Sire by environment interaction plot for median lifespan showing the median lifespan for each sire family in each of the three diets. Each line represents one sire family. **d.** Sire by environment interaction plot for

483 total egg count per female (summed across weekly measures) showing the median
484 lifespan for each sire family in each of the three diets. Each line represents one sire
485 family.

486 **Discussion**

487 In this study, we used an admixed multiparent population of *D. melanogaster* to
488 characterize the quantitative genetics of fitness components in different nutritional
489 conditions. We characterized the effect of three different diets on survival and fecundity,
490 measured over the course of the entire lifetime of the flies. Using a quantitative genetic
491 breeding design, we show that these traits harbor substantial genetic variation both
492 within diets and in the response to diet. This result has important implications for our
493 understanding of the evolution of these traits in wild populations and for strategies to
494 uncover the genetic basis of these traits, which we discuss below.

495 *Evolutionary potential of the coordination between nutrition and fitness components*

496 We characterized the effects of a restricted diet in the form of a low protein diet
497 (DR) and of a calorie rich diet in the form of a high sugar diet (HS). Dietary restriction
498 has been studied extensively in *D. melanogaster* and other metazoans (e.g.,
499 (Chippindale *et al.*, 1993a; Good and Tatar, 2001; Vrtílek and Reichard, 2014);
500 reviewed in (Sohal and Weindruch, 1996; Kenyon, 2005; Kirkwood and Shanley, 2005;
501 Piper *et al.*, 2011; Tatar, 2011; Tatar *et al.*, 2014)). A general finding of these studies is
502 that reduced nutrient intake without starvation results in lifespan extension, and that this
503 effect is often coupled with reduced reproductive output, though there are exceptions to
504 this pattern (e.g., (Kaitala, 1991; Weindruch *et al.*, 1995; Kirk *et al.*, 2001; Stelzer, 2001;
505 Cooper *et al.*, 2004); see Ng'oma *et al.*, (2017)). Even within species that tend to show

506 this effect such as *D. melanogaster*, there is variability in this pattern among different
507 genotypes. For example, (Stanley *et al.*, 2017) found variation among inbred lines for
508 the amount of lifespan extension, with some lines showing little to no extension in a DR
509 diet, and a few showing reduced lifespan in a DR diet, a result that has also been
510 shown in mice (Rikke *et al.*, 2003). The effects of a high sugar diet have not been
511 studied as extensively, though recently some studies have shown flies reared on high
512 sugar diets as larvae show obesity-like phenotypes (Musselman *et al.*, 2011; Rovenko
513 *et al.*, 2015), and adults reared on high sugar diets have elevated triglyceride levels,
514 reduced lifespan, and reduced fecundity (Skorupa *et al.*, 2008), a pattern that is
515 consistent with our findings.

516 These types of phenotypic patterns in different diet conditions have been
517 interpreted in different ways. The widespread observation of lifespan extension
518 observed in response to dietary restriction in many taxa has led to competing
519 hypotheses for this pattern. It is often argued that this pattern is the result of natural
520 selection acting in environments with fluctuating resources where it should be adaptive
521 to increase allocation of nutrient resources to the soma when nutrients are limiting,
522 however it is also possible it is the result of an unavoidable physiological constraint
523 (Holliday, 1989; Kirkwood and Shanley, 2005). Additionally, studies employing high
524 sugar and high fat diets often view the results of those studies in the context of human
525 populations where it is hypothesized the negative health consequences of a
526 Westernized diet result from a mismatch between that diet and the diet humans adapted
527 to in the past over the majority of their evolutionary history (Neel, 1962; Wells, 2009),
528 though this hypothesis has also been challenged (Speakman, 2008). In general,

529 assessing the potential for phenotypic effects of different diets to be adaptive patterns or
530 to be highly constrained physiological responses requires an understanding of the
531 underlying genetic variation for the response to diet in order to understand if this
532 response has the potential to evolve.

533 Here, we showed there is heritable variation for both phenotypes within diets,
534 and for the response the phenotypes to diet (Table 1; Fig. 4a,b). While many studies
535 have demonstrated GEI for different traits (Reed *et al.*, 2010; Qi *et al.*, 2012; Ingleby *et*
536 *al.*, 2013), few studies have estimated GEI specifically for life history traits in response
537 to diet, and not all previous studies of diet have found significant GEI. For example,
538 (King *et al.*, 2011) estimated quantitative genetic parameters for allocation to flight
539 capability and to reproduction in the sand cricket, *Gryllus firmus.* These authors found
540 no GEI for these traits, with all genotypes allocating more to reproduction when food
541 levels increase, suggesting important evolutionary constraints can exist for the response
542 to diet and that significant GEI is not a foregone conclusion. Our results show that this
543 constraint does not exist for this population of *D. melanogaster* and that the response to
544 diet will readily evolve in response to selection. The founder lines of the DSPR have a
545 global distribution and were derived from wild-caught flies and thus, our population
546 encompases some of the genetic variation that exists among natural populations of *D.*
547 *melanogaster.* It is plausible, therefore, that the diet dependent effects observed in this
548 study and others represent adaptive patterns that have evolved. However, a major
549 challenge that remains in interpreting these patterns is the extent to which laboratory
550 diets represent the nutritional conditions experienced by flies in the wild.

551 *Prospects for multiparent population approaches*

552 One of the most fundamental goals in biology is to understand the genetic basis
553 of complex phenotypes. This goal has proved to be quite challenging. For most traits,
554 some of which have been the focus of study for many years, the causative genetic
555 variants that have been discovered explain only a small percentage of the trait
556 heritability (for reviews see McCarthy *et al.*, 2008; Manolio *et al.*, 2009; Rockman, 2012;
557 Visscher *et al.*, 2012). For traits that are expected to be highly polygenic, such as
558 lifespan and fecundity, estimating quantitative genetic parameters as we have done in
559 this study, can be as informative about the genetic basis of a trait and its evolutionary
560 potential as studies aimed at uncovering specific genetic variants.

561 In recent years, multiparent populations, in which multiple inbred founder lines
562 are crossed for a number of generations to create a recombinant mapping population,
563 have increasingly been employed in studies aiming to identify the causative genetic
564 variants underlying complex traits (e.g. (Kover *et al.*, 2009; McMullen *et al.*, 2009;
565 Huang *et al.*, 2011; Aylor *et al.*, 2011; Threadgill and Churchill, 2012; King, Macdonald,
566 *et al.*, 2012; King, Merkes, *et al.*, 2012; Cubillos *et al.*, 2013); reviewed in (Long *et al.*,
567 2014; de Koning and McIntyre, 2017). One reason for employing a mapping population
568 consisting of multiple parent lines is the inclusion of additional genetic variation when
569 compared to traditional two-line QTL mapping panels. Even with multiple parent lines
570 contributing, a potential criticism of these kinds of approaches when compared to
571 mapping approaches that sample from natural populations is that they contain limited
572 amounts of genetic variation. The DSPR population used in this study was started from
573 8 inbred parent lines. Therefore, while every individual in our admixed population has a

574 unique mosaic genome, at any given position, there are only 8 possible haplotypes.
575 Despite this simplicity, we find moderate heritabilities for lifespan in all diets (ranging
576 from ~0.3 - 0.5) that are comparable to estimates from natural populations. A meta-
577 analysis of heritability estimates from natural populations found life history traits typically
578 have values around 0.25 and values higher than 0.3 are not infrequent (Rose and
579 Charlesworth, 1981; Mousseau and Roff, 1987; Rikke *et al.*, 2010). We also found
580 strong evidence for a genotype by diet interaction for lifespan, indicating there is also
581 segregating genetic variation for the response to diet. While our evidence of a genotype
582 by diet interaction is not as strong for fecundity, our model comparison for total fecundity
583 supports a model including this interaction.
584 In addition to their utility for QTL mapping, multiparent populations have also
585 been used in evolve and resequence approaches (e.g. (Turner and Miller, 2012; Burke,
586 King, *et al.*, 2014; Burke, Liti, *et al.*, 2014) to identify the underlying genetic basis of
587 complex traits. These studies impose selection and track the resulting changes in
588 phenotype and genotype. This approach is potentially very powerful because 1) entire
589 haplotypes can be tracked over evolutionary time, 2) the simplified genetic architecture
590 with only a few potential haplotypes at any given location may lead to a more repeatable
591 response to selection increasing the likelihood of identifying causative loci, and 3) it may
592 allow for validation of QTL identified in mapping studies using these same populations.
593 A potential downside to such an approach is if the relatively simple genetic architecture
594 of such a population results in limited genetic variation for the trait of interest, thereby
595 constraining evolution. Our study shows there is substantial genetic variation for our
596 focal phenotypes in our admixed DSPR population, which establishes this approach as

597 a viable strategy for investigating both how a focal trait evolves and the underlying
598 genetic basis. In general, MPPs have great potential to advance our understanding of
599 the genetic basis of complex traits via these kinds of multi-pronged approaches.

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608 **Conflict of interest**

609 The authors declare no conflict of interest.

610 **Data availability**

611 Our raw data, including data on lifespan, fecundity, and all egg image files, have been
612 uploaded to Zenodo and may be retrieved at http://doi.org/10.5281/zenodo.1285237. All
613 scripts to reproduce all of our analyses are available at GitHub:
614 https://github.com/EGKingLab/h2lifespan. Supplementary information is available at
615 https://www.nature.com/hdy/

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