Dissection of complex, fitness-related traits in multiple *Drosophila* mapping populations offers insight into the genetic control of stress resistance

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Abstract

We leverage two complementary *Drosophila melanogaster* mapping panels to genetically dissect starvation resistance, an important fitness trait. Using >1600 genotypes of the multiparental *Drosophila* Synthetic Population Resource (DSPR) we map numerous starvation stress QTL that collectively explain a substantial fraction of trait heritability. QTL effects further allowed us to estimate DSPR founder phenotypes, predictions that were correlated with the actual founder phenotypes. Starvation resistance has been linked to triglyceride level, and while we observe a modest phenotypic correlation between the traits in the DSPR, overlap among the QTL identified for each trait is low. Since we show that DSPR strains with extreme starvation phenotypes also differ in desiccation resistance and activity level, our data imply that multiple physiological mechanisms contribute to starvation variability. We also exploit the *Drosophila* Genetic Reference Panel (DGRP), identifying a number of sequence variants associated with starvation resistance. Consistent with prior work these sites rarely fall within QTL intervals mapped in the DSPR. Two other groups previously measured starvation resistance in the DGRP, offering a unique opportunity to directly compare mapping results across labs. We found strong phenotypic correlations among studies, but extremely low overlap in the sets of genomewide significant sites. Despite this, our analyses revealed that the most highly-associated variants from each study typically showed the same additive effect sign in independent studies, in contrast to otherwise equivalent sets of random variants. This consistency provides evidence for reproducible trait-associated sites in a widely-used mapping panel, and highlights the polygenic nature of starvation resistance.

Introduction

Starvation resistance is a classic quantitative fitness-related trait that is associated with several other phenotypes that influence survival, lifespan, and reproduction (Murphey and Hall 1969; Service and Rose 1985; Da Lage *et al.* 1990; Rose *et al.* 1992; Toda and Kimura 1997; Karan and Parkash 1998; Hoffmann *et al.* 2005b; Sørensen *et al.* 2007; Telonis-Scott *et al.* 2009). In particular, increased starvation resistance is often negatively correlated with fecundity and positively correlated with longevity, energy storage, and other stress response traits (Service *et al.* 1985; Rose *et al.* 1992; Hoffmann and Parsons 1993; Chippindale *et al.* 1993; Harshman *et al.* 1999; Bochdanovits and de Jong 2003; Bubliy and Loeschcke 2005; Sørensen *et al.* 2007; Schwasinger-Schmidt *et al.* 2012). Because of pervasive phenotypic and genetic correlations between starvation resistance and other traits, the evolution of starvation resistance in natural populations is complex and population-specific, and is driven by adaptation to heterogeneous environments, phenotypic plasticity, and extensive pleiotropy (Service and Rose 1985; Hoffmann and Parsons 1991; Chippindale *et al.* 1993; Falconer and Mackay 1996; Toda and Kimura 1997; Karan and Parkash 1998; Harshman *et al.* 1999; Harbison *et al.* 2004; Pijpe *et al.* 2007; Rion and Kawecki 2007; Bauerfeind *et al.* 2014; Colinet *et al.* 2015; Everman and Morgan 2018).

Artificial selection for increased starvation resistance often results in a corresponding increase in lipid levels in *D. melanogaster* (Chippindale *et al.* 1996, 1998; Djawdan *et al.* 1998; Harshman *et al.* 1999; Goenaga *et al.* 2013; Hardy *et al.* 2018), suggesting that energy storage is one important mechanism that contributes to starvation resistance. However, variation in the association between these traits has also been observed (Hoffmann *et al.* 2001; Schwasinger-Schmidt *et al.* 2012). For example, while Chippindale *et al.* (1996) provided evidence of a strong positive correlation between starvation resistance and lipid concentration following 60 generations of selection for starvation resistance, Hoffmann *et al.* (2001) found that total lipid concentration and starvation resistance in isofemale lines derived from natural populations were not correlated. The correlation between starvation resistance and lipid level is likely dependent upon genetic background and the evolutionary history of a population, resulting in across-population variation in the strength and direction of the correlation between these traits. Furthermore, starvation resistance is likely influenced by physiological factors in addition to lipid

level, including metabolic rate (Hoffmann and Parsons 1993; Rion and Kawecki 2007; Schwasinger-Schmidt *et al.* 2012) and differences in overall activity level.

Similar to lipid levels, selection for starvation resistance often results in a concomitant change in desiccation resistance (Hoffmann and Parsons 1989; Chippindale *et al.* 1996; Hoffmann and Harshman 1999; Harshman *et al.* 1999; Hoffmann *et al.* 2001). Nonetheless, surveys of natural populations in several *Drosophila* species have shown that starvation and desiccation resistance can also independently vary (Davidson 1990; Karan and Parkash 1998; Karan *et al.* 1998; Chippindale *et al.* 1998; Hoffmann and Harshman 1999; Gilchrist *et al.* 2008; Goenaga *et al.* 2013). In addition, opposing clines in starvation and desiccation resistance were observed across tropical and temperate climates in several *Drosophila* species (reviewed in Hoffmann and Harshman 1999). Given these variable patterns, Karan and Parkash (1998) and Da Lage *et al.* (1990) suggest that desiccation and starvation resistance are not physiologically constrained. Rather, they are both directly and indirectly influenced by climate variability and selection on other correlated traits such as diapause or thermal tolerance in seasonally variable temperate environments (Schmidt *et al.* 2005; Sørensen *et al.* 2007; Rion and Kawecki 2007; Goenaga *et al.* 2013).

Genetic dissection of starvation resistance using diverse mapping panels can facilitate a deeper understanding of the evolution of, and mechanisms underlying, starvation resistance and its phenotypic association with other traits such as lipid level and desiccation resistance. Previous studies have examined the genetic basis of starvation resistance in *D. melanogaster* using a combination of selection experiments (Rose *et al.* 1992; Chippindale *et al.* 1996; Harshman *et al.* 1999; Bochdanovits and de Jong 2003; Bubliy and Loeschcke 2005; Schwasinger-Schmidt *et al.* 2012), gene expression studies following exposure to starvation stress (Harbison *et al.* 2005; Sørensen *et al.* 2007), and genetic mapping (Harbison *et al.* 2004; Mackay *et al.* 2012; Huang *et al.* 2014; Everman and Morgan 2018). These studies have provided extensive lists of candidate genes, some of which have been functionally validated (Lin *et al.* 1998; Clancy *et al.* 2001; Harbison *et al.* 2004, 2005; Nuzhdin *et al.* 2005; Sørensen *et al.* 2007). However, up to this point few studies have undertaken an examination of the genetic architecture of triglyceride or lipid content in the same genetically diverse panel used to examine variation in starvation resistance.

123 Doing so would allow a detailed comparison of quantitative trait loci (QTL) that contribute to
124 variation in each trait, provide important insight into the similarity of the genetic architectures
125 of starvation resistance and correlated traits, and facilitate a better understanding of the
126 coevolutionary dynamics underlying these traits.

127 In this study, we used two powerful, complementary *D. melanogaster* mapping panels -
128 the *Drosophila* Synthetic Population Resource (DSPR) and the *Drosophila* Genetic Reference
129 Panel (DGRP) - to genetically dissect starvation resistance. In addition to mapping loci that
130 contribute to variation in this trait, we measured and mapped triglyceride level in the DSPR. Our
131 approach allows us to accomplish three primary objectives. First, by measuring starvation
132 resistance and triglyceride level in the same mapping panel, we can assess overlap in the loci that
133 contribute to variation in each trait, thus gaining insight into the level of pleiotropy between
134 these traits. Second, by measuring starvation resistance in both the DSPR and the DGRP, we are
135 able to address variation in the genetic architecture of this trait between two populations that
136 have unique evolutionary histories. Finally, one advantage of a stable reference panel of inbred
137 lines is the ability to repeatedly measure trait variation on the same set of genotypes. Here, in
138 addition to our new screen, we leveraged two previous starvation resistance screens using the
139 DGRP (Mackay *et al.* 2012; Everman and Morgan 2018) to resolve SNP variants with consistent,
140 across-study phenotypic effects. This across-study comparison of the genetic architecture of
141 starvation resistance in the DGRP provides both technical insight into the use of genomewide
142 association studies (GWAS) to understand the genetic basis of complex traits, and biological
143 insight into the phenotypic effects of loci that contribute to trait variation.

144 Overall, despite a significant phenotypic correlation, we report limited overlap among
145 mapped loci that contribute to variation in starvation resistance and triglyceride level in the
146 DSPR, suggesting that the genetic basis of these traits is largely independent. This highlights the
147 role other physiological mechanisms, such as activity level and desiccation resistance that we
148 explore here, may have in influencing starvation resistance. In common with previous studies
149 using both the DSPR and DGRP, we also find little overlap in the loci associated with starvation
150 resistance between mapping panels, likely the combined result of unique genetic backgrounds
151 and evolutionary histories, as well as differences in power to detect causative loci. A comparison

152 of the genetic architecture of starvation resistance across three DGRP datasets supports this
153 interpretation. We found that the sign of the additive effects of the most strongly-associated
154 SNPs were consistent across datasets, suggesting these SNPs likely contribute to variation in
155 starvation resistance in the DGRP, but have sufficiently small effects that they are regularly not
156 identified following genomewide multiple testing correction.

157 Materials and Methods

158

159 Mapping populations

160 Drosophila *Synthetic Population Resource*

161 The DSPR is a multiparental population that consists of two synthetic populations (pA and
162 pB) that were each established following an intercross of eight highly-inbred founder lines, with
163 one founder line shared between the two populations (King *et al.* 2012a). Flies were maintained
164 in pairs of subpopulations (pA1, pA2, pB1, pB2) at high population density for 50 generations
165 prior to the establishment of > 1600 genotyped recombinant inbred lines (RILs) generated via 25
166 generations of full-sib inbreeding (King *et al.* 2012a; b). Founder lines for the pA and pB panels
167 have also been sequenced at 50x coverage, enabling inference of the haplotype structure of each
168 RIL via a hidden Markov model (described in King *et al.* (2012a).

169

170 Drosophila *Genetic Reference Panel*

171 The DGRP was established from mated females collected from a natural population in
172 Raleigh, North Carolina, with inbred lines derived from 20 generations of full-sib mating (Mackay
173 *et al.* 2012). Each of the 205 DGRP lines have been fully re-sequenced and genotyped allowing
174 GWA mapping to be carried out in the panel (Mackay *et al.* 2012; Huang *et al.* 2014).

175

176 Phenotyping assays and analysis

177 *Large-scale starvation resistance assay*

178 Strains from the DSPR and DGRP were duplicated from stocks, and adults were discarded
179 following 1-2 days of oviposition to maintain a relatively even egg density across all experimental
180 vials. In the following generation, experimental flies (2-4 days old) were sorted by sex over light

181 CO_2 anesthesia and placed in groups of same-sex individuals on new cornmeal-molasses-yeast
182 media for 1 day until the start of the starvation assay. The assay was initiated by placing flies on
183 1% agar media that additionally contained propionic acid and benzoic acid preservatives.
184 Starvation media was made within 24 hours of the initiation of each block of the assay and was
185 not replaced throughout its duration. In both mapping panels, survival was measured across 2
186 vial replicates per sex in ~85% of strains, with ~90% of vials containing 10 flies (minimum flies per
187 vial = 6). Some strains were replicated 3-4 times for a maximum of 40 flies screened per strain.
188 Vials were barcoded during the screen, blinding experimenters to strain identification number,
189 and assisting with efficient data collection and analysis.

190 Death was assessed for each vial twice per day at approximately 0900 and 2100 hrs. The
191 first assessment of survival was made 24 hours after flies were transferred to starvation media.
192 Dead flies at this initial assessment point were not included in the analysis as their death may
193 have resulted from handling during the initial transfer to experimental vials rather than
194 starvation stress. Vials with flies that had become entangled in the cotton vial plug at any point
195 in the duration of the assay were also excluded from the analysis. Flies were considered dead if
196 they were not moving or unable to dislodge themselves from the starvation media. The
197 phenotype used for mapping was the mean time to death in hours per strain across the vial
198 replicates.

199 We screened the DSPR (861 pA1/pA2 and 864 pB1/pB2 RILs) in a series of batches across
200 a seven-month period in 2010. Each batch included the majority of RILs that belonged to a
201 particular subpopulation. Starvation resistance was measured in 168 DGRP lines in a single batch
202 in 2012. Finally, we measured starvation resistance in the 15 DSPR founder lines, using 5 vial
203 replicates per founder, in one batch. In all cases flies were reared and tested at approximately
204 23°C, 30-60% humidity, with constant light.

205 We assessed variation in starvation resistance due to mapping subpopulation and sex in
206 the DSPR with a two-way ANOVA, including the interaction, and treated subpopulation (pA1, pA2,
207 pB1, and pB2) and sex as fixed factors. Male and female-specific differences among the four
208 subpopulations were tested using Tukey's HSD post hoc comparisons with an experiment-wide

209 α = 0.05. Differences in starvation resistance due to sex among the DGRP lines were analyzed
210 with a one-way ANOVA, treating sex as a fixed factor.
211
212 *Desiccation resistance assay*
213 To investigate the correlation between starvation and desiccation resistance, we
214 measured desiccation resistance in a subset of pA1/pA2 RILs that had very low (17 RILs) or very
215 high (16 RILs) average female starvation resistance in the large-scale screen. Desiccation
216 resistance was assessed in females only with two vial replicates per RIL, 92.9% of which contained
217 10 flies (minimum flies per vial = 8). We placed experimental flies in empty vials plugged with
218 cotton inside an airtight container. Relative humidity was reduced to close to 0% by adding a
219 large quantity of Drierite (calcium sulfate) to the chamber. Survival was assessed every hour
220 following initiation of the experiment, and mean desiccation resistance per RIL was used in all
221 analyses.
222
223 *Activity monitor starvation resistance assay*
224 The *Drosophila* Activity Monitoring System (DAM2, TriKinetics, Inc.) was used to measure
225 starvation resistance and activity level prior to starvation under environmental conditions similar
226 to those used in previous starvation resistance studies in *D. melanogaster* (Mackay *et al.* 2012;
227 Everman and Morgan 2018). For this experiment we selected a subset of pA1/pA2 RILs from the
228 two tails of the distribution of female starvation resistance measured in the large-scale screen
229 described above, testing 16 flies of each sex from 16 (19) lines with high (low) female starvation
230 resistance.
231 One day prior to adding flies to monitor tubes, cornmeal-yeast-dextrose media was
232 poured into 100mm diameter petri dishes. Once set, polycarbonate activity monitor tubes (5mm
233 diameter x 65mm length) were filled to approximately 10mm by pushing them into the media.
234 The food plug in each tube was sealed with paraffin wax. A single fly was aspirated into each
235 tube, and the tubes were capped with small pieces of Droso-Plugs (Genesee Scientific
236 Corporation). Flies were allowed to acclimate to the tubes and incubator conditions for 24 hours
237 at 25°C and ~50% relative humidity with a 12:12 hr photoperiod. Following the acclimation

238 period, we measured activity for 24 hours. After the pre-starvation period, each fly was tipped to
239 a second monitor tube containing starvation media (agar with preservative prepared as described
240 above) and activity was monitored every day until each fly died.

241 To assess the effect of the different environmental conditions on starvation resistance,
242 mean resistance per RIL from the DAM starvation assay was compared to mean resistance per
243 RIL from the large-scale screen using general linear regression. To determine differences in
244 activity patterns due to starvation resistance rank (high versus low) during the pre-starvation
245 period, we used a full three-way ANOVA model with interactions, and treated starvation rank,
246 sex, and the light status of the incubator (light versus dark) as fixed effects. The effect size of the
247 main effects and interactions were calculated using Cohen's F, which determines the effect size
248 as a ratio of the between-group and between-replicate standard deviations (R package: sjstats)
249 (Cohen 1988; Quinn and Keough 2002; Lüdecke 2018).

250

251 *Triglyceride level assay*

252 We duplicated 311 pA1/pA2 and 628 pB1/pB2 DSPR RILs from stocks to two replicate
253 vials, allowing parental flies to lay eggs for 1-2 days. Eleven days following the start of egg laying
254 we collected two sets of 10-12 females from each parental vial, resulting in four collection vials
255 from each RIL. Flies were aged for three additional days before measuring triglyceride level.

256 Experimental females from each collection vial were anesthetized using CO_2, and groups
257 of 5 were arrayed into deep well plates (Axygen, P-DW-11-C) over ice, with each well pre-loaded
258 with a single glass bead. This resulted in 8 replicate samples of 5 females per RIL. Immediately
259 after finishing a plate, we added 400µl of cold homogenization buffer (10mM potassium
260 phosphate monobasic, 1mM EDTA, 0.05% Tween-20) to each well, homogenized for 45sec in a
261 Mini-BeadBeater-96 (Bio Spec Products, Inc.), and centrifuged for 4min at 2,500g. We then
262 moved 50µl of the supernatant to a standard PCR plate, incubated the plate in a thermocycler at
263 70°C for 5min, and then placed the plate on ice for 5min.

264 During the incubation steps, we added 30µl of homogenization buffer to 92 of the 96
265 wells of a flat-bottom, polystyrene assay plate (Greiner, 655101), and subsequently added 20µl
266 of the heat-deactivated fly homogenate to each. The four remaining wells of every assay plate

267 were dedicated to controls; one blank well contained 50µl of homogenization buffer only, and
268 three wells contained 5µl of Glycerol Standard Solution (SigmaAldrich, G7793, 2.5mg/ml) along
269 with 45µl of homogenization buffer.

270 The assay plate was then inserted into a BioTek Powerwave XS2 instrument pre-heated
271 to 37°C and read at 540nm (baseline absorbance scan). After the scan, and within 10min, we
272 added 100µl of Free Glycerol Reagent (SigmaAldrich, F6428) to each well. The plate was then re-
273 inserted into the instrument, incubated at 37°C for 5min, and read again at 540nm (free glycerol
274 absorbance scan). After this second scan, and again within 10min, we added 25µl of Triglyceride
275 Reagent (SigmaAldrich, T2449) to each well. The plate was again incubated at 37°C for 5min in
276 the machine and read for a third time at 540nm (triglyceride, or final absorbance scan).

277 For each sample, we obtained the final absorbance for each sample (FA_{sample}) and
278 calculated the initial absorbance (IA_{sample}) as the free glycerol measurement minus the baseline
279 measurement. We also generated the average final absorbance for the three standard wells
280 (FA_{std}) and the initial absorbance for the one blank well (IA_{blank}). We then estimated the true
281 serum triglyceride level as

282

283 $$\left[FA_{sample} - \left(IA_{sample} \times F\right)\right] / \left[FA_{std} - \left(IA_{blank} \times F\right)\right]$$

284

285 where F = 0.8. We then multiplied this value by the concentration of the glycerol standard
286 solution (2.5mg/ml) and used the average value across all 8 replicate samples as the RIL mean
287 triglyceride level for mapping and analysis. For precise details of the enzyme assay and
288 triglyceride calculation, see the SigmaAldrich Serum Triglyceride Determination kit (TR0100).
289 Differences in triglyceride level due to DSPR subpopulation were investigated with a one-way
290 ANOVA followed by post hoc comparisons using Tukey's HSD (experiment-wide α = 0.05).

291

292 ## Correlations among traits

293 The relationships between starvation resistance measured in the large-scale starvation
294 assay and DAM starvation resistance, desiccation resistance, and triglyceride level were assessed

295 with general linear models using the mean trait value per RIL. Subpopulation (pA1, pA2, pB1,
296 pB2) was included as a factor in the analysis of starvation resistance and triglyceride content.
297 Correlations among the three DGRP starvation datasets - that from Mackay et al. (2012),
298 Everman and Morgan (2018), and the new screen we report here - were examined in a pairwise
299 manner using line means, accounting for multiple comparisons with a Bonferroni-adjusted alpha
300 level (three comparisons: α = 0.0167). Any difference in overall mean starvation resistance
301 measured over experiments was analyzed with a one-way ANOVA, treating study as a fixed
302 factor.
303

304 ## Heritability

305 The genetic and phenotypic variances of starvation resistance and triglyceride content for
306 the pA and pB DSPR panels, and of starvation resistance for the DGRP panel, were estimated with
307 a linear mixed model using the lme and varcomp functions in R (R package: APE, Paradis *et al.*
308 2004; R package: nlme, Pinheiro *et al.* 2017). We calculated broad sense heritability as the
309 proportion of the total variance of the strain-specific response explained by the estimated
310 genetic variance component (Lynch and Walsh 1998).
311

312 ## QTL mapping in the DSPR

313 QTL mapping analysis and power using the DSPR panel are discussed in detail in King *et*
314 *al.* (2012a,b). Briefly, QTL mapping and peak analysis was performed on starvation resistance and
315 triglyceride data using the DSPRqtl R package (github.com/egking/DSPRqtl; FlyRILs.org) by
316 regressing the mean trait response for each RIL on the additive probabilities that each of the
317 panel founders contributed to the haplotype of the RIL at each mapped position. Significance
318 thresholds were assigned following 1000 permutations of the data, and positions of putative
319 causative loci were estimated with 2-LOD support intervals, which approximate 95% confidence
320 intervals for QTL position in the DSPR (King *et al.* 2012a). Mean starvation resistance varied
321 between sexes in both the pA and pB panel ($F_{3,3440}$ = 18.317; $p < 0.0001$; Fig S1; Table S1), and
322 subpopulation influenced female starvation resistance in the pA panel (Tukey's HSD $p < 0.0001$;
323 Fig S1). Therefore, QTL mapping was performed for males and females of each panel separately,

324 and subpopulation was included as a covariate in the analysis of pA females. Mean female
325 triglyceride level was similar between the pA1 and pA2 subpopulations (Tukey's HSD $p = 0.75$; Fig
326 S2; Table S2) but varied between the pB1 and pB2 subpopulations (Tukey's HSD $p < 0.0001$; Fig
327 S2; Table S2), so subpopulation was included as a covariate in QTL analysis of the pB triglyceride
328 data.
329

330 ## Genomewide association mapping in the DGRP

331 Variants associated with male and female starvation resistance in the DGRP were
332 identified using the DGRP2 web-based GWA mapping tool (http://dgrp2.gnets.ncsu.edu), which
333 takes into account variable *Wolbachia* infection status and large inversions that segregate among
334 the lines (Mackay *et al.* 2012; Huang *et al.* 2014). We performed GWA on data collected in this
335 study and reanalyzed data from Mackay *et al.* (2012) and for starvation resistance data obtained
336 from young flies (5 – 7 days old) from Everman and Morgan (2018). We additionally assigned the
337 150 DGRP lines that are shared between the three datasets an across-study mean and performed
338 GWA on this summary measure of starvation resistance.
339 SNPs associated with starvation resistance were identified within each of the four
340 datasets following correction for multiple comparisons using FDR (Benjamini and Hochberg 1995)
341 in R (p.adjust; R Core Team 2017). Since we found no significantly associated SNPs with an FDR
342 adjusted p-value < 0.05 for any starvation resistance dataset in either sex, we relaxed the
343 significance threshold to an FDR adjusted p-value < 0.2. As a significance threshold of $P < 10^{-5}$ is
344 commonly used in the DGRP (e.g. Mackay *et al.* 2012; Morozova *et al.* 2014; Huang *et al.* 2014;
345 Everman and Morgan 2018), we also present SNPs associated with starvation resistance in each
346 of the four datasets using this threshold.
347 There was minimal overlap in the identity of the above-threshold, starvation-associated
348 variants in each study. Thus, we sought to examine whether the sign of the additive effects of
349 these sets of variants was preserved across studies. Additive effects were calculated as one-half
350 the difference in starvation resistance between lines homozygous for the major allele and lines
351 homozygous for the minor allele (major allele frequency > 0.5), after accounting for *Wolbachia*
352 infection and TE insertions (Falconer and Mackay 1996; Huang *et al.* 2014). To determine the

353 proportion of SNPs that are expected by chance to have additive effects of the same sign across
354 studies, we obtained random samples of 50 SNPs from all of the DGRP SNP calls (> 2 million SNPs)
355 and calculated the additive effects of the random SNPs across pairs of datasets for each sex. To
356 account for the possibility that the frequency spectrum of above-threshold ($P < 10^{-5}$), associated
357 SNPs is not represented by a set of randomly-selected variants, we stratified the random subsets
358 of 50 SNPs according to the distribution of allele frequencies of the top 50 SNPs associated with
359 starvation resistance for each sex in each study. Allele frequency bins used in this stratification
360 were 0.05 – 0.1, > 0.1 – 0.2, > 0.2 – 0.3, > 0.3 – 0.4, and > 0.4 – 0. 5. The exact stratification for
361 each sex and dataset is provided in Table S3. This process was repeated 1000 times for each
362 paired comparison of datasets (6 comparisons total) using an ordinary nonparametric
363 bootstrapping procedure with the R package boot (Davison and Hinkley 1997; Canty and Ripley
364 2017). For each iteration, we used a custom R function (see File S2) to calculate the proportion
365 of the 50 random stratified SNPs that had positive additive effects in both of the datasets being
366 compared.
367

368 ### Data availability

369 DGRP data from Mackay *et al.* (2012) are available online from
370 http://dgrp2.gnets.ncsu.edu, and DGRP data from Everman and Morgan (2018) are available
371 from Dryad (DOI: https://doi.org/10.5061/dryad.vq087). All phenotypes collected in this study
372 are available in File S1. R code for the bootstrapping analysis and calculation of additive effects
373 for DGRP starvation resistance is available in File S2.

374 ## Results and Discussion

375 ### Phenotypic variation in starvation resistance

376 Starvation resistance in both the DSPR and DGRP was highly variable among strains (Fig
377 1, Fig 2). In the DSPR, pB RILs tended to have higher starvation resistance than pA RILs across
378 sexes (Fig S1), and a narrower range of phenotypic variation in mean starvation resistance (Fig
379 1). These differences likely reflect the different sets of founder lines used to establish the two
380 sets of RILs. Males and females in both the DSPR and DGRP differed in mean starvation resistance,

381 with males being less starvation resistant overall (Fig S1, Fig S3, Table S1, Table S4). Nonetheless,
382 male and female starvation resistance was significantly correlated in the DSPR (pA: R^2 = 53.0%;
383 pB: R^2 = 57.0%; Fig S4) and DGRP (R^2 = 68.0%; Fig S5). Any sex-specific differences in starvation
384 resistance are likely influenced by a combination of higher glycogen and triglyceride levels and
385 larger body size, which are often observed for females relative to males (Chippindale *et al.* 1996;
386 Toda and Kimura 1997; Schwasinger-Schmidt *et al.* 2012; Goenaga *et al.* 2013). Finally, the broad
387 sense heritability for starvation resistance measured for both sexes in the DSPR and DGRP was
388 routinely high (Table 1).
389
390
391
392 ***Table 1.*** *Broad sense heritability by trait, sex, and mapping panel.*

Panel	Trait	Sex	Heritability
DSPR pA	SR	Female	80.0%
DSPR pA	SR	Male	73.1%
DSPR pB	SR	Female	74.4%
DSPR pB	SR	Male	71.5%
DGRP	SR	Female	87.1%
DGRP	SR	Male	87.0%
DSPR pA	TL	Female	77.5%
DSPR pB	TL	Female	82.3%

SR = Starvation Resistance; TL = Triglyceride Level

393

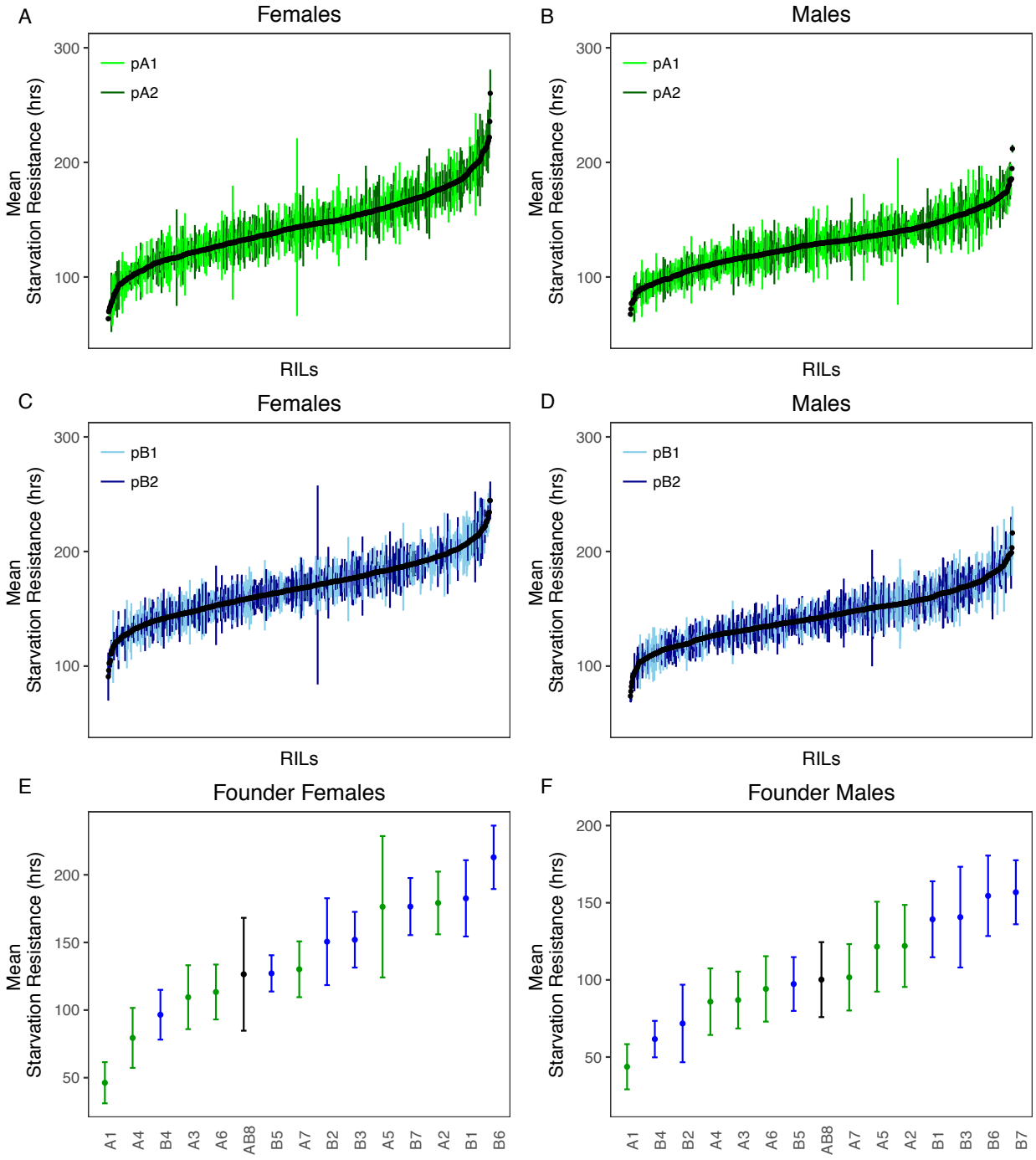
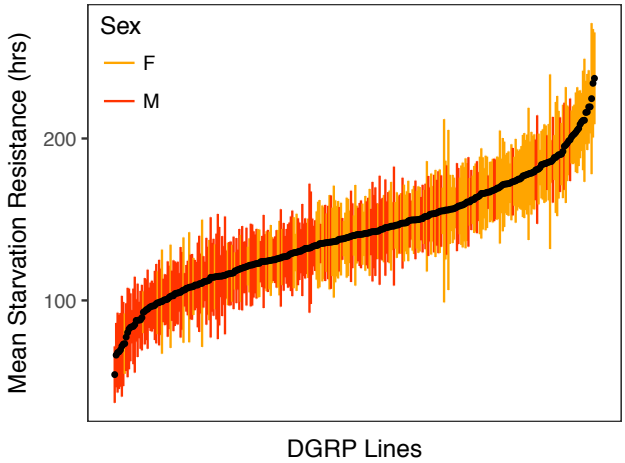


Figure 1. *Variation in mean (±SD) starvation resistance for each sex. A-D shows data for DSPR RIL panels pA and pB. E and F show data for the founder lines. In E and F, names of the founder lines are shown on the x-axis; the founder line AB8 is the founder shared by the two mapping panels.*



398

399 ***Figure 2.*** *Variation in mean DGRP (±SD) starvation resistance in females (orange) and males (red).*

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401
402
403 We screened the DSPR and DGRP at 23°C and under constant light conditions, whereas
404 others have measured starvation resistance in *D. melanogaster* at a higher temperature and with
405 a light cycle (e.g. Mackay *et al.* (2012) and Everman and Morgan (2018)). Since starvation
406 resistance is sensitive to the thermal environment (van Herrewege and David 1997; Karan and
407 Parkash 1998; Karan *et al.* 1998; Hoffmann *et al.* 2005a; Bauerfeind *et al.* 2014) and may vary
408 under different photoperiods (Sheeba *et al.* 2000; Xu *et al.* 2008; Seay and Thummel 2011), we
409 sought to re-measure starvation resistance for a subset of DSPR RILs at 25°C and with a 12 hour
410 : 12 hour light/dark cycle. Overall, starvation resistance of the re-tested RILs was lower in both
411 sexes compared to that measured in the original large-scale starvation assay (effect of assay:
412 $F_{1,136} = 31.60$, $p < 0.0001$; Fig S6A). Despite this, starvation resistance in the subset of RILs was
413 significantly correlated between the two experiments (Females: $\beta = 0.43 \pm 0.04$, $t = 9.7$, $p <$
414 0.0001, $R^2 = 73.9\%$; Males: $\beta = 0.59 \pm 0.05$, $t = 10.9$, $p < 0.0001$, $R^2 = 78.3\%$; Fig S6B).
415 We similarly compared starvation resistance phenotypes for the DGRP measured in the
416 current study with those generated by Mackay *et al.* (2012) and Everman and Morgan (2018). On
417 average, starvation resistance in the DGRP measured in this study was much higher compared to
418 estimates from Mackay *et al.* (2012) and Everman and Morgan (2018) ($F_{2,532} = 1457.5$, $p < 0.0001$;
419 Fig S7). We can only speculate as to the cause of this major difference, but we did use a lower
420 rearing/testing temperature than these other studies (23°C versus 25°C), our flies were not

421 exposed to a light cycle, humidity varied across studies, and the fly culture media is likely
422 different. Notably, we included preservatives in the starvation media we used here, in contrast
423 to that used in Mackay *et al*. (2012) and Everman and Morgan (2018). The addition of
424 preservatives may have contributed to the increased starvation resistance we observed via
425 suppression of fungal and bacterial growth.

426 Even given the large across-study difference in mean starvation resistance in the DGRP,
427 we found moderately strong correlations in both sexes among each dataset, ranging from 50.8%
428 to 64.4% (Fig S8). The high correspondence among these three DGRP datasets, coupled with the
429 phenotypic correlation between the subset of DSPR strains assayed using two different
430 approaches (see above), suggests that fundamental aspects of the genetic control of starvation
431 resistance are generally consistent over experiments, even when environmental conditions such
432 as temperature are quite different. The differences we observed in starvation resistance between
433 studies may reflect ecologically-relevant phenotypic plasticity. The temporally variable thermal
434 environment is a particularly important source of selection for ectothermic organisms (Bell 2010;
435 Bergland *et al.* 2014). Plastic shifts in starvation resistance in response to temperature can have
436 important fitness benefits, including seasonal adaptation to fluctuating resource availability as
437 has been reported in the butterfly *Bicyclus anynana* (Pijpe *et al.* 2007) and following the induction
438 of diapause in *D. melanogaster* (Schmidt *et al.* 2005; Rion and Kawecki 2007). Collectively, these
439 previous studies and our data speak to the important influence of both phenotypic plasticity and
440 genotype on variation in starvation resistance in natural populations.

441

442 Desiccation resistance and activity levels in DSPR lines with extreme starvation resistance

443 Several artificial selection studies for starvation resistance have observed a correlated
444 change in desiccation resistance, suggesting these stress traits are related (Hoffmann and
445 Parsons 1989; Chippindale *et al.* 1996; Hoffmann and Harshman 1999; Harshman *et al.* 1999;
446 Hoffmann *et al.* 2001). We investigated this correlation in the DSPR by measuring female
447 desiccation resistance in RILs chosen from the two tails of the phenotypic distribution of female
448 starvation resistance (17 high starvation RILs and 16 low). We found that desiccation and
449 starvation resistance were significantly correlated ($R^2 = 43.8$, $F_{1,31} = 24.11$, $p < 0.0001$; Fig 3). We

also found that mean desiccation resistance was considerably lower than starvation resistance in the DSPR (Figures 1 versus 3), replicating previous studies (Da Lage *et al.* 1990; Hoffmann and Parsons 1993; van Herrewege and David 1997; Karan and Parkash 1998; Karan *et al.* 1998; Hoffmann *et al.* 2001). This pattern is most likely due to the much higher risk of death due to water loss compared to risk of death due to lack of food (Fairbanks and Burch 1970; Da Lage *et al.* 1990; Gibbs 2003). For instance, flies that die due to desiccation still have the majority of their lipid stores (Da Lage *et al.* 1989), whereas, under sufficiently humid conditions, flies remain alive until their fat stores are depleted (Da Lage *et al.* 1990; Karan *et al.* 1998).

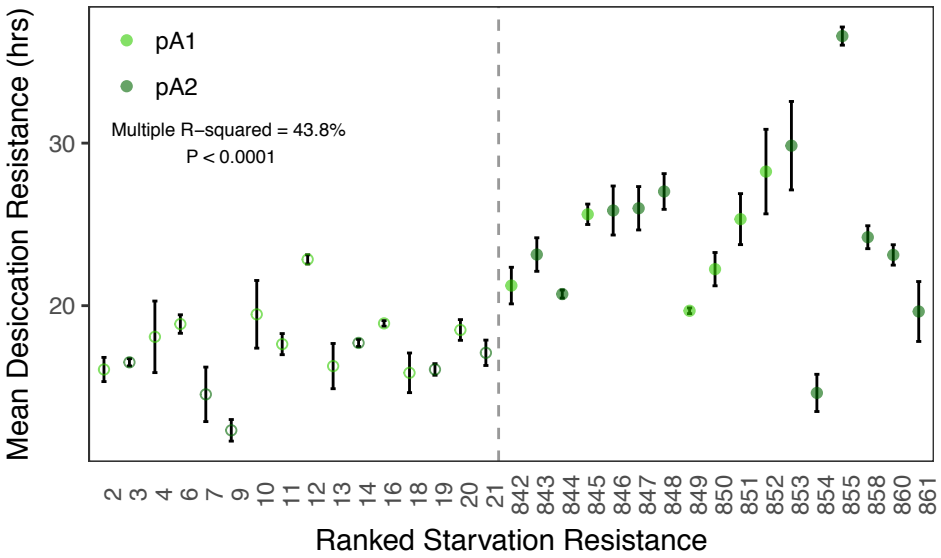


Figure 3. *Mean starvation and desiccation resistance are correlated in the DSPR ($F_{1,31}$ = 21.11, $p < 0.0001$). Desiccation resistance is presented as RIL means (±SD). Open symbols indicate "low" starvation resistance RILs; filled symbols indicate "high" starvation resistance RILs, and the dashed vertical line also separates these RIL classes.*

The relationship between starvation and desiccation resistance in this study provides support for the genetic correlation that has been proposed to exist between these traits. However, it does not rule out the potential that the covariation observed between starvation and desiccation resistance may be influenced by genetic variation in one or more other traits that are related to these resistance phenotypes, such as body size or overall lifespan (Rose *et al.* 1992; Parsons 1995; Chippindale *et al.* 1996; Toda and Kimura 1997; Karan and Parkash 1998; Hoffmann

472 and Harshman 1999; Bubliy and Loeschcke 2005). Indeed, instances of positively and negatively
473 correlated levels of desiccation and starvation resistance in natural and selected populations
474 have been described, suggesting that these stress traits can vary independently across different
475 environments and selection regimes (Da Lage *et al.* 1990; van Herrewege and David 1997; Karan
476 and Parkash 1998; Karan *et al.* 1998; Kennington *et al.* 2001; Harbison *et al.* 2004). Physiological
477 independence of desiccation and starvation resistance likely contributes to the varying strength
478 of the correlation between these traits across multiple studies (Hoffmann and Harshman 1999).
479 A more intensive sampling of the DSPR would be necessary to thoroughly investigate the genetic
480 correlation between starvation and desiccation resistance.

481 One physiological mechanism that may increase tolerance to environmental stressors
482 (such as starvation or desiccation) is a reduction in metabolic rate (Lighton and Bartholomew
483 1988; Hoffmann and Parsons 1989, 1991; Chippindale *et al.* 1996; Djawdan *et al.* 1997; Marron
484 *et al.* 2003; Rion and Kawecki 2007; Schwasinger-Schmidt *et al.* 2012; Slocumb *et al.* 2015).
485 Indeed, selection for starvation resistance has been shown to lead to a correlated change in
486 activity level, an indirect proxy for metabolic rate (Hoffmann and Parsons 1993; Schwasinger-
487 Schmidt *et al.* 2012). Here, we assessed activity of a subset of high (N = 16) and low (N = 19)
488 starvation resistance RILs to understand how genetic variability in starvation resistance relates
489 to activity levels under non-stressful conditions. Prior to starvation, males and females differed
490 in activity level across the light and dark period ($F_{1,132} = 16.9$, $p < 0.0001$; Fig 4B; Table S5), and
491 high starvation resistance RILs were less active than low starvation resistance RILs ($F_{1,132} = 12.5$,
492 $p < 0.001$; Fig 4A; Table S5). The effects of starvation resistance rank (high vs low), sex, and the
493 light status (light versus dark) on activity were similar in magnitude (Cohen's F: 0.21-0.36; Table
494 S5), suggesting that these factors contribute similarly to variation in waking activity levels.

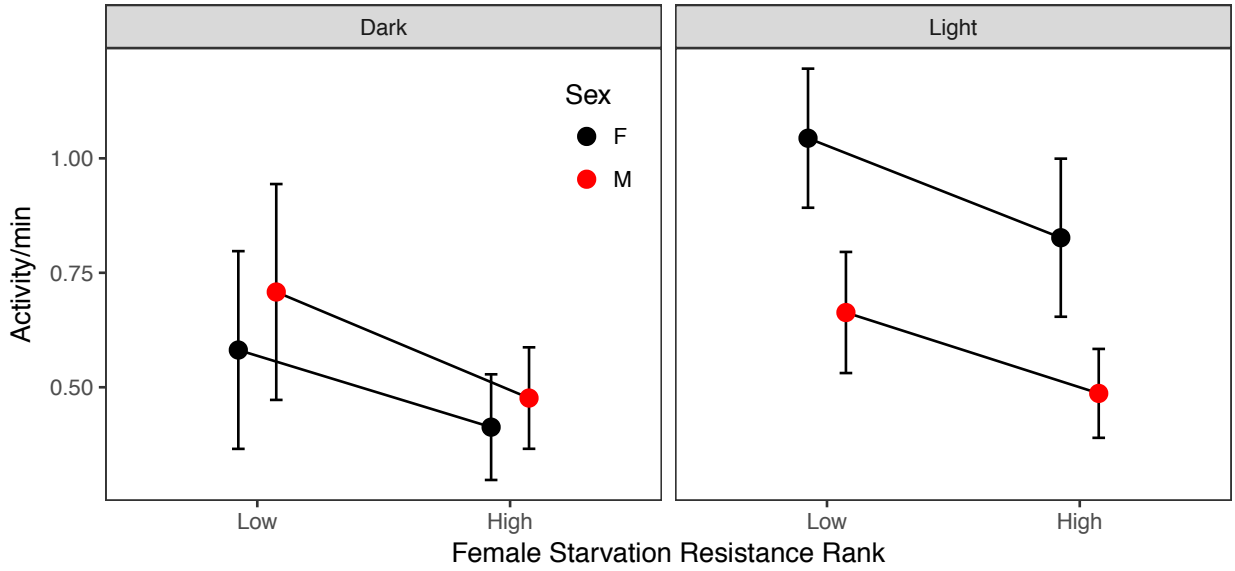


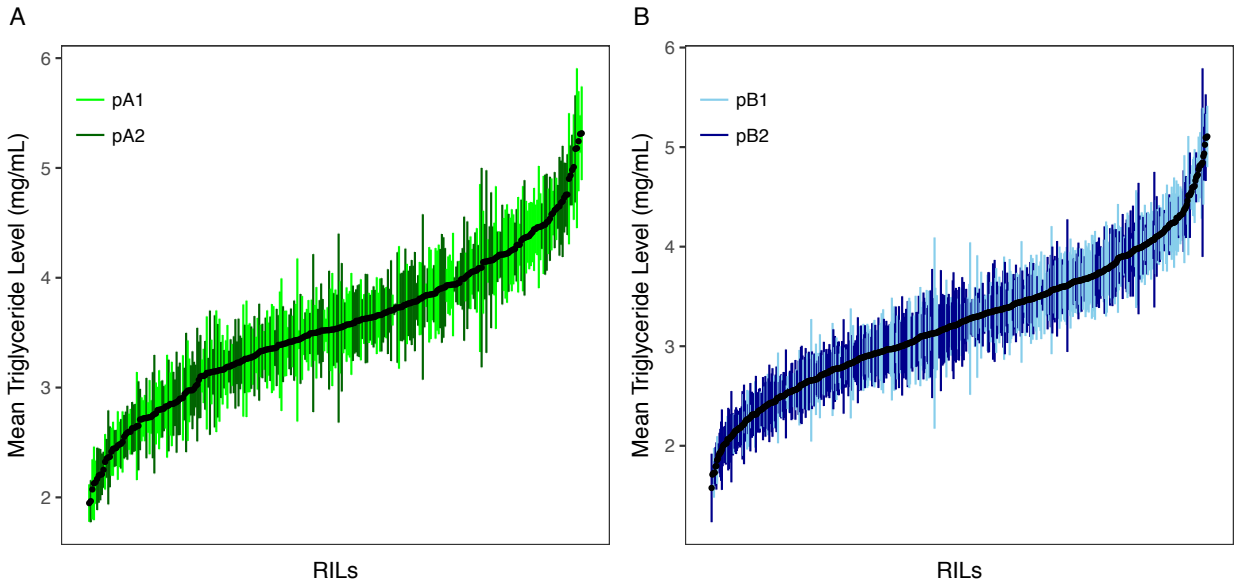
Figure 4. *Activity level on regular media for males (red) and females (black) from a subset of high and low female starvation resistance RILs. Panels in each figure indicate the light and dark periods of a 24-hour monitoring period. Activity while awake was influenced by both a sex-by-light interaction ($F_{1,132}$ = 16.9, P < 0.001) and by starvation resistance class (i.e. high or low; $F_{1,132}$ = 12.5, P < 0.001).*

The differences in activity between high and low starvation resistance lines on regular, nutritive media (Figure 4) were preserved under starvation conditions, with the high starvation resistant lines being less active than the low starvation resistant lines throughout the starvation process (Fig S9; Table S6). This pattern aligns with those from previous studies. For instance, Schwasinger-Schmidt *et al.* (2012) found that activity of flies with high starvation resistance was reduced following 15 generations of selection for starvation resistance in both males and females. Slocumb *et al.* (2015) also found that waking activity was reduced in lines selected to have high starvation resistance. Although previous associations between increased starvation tolerance and lower activity levels, metabolic rate, and changes in behavior have been observed (Murphey and Hall 1969; Hoffmann and Parsons 1989; Blows and Hoffman 1993; Djawdan *et al.* 1997; Karan *et al.* 1998; Schwasinger-Schmidt *et al.* 2012; Masek *et al.* 2014), our findings present a novel addition to our understanding of how increased starvation resistance may occur. Behavioral components of energy conservation are likely to play a role in how individuals

516 compensate for stressful conditions (van Dijk *et al.* 2002; McCue 2010; Masek *et al.* 2014) and
517 represent an additional facet of the complex nature of phenotypic variability in starvation
518 resistance.
519

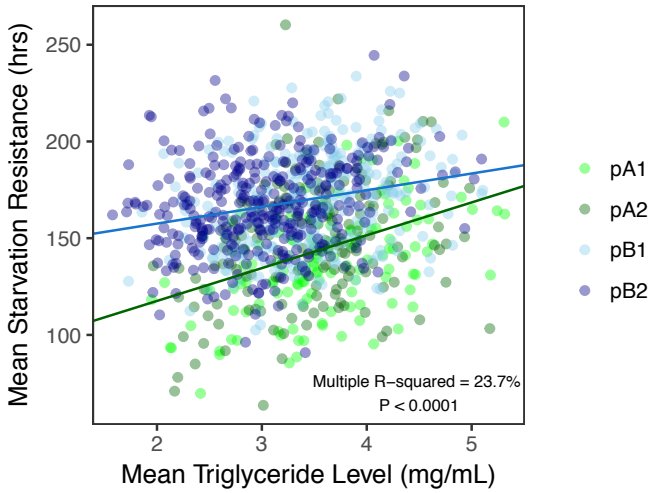
520 ## Phenotypic variation in triglyceride level

521 Periods of starvation have been shown to significantly reduce triglyceride levels in both
522 males and females (Schwasinger-Schmidt *et al.* 2012), and others have suggested that fat stores
523 and starvation resistance may be genetically correlated (Service *et al.* 1985; Rose *et al.* 1992;
524 Chippindale *et al.* 1996; Harshman *et al.* 1999; Schwasinger-Schmidt *et al.* 2012; Slocumb *et al.*
525 2015). To investigate the relationship between these traits in the DSPR, we measured mean
526 female triglyceride level in a subset of the pA and pB DSPR RILs and found substantial phenotypic
527 variation among RILs (pA: N = 311, 1.95 – 5.32 mg/mL; pB: N = 628, 1.58 – 5.11 mg/mL; Fig 5).
528 Broad sense heritability estimates for triglyceride level were high for each panel (Table 1). Mean
529 starvation resistance and triglyceride level were positively correlated in both DSPR panels,
530 although the correlation in the pA and pB panels was significantly different (Fig 6). Overall,
531 variation in mean starvation resistance explained 23.7% of the variation observed in mean
532 triglyceride level among the DSPR RILs across the two mapping panels (R^2 = 23.7%, $F_{3,929}$ = 95.96,
533 $p < 0.0001$; Fig 6), suggesting that a proportion of variation in the starvation resistance response
534 can be explained by variation in triglyceride level in the DSPR.



535

536 ***Figure 5.*** *Variation in mean DSPR triglyceride level (±SD) for females in the pA panel (A) and pB panel (B).*



537

538 ***Figure 6.*** *Mean female starvation resistance and female triglyceride level are positively correlated ($F_{3,929}$ =*
539 *95.96, p < 0.0001). The strength of the correlation varied between the two mapping panels (interaction:*
540 *$F_{1,929}$ = 9.32, p < 0. 01). Points are colored to indicate subpopulation for each mapping panel, although*
541 *subpopulation was not included in the regression analysis.*

542
543
544
545 The correlation between triglyceride level (or total lipid level, depending on the study)
546 and starvation resistance has been measured in numerous natural and artificially selected
547 *Drosophila* populations, and a positive relationship is often described (Chippindale *et al.* 1996;
548 Djawdan *et al.* 1997; van Herrewege and David 1997; Harshman *et al.* 1999; Schwasinger-Schmidt

549 *et al.* 2012; Goenaga *et al.* 2013; Slocumb *et al.* 2015; Hardy *et al.* 2018). For example, in
550 isofemale lines derived from populations distributed across approximately 14.4 degrees of
551 latitude, Goenaga *et al.* (2015) found that 12% of the variation in female starvation resistance
552 was accounted for by lipid content. Similarly, Chippindale *et al.* (1996) found a very strong
553 positive relationship between total lipids and starvation resistance following extended selection
554 for increased starvation resistance and suggested that lipid levels may directly determine
555 starvation resistance. Hoffmann and Harshman (1999) also inferred that lipid content is the
556 primary influencer of starvation resistance. However, a strong correspondence between lipid
557 content and starvation resistance is not always observed in strains derived from natural
558 populations (Da Lage *et al.* 1990; Robinson *et al.* 2000; Schmidt *et al.* 2005). Hoffman *et al.* (2001)
559 suggested that variation in the strength of the correlation between triglycerides and starvation
560 resistance may be due to the evolutionary history of the study population. Evolutionary tradeoffs
561 between increased lipid storage and other aspects of fitness may also influence the correlation
562 between starvation resistance and lipid levels (Hardy *et al.* 2015, 2018). Furthermore, artificial
563 selection may increase starvation resistance via mechanisms that preferentially modify lipid
564 accumulation or metabolism, rather than by impacting energy level (Hoffmann and Parsons 1989;
565 Blows and Hoffman 1993; Hoffmann *et al.* 2001; Marron *et al.* 2003; Rion and Kawecki 2007). The
566 relationship observed between triglyceride levels and starvation resistance in our study supports
567 the hypothesis that triglyceride levels and starvation resistance are physiologically and
568 potentially genetically related. Equally, it is evident from our DSPR data that triglyceride level
569 likely influences starvation resistance to a lesser degree than proposed by Chippindale *et al.*
570 (1996) and Hoffmann and Harshman (1999).
571

572 ## QTL mapping of starvation resistance and triglyceride level in the DSPR

573 Following characterization of the extensive phenotypic variation for starvation resistance
574 and triglyceride level in the DSPR, we used QTL mapping to investigate and compare the genetic
575 architectures of these traits in this multiparental panel that offers high power to map QTL to
576 relatively modest genetic intervals (see King *et al.* (2012a)).

577 We mapped 8 QTL for starvation resistance in the pA panel and 7 QTL in the pB panel,
578 several of which overlapped between sexes (Fig S10). Both sets of QTL explained a substantial
579 amount of variation in starvation resistance, with individual peaks accounting for 3.7-13.2% of
580 the variation (Table 2). The total variance explained by QTL in the pA (pB) population was 26.1%
581 (32.8%) in females and 17.5% (37.9%) in males, assuming QTL are independent and additive
582 (Table 2). None of the QTL identified in the pA and pB mapping panels overlapped, and since
583 power to detect 5% QTL is expected to be high in our study (King *et al.* 2012a) and all DSPR
584 phenotyping was completed within seven months using the same design and environmental
585 conditions, this likely reflects genetic variation among the different sets of founders used to
586 establish the two sets of lines.
587
588
589 ***Table 2.*** *Summary of QTL identified for starvation resistance and triglyceride level in the DSPR, divided by*
590 *mapping panel (pA, pB). No QTL were identified for triglyceride level in the pA panel. Genes and functions*
591 *associated with starvation resistance are listed in Table S7. In the last column, % genes associated with*
592 *starvation resistance was determined by cross-referencing genes within each QTL interval with previously*
593 *reported candidates and biological functions reported by FlyBase (Lin* et al. *1998; Clancy* et al. *2001;*
594 *FlyBase Curators* et al. *2004; Harbison* et al. *2004; Sørensen* et al. *2007; Nuzhdin* et al. *2007).*

Starvation Resistance QTL: pA DSPR panel

QTL	Sex	Peak LOD	Chr	Physical Interval (Mb)	Genetic Interval (cM)	Variance Explained	No. Genes	No. Genes assoc. with SR
SA1	M	7.41	2L	0.34-0.64	0.36-1.02	3.90	52	3
SA2	F	10.85	2R	4.90-5.53	59.39-60.64	6.01	122	10
SA2	M	10.59	2R	4.87-5.58	59.32-60.74	5.52	126	10
SA3	F	8.05	2R	12.85-13.43	80.75-82.81	4.23	110	9
SA4	F	7.03	2R	14.18-14.72	84.14-86.68	3.70	105	7
SA5	F	10.13	3L	3.40-3.92	6.89-8.88	5.29	52	4
SA6	F	13.21	3R	0.79-2.37	47.10-47.52	6.85	244	11
SA7	M	7.66	3R	15.55-15.86	66.52-67.36	4.03	52	4
SA8	M	7.73	3R	21.07-21.48	86.77-88.20	4.06	80	3

Starvation Resistance QTL: pB DSPR panel

QTL	Sex	Peak LOD	Chr	Physical Interval (Mb)	Genetic Interval (cM)	Variance Explained	No. Genes	No. Genes assoc. with SR
SB1	F	7.68	2L	7.02-7.30	23.69-25.20	4.05	33	1
SB2	M	8.35	2L	10.10-10.50	39.24-40.93	4.39	110	13
SB3	F	7.13	2L	12.14-12.65	45.94-46.96	3.77	44	4
SB3	M	7.05	2L	12.13-12.63	45.92-46.93	3.72	45	4
SB4	F	12.28	2R	1.10-2.70	54.93-55.73	6.41	148	10

SB4	M	19.40	2R	2.01-2.69	55.21-55.72	9.91	81	8
SB5	F	18.98	3L	18.94-19.42	45.48-45.75	9.73	54	5
SB5	M	26.40	3L	19.00-19.43	45.51-45.76	13.24	46	3
SB6	F	9.07	3R	8.86-9.20	52.50-53.29	4.77	48	7
SB6	M	12.77	3R	8.57-9.21	52.33-53.30	6.64	90	9
SB7	F	7.72	3R	25.65-26.15	99.12-100.11	4.08	111	8
Triglyceride Level QTL: pB DSPR panel								
QTL	**Sex**	**Peak LOD**	**Chr**	**Physical Interval (Mb)**	**Genetic Interval (cM)**	**Variance Explained**	**No. Genes**	**No. Genes assoc. with SR**
TB1	F	8.73	3L	3.45-4.09	7.08-9.52	6.22	84	7
TB2	F	7.90	3R	7.18-8.01	50.50-51.54	5.64	118	5
TB3	F	8.45	3R	8.31-8.71	51.96-52.54	6.03	54	8
TB4	F	7.71	X	17.34-17.43	57.17-57.44	5.51	4	0

SR = Starvation Resistance

We mapped four distinct QTL for triglyceride level in the pB population, each of which accounted for 5.5-6.2% of the variation in this trait (Table 2; Fig S11), in total explaining 23.4% of the variation in pB. No QTL for triglyceride level were detected in the pA panel, likely due to the reduced number of pA RILs assessed (pA N = 311; pB N = 628). However, even with this reduced power, the QTL map for pA suggests that the genetic architecture for triglyceride level is different between the two mapping panels, as there is no evidence of near-significant peaks in pA within QTL intervals statistically identified in the pB panel (Fig S11). As has been seen in a number of studies using the DSPR and other multiparental populations (King *et al.* 2012b; Giraud *et al.* 2014; Najarro *et al.* 2015), the estimated phenotypic effects of each founder haplotype suggest that multiple alleles may be present at our starvation and triglyceride QTL, since the effects do not fall into two clear "high" and "low" classes (Figs S10 and S11).

Given the phenotypic correlation between triglyceride level and starvation resistance in the DSPR (Fig 6) and similar correlations previously reported in other studies (Chippindale *et al.* 1998; Hoffmann and Harshman 1999), one might predict overlap of QTL associated with these traits. Indeed, the intervals of two QTL identified for triglyceride level, TB1 and TB3, do overlap with the female SA5 and male SB6 QTL intervals, respectively, identified for starvation resistance (Table 2). However, given the complete lack of evidence for QTL for the same trait co-localizing in both the pA and pB DSPR mapping panels, it is unlikely the variant(s) underlying TB1 (mapped

616 in the pB population) and SA5 (mapped in the pA population) are the same. The limited overlap
617 in the QTL intervals associated with starvation and triglycleride level suggests that the genetic
618 bases of this pair of traits are largely independent.

619 To investigate the relationship between the two QTL that do overlap within the same
620 panel (SB6 and TB3), we assessed the influence of haplotype structure at the overlapping QTL on
621 the positive phenotypic correlation between triglyceride level and starvation resistance (Fig 6).
622 In this analysis, we first identified the founder haplotype for each RIL at the positions of the
623 overlapping QTL peaks, then calculated the mean trait response of these haplotype groups (i.e.
624 the average phenotypes of RILs carrying each of the founder haplotypes). We then assessed the
625 correlation between haplotype-specific mean triglyceride level and starvation resistance with a
626 general linear model. After accounting for the haplotype structure at the overlapping peaks, we
627 found that mean starvation resistance and triglyceride level were significantly correlated ($F_{1,7}$ =
628 7.72, $p < 0.05$, R^2 = 52.4%; Fig S12), suggesting some pleiotropic variants may be responsible for
629 this pair of overlapping starvation resistance and triglyceride level QTL.

630 Across all QTL identified for starvation resistance and triglyceride level in this study,
631 several genes within the QTL intervals have functions related to these and other correlated traits
632 (Table 2, Table S7). Of particular interest are the 30 genes that fall within our QTL intervals that
633 were identified in previous starvation resistance studies (Clancy *et al.* 2001; Harbison *et al.* 2005;
634 Sørensen *et al.* 2007) (Table S7). Gene ontology analyses performed for each trait and panel also
635 revealed enrichment of genes within pA QTL related to glutathione metabolic process (6.91-fold
636 enrichment, FDR corrected P = .000146; Table S8), as well as several categories that were
637 enriched for pB triglyceride level (Table S8). However, it should be noted that the sets of genes
638 implicated by QTL mapping in the DSPR (3-244 genes per interval in this study) are extremely
639 unlikely to all contribute to trait variation, and their presence within QTL intervals cannot alone
640 be taken as evidence for causality, particularly if the number of genes that actually contribute to
641 trait variation is high.

642 Upon examination of the biological processes assigned to genes within the overlapping
643 starvation resistance and triglyceride intervals (TB3 and SB6), we found several genes that have
644 either been predicted or experimentally demonstrated to be associated with traits related to

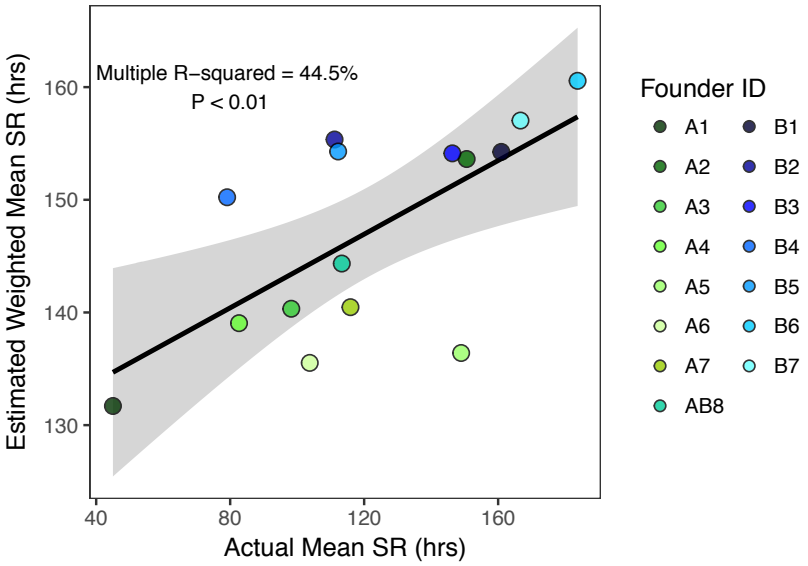
645 starvation resistance and triglycerides or metabolism (Table S7 and references therein). Genes
646 that fall within the intervals of the overlapping peaks include those that influence adult lifespan
647 (e.g. *ry*, *Men*, *Gnmt* (Simonsen *et al.* 2006; Paik *et al.* 2012; Obata and Miura 2015)), lipid
648 metabolic processes (including *Lip3*, *CG11598*, *CG11608*, *CG18530* (FlyBase Curators *et al.* 2004)),
649 insulin signaling (e.g. *poly* (Bolukbasi *et al.* 2012)), response to starvation (e.g. *mthl12*, *Gnmt*
650 (UniProt Curators 2002; Obata *et al.* 2014)), larval feeding behavior (e.g. *Hug* (Melcher and
651 Pankratz 2005)), circadian rhythm and sleep (e.g. *timeout*, *Men* (Harbison *et al.* 2004; Benna *et*
652 *al.* 2010)), and triglyceride homeostasis (*Gnmt* (Obata *et al.* 2014)) (Table S7). These genes are
653 promising candidates for future studies seeking to examine the functional genetic relationship
654 between these two traits.
655

656 Predicting starvation resistance in the DSPR founders with mapped QTL haplotype effects

657 We can estimate the effect associated with each founder haplotype at each mapped QTL
658 in the DSPR. It follows that a combination of the estimates across all QTL can be used to predict
659 the actual phenotypes of the original founder strains. We measured starvation resistance in the
660 15 founder lines in addition to the DSPR RILs (Fig 1E, F) to test this prediction. It is likely that the
661 strength of the correlation between the estimated and actual trait response is influenced by the
662 number and effect size of each QTL mapped for the trait. To account for differences in the degree
663 to which starvation resistance is influenced by each QTL, we calculated the predicted mean trait
664 response for each founder line weighted by the variance explained by each QTL. By weighting the
665 estimates by the variance, QTL that have smaller effects will also have less influence on the
666 overall mean predicted trait response for each founder line, while QTL with large effects will
667 influence the predicted trait response for each founder line to a proportionately larger degree.

668 As predicted, using a general linear model the weighted mean predicted starvation
669 resistance of the founders based on QTL effects was significantly correlated with the sex-
670 averaged mean starvation resistance measured for the founder lines (R^2 = 44.5%, $F_{1,13}$ = 10.43, p
671 < 0.01; Fig 7). The slope of this relationship is relatively small (β = 0.16 +/- 0.05), suggesting that
672 while a large component of variation in starvation resistance is clearly genetic (as supported by
673 heritability estimates for each panel), substantial variation in the phenotype response is

674 unaccounted for by additive genetic effects at mapped QTL. This unaccounted-for genetic
675 variation in starvation resistance is likely due to many QTL with very small effects beyond our
676 power to detect them (King *et al.* 2012a). However, the strength of the correlation between
677 predicted and actual responses does suggest that QTL identified from the DSPR mapping panels
678 identify causative loci that influence the level of starvation resistance among the progenitors of
679 the RILs.



680

681 ***Figure 7.*** *Estimated starvation resistance weighted by the variance explained by each QTL and actual*
682 *starvation resistance measured for the 15 founder lines of the DSPR were significantly correlated (β = 0.16*
683 *$\pm$ 0.05; $F_{1,13}$ = 10.43, p < 0.01). AB8 identifies the founder line shared between the pA and pB mapping*
684 *panels. Grey shading indicates the 95% CI of the regression.*

685
686
687

688 Genomewide association mapping in the DGRP

689 Dissection of a quantitative genetic trait in multiple mapping panels can allow greater
690 resolution of the genetic architecture, and provide insight into how alleles unique to each panel
691 contribute to phenotypic variation. To gain this additional understanding we measured starvation
692 resistance in the DGRP and assessed the genetic architecture of this trait using GWA mapping in
693 four starvation resistance datasets: data collected in this study, data from Mackay *et al.* (2012),
694 data from Everman and Morgan (2018), and a consensus, across-study starvation resistance
695 measure calculated as the mean response across the three starvation datasets (N shared lines =

696 150). Using an FDR threshold of 20%, between 0 and 12 SNPs were associated with starvation
697 resistance for each dataset and sex (Table 3; Table S9). Aside from 3 SNPs that overlap between
698 the across-study mean dataset and the Mackay *et al.* (2012) dataset, none of these above-
699 threshold SNPs were the same (Table 3; Table S9). Using the more lenient significance threshold
700 of $P < 10^{-5}$, between 17 and 48 SNPs were associated with starvation resistance for each dataset
701 and sex (Table 3; Table S9); however, overlap in associated SNPs among datasets was still minimal
702 (Fig S13). With this significance threshold, our sex-specific GWA results for the Mackay *et al.*
703 (2012) and Everman and Morgan (2018) datasets matched those reported in the original studies
704 (Table 3; Table S9). The SNPs identified using the more lenient significance threshold include all
705 those identified at the FDR threshold of 20%, so all subsequent analyses are performed on the
706 larger set of associated SNPs, and we acknowledge that these sets may include larger fractions
707 of false-positive associations. The equivalent FDR for the $P < 10^{-5}$ thresholds are provided in Table
708 3.
709
710
711 ***Table 3.*** *Summary of DGRP GWA results and lines measured in this study, Mackay* et al. *(2012) and*
712 *Everman and Morgan (2018). Text in italics indicates results from the reanalysis of starvation data*
713 *previously presented by Mackay* et al. *(2012) and Huang* et al. *(2014) and Everman and Morgan (2018).*
714 *Numbers noted with an asterisk indicate results that match those reported in the original studies.*

Study	Significance Threshold	Equivalent FDR Female	Equivalent FDR Male	No. Strains	No. SNPs: Female	No. SNPs: Male
This Study	FDR 0.2	-	-	168	2	3
	$P < 10^{-5}$	0.78	0.79	168	23	19
Mackay et al. (2012)	*FDR 0.2*	*-*	*-*	*203*	*11*	*0*
	$P < 10^{-5}$	*0.49*	*0.82*	*203*	*39**	*17**
Everman and Morgan (2018)	*FDR 0.2*	*-*	*-*	*164*	*0*	*0*
	$P < 10^{-5}$	*0.67*	*0.65*	*164*	*25**	*22**
Across-Study Mean Response	FDR 0.2	-	-	150	12	0
	$P < 10^{-5}$	0.43	0.80	150	48	22

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718 Compared to genes implicated by QTL identified in the DSPR, which include several that
719 have been previously associated with starvation or related phenotypes (e.g., lifespan or lipid

720 content), DGRP GWAS hits implicate far fewer *a priori* strong candidate genes. Additionally, we
721 did not observe any GO enrichment following analysis of SNPs associated with each dataset,
722 although we acknowledge that the limited number of implicated genes likely compromised the
723 power of such enrichment analyses. Of the total 127 unique genes associated with starvation
724 resistance in the DGRP across studies and sexes, only two have been previously identified as
725 influencing starvation resistance in other mapping populations (*CG30118*, *scaf6*; Table S8;
726 (Sørensen *et al.* 2007)). More generally, five genes identified through our GWA had biological
727 processes linked to the determination of adult lifespan (e.g. *cnc* and *Egfr*; Table S8; (Sykiotis and
728 Bohmann 2008; Kamakura 2011)), and 5 have been previously associated with lipid metabolism
729 or metabolic processes (e.g. *GlcAT-P* and *Ugt86Dj*; Table S8; (FlyBase Curators *et al.* 2004; Gaudet
730 *et al.* 2010)). Given the relative lack of power in the DGRP (Long *et al.* 2014), and our use of a
731 permissive genomewide threshold, it could be that many of the GWAS associations are incorrect.
732 Equally, it could be the case that a series of novel pathways are involved in natural variation for
733 starvation resistance, and that traditional candidates - often identified via mutagenesis screens
734 rather than through examination of segregating allelic variation - typically do not harbor the
735 functional natural variants detectable in a GWAS (Mackay *et al.* 2009).
736

737 Repeatability in the SNPs associated with of starvation resistance across studies

738 The public availability of starvation resistance data for the DGRP from multiple studies
739 provides a novel opportunity to investigate the reliability and repeatability of associations
740 identified for a classic quantitative trait in the same mapping panel across independent
741 phenotypic screens (Lithgow *et al.* 2017). Despite the moderately high phenotypic correlation
742 between starvation resistance measured in the three studies (Fig S8), overlap in the variants
743 implicated in starvation resistance in the DGRP was very limited. Only one SNP (associated with
744 male starvation resistance) overlapped between starvation resistance measured in Mackay *et al.*
745 (2012) and Everman and Morgan (2018), and 2-4 overlapped between the across-study mean
746 response and data from this study, Mackay *et al.* (2012), and Everman and Morgan (2018) (Fig
747 S13).

748 The lack of overlap in SNPs associated with starvation resistance could be due to
749 differences in screen environments (discussed above), where genotype-by-environment effects
750 (often pervasive for complex traits (Gurganus *et al.* 1998)) could lead to different sites impacting
751 variation in different studies. However, it is potentially more likely that the sets of associated
752 SNPs have real, but extremely small effects on starvation resistance variation, and power in a
753 GWA panel of modest size is too low to consistently detect them (Boyle *et al.* 2017). If true, one
754 would predict that - in contrast to random SNPs - the "top SNPs" identified within each starvation
755 resistance dataset would have additive effects of the same sign across all studies. In essence,
756 significantly associated SNPs with positive effects on starvation resistance from data collected in
757 this study would be expected to have positive additive effects on starvation resistance measured
758 in Mackay *et al.* (2012) and Everman and Morgan (2018) more often than expected by random
759 chance.

760 To test this prediction, we first collected the sign of the additive effects of SNPs that
761 survived the significance threshold ($P < 10^{-5}$) associated with each measure of starvation
762 resistance in each dataset for both sexes (Table S9), and determined whether these top SNPs had
763 additive effects of the same sign in every other data set. We then established a null distribution
764 of SNP additive effect signs across pairs of datasets. This was accomplished by taking samples of
765 50 SNPs segregating in the DGRP and extracting the sign of the additive effect of each SNP in the
766 pair of datasets, regardless of the association statistic for that SNP. The proportion of the 50 SNPs
767 that had a positive additive effect on the trait was recorded for each of 1000 iterations and used
768 to build a distribution of additive effect shifts between the data collected in this study and that
769 presented by Mackay *et al.* (2012) and Everman and Morgan (2018) (see Methods). We note that
770 to compensate for any allele frequency bias in the variants that are actually most associated with
771 phenotype we ensured that the frequencies of the sampled SNPs were stratified to match the
772 top 50 SNPs associated with each sex and dataset (see Methods; Table S3).

773 Finally, we compared the proportion of top SNPs for which the sign stayed the same
774 across studies to our expected distribution for each pair of datasets (Fig 8). We found that for the
775 random samples of SNPs, the probability that the additive effects were positive in both datasets
776 compared was greater than 50% for most comparisons (distribution means ranging from 45.9-

777 72.1%; Fig 8). This implies that a random SNP is slightly more likely to have the same sign effect
778 across data sets, which may be explained in part by the phenotypic correlation between the
779 datasets (Fig S8). Even given this skew, far more top SNPs than expected by chance had additive
780 effects of the same sign in each of the starvation resistance studies (i.e. a SNP with a positive
781 effect on starvation resistance in this study had positive effects on starvation resistance obtained
782 by Mackay *et al.* (2012) and Everman and Morgan (2018); Fig 8; Table S8; Fig S14). This may
783 suggest that there is phenotypic signal even in SNPs that have very small effects, and that are not
784 clearly associated with starvation resistance in the GWAS (see Yang *et al.* 2010). Generally, the
785 consistency of the additive effects of SNPs associated with starvation resistance in the DGRP
786 calculated across data sets implies that starvation resistance is a highly polygenic trait, with a
787 large number of QTL with very small effects that influence variation in this trait (Mackay 2004;
788 Boyle *et al.* 2017).
789

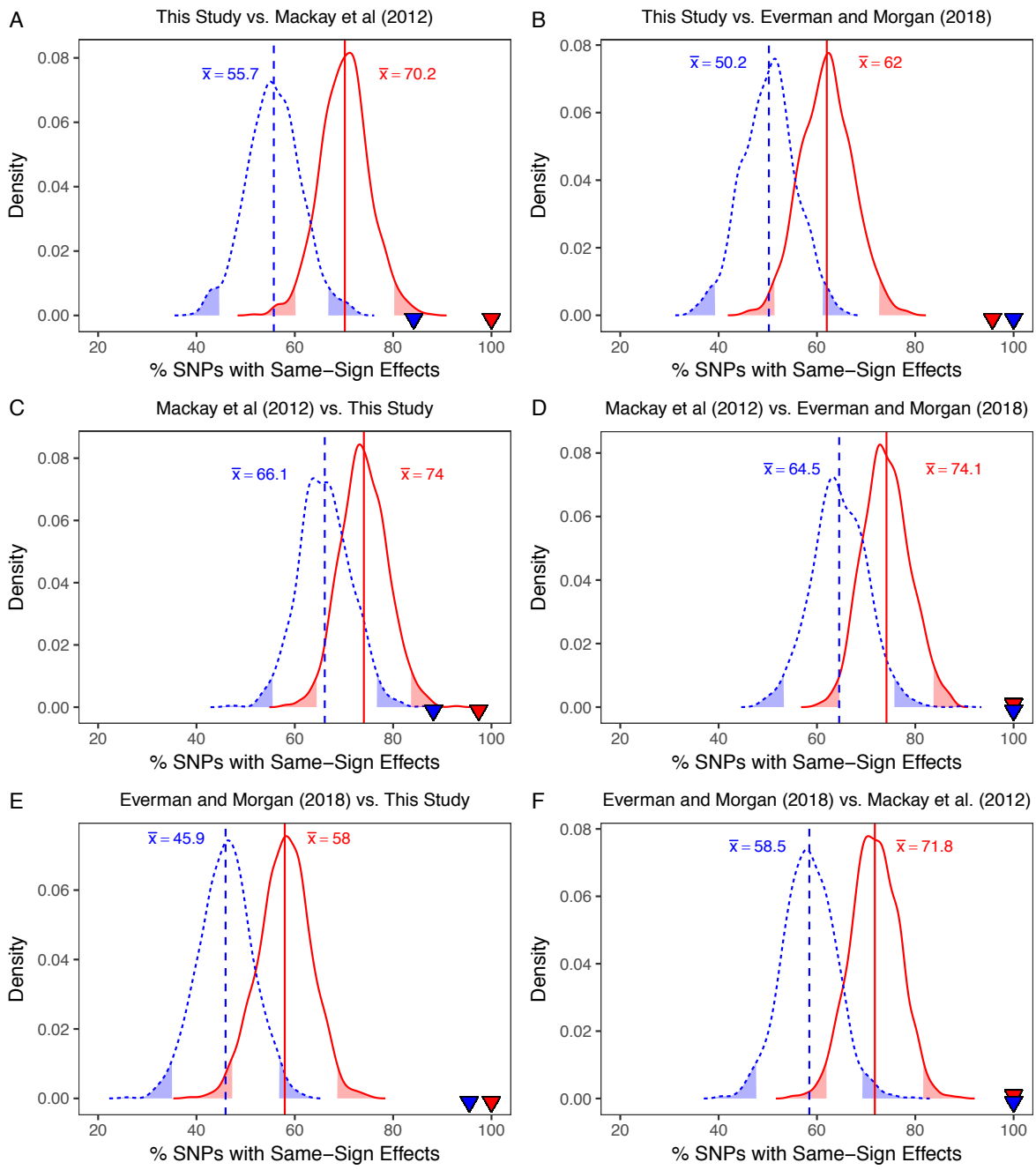

Figure 8. *Distribution of the proportion of SNPs that are expected to have additive effects of the same sign in pairwise comparisons of data from this study, Mackay* et al. *(2012) and Everman and Morgan (2018). Data shown were obtained for random samples of 50 SNPs with 1000 iterations. In each plot, red indicates female data, blue indicates male data, and corresponding vertical lines and text annotation indicate the mean of the sex-specific samples. The shaded tails represent the upper and lower 95% confidence intervals of each distribution. The triangles in each plot represent the sex-specific observed proportion of top SNPs from each GWA analysis ($P < 10^{-5}$) that had additive effects that were the same sign across studies (Fig S14). For example, in A, 100% of the SNPs associated with female starvation resistance in this study had additive effects of the same sign when calculated for starvation resistance reported in Mackay* et al. *(2012). The dataset comparison is indicated above each plot.*

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QTL associated with starvation resistance across mapping panels

804 805 806 807 808 809 810 Across all datasets reporting starvation resistance in the DSPR and DGRP, four SNPs identified using the $P < 10^{-5}$ significance threshold fell within QTL intervals identified for starvation resistance in the DSPR (Fig S15). In females, QTL SB4 and SA4 overlapped with two SNPs associated with starvation resistance from Mackay *et al.* (2012), and one SNP associated with starvation resistance in this study overlapped with QTL SA4. In males, one SNP associated with starvation resistance from Mackay *et al.* (2012) overlapped with QTL SB3.

811 812 813 814 815 816 817 818 819 820 821 822 823 The general lack of correspondence among the loci associated with starvation resistance in each mapping panel does not invalidate either approach as a strategy to uncover functional variation. It is likely that many genes contribute to variation in this trait with effects that are either fairly small or that vary in different genetic backgrounds, and we would not expect to routinely identify such loci. In addition, comparison of the genetic architecture of quantitative genetic traits across multiple panels is complicated by a number of additional factors. The genetic structure of the mapping panel (e.g., whether it is a multiparental panel like the DSPR or a population-based association study panel like the DGRP) influences the analytical strategy, and the power and resolution of mapping (Long et al. 2014). The complement of alleles present in the panel, and the frequency with which they segregate, will also affect the ability to identify the same locus across mapping panels (e.g., King and Long 2017). Therefore, a lack of overlap in the identified QTL for a complex, highly-polygenic trait between the DSPR and the DGRP is perhaps not unexpected.

824

825 Conclusions

826 827 828 829 830 In this study, we described the complex quantitative genetics of starvation resistance in two large *D. melanogaster* mapping panels that have been thoroughly genomically characterized. The DSPR and DGRP panels have the advantage of increased, stable genetic diversity and provide a unique comparison to many previous quantitative genetic studies of starvation resistance that may be limited by genetic diversity or mapping power. Correlations between starvation

831 resistance and the additional traits described in this study offer insight into the genetic control
832 of related stress response traits and provide support for the hypothesis that the genetic
833 architecture of stress traits varies by population and is dependent upon sex, environment, and
834 the evolutionary history of the populations studied. The relationships between the traits
835 analyzed in this study also offer insight into the broader responses of organisms to starvation
836 stress, given the high conservation of mechanisms related to starvation resistance in diverse
837 species (Partridge *et al.* 2005; Rion and Kawecki 2007). Here, we have demonstrated that traits
838 related to survival under starvation conditions, energy storage, activity levels, and survival under
839 desiccation stress are phenotypically correlated in the DSPR, consistent with previous artificial
840 selection studies as well as some natural populations. However, we also clearly demonstrate that
841 starvation resistance and triglyceride level are largely genetically independent traits, indicating
842 that evolutionary constraint between these two traits is unlikely. We additionally describe the
843 highly polygenic nature of starvation resistance using the DGRP, leveraging previously published
844 phenotypes on the same lines to compare the genetic architecture of the trait across three
845 studies. Our work shows that despite a lack of overlap across studies in the identity of the variants
846 associated with phenotype at a nominal genomewide threshold, the sign of the additive effects
847 of such top SNPs are conserved across studies. In turn this suggests that these variants do
848 contribute to phenotype but have sufficiently small effects that they are not routinely captured
849 following a severe, genomewide correction for multiple tests. From this, we gain a much more
850 detailed understanding of the genetic control of starvation resistance in a genetically diverse
851 panel and provide insight into the utility of across-study and across-panel comparisons.

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853

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Dissection of complex, fitness-related traits in multiple *Drosophila* mapping populations offers insight into the genetic control of stress resistance

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Supplemental Figures, Tables, and Supplemental File descriptions

Supplemental Figures

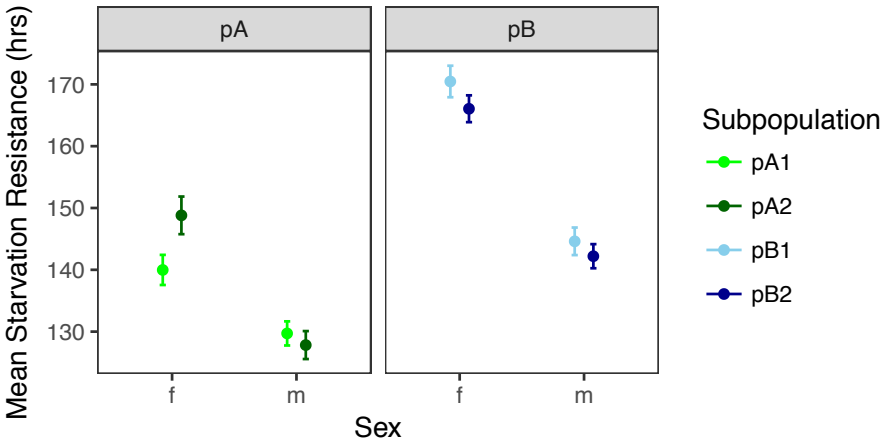

Figure S1. *Mean (± 95% CI) starvation resistance per subpopulation and sex. Sex and subpopulation interacted to influence starvation resistance ($F_{3,3440}$ = 18.317; $p < 0.0001$), though only females from the pA1 and pA2 subpopulations were significantly different within a panel (Tukey's HSD adj. $p < 0.0001$).*

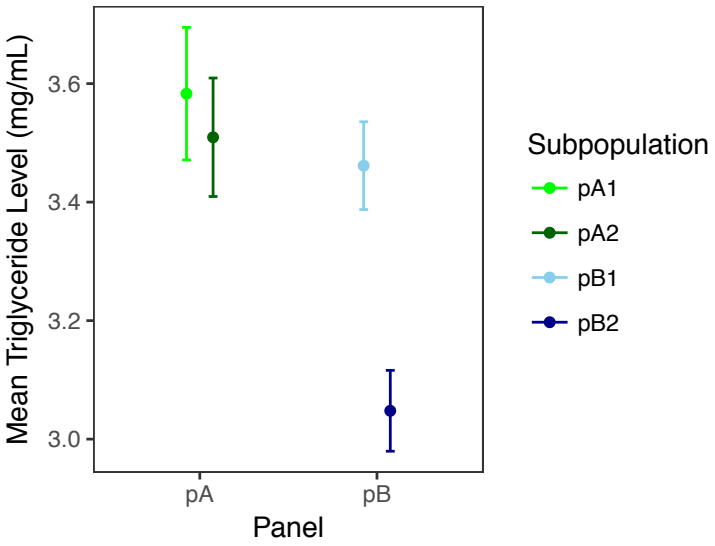

Figure S2. *Mean female triglyceride level per subpopulation (± 95% CI). Subpopulation influenced triglyceride level ($F_{3,935}$ = 37.099; $p < 0.0001$), though this was driven by differences between the pB subpopulations (Tukey's HSD adj. $p < 0.0001$)*

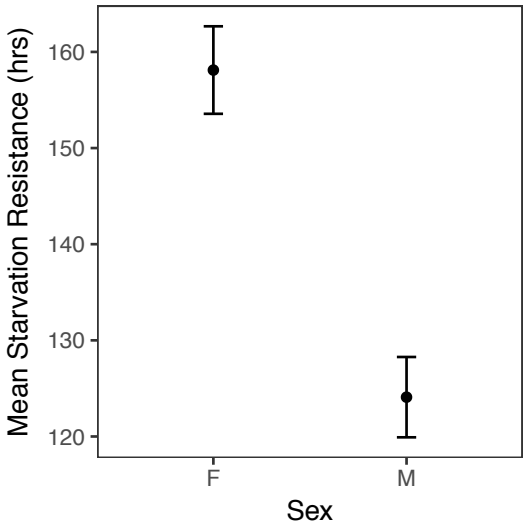

Figure S3. *Mean starvation resistance (±95% CI) for males and females in the DGRP ($F_{1,334}$ = 118.21, $p < 0.0001$).*

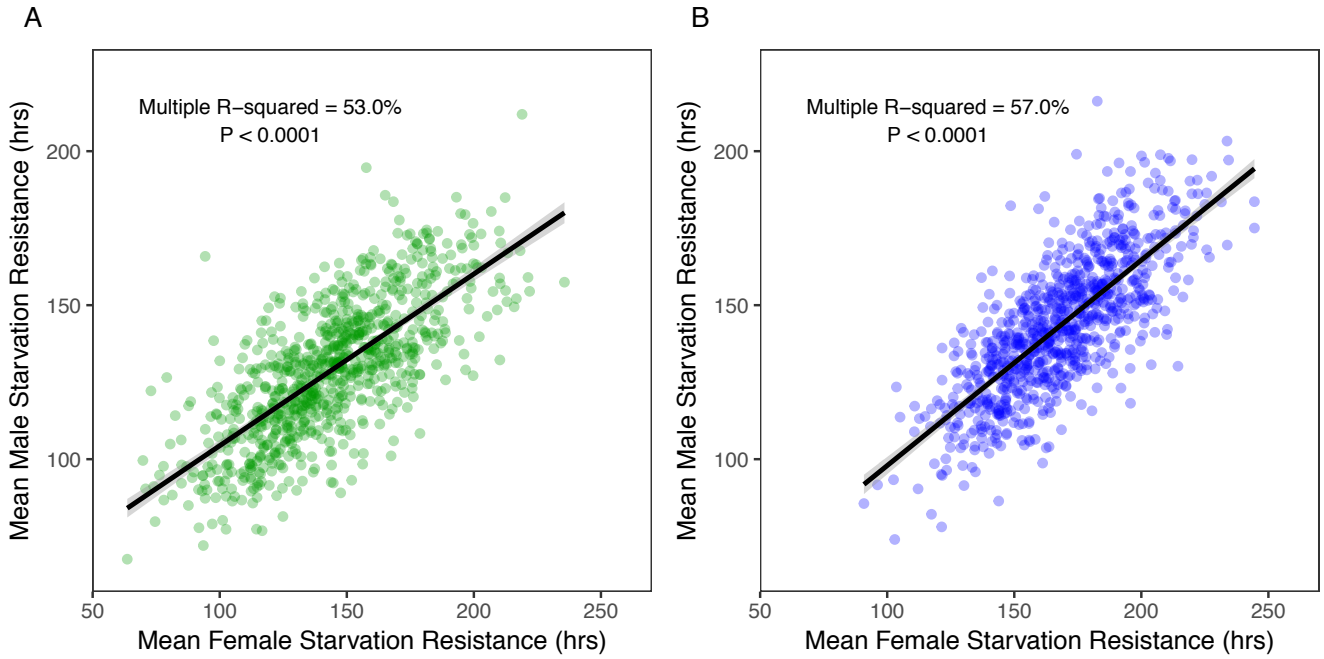

Figure S4. *Correlation between sex-specific mean starvation resistance for the DSPR pA mapping panel (A) and pB mapping panel (B). Sex-specific responses were significantly correlated for both panels (pA: $F_{1,859}$ = 968.5, P < 0.0001; pB: $F_{1,860}$ = 1138, p < 0.0001). Grey shading around the regression line in both plots indicates the 95% confidence interval.*

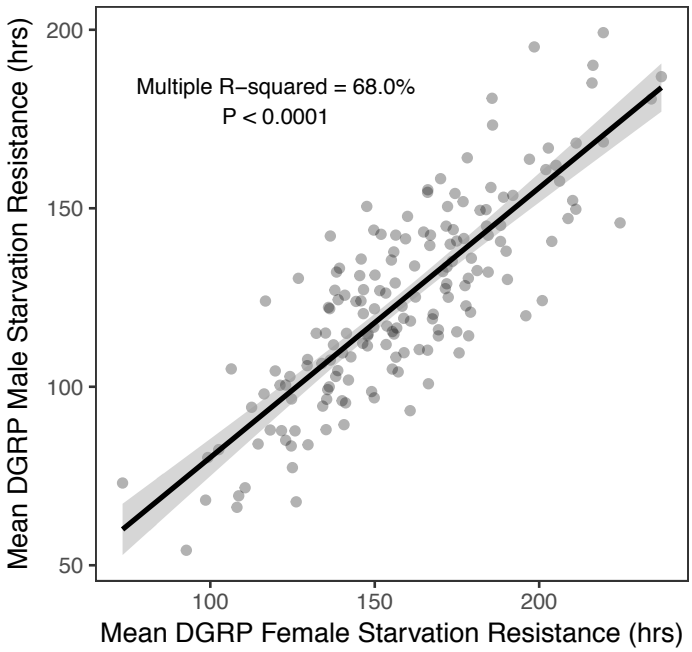

Figure S5. *Male and female mean starvation resistance was significantly correlated in the DGRP ($F_{1,334}$ = 118.21, P < 0.0001). Grey shading around the regression line indicates the 95% confidence interval.*

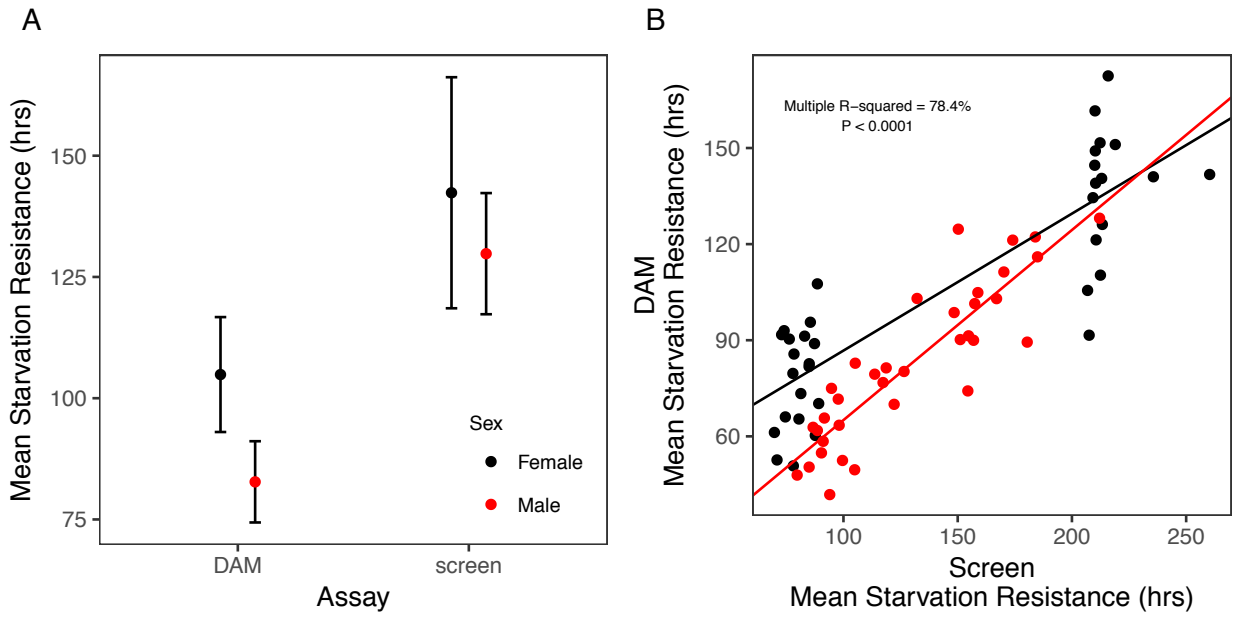

Figure S6. *A. Starvation resistance was significantly higher in the large-scale starvation screen of all DSPR RILs compared to the DAM starvation assay for the selected subset of RILs (Assay: $F_{1,136} = 31.60$, $p < 0.0001$). Mean starvation resistance across RIL means is presented ($\pm$ 95% CI). B. Mean starvation resistance measured in the large-scale starvation resistance screen (x-axis) was correlated with mean starvation resistance measured in the DAM assay (y-axis) in the DSPR (Females: $\beta = 0.43 \pm 0.04$, $t = 9.7$, $p < 0.0001$, $R^2 = 73.9\%$; Males: $\beta = 0.59 \pm 0.05$, $t = 10.9$, $p < 0.0001$, $R^2 = 78.3\%$). The multiple R^2 value in the plot includes the interaction between starvation resistance measured under different assay conditions with sex.*

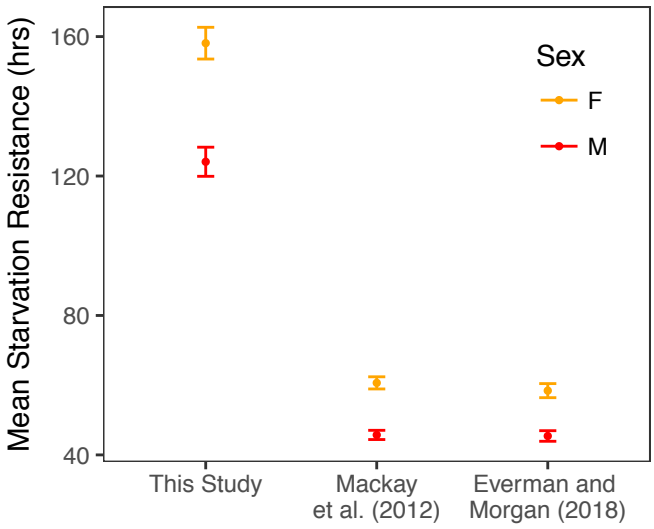

Figure S7. *Mean starvation resistance (±95% CI) was significantly higher in this study compared to Mackay* et al. *(2012) and Everman and Morgan (2018) ($F_{2,532}$ = 1457.5, P < 0.0001).*

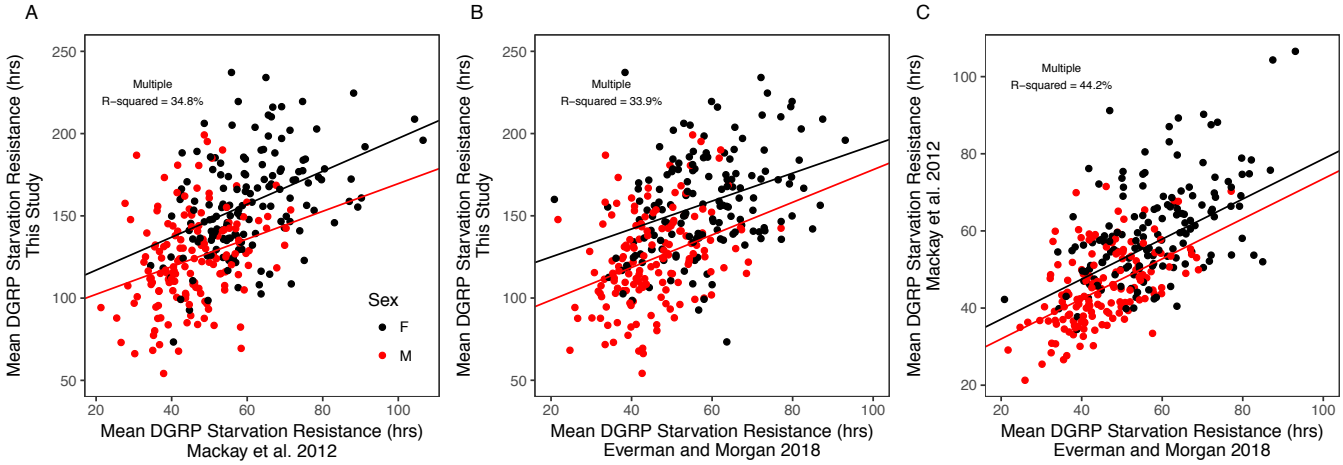

Figure S8. *Correlation between sex-specific mean starvation resistance in the DGRP panel for 150 lines that overlap between this study, Mackay* et al. *2012, and Everman and Morgan 2018. Red points indicate males and black points indicate females. All comparisons showed that the three independent measures of starvation resistance were significantly correlated (A: $F_{3,296} = 52.69$, $p < 0.0001$; B: $F_{3,296} = 50.66$, $p < 0.0001$; C: $F_{3.296} = 78.18$, $p < 0.0001$).*

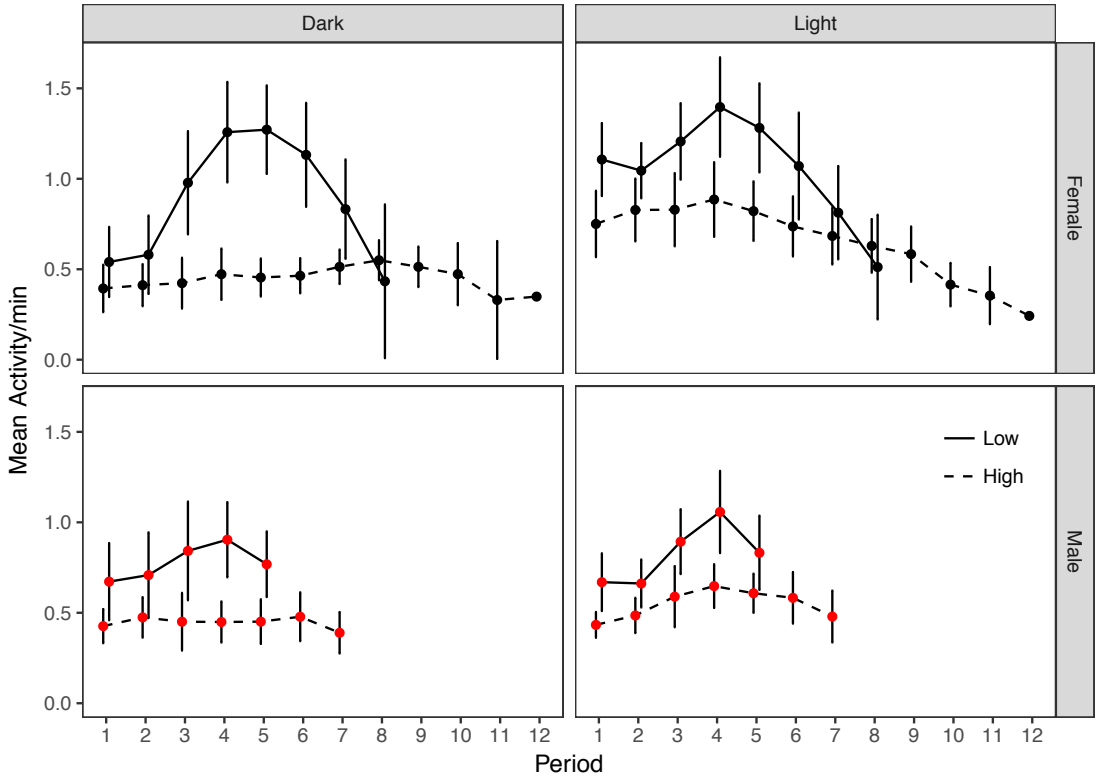

Figure S9. *Mean activity levels for females (top panel, black points) and males (bottom panel, red points) (± 95% CI) across the sampling periods during starvation in the DAM assay until death. Low starvation resistance RILs (solid line) tended to be more active during starvation compared to high starvation resistance RILs (dashed line) under both light and dark conditions. Light status (dark versus light) influenced the overall activity level of females but did not influence male activity. Data were analyzed with a repeated measures ANOVA; results are presented in Table S6. Similar to the pre-starvation period (Figure 4), waking activity levels of individuals during the DAM starvation experiment were primarily driven by starvation resistance rank in both sexes (females: $F_{1,8} = 14.87$, $p < 0.01$; males: $F_{1,6} = 13.87$, $p < 0.01$; Table S6). Light status did not influence activity between sampling periods for either sex (females: $F_{1,8} = 0.84$, $p = 0.39$; males: $F_{1,6} = 0.23$, $p = 0.65$; Table S6). However, light status did significantly influence activity in females within each sampling period (females: $F_{1,8} = 43.09$, $p < 0.0001$; Table S6), indicating that female activity in both high and low starvation resistance RILs was consistently higher during times when lights were on. Male activity of high and low starvation resistance RILs tended to remain constant under different light conditions. The larger influence of light status on females is consistent with patterns observed in activity during the pre-starvation period (Fig 4).*

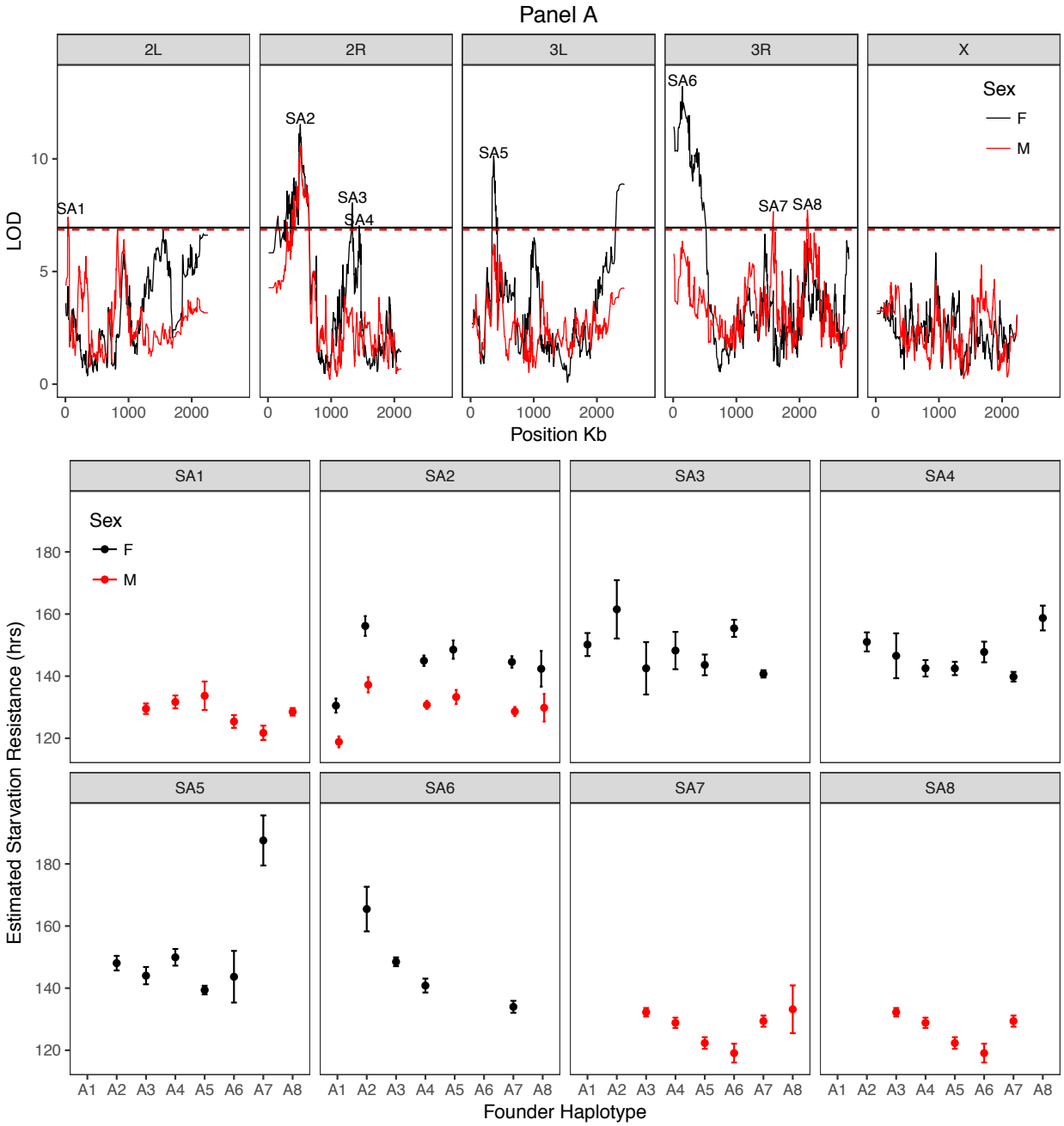

Figure S10. *Starvation resistance QTL and estimated allele effects at each QTL. Data are presented as RIL means (±SE) for estimated starvation resistance when the founder haplotype was present in more than 5 RILs.*

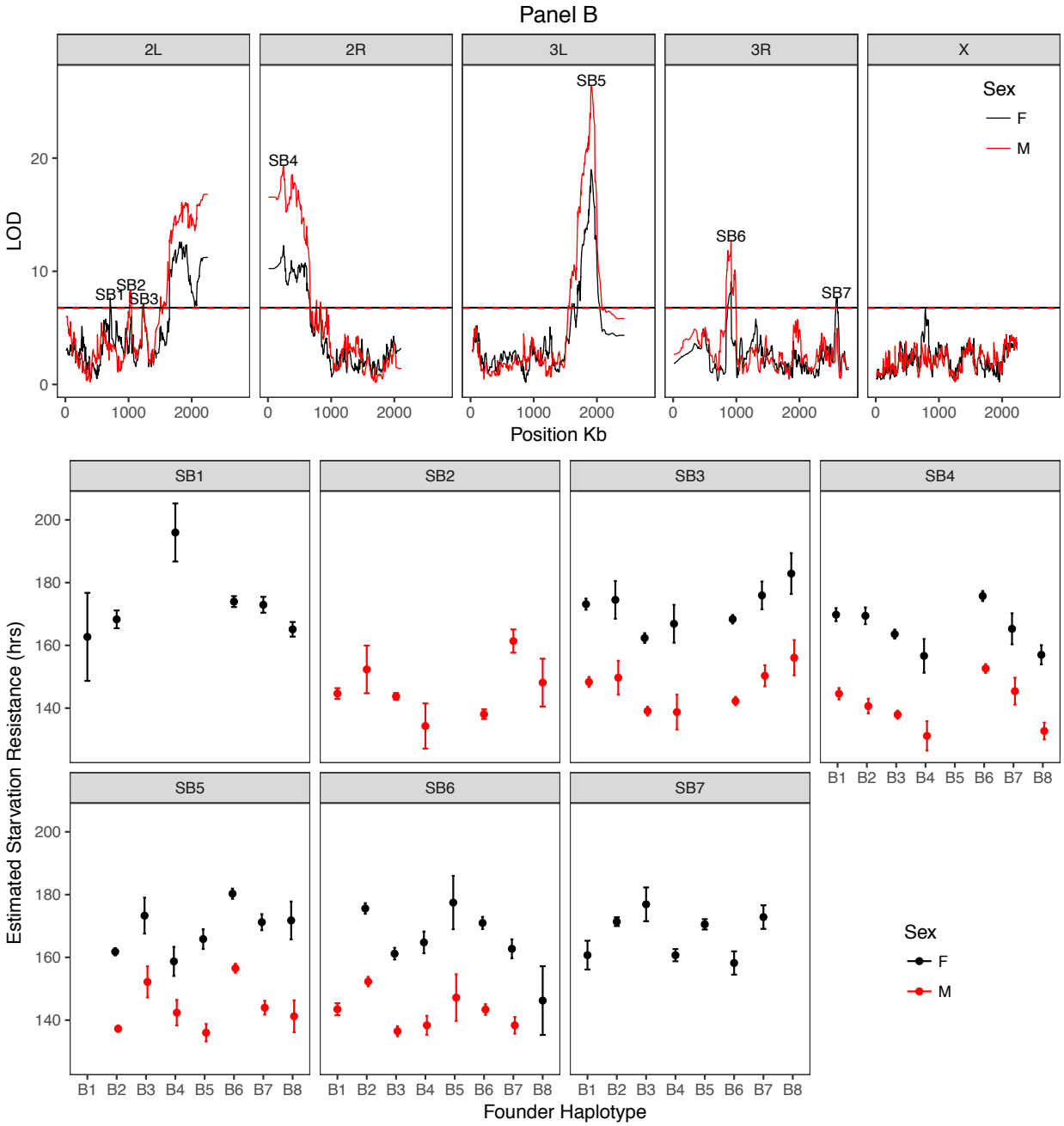

Figure S10 continued.

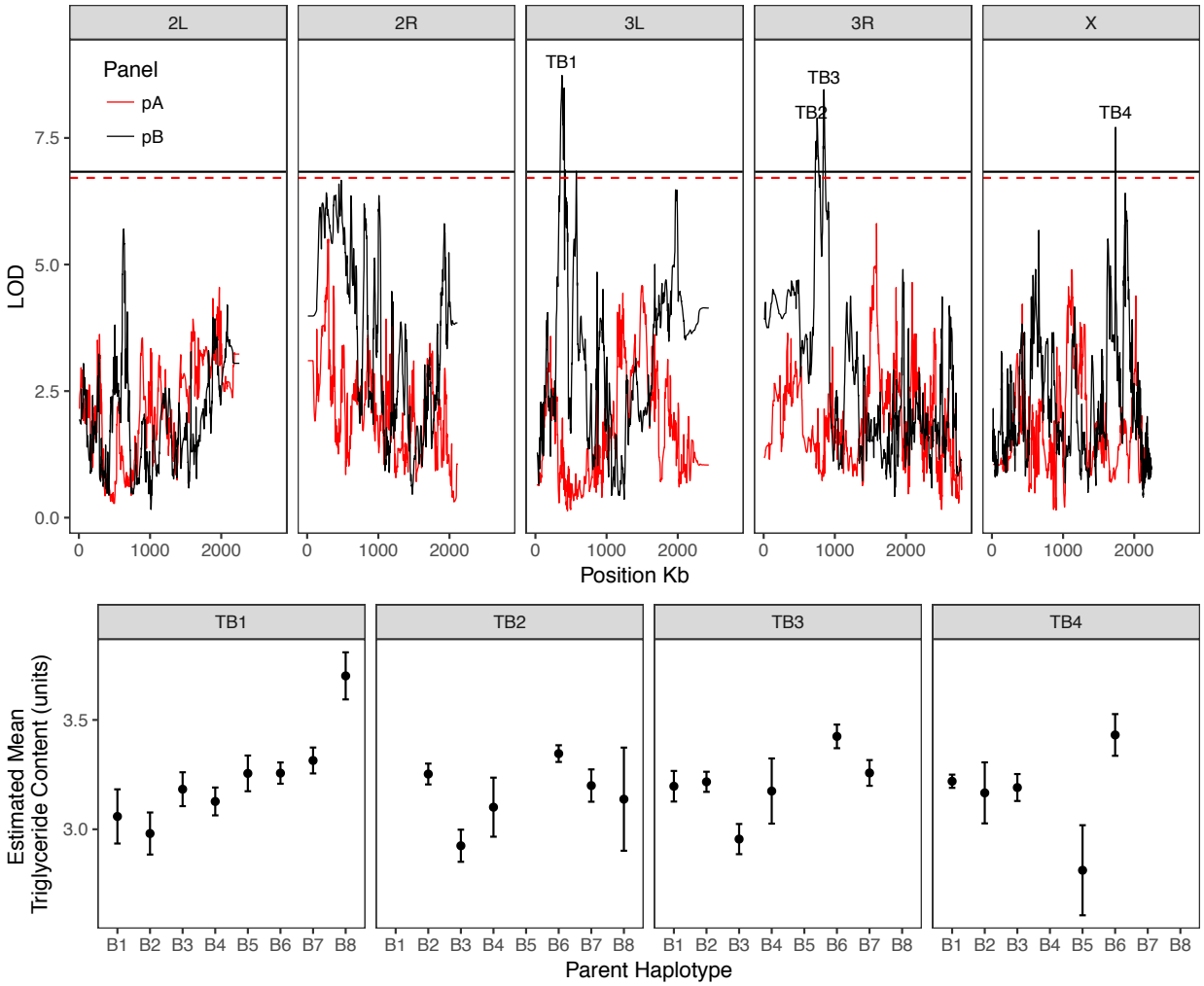

Figure S11. *Triglyceride level QTL and estimated allele effects of founders at each QTL. Data are presented as RIL means (±SE) for estimated triglyceride level when the founder haplotype was present in more than 5 RILs.*

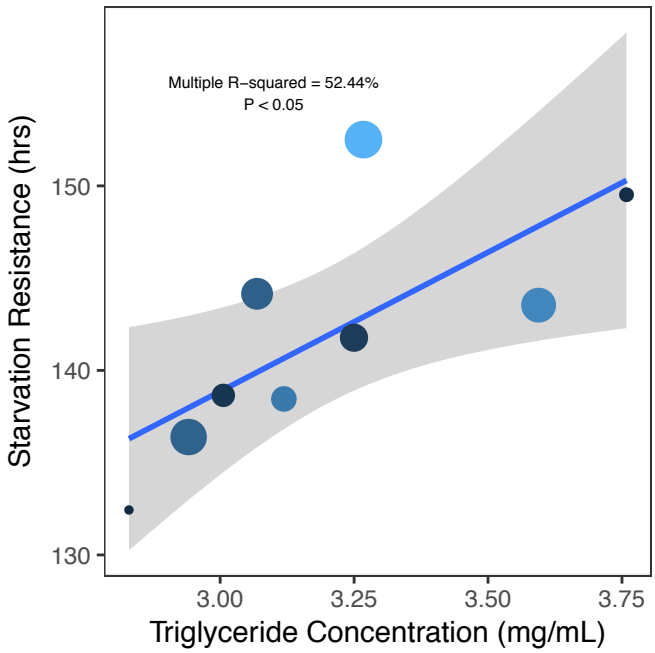

Figure S12. *Triglyceride level and starvation resistance were correlated after accounting for variation due to founder haplotype at the overlapping peaks TB3 and SB6 ($F_{1,7}$ = 7.72, P < 0.05). Data presented are averages for each founder haplotype in the pB panel, including "NA" for RILs that could not be assigned with confidence to a known haplotype. Point size relates the number of RILs per haplotype for the starvation resistance peak (smallest = 1 RIL; largest = 193 RILs); point color relates the number of RILs per haplotype for the triglyceride level peak (black = 1 RIL; lightest blue = 181 RILs). Grey shading around the regression line indicates the 95% confidence interval.*

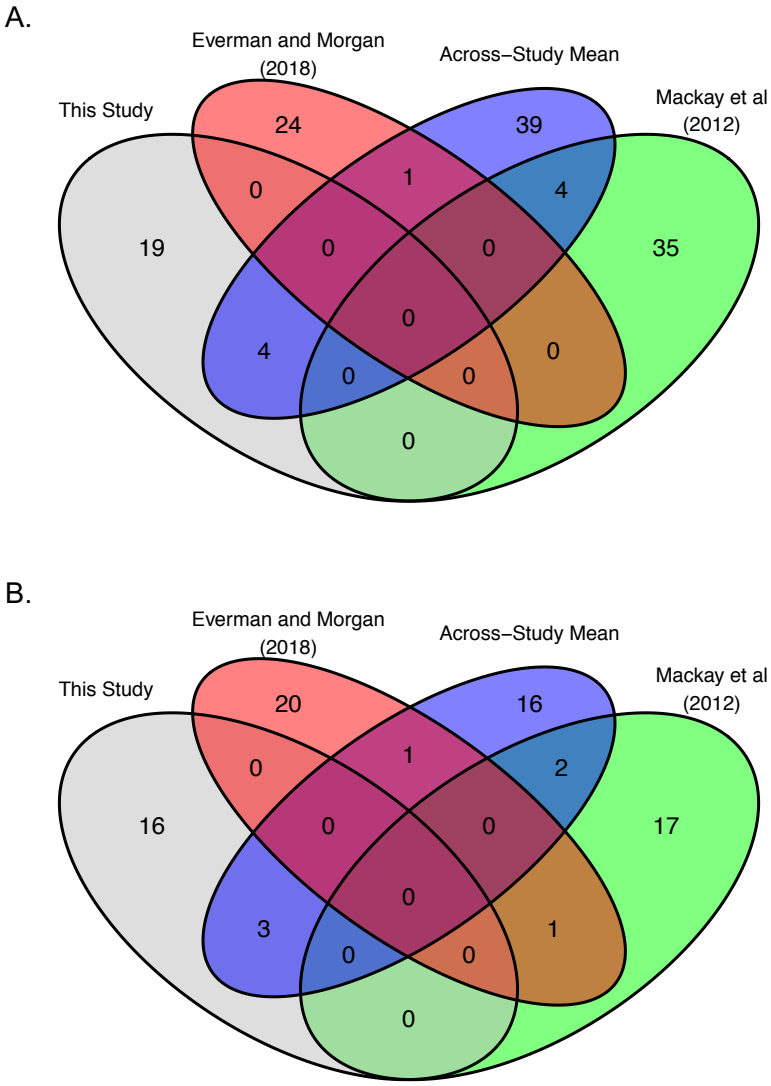

Figure S13. *Overlap in SNPs associated with starvation resistance for each data set using the $P < 10^{-5}$ significance threshold. Overlap between data sets was minimal. Plot A presents results for females; plot B presents results for males.*

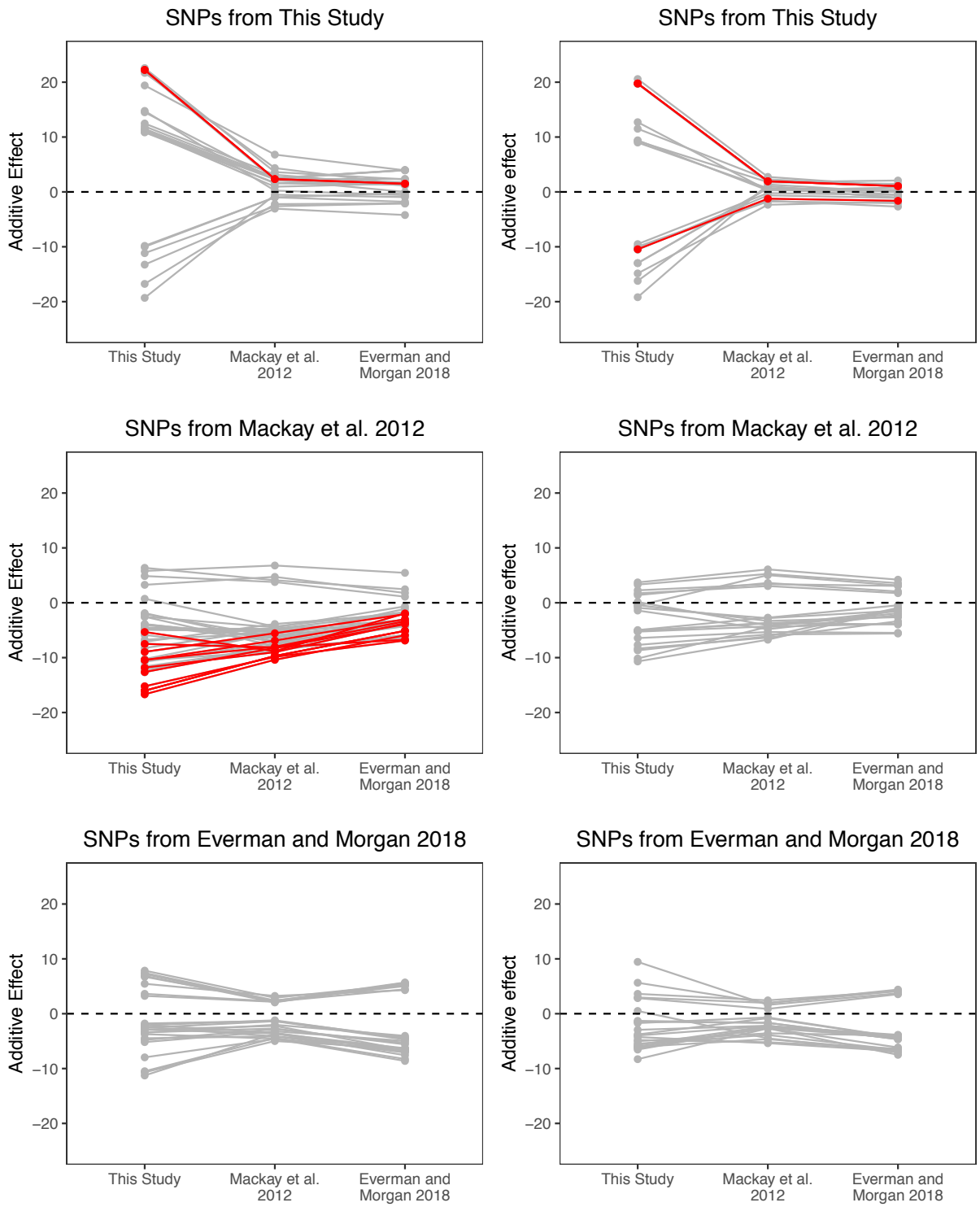

Figure S14. *Additive effects of SNPs associated with starvation resistance in each study with a threshold of $P < 10^{-5}$ calculated for starvation resistance measured in every other dataset. Female data is presented in the left column; male data is presented in the right column. SNPs that passed the FDR threshold of 0.2 are highlighted in red. Generally, SNPs had similar effects (of the same +/- sign) on starvation resistance as measured in each experiment.*

Figure S15. *Manhattan plots of mean starvation resistance in the DGRP with SNPs that were associated with starvation resistance in previous studies and intervals of sex-specific QTL identified for starvation resistance in the DSPR highlighted. Plots are broken up by chromosome arm in A for females and in B for males. In all plots, points highlighted in black indicate SNPs that are associated with starvation resistance in the DGRP from data obtained in this study; red points indicate SNPs associated with starvation resistance in Everman and Morgan 2018; green points indicate SNPs associated with starvation resistance in Mackay et al. 2012; blue points indicate SNPs associated with starvation resistance averaged across the three datasets. A genomewide significance threshold of* $P < 10^{-5}$ *is shown with the blue line. Yellow shaded boxes and labels correspond to QTL intervals around peaks mapped in the pB DSPR panel; orange shaded boxes correspond to QTL intervals around peaks mapped in the pA DSPR panel. [Figure is provided as an independent file]*

Supplemental Tables

Table S1. *Analysis of variance of mean starvation resistance in the DSPR.*

Source	df	SS	MS	F	P
Subpopulation	3	330782	110261	183.702	< 0.0001
Sex	1	342197	342197	570.121	< 0.0001
Subpopulation x Sex	3	32982	10994	18.317	< 0.0001
Residual	3440	2064747	600		

Table S2. *Analysis of variance of mean triglyceride level in the DSPR.*

Source	**df**	**SS**	**MS**	**F**	**P**
Subpopulation	3	46.81	15.6020	37.099	< 0.0001
Residual	935	393.22	0.4206		

Table S3. *Stratification of the top 50 SNPs associated with starvation resistance across five frequency bins.*

Study	Sex	Allele Frequency Bin	No. SNPs
This Study	F	0.05 - 0.1	14
This Study	F	> 0.1 - 0.2	9
This Study	F	> 0.2 - 0.3	13
This Study	F	> 0.3 - 0.4	6
This Study	F	> 0.4 - 0.5	8
This Study	M	0.05 - 0.1	11
This Study	M	> 0.1 - 0.2	12
This Study	M	> 0.2 - 0.3	13
This Study	M	> 0.3 - 0.4	3
This Study	M	> 0.4 - 0.5	11
Mackay *et al.* 2012	F	0.05 - 0.1	28
Mackay *et al.* 2012	F	> 0.1 - 0.2	11
Mackay *et al.* 2012	F	> 0.2 - 0.3	5
Mackay *et al.* 2012	F	> 0.3 - 0.4	2
Mackay *et al.* 2012	F	> 0.4 - 0.5	4
Mackay *et al.* 2012	M	0.05 - 0.1	21
Mackay *et al.* 2012	M	> 0.1 - 0.2	13
Mackay *et al.* 2012	M	> 0.2 - 0.3	7
Mackay *et al.* 2012	M	> 0.3 - 0.4	2
Mackay *et al.* 2012	M	> 0.4 - 0.5	7
Everman and Morgan 2018	F	0.05 - 0.1	11
Everman and Morgan 2018	F	> 0.1 - 0.2	13
Everman and Morgan 2018	F	> 0.2 - 0.3	10
Everman and Morgan 2018	F	> 0.3 - 0.4	8
Everman and Morgan 2018	F	> 0.4 - 0.5	8
Everman and Morgan 2018	M	0.05 - 0.1	18
Everman and Morgan 2018	M	> 0.1 - 0.2	13
Everman and Morgan 2018	M	> 0.2 - 0.3	12
Everman and Morgan 2018	M	> 0.3 - 0.4	4
Everman and Morgan 2018	M	> 0.4 - 0.5	3

Table S4. *Analysis of variance of mean starvation resistance in the DGRP.*

Source	**df**	**SS**	**MS**	**F**	**P**
Sex	1	97213	97213	118.21	< 0.0001
Residual	334	274680	822		

Table S5. *Analysis of variance of activity during the 24-hour period prior to the DAM starvation assay with effect sizes (Cohen's F) for each variable.*

Source	df	SS	MS	F value	P	Effect Size
Starvation Resistance Rank (High vs. Low)	1	1.37	1.37	12.48	< 0.001	0.31
Sex	1	0.61	0.61	5.57	< 0.05	0.21
Lights On/Off	1	1.55	1.55	14.11	< 0.001	0.33
Starvation Resistance Rank x Sex	1	0.00	0.00	0.01	0.92	0.01
Starvation Resistance Rank x Lights On/Off	1	0.00	0.00	0.00	0.98	0.00
Sex x Lights On/Off	1	1.85	1.85	16.87	< 0.0001	0.36
Starvation Resistance Rank x Sex x Lights On/Off	1	0.02	0.02	0.21	0.64	0.04
Residuals	132	14.49	0.11	0.00		

Table S6. *Repeated measures analysis of variance across sampling period for activity during the DAM starvation assay for males and females.*

Female Activity During Starvation
Between Periods

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	11.15	11.15	14.19	< 0.01
Lights On/Off	1	0.66	0.66	0.84	0.39
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.48	0.48	0.61	0.46
Residuals	8	6.29	0.79		

Within Periods

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	19.06	19.06	115.58	< 0.0001
Lights On/Off	1	7.10	7.11	43.09	< 0.0001
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.16	0.16	0.99	0.32
Residuals	575	94.81	0.17		

Male Activity During Starvation
Between Periods

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	5.47	5.47	13.79	< 0.01
Lights On/Off	1	0.09	0.09	0.23	0.65
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.67	0.67	1.69	0.24
Residuals	6	2.38	0.40		

Within Periods

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	5.03	5.04	46.70	< 0.0001
Lights On/Off	1	0.32	0.32	2.93	0.09
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.12	0.12	1.14	0.29
Residuals	471	50.78	0.11		

Table S7. *Data from genes mapped to the region under QTL intervals for starvation resistance in the pA and pB DSPR mapping panels and triglyceride level in the pB DSPR mapping panel based on Flybase release version FB2018_1. Highlighted genes indicate those previously identified in QTL mapping studies of starvation resistance. [Table is provided as an independent file]*

Table S8. *Gene ontology analysis of genes that are included within QTL intervals for starvation resistance and triglyceride level. Gene lists used in each analysis included all genes unique to male and female analyses for each trait. GO analysis was performed using the PANTHER Overrepresentation Analysis (Released 2018-05-21) with Fisher's Exact test with FDR multiple test correction. No enrichment was found for starvation resistance in the pB panel or for starvation resistance in the DGRP in any dataset.*

Starvation Resistance GO Analysis: pA DSPR Panel

Category	Fold Enrichment	FDR
glutathione metabolic process (GO:0006749)	6.91	1.46E-04
cellular modified amino acid metabolic process (GO:0006575)	3.85	1.79E-02

Triglyceride Level GO Analysis: pB DSPR Panel

Category	Fold Enrichment	FDR
heat shock-mediated polytene chromosome puffing (GO:0035080)	42.66	4.25E-04
polytene chromosome puffing (GO:0035079)	38.39	3.35E-04
sensory perception of sweet taste (GO:0050916)	27.42	7.71E-04
detection of chemical stimulus involved in sensory perception of taste (GO:0050912)	19.47	7.16E-04
chaperone cofactor-dependent protein refolding (GO:0051085)	16	5.94E-03
'de novo' posttranslational protein folding (GO:0051084)	16	5.19E-03
protein refolding (GO:0042026)	15.36	5.65E-03
'de novo' protein folding (GO:0006458)	13.24	9.68E-03
cellular response to unfolded protein (GO:0034620)	12.8	4.87E-03
response to unfolded protein (GO:0006986)	12.44	4.78E-03
cellular response to topologically incorrect protein (GO:0035967)	9.95	1.06E-02
response to topologically incorrect protein (GO:0035966)	9.74	1.00E-02
cellular response to heat (GO:0034605)	8.93	5.11E-02
chaperone-mediated protein folding (GO:0061077)	8.93	4.79E-02
response to hypoxia (GO:0001666)	7.22	4.89E-02
sensory perception of taste (GO:0050909)	6.65	2.84E-02

Table S9. *Data from GWA, generated from the DGRP Freeze 2.0 pipeline, based on Flybase release version FB2018_1. All SNPs shown passed the* $P < 10^{-5}$ *significance threshold; highlighted SNPs passed the FDR threshold of 0.2. [Table is provided as an independent file]*

Supplemental File Descriptions

All are provided as independent files

File S1. Data generated in the analysis of starvation resistance, desiccation resistance, triglyceride level, and activity.

File S2. All code associated with the bootstrapping analysis of SNPs associated with starvation resistance measured in the DGRP in this study, Mackay *et al.* (2012), and Everman and Morgan (2018).

File S3. Raw LOD scores for starvation resistance in the DSPR pA panel.

File S4. Raw LOD scores for starvation resistance in the DSPR pB panel.

File S5. Raw LOD scores for triglyceride level in the DSPR pA panel.

File S6. Raw LOD scores for triglyceride level in the DSPR pB panel.

File S7. GWA results for DGRP starvation resistance collected in this study.

File S8. GWA results for DGRP starvation resistance collected in Mackay *et al.* (2012).

File S9. GWA results for DGRP starvation resistance collected in Everman and Morgan (2018).

File S10. GWA results for average DGRP starvation resistance across the three datasets.