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 from the multiparental Drosophila Synthetic Population Resource (DSPR), we map numerous starvation stress QTL that collectively explain a substantial fraction of trait heritability. Mapped QTL effects allowed us to estimate DSPR founder phenotypes, predictions that were correlated with the actual phenotypes of these lines. We observe a modest phenotypic correlation between starvation resistance and triglyceride level, traits that have been linked in previous studies. However, overlap among QTL identified for each trait is low. Since we also show that DSPR strains with extreme starvation phenotypes differ in desiccation resistance and activity level, our data imply multiple physiological mechanisms contribute to starvation variability. We additionally exploited the Drosophila Genetic Reference Panel (DGRP) to identify sequence variants associated with starvation resistance. Consistent with prior work these sites rarely fall within QTL intervals mapped in the DSPR. We were offered a unique opportunity to directly compare association mapping results across laboratories since two other groups previously measured starvation resistance in the DGRP. 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.

KEYWORDS starvation resistance; triglyceride level; DSPR; DGRP; MPP; Multiparent Advanced Generation Inter-Cross (MAGIC); multiparental populations

PERIODS of food scarcity and suboptimal nutrient resources present an important challenge for nearly all species (McCue 2010), and this form of environmental stress can limit the survival of individuals with poor nutritional status and reduced stress resistance (Harshman et al. 1999; Lee and Jang 2014). As a result, starvation stress resistance has direct implications for the fitness of individuals as they experience resource variability in natural populations. Starvation resistance is a classic quantitative, fitness-related trait that is associated with several other phenotypes that influence survival, lifespan, and reproduction (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; Lee and Jang 2014). 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; Chippindale et al. 1993; Hoffmann and Parsons 1993; Harshman et al. 1999; Bochdanovits and de Jong 2003; Bubliy and Loschcke 2005; Sørensen et al. 2007; Schwasinger-Schmidt et al. 2012; Lee and Jang 2014).
Because of pervasive phenotypic and genetic correlations between starvation resistance and these other traits, the evolution of starvation resistance in natural populations is complex, and is driven by adaptation to heterogeneous environments, phenotypic plasticity, and extensive pleiotropy (Service and Rose 1985; Hoffmann and Parsons 1991, 1993; Chippindale et al. 1993; Toda and Kimura 1997; Karan and Parkash 1998; Harshman et al. 1999; Harbison et al. 2004; Pijpe et al. 2007; Rion and Kawecki 2007; Bauerfénd et al. 2014; Colinet et al. 2016; Everman and Morgan 2018).

Artificial selection for starvation resistance often results in a concomitant increase in desiccation resistance (Hoffmann and Parsons 1989a; Chippindale et al. 1996; Harshman et al. 1999; Hoffmann and Harshman 1999; Hoffmann et al. 2001), and selection specifically on desiccation resistance can also result in a corresponding rapid increase in starvation resistance (Hoffmann and Parsons 1989b). Nonetheless, surveys of natural populations in several Drosophila species have shown that starvation and desiccation resistance can also vary independently (Davidson 1990; Chippindale et al. 1998; Karan and Parkash 1998; Karan et al. 1998; Hoffmann and Harshman 1999; Gilchrist et al. 2008; Goenaga et al. 2013). Given these variable patterns, Karan and Parkash (1998) and Da Lage et al. (1990) suggest that desiccation and starvation resistance may not routinely be associated. Rather, both traits may be directly and indirectly influenced by climate variability, and selection on other correlated traits such as diapause or thermal tolerance in seasonally variable temperate environments (Hoffmann and Parsons 1989b; Schmidt et al. 2005; Rion and Kawecki 2007; Sørensen et al. 2007; Goenaga et al. 2013; Rajpurohit et al. 2018).

Similar to desiccation, artificial selection for increased starvation resistance often results in an increase in lipid levels in Drosophila melanogaster (Chippindale et al. 1996, 1998; Djawdan et al. 1998; Harshman et al. 1999; Schwasinger-Schmidt et al. 2012; 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. 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. Thus, the association 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.

Genetic dissection of starvation resistance can both lead to the identification of loci impacting phenotypic variation and help understand how this trait is associated with desiccation resistance and lipid level. Several 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; Bubly and Loeschke 2005; Schwasinger-Schmidt et al. 2012; Hardy et al. 2018; Michalak et al. 2018), 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 and variants, some of which have been functionally validated (Lin et al. 1998; Clancy et al. 2001; Harbison et al. 2004, 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. Doing so would allow a detailed comparison of quantitative trait loci (QTL) that contribute to variation in each trait, provide insight into the similarity of the genetic architectures of starvation resistance and correlated traits, and facilitate a better understanding of their evolution, and the mechanisms underlying their variation.

In this study we use two powerful D. melanogaster mapping panels—the Drosophila Synthetic Population Resource (DSPR) and the Drosophila Genetic Reference Panel (DGRP)—to genetically dissect phenotypic variation, and to explore the phenotypic and genetic relationships among traits, among mapping panels, and among laboratories. Our approach allows us to accomplish three primary objectives. First, by measuring starvation resistance and triglyceride level in the DSPR, we assess overlap in the loci that contribute to variation in each trait. Prior work on these traits in flies suggests they would show similar genetic architectures with many pleiotropic loci. However, despite a significant phenotypic correlation, we report limited overlap among mapped loci contributing to variation in starvation resistance and triglyceride level, suggesting that the genetic basis of these traits is largely independent in the DSPR. This highlights the role that other physiological mechanisms, such as activity level and desiccation resistance that also we explore here, may have in influencing starvation resistance.

Second, by measuring starvation resistance in both the DSPR and the DGRP under the same environmental conditions, we address variation in the genetic architecture of this trait between two distinct populations. In common with some previous studies using both panels to dissect a trait (e.g., Najarro et al. 2015, 2017), we also find little overlap in the loci associated with starvation resistance between mapping panels. This is likely the combined result of the populations having unique genetic backgrounds (King and Long 2017), distinct evolutionary histories, and differences in power to detect causative loci (Long et al. 2014).

Third, we leverage the ability to repeatedly measure trait variation on the same, stable set of inbred genotypes to compare our DGRP starvation data to two previous starvation
resistance datasets collected by different laboratories (Mackay et al. 2012; Everman and Morgan 2018). We found that the sign of the additive effects of the most strongly associated SNPs were consistent across datasets. This suggests these SNPs contribute to variation in starvation resistance in the DGRP, but have sufficiently small effects that they are regularly not identified following genomewide multiple testing correction. This across-study comparison of the genetic architecture of starvation resistance provides both technical insight into the use of genomewide association (GWA) studies to understand the genetic basis of complex traits, and biological insight into the phenotypic effects of loci that contribute to trait variation.

Materials and Methods

Mapping populations

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

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

Phenotyping assays and analysis

Large-scale starvation resistance assay: Strains from the DSPR and DGRP were duplicated from stocks, and flies were allowed to lay eggs for up to 2 days. Vials were inspected twice daily, and laying adults were cleared when necessary to maintain a relatively even egg density across experimental vials. While this visual method of density control is less precise than counting eggs, experiments with 20 randomly selected DSPR RILs showed that the effect on starvation resistance of rearing flies via egg counting or by visually assessing egg number is extremely limited (variance explained = 0.9%; Supplemental Material, Figure S1; see Table S1 for full breakdown of variance components).

In the following generation, experimental flies (2–4 days old) were sorted by sex over light CO₂ anesthesia and placed in groups of same-sex individuals on new cornmeal-molasses-yeast media for 1 day until the start of the starvation assay. The assay was initiated by placing flies on 1.5% agar media that additionally contained preservatives—a mix of propionic and phosphoric acids, and benzoic acid (Tegosept; Genesee Scientific) dissolved in ethanol (see starvation media recipe Text S1). Starvation media was made within 24 hr of the initiation of each block of the assay, and was not replaced throughout its duration. Vials were barcoded during the screen, blinding experimenters to strain identification number, and assisting with efficient data collection and analysis.

Death was assessed for each vial twice per day at ~0900 and 2100 hr. The first assessment of survival was made 24 hr after flies were transferred to starvation media. Dead flies at this initial assessment point were not included in the analysis as their death may have resulted from handling during the initial transfer to experimental vials rather than starvation stress. Vials containing flies that had become entangled in the cotton vial plug at any point during the assay were also excluded from the analysis. Flies were considered dead if they were not moving, or were unable to dislodge themselves from the starvation media. The phenotype used for mapping was the mean time to death in hours per strain across the vial replicates. Flies for this screen were reared and tested at ~23°, 30–60% humidity, with constant light.

We screened the DSPR (861 pA1/pA2 and 864 pB1/pB2 RILs) in a series of batches across a 7-month period in 2010. Each batch included the majority of RILs that belonged to a particular subpopulation. Starvation resistance was measured in 168 DGRP lines in a single batch in 2012. In both mapping panels, survival was measured across two vial replicates per sex in ~85% of strains, with ~90% of vials containing 10 flies (minimum flies per vial = 6). Finally, we measured starvation resistance in the 15 DSPR founder lines, using five vial replicates per founder, in one batch.

We assessed variation in starvation resistance due to subpopulation and sex in the DSPR with a two-way ANOVA, including the interaction, and treated subpopulation (pA1, pA2, pB1, pB2) and sex as fixed factors. Male and female-specific differences among the four subpopulations were tested using Tukey’s HSD post hoc comparisons with an experiment-wide α = 0.05. Differences in starvation resistance due to sex among the DGRP lines were analyzed with a one-way ANOVA, treating sex as a fixed factor.

Desiccation resistance assay: To investigate the correlation between starvation and desiccation resistance, we measured desiccation resistance in a subset of pA1/pA2 RILs that exhibited very low (17 RILs) or very high (16 RILs) average female starvation resistance in the large-scale screen. Desiccation resistance of female flies from all 33 strains was assessed in a single batch with two vial replicates per RIL, where 92.9% of vials contained 10 flies (minimum flies per vial = 8). We placed experimental flies, reared as described above, in empty vials plugged with cotton inside an airtight desiccator.
(Cleatech, LLC). Relative humidity was reduced to <5% throughout the experiment by adding a large quantity of Drierite (calcium sulfate) to the chamber. Survival was assessed every hour following initiation of the experiment, and mean desiccation resistance per RIL was used in all analyses.

**Activity assay:** We employed the *Drosophila* Activity Monitoring System (DAM2; TriKinetics) to assess activity levels both prior to, and during, starvation, for a subset of DSPR RILs, selecting 16 (19) pA1/pA2 RILs that exhibited high (low) female starvation resistance in the large-scale screen; 16 flies of each sex were tested per RIL. Flies for these assays were reared and tested at 25ºC, and groups of 6–10 females per RIL. Flies were aged for an additional 3 days before measuring activity for the next 24 hr under nonstressful conditions. At 11 days following the start of egg laying, we collected two sets of 10–12 females from each parental vial, resulting in four collection vials from each RIL. Flies were aged for an additional 3 days before measuring activity under nonstressful conditions. These environment conditions are different from our large-scale screen, but in line with those used in previous starvation resistance studies in *D. melanogaster* (Mackay et al. 2012; Everman and Morgan 2018). This change allowed us to examine the stability of DSPR starvation phenotypes across assay environments.

One day prior to adding flies to monitor tubes, cornmeal-yeast-dextrose media was poured into 100 mm diameter Petri dishes and allowed to set. Polycarbonate activity monitor tubes (5 mm diameter × 65 mm length) were filled to ~10 mm by pushing them into the media, and the food plug in each tube was sealed with parafilm wax. A single fly was aspirated into each tube, and the tubes were capped with small pieces of Droso-Plugs (Genesee Scientific). Flies were allowed to acclimate to the tubes for 24 hr, and then we measured activity for the next 24 hr under nonstressful conditions. Subsequently, each fly was tipped to a second monitor tube containing starvation media (Text S1) and activity was continuously monitored until each fly died.

To determine differences in activity under nonstressful conditions due to starvation resistance rank (high vs. low), we used a full three-way ANOVA model with interactions, and treated starvation rank, sex, and light status (light vs. dark) as fixed effects. The effect size of the main effects and interactions were calculated using Cohen’s F, which determines the effect size as a ratio of the between-group and between-replicate SD (R package: sjstats) (Cohen 1988; Quinn and Keough 2002; Lüdecke 2018). This allowed us to examine the stability of DSPR starvation phenotypes across assay environments.

**Triglyceride level assay:** We duplicated 311 pA1/pA2 and 628 pB1/pB2 DSPR RILs from stocks to two replicate vials, clearing parental flies when necessary to maintain relatively even egg density over test vials. At 11 days following the start of egg laying, we collected two sets of 10–12 females from each parental vial, resulting in four collection vials from each RIL. Flies were aged for an additional 3 days before measuring triglyceride level.

Experimental females from each collection vial were anesthetized using CO₂, and groups of five were arrayed into deep well plates (P-DW-11C; Axygen) over ice, with each well preloaded with a single glass bead. This resulted in eight replicate samples of five females per RIL. Immediately after finishing a plate, we added 400 μl of cold homogenization buffer (10 mM potassium phosphate monobasic, 1 mM EDTA, 0.05% Tween-20) to each well, homogenized for 45 sec in a Mini-BeadBeater-96 (BioSpec Products), and centrifuged for 4 min at 2500 × g. We then moved 50 μl of the supernatant to a standard PCR plate, incubated the plate in a thermocycler at 70ºC for 5 min, and then placed the plate on ice for 5 min.

During the incubation steps, we added 30 μl of homogenization buffer to 92 of the 96 wells of a flat-bottom, polystyrene assay plate (655101; Greiner), and subsequently added 20 μl of the heat-deactivated fly homogenate to each. The four remaining wells of every assay plate were dedicated to controls; one blank well contained 50 μl of homogenization buffer only, and three wells contained 5 μl of Glycerol Standard Solution (G7793, 2.5 mg/ml; Sigma-Aldrich) along with 45 μl of homogenization buffer.

The assay plate was then inserted into a BioTek Powerwave XS2 instrument preheated to 37ºC and read at 540 nm (baseline absorbance scan). After the scan, and within 10 min, we added 100 μl of Free Glycerol Reagent (F6428; Sigma-Aldrich) to each well. The plate was then reinserted into the instrument, incubated at 37ºC for 5 min, and read again at 540 nm (free glycerol absorbance scan). After this second scan, and again within 10 min, we added 25 μl of Triglyceride Reagent (T2449; SigmaAldrich) to each well. The plate was again incubated at 37ºC for 5 min in the machine and read for a third time at 540 nm (triglyceride, or final absorbance scan).

For each sample, we obtained the final absorbance for each sample (FA samp t e ) and calculated the initial absorbance (IA samp t e ) as the free glycerol measurement minus the baseline measurement. We also generated the average final absorbance for the three standard wells (FA std ) and the initial absorbance for the one blank well (IA blank ). We then estimated the true serum triglyceride level as

\[
[F_A^{\text{sample}} - (I_A^{\text{sample}} \times F)] / [F_A^{\text{std}} - (I_A^{\text{blank}} \times F)]
\]

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

**Correlations among traits**

We assessed the relationship between the DSPR RIL mean starvation phenotypes from the large-scale screen with those from the activity monitor experiment, the desiccation resistance measures, and triglyceride level using general linear models. Subpopulation (pA1, pA2, pB1, pB2) was included as
a factor in the analysis examining starvation resistance and triglyceride content.

Correlations among three DGRP starvation datasets—that from Mackay et al. (2012), Everman and Morgan (2018), and the new screen we report here—were examined in a pairwise manner using line means, accounting for multiple comparisons with a Bonferroni-adjusted \( \alpha \) level. Differences in the overall mean starvation resistance among the three datasets were analyzed with a one-way ANOVA, treating study as a fixed factor.

**Heritability**

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

**QTL mapping in the DSPR**

Methods for QTL analysis, and the power and resolution of mapping using the DSPR panel are discussed in detail in King et al. (2012a,b). Briefly, QTL mapping and peak analysis were executed for starvation resistance and triglyceride data using the DSPRQTL R package (github.com/egking/DSPRqtl; FlyRILs.org), regressing the mean trait response for each RIL on the additive probabilities that each of the eight founders contributed the haplotype of the RIL at each mapped position. Significance thresholds were assigned following 1000 permutations of the data, and positions of putative causative loci were estimated with 2-LOD support intervals, which \( \sim 95\% \) confidence intervals for QTL position in the DSPR (King et al. 2012a). Mean starvation resistance varied between sexes in both the pA and pB panel \( (F_{3,3440} = 18.317; P < 0.0001; \text{Figure S2 and Table S2}) \), and subpopulation influenced female starvation resistance in the pA panel \( (\text{Tukey’s HSD} P < 0.0001; \text{Figure S2}) \). Therefore, QTL mapping was performed for males and females of each panel separately, and subpopulation was included as a covariate in the analysis of pA females. Mean female triglyceride level was similar between the pA1 and pA2 subpopulations (Tukey’s HSD \( P = 0.75; \text{Figure S3 and Table S3} \)) but varied between the pB1 and pB2 subpopulations (Tukey’s HSD \( P < 0.0001; \text{Figure S3 and Table S3} \)), so subpopulation was included as a covariate in QTL analysis of the pB triglyceride data.

**Analysis of DGRP starvation data**

Variants associated with male and female starvation resistance in the DGRP were identified using the DGRP2 web-based GWA mapping tool (http://dgrp2.gnets.ncsu.edu), which takes into account variable Wolbachia infection status and large inversions that segregate among the lines (Mackay et al. 2012; Huang et al. 2014). We performed GWA analysis on data collected in this study and additionally reanalyzed starvation data from Mackay et al. (2012) and starvation of young flies (5–7 days old) from Everman and Morgan (2018). We additionally assigned the 150 DGRP lines that are shared between the three datasets an across-study mean, and performed GWA analysis on this summary measure of starvation resistance.

SNPs associated with starvation resistance were identified within each of the four datasets following FDR correction for multiple comparisons (Benjamini and Hochberg 1995) in R (p.adjust; R Core Team 2017). Since we found no significantly associated SNPs with an FDR adjusted \( P \)-value \( <0.05 \) for any starvation resistance dataset in either sex, we relaxed the significance threshold to an FDR adjusted \( P \)-value \( <0.2 \). As a significance threshold of \( P < 10^{-5} \) is commonly used in the DGRP (e.g., Mackay et al. 2012; Huang et al. 2014; Morozova et al. 2014; Everman and Morgan 2018), we also present variants associated with starvation resistance in each of the four datasets using this threshold.

There was minimal overlap in the identity of the above-threshold, starvation-associated variants in each study. Thus, we sought to examine whether the sign of the additive effects of these sets of variants was preserved across studies. Additive effects were calculated as one-half the difference in starvation resistance between lines homozygous for the major allele and lines homozygous for the minor allele (major allele frequency \( >0.5 \)), after accounting for Wolbachia infection and TE insertions (Falconer and Mackay 1996; Huang et al. 2014). To determine the proportion of SNPs that are expected by chance to have additive effects of the same sign across studies, we obtained random samples of 50 SNPs from all of the DGRP SNP calls (~2 million SNPs) and calculated the additive effects of the sampled SNPs across pairs of datasets for each sex. To account for the possibility that the frequency spectrum of above-threshold \( (P < 10^{-5}) \), associated SNPs is not represented by a set of randomly selected variants, we stratified the random subsets of 50 SNPs according to the distribution of allele frequencies of the top 50 SNPs associated with starvation resistance for each sex in each study. Allele frequency bins used in this stratification 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 each sex and dataset is provided in Table S4. This process was repeated 1000 times for each pairwise comparison of datasets (six comparisons total) using an ordinary nonparametric bootstrapping procedure with the R package boot (Davison and Hinkley 1997; Canty and Ripley 2017). For each iteration, we used a custom R function (see File S1) to calculate the proportion of the 50 random stratified SNPs that had positive additive effects in both of the datasets being compared.

**Data availability**

DGRP data from Mackay et al. (2012) are available online from http://dgrp2.gnets.ncsu.edu, and DGRP data from
Everman and Morgan (2018) are available from Dryad (DOI: https://doi.org/10.5061/dryad.vq087). Data collected in this study is available from FigShare, and includes all raw data for starvation resistance in the DSPR and DGRP, raw desiccation resistance, triglyceride level, and activity data collected using the DSPR, and all mapping results (see File S2 for complete details). R code for bootstrapping analysis and additive effect calculations in the DGRP is available in File S1. Supplemental material available at Figshare: https://doi.org/10.25386/genetics.7713173.

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.
Results and Discussion

Extensive phenotypic variation in starvation resistance in the DSPR and DGRP

Starvation resistance in both the DSPR and DGRP was highly variable among strains (Figure 1 and Figure 2), and the broad sense heritability for starvation resistance was routinely high (Table 1). Males were typically less starvation resistant than females (Figure S2, Figure S4, Table S2, and Table S5), although, despite this, male and female starvation resistance were significantly correlated in both the DSPR (pA: $R^2 = 53.0\%$; pB: $R^2 = 57.0\%$; Figure S5) and DGRP ($R^2 = 68.0\%$; Figure S6). Such sex-specific differences in starvation resistance are likely influenced by a combination of higher glycogen and triglyceride levels and larger body size, which are often observed for females relative to males (Chippindale et al. 1996; Toda and Kimura 1997; Schwasinger-Schmidt et al. 2012; Goenaga et al. 2013).

We screened the DSPR and DGRP for starvation resistance at 23° and under constant light conditions. Since starvation resistance is sensitive to the thermal environment (van Herreweg and David 1997; Karan and Parkash 1998; Karan et al. 1998; Hoffmann et al. 2005a; Bauerfeind et al. 2014), and may vary under different photoperiods (Sheeba et al. 2000; Xu et al. 2008; Seay and Thummel 2011), we sought to remeasure starvation resistance for a subset of DSPR RILs at 25° and with a 12 hr/12 hr light/dark cycle—conditions that have been used in other starvation studies (e.g., Mackay et al. 2012; Everman and Morgan 2018). Overall, starvation resistance of the retested RILs was lower in both sexes compared to that measured in the original large-scale starvation assay (effect of assay: $F_{1,136} = 31.60$, $P < 0.0001$; Figure S7A). Despite this, starvation resistance in the subset of RILs was significantly correlated between the two experiments (Females: $\beta = 0.43 \pm 0.04$, $t = 9.7$, $P < 0.0001$, $R^2 = 73.9\%$; Males: $\beta = 0.59 \pm 0.05$, $t = 10.9$, $P < 0.0001$, $R^2 = 78.3\%$; Figure S7B).

We similarly compared starvation resistance phenotypes for the DGRP measured in the current study with data generated by Mackay et al. (2012) and Everman and Morgan (2018). In our study, the DGRP exhibited considerably higher resistance than in these previous works ($F_{2,532} = 1457.5$, $P < 0.0001$; Figure S8). This discrepancy was not due to differences across studies in the frequency with which flies were counted (every 4, 8, or 12 hr depending on the study, Figure S8). To investigate whether the difference was due to the environmental conditions experienced by the experimental animals, we raised and tested 12 randomly selected DGRP lines under the same conditions as described for our initial screen (i.e., 23°, 30–60% relative humidity, and constant light) and under conditions that more closely mimic those described in Mackay et al. (2012) and Everman and Morgan (2018) (i.e., 25°, 50% relative humidity, and 12:12 hr light: dark). Furthermore, for both environments, we assayed starvation on agar media containing preservatives (see Text S1), and on media lacking preservatives, as used by Everman and Morgan (2018) and Mackay et al. (2012). The inclusion of preservatives in the assay media had the largest effect on variation in starvation resistance among studies (Preservatives: $F_{1,327} = 1628.9$, $P < 0.0001$; variance explained = 81.2%; Figure S9), with rearing/testing environment explaining very little of the variation (see Table S6 for the full breakdown of ANOVA variance components). We speculate that the antibiotic properties of the preservatives extend lifespan under starvation conditions by limiting growth of pathogenic microorganisms.

Even given the large across-study difference in mean starvation resistance in the DGRP, we found moderately strong correlations in both sexes over datasets, ranging from 50.8 to 64.4% (Figure S10). The high correspondence among these three DGRP datasets, coupled with the phenotypic correlation between the subset of DSPR strains assayed using two different approaches (see above), suggests that fundamental aspects of the genetic control of starvation resistance are generally consistent over experiments, even when environmental conditions such as temperature are quite different. The differences we observe in starvation resistance between studies may reflect ecologically relevant phenotypic plasticity. The temporally variable thermal environment is a particularly important source of selection for ectothermic organisms.

| Panel | Trait | Sex | Heritability (%) |
|-------|-------|-----|------------------|
| DSPR pA | Starvation | Female | 80.0 |
| DSPR pA | Starvation | Male | 73.1 |
| DSPR pB | Starvation | Female | 74.4 |
| DSPR pB | Starvation | Male | 71.5 |
| DGRP | Starvation | Female | 87.1 |
| DGRP | Starvation | Male | 87.0 |
| DSPR pA | Triglycerides | Female | 77.5 |
| DSPR pB | Triglycerides | Female | 82.3 |

Table 1 Broad sense heritability for starvation resistance and triglyceride level
Plastic shifts in starvation resistance in response to temperature can have important fitness benefits, including seasonal adaptation to fluctuating resource availability as has been reported in the butterfly Bicyclus anynana (Pijpe et al. 2007), and following the induction of diapause in D. melanogaster (Schmidt et al. 2005; Rion and Kawecki 2007). Collectively, these previous studies and our data speak to the important influence of both phenotypic plasticity and genotype on variation in starvation resistance in natural populations.

**Starvation resistance is associated with desiccation resistance and low activity in the DSPR**

Environmental stress can exert selection pressure on energy use and storage, and environmental stressors that impact one type of stress resistance often impact a suit of other stress-related traits (Hoffmann and Parsons 1989b). Several artificial selection studies for starvation resistance have shown a correlated change in desiccation resistance, suggesting these stress traits are related (Hoffmann and Parsons 1989a,b; Chippindale et al. 1996; Harshman et al. 1999; Hoffmann and Harshman 1999; Hoffmann et al. 2001). For instance, a detailed study of this correlated response by Hoffmann and Parsons (1989b) demonstrated a rapid phenotypic response in both desiccation and starvation resistance following four generations of strong selection for increased desiccation resistance, and, in part, this was attributed to selection acting on a general stress response mechanism. Subsequent genomics studies have suggested that this rapid phenotypic response is accompanied by rapid and extensive genomic change (Kang et al. 2016), and that extensive pleiotropy underlies desiccation resistance (Talonis-Scott et al. 2012, 2016; Kang et al. 2016; Griffin et al. 2017).

We investigated the association between starvation and desiccation resistance in the DSPR by measuring female desiccation resistance in RILs chosen from the two tails of the phenotypic distribution of female starvation resistance. We found that desiccation and starvation resistance were significantly correlated \( R^2 = 43.8, F_{1,31} = 24.11, P < 0.0001 \) (Figure 3). Since mean desiccation resistance was considerably lower than mean starvation resistance for all lines tested (compare Figure 1 and Figure 3), flies experiencing desiccation conditions are unlikely to be dying from starvation. In addition, since DSPR lines with very low starvation resistance do not also have low larval viability (data from Marriage et al. 2014) or reduced adult lifespan (data from Highfill et al. 2016) it does not appear that DSPR lines with very low resistance to starvation and desiccation are simply “sick” (Figure S11). The relationship between starvation and desiccation resistance in the present study provides support for the genetic correlation and shared physiological mechanisms that have been proposed to exist between these traits (Hoffmann and Parsons 1989a,b, 1993; Harshman et al. 1999; Kennington et al. 2001). However, the correlation we observed is modest, and does not rule out the possibility that the covariation observed between starvation and desiccation resistance may be influenced by genetic variation in one or more other resistance-associated traits. A more intensive sampling of the DSPR would be necessary to thoroughly
investigate the genetic correlation between starvation and desiccation resistance.

One physiological mechanism that may increase tolerance to environmental stressors is a reduction in metabolic rate (Lighton and Bartholomew 1988; Hoffmann and Parsons 1989a,b, 1991; Chippindale et al. 1996; Djawdan et al. 1997; Marron et al. 2003; Rion and Kawecki 2007; Schwasinger-Schmidt et al. 2012; Slocumb et al. 2015). Indeed, selection for both starvation and desiccation resistance has been shown to lead to a correlated change in activity level, an indirect proxy for metabolic rate (Hoffmann and Parsons 1989b, 1993; Schwasinger-Schmidt et al. 2012). Here, we assessed activity of a subset of RILs exhibiting high and low starvation resistance to understand how genetic variability in starvation resistance relates to activity levels under nonstressful conditions. In the presence of nutritive media, males and females differed in activity level across the light and dark period ($F_{1,132} = 16.9, P < 0.0001$; Figure 4 and Table S7), with high starvation resistance RILs exhibiting significantly lower activity levels than low starvation resistance RILs ($F_{1,132} = 12.5, P < 0.001$; Figure 4 and Table S7). The effects of starvation resistance rank (high vs. low), sex, and the light status (light vs. dark) on activity were similar in magnitude (Cohen’s F: 0.21–0.36; Table S7), suggesting that these factors contribute similarly to variation in waking activity levels.

The differences in activity between high and low starvation resistance lines on regular, nutritive media (Figure 4) were preserved under starvation stress conditions, with the high starvation resistant lines being less active than the low starvation resistant lines throughout the starvation process (Figure S13 and Table S8). This pattern aligns with that 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 1989a; 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 compensate for stressful conditions (van Dijk et al. 2002; McCue 2010; Masek et al. 2014) and represent an additional facet of the complex nature of phenotypic variability in starvation resistance.

**Starvation resistance and triglyceride level are correlated in the DSPR**

Periods of starvation have been shown to significantly reduce triglyceride levels in both males and females (Schwasinger-Schmidt et al. 2012), and others have suggested that fat stores and starvation resistance may be genetically correlated (Service et al. 1985; Rose et al. 1992; Chippindale et al. 1996; Harshman et al. 1999; Schwasinger-Schmidt et al. 2012; Slocumb et al. 2015). To investigate the relationship between these traits in the DSPR, we measured mean female triglyceride level in a subset of the pA and pB DSPR RILs and found substantial phenotypic and genetic variation among RILs (Figure 5 and Table 1). Mean starvation resistance and triglyceride level were positively correlated in both DSPR panels, although the correlation in the pA and pB panels was significantly different (Figure 6). Overall, variation in mean
starvation resistance explained 23.7% of the variation observed in mean triglyceride level among the DSPR RILs across the two mapping panels \( (R^2 = 23.7\%, F_{3,929} = 95.96, P < 0.0001; \) Figure 6), suggesting that a proportion of variation in female starvation resistance can be explained by variation in triglyceride level in the DSPR.

The correlation between triglyceride level (or total lipid level, depending on the study) and starvation resistance has been measured in numerous natural and artificially selected Drosophila populations, and a positive relationship is often described (Chippindale et al. 1996; Djawdan et al. 1997; van Herrewege and David 1997; Harshman et al. 1999; Schwasinger-Schmidt et al. 2012; Goenaga et al. 2013; Slocumb et al. 2015; Hardy et al. 2018). For example, in isofemale lines derived from populations distributed across \( \sim 14.4 \) degrees of latitude, Goenaga et al. (2013) found that 12% of the variation in female starvation resistance was accounted for by lipid content. Similarly, Chippindale et al. (1996) found a very strong positive relationship between total lipids and starvation resistance following extended selection for increased starvation resistance, and suggested that lipid levels may directly determine starvation resistance. However, a strong correspondence between lipid content and starvation resistance is not always observed in strains derived from natural populations (Robinson et al. 2000; Hoffmann et al. 2001; Jumbo-Lucioni et al. 2010). For example, Jumbo-Lucioni et al. (2010) found no correlation between triacylglycerol levels and starvation resistance measured in inbred lines derived from a natural population. Hoffman et al. (2001) suggested that variation in the strength of the correlation between triglycerides and starvation resistance may be due to the evolutionary history of the study population. Evolutionary tradeoffs between increased lipid storage and other aspects of fitness may also influence the correlation between starvation resistance and lipid levels (Huang et al. 2014; Hardy et al. 2015, 2018). Furthermore, artificial selection may increase starvation resistance via mechanisms that preferentially modify lipid accumulation or metabolism, rather than by impacting energy level or energy-saving behavioral strategies (Hoffmann and Parsons 1989a; Blows and Hoffman 1993; Hoffmann et al. 2001; Marron et al. 2003; Rion and Kawecki 2007; Masek et al. 2014; Slocumb et al. 2015). The relationship observed between triglyceride levels and starvation resistance in our study supports the hypothesis that triglyceride levels and starvation resistance are likely physiologically related. Equally, it is evident from our data that triglyceride level likely influences starvation resistance to a lesser degree than proposed by Chippindale et al. (1996) and Hoffmann and Harshman (1999), and that starvation resistance and triglyceride level have the potential to evolve independently under natural selection.

**Starvation resistance QTL allow prediction of DSPR founder phenotypes**

We identified eight QTL for starvation resistance in the pA panel and seven QTL in the pB panel, several of which overlapped between sexes (Figure S14). Both sets of QTL explained a substantial amount of variation in starvation resistance, with individual peaks accounting for 3.7–13.2% of the variation (Table 2). The total variance explained by QTL in the pA (pB) population was 26.1% (32.8%) in females and 17.5% (37.9%) in males, assuming QTL are independent and additive (Table 2). None of the QTL identified in the pA and pB mapping panels overlapped, and, since power to detect 5% QTL is expected to be high in our study (King et al. 2012a) and all DSPR phenotyping was completed within 7 months using the same design and environmental conditions, this likely reflects genetic variation among the different sets of founders used to establish the two sets of lines.

Because we can estimate the effect associated with each founder haplotype at each mapped QTL in the DSPR, it follows
that a combination of the estimates across all QTL can be used to predict the actual phenotypes of the original founder strains. We measured starvation resistance in the 15 DSPR founder lines (Figure 1, E and F) to test this prediction. It is likely that the strength of the correlation between the estimated and actual trait response is influenced by the number and effect size of each QTL mapped for the trait. To account for differences in the degree to which starvation resistance is influenced by QTL of varying effect sizes, we calculated the predicted mean trait for each founder line weighted by the variance explained by each QTL.

As anticipated, using a general linear model, the weighted mean predicted starvation resistance of the founders based on QTL effects was significantly correlated with the sex-averaged mean starvation resistance measured for the founder lines ($R^2 = 60.8\%$, $F_{3,13} = 6.12$, $P < 0.01$; Figure 7). The slope of this relationship is relatively small ($\beta = 0.13 \pm 0.07$), suggesting that, while a large component of variation in starvation resistance is clearly genetic (supported by heritability estimates for each panel, Table 1), substantial variation in the phenotype is unaccounted for by additive genetic effects at mapped QTL. This unaccounted-for genetic variation in starvation resistance is likely due to many QTL with very small effects beyond our power to detect them (King et al. 2012a) and/or epistatic interactions among QTL (Mackay 2014; Evans et al. 2018). Epistasis may be especially important when comparing actual founder strain phenotypes with those inferred via QTL effects due to the many generations of recombination employed while establishing the DSPR from the inbred founders. However, the strength of the correlation between predicted and actual responses does suggest that QTL identified from the DSPR mapping panels identify causative loci that influence the level of starvation resistance among the progenitors of the RILs.

**Limited overlap between the genetic architecture of starvation resistance and triglyceride level in the DSPR**

To further understand the relationship between starvation resistance and triglyceride level in the DSPR, we compared the genetic architectures of these two traits. 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 (Figure S15 and Table 2), 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 (Figure S15).

Given the phenotypic correlation between triglyceride level and starvation resistance in the DSPR (Figure 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. However, we see only limited evidence for this. Triglyceride QTL TB1 (mapped in the pB panel) and starvation QTL SA5 (mapped in the pA panel) do physically overlap, but, given the complete lack of evidence for QTL for the same trait colocalizing in both the pA and pB DSPR mapping panels, it is unlikely the variant(s) underlying these QTL are the same. To investigate the relationship between the two QTL that do overlap within the same panel (SB6 and TB3), we assessed the influence of haplotype structure at the overlapping QTL on the positive phenotypic correlation between triglyceride level and starvation resistance (Figure 6). In this analysis, we first identified the founder haplotype for each RIL at the positions of the overlapping QTL peaks, and calculated the average phenotype of each of the founder haplotypes. We then assessed the correlation between haplotype-specific mean triglyceride level and starvation resistance with a general linear model. After accounting for the haplotype structure at the overlapping peaks, we found that mean starvation resistance and triglyceride level were significantly correlated ($F_{1,7} = 7.72$, $P < 0.05$, $R^2 = 52.4\%$; Figure S16), suggesting some pleiotropic variants may be responsible for this pair of overlapping starvation resistance and triglyceride level QTL.

The limited overlap in the QTL intervals associated with starvation and triglyceride level suggests that the genetic bases of this pair of traits are largely independent, or at least not tightly linked at QTL with moderate to large effects. In natural populations, increased starvation resistance may evolve as a result of selection on diverse traits, including metabolic rate, activity level, lifespan, development rate, thermal tolerance, and fecundity (Service et al. 1985; Hoffmann and Parsons 1989b, 1993; Rose et al. 1992; Chippindale et al. 1993; Djawdan et al. 1997; Harshman...
et al. 1999; Bochdanovits and de Jong 2003; Marron et al. 2003; Bubliy and Loeschcke 2005; Rion and Kawecki 2007; Schwasinger-Schmidt et al. 2012), and triglyceride levels may be influenced by genetic variation in each of these traits. Our evidence of minimal overlap between the genetic architectures of starvation resistance and triglyceride levels, coupled with a phenotypic correlation between these traits, may be indicative of a series of complex correlations between traits that influence stress tolerance, energy metabolism, and life history in the DSPR.

Candidate genes underlying fitness trait variation

Across all QTL identified for starvation resistance and triglyceride level in this study, several genes within mapped QTL intervals have functions related to these and other correlated traits (Table 2 and Table S9). Of particular interest are the 30 genes that fall within our QTL intervals that were identified in previous starvation resistance studies (Clancy et al. 2001; Harbison et al. 2005; Sørensen et al. 2007) (Table S9). Gene ontology analyses performed for each trait and panel revealed enrichment of genes within pA starvation QTL related to glutathione metabolic process (6.91-fold enrichment, FDR corrected \( P = 0.000146 \); Table S10), as well as several categories that were enriched for genes implicated by mapped triglyceride QTL (Table S10). This enrichment could assist with the resolution of the functional genes within QTL regions. However, it should be noted that the sets of genes implicated by QTL mapping in the DSPR (3–244 genes per interval in this study) are extremely unlikely to all contribute to trait variation, and their presence within QTL intervals cannot alone be taken as evidence for causality.

Upon examination of the genes within the overlapping starvation resistance and triglyceride interval (TB3 and SB6), we found several genes that have either been predicted or demonstrated experimentally to be associated with traits related to starvation resistance and triglycerides or metabolism (Table S9 and references therein). Genes that fall within the intervals of the overlapping peaks include those that influence adult lifespan [e.g., *ry*, *Men*, *Gnmt* (Simonsen et al. 2006; Paik et al. 2012; Obata and Miura 2015)], lipid metabolic processes [including *Lip3*, *CG11598*, *CG11608*, *CG18530* (FlyBase Curators et al. 2004; Harbison et al. 2004; Nuzhdin et al. 2007; Sørensen et al. 2007)], antioxidant defense [e.g., *NQO1* (Nakamura et al. 2007; Natori et al. 2014)], circadian rhythm and sleep [e.g., *tau* (Bienz and囤t 2001; Harbison et al. 2004; Clancy et al. 2001; FlyBase Curators et al. 2004; Harbison et al. 2004; Nuzhdin et al. 2007; Sørensen et al. 2007)], and metabolic processes.

### Table 2 Summary of QTL identified for starvation resistance and triglyceride level in the DSPR

| QTL                  | Sex | Peak LOD | Chr | Physical interval (Mb) | Genetic interval (cM) | Variance explained | No. potential starvation candidate genesa |
|----------------------|-----|----------|-----|------------------------|-----------------------|-------------------|-------------------------------------------|
| Starvation Resistance QTL: pA DSPR panel |     |          |     |                        |                       |                   |                                           |
| SA1                  | M   | 7.41     | 2L  | 0.34–0.64              | 0.36–1.02             | 3.90              | 52                                        |
| SA2                  | F   | 10.85    | 2R  | 4.90–5.53              | 59.39–60.64           | 6.01              | 122                                       |
| SA2                  | M   | 10.59    | 2R  | 4.87–5.58              | 59.32–60.74           | 5.52              | 126                                       |
| SA3                  | F   | 8.05     | 2R  | 12.85–13.43            | 80.75–82.81           | 4.23              | 110                                       |
| SA4                  | F   | 7.03     | 2R  | 14.18–14.72            | 84.14–86.68           | 3.70              | 105                                       |
| SA5                  | F   | 10.13    | 3L  | 3.40–3.92              | 6.89–8.88             | 5.29              | 52                                        |
| SA6                  | F   | 13.21    | 3R  | 0.79–2.37              | 47.10–47.52           | 6.85              | 244                                       |
| SA7                  | M   | 7.66     | 3R  | 15.55–15.86            | 66.52–67.36           | 4.03              | 52                                        |
| SA8                  | M   | 7.73     | 3R  | 21.07–21.48            | 86.77–88.20           | 4.06              | 80                                        |
| Starvation Resistance QTL: pB DSPR panel |     |          |     |                        |                       |                   |                                           |
| SB1                  | F   | 7.68     | 2L  | 7.02–7.30              | 23.69–25.20           | 4.05              | 33                                        |
| SB2                  | M   | 8.35     | 2L  | 10.10–10.50            | 39.24–40.93           | 4.39              | 110                                       |
| SB3                  | F   | 7.13     | 2L  | 12.14–12.65            | 45.94–46.96           | 3.77              | 44                                        |
| SB4                  | M   | 7.05     | 2L  | 12.13–12.63            | 45.92–46.93           | 3.72              | 45                                        |
| SB4                  | F   | 12.28    | 2R  | 1.10–2.70              | 54.93–55.73           | 6.41              | 148                                       |
| SB5                  | F   | 19.40    | 2R  | 2.01–2.69              | 55.21–55.72           | 9.91              | 81                                        |
| SB5                  | M   | 18.98    | 3L  | 18.94–19.42            | 45.48–45.75           | 9.73              | 54                                        |
| SB6                  | F   | 26.40    | 3L  | 19.00–19.43            | 45.51–45.76           | 13.24             | 46                                        |
| SB6                  | M   | 9.07     | 3R  | 8.86–9.20              | 52.50–53.29           | 4.77              | 48                                        |
| SB7                  | F   | 12.77    | 3R  | 8.57–9.21              | 52.33–53.30           | 6.64              | 90                                        |
| SB7                  | F   | 7.72     | 3R  | 25.65–26.15            | 99.12–100.11          | 4.08              | 111                                       |
| Triglyceride Level QTL: pB DSPR panel |     |          |     |                        |                       |                   |                                           |
| TB1                  | F   | 8.73     | 3L  | 3.45–4.09              | 7.08–9.52             | 6.22              | 84                                        |
| TB2                  | F   | 7.90     | 3R  | 7.18–8.01              | 50.50–51.54           | 5.64              | 118                                       |
| TB3                  | F   | 8.45     | 3R  | 8.31–8.71              | 51.96–52.54           | 6.03              | 54                                        |
| TB4                  | F   | 7.71     | X   | 17.34–17.43            | 57.17–57.44           | 5.51              | 4                                         |

* a Genes and functions associated with starvation resistance are listed in Table S9. The number of genes associated with starvation resistance (SR) was determined by cross-referencing genes within each QTL interval with previously reported candidates and biological functions reported by FlyBase (Lin et al. 1998; Clancy et al. 2001; FlyBase Curators et al. 2004; Harbison et al. 2004; Nuzhdin et al. 2007; Sørensen et al. 2007).
genes are promising candidates for future studies seeking to examine the functional genetic relationship between these two traits.

**Different mapping approaches reveal unique genetic architectures for starvation resistance**

Dissection of a quantitative genetic trait using different approaches can allow greater resolution of the genetic architecture, and provide insight into how alleles unique to different mapping panels contribute to phenotypic variation. To gain this additional understanding, we assessed the genetic architecture of starvation resistance in the DGRP using GWA mapping of four starvation resistance datasets: new data collected in this study, data from Mackay et al. (2012), data from Everman and Morgan (2018), and a consensus, across-study starvation resistance measure calculated as the mean response across the three starvation datasets (150 lines were measured across the three studies). Using an FDR threshold of 20%, between 0 and 12 SNPs were associated with starvation resistance in each dataset and sex (Table 3 and Table S11). Aside from three SNPs that overlap between the across-study mean dataset and the Mackay et al. (2012) dataset, none of these above-threshold SNPs were the same (Table 3 and Table S11). Using the more lenient significance threshold of \( P < 10^{-5} \) (see Table 3 for the equivalent FDR values), between 17 and 48 SNPs were associated with starvation resistance for each dataset and sex (Table 3). However, overlap in associated SNPs among datasets was still minimal (Figure S17). The SNPs identified using the more lenient significance threshold included all those identified at the FDR threshold of 20%, so all subsequent analyses were performed on the larger set of associated SNPs, and we acknowledge that these sets may include larger fractions of false-positive associations.

Across all four datasets reporting starvation resistance in the DGRP, 12 SNPs (associated with seven genes) identified using the \( P < 10^{-5} \) significance threshold fell within QTL intervals identified for starvation resistance in the DSPR (Figure S18 and Table S11). In females, one SNP associated with starvation resistance from Mackay et al. (2012) is within QTL SA4 (gene *CG30118*), and one SNP associated with starvation resistance in this study is present in QTL SA3 (gene *mbl*). One SNP associated with starvation resistance from Everman and Morgan (2018) is within QTL SB7 (gene *hdc*), and one SNP from the average of starvation resistance across the DGRP datasets is within QTL SB5 (gene *Gbs-76A*). In males, one SNP associated with starvation resistance from Mackay et al. (2012) is within QTL SB3 and was not associated with a gene. Four SNPs (one from this study and three from the average of starvation resistance) fell within QTL SB5 and were all near the gene *fs2*; one additional SNP associated with the average of starvation resistance was also within SB5 and was associated with the gene *pip*. One SNP associated with starvation resistance from Everman and Morgan (2018) was within QTL SB6 (gene *beat-Vc*). Only one of these seven genes has been previously associated with starvation resistance (*CG30118*; (Sørensen et al. 2007)), and none have reported functions specifically related to starvation resistance or general stress response (FlyBase Curators et al. 2004). Furthermore, none of the overlapping genes survived an FDR threshold of 0.2, increasing the possibility that these genes may be false positives. Therefore, with the possible exception of *CG30118*, these genes may not be promising candidates, despite their overlap among studies.

Compared to genes implicated by QTL identified in the DSPR, which include several that have been previously associated with starvation or related phenotypes (e.g., lifespan or lipid content), DGRP GWAS hits implicate fewer a priori strong candidate genes. Additionally, we did not observe any GO enrichment following analyses of SNPs associated with the four starvation resistance datasets, although we acknowledge that the limited number of implicated genes likely compromised the power of these analyses. Of the total 127 unique genes associated with starvation resistance in the DGRP across studies and sexes, only two have been previously identified as associated with starvation resistance in other mapping populations [*CG30118*, *scaf6*; Table S11; (see Table S2 in Sørensen et al. 2007)]. More generally, five had previously been associated with the determination of adult lifespan (e.g., *cnc* and *Egfr*; Table S11; (Sykiotis and Bohmann 2008; Kamakura 2011)), and five have been previously associated with lipid metabolism or metabolic processes (e.g., *GlcAT-P* and *Ugt86D*; Table S11; (FlyBase Curators et al. 2004; Gaudet et al. 2010)). Given the relative lack of power of a GWA study using <200 genotypes (Long et al. 2014), and our use of a permissive genomewide threshold, it could be that many of the GWAS associations are incorrect, explaining why associations do not typically tag known candidates. Equally, it could be the case that a series of novel pathways are involved in natural variation for starvation resistance,
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 have effects only in a specific genetic background (i.e., exhibit genetic epistasis), 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). This point is especially true for the comparisons made here, since the DSPR represents a global sampling of genetic variation represented by the 15 founder strains, whereas the variation present in the DGRP is a direct reflection of the genetic variability in a single population at a single point in time. 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.

### Repeatability in the SNPs associated with starvation resistance across DGRP studies

The public availability of starvation resistance data for the DGRP from multiple studies provides a novel opportunity to investigate the reliability and repeatability of associations identified for a classic quantitative trait in the same mapping panel across independent phenotypic screens (Lithgow et al. 2017). Despite the moderately high phenotypic correlation between starvation resistance measured in the three studies (Figure S8), only a single variant was implicated in more than one of the three studies (Figure S17).

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

To test this prediction, we first collected the sign of the additive effects of SNPs that survived the significance threshold ($P < 10^{-5}$) in each dataset for both sexes (Table S11), and determined whether these top SNPs had additive effects of the same sign in every other dataset. We then established a null distribution of SNP additive effect signs across pairs of datasets. This was accomplished by taking samples of 50 SNPs segregating in the DGRP and extracting the sign of the additive effect of each SNP in the pair of datasets, regardless of the association statistic for that SNP. The proportion of the 50 SNPs that had a positive additive effect on the trait was recorded for each of 1000 iterations and used to build an expected distribution of SNP effect sign sharing for each pair of datasets. We note that to compensate for any allele frequency bias in the variants that are actually most associated with phenotype, we ensured that the frequencies of the randomly sampled SNPs were stratified to match the top 50 SNPs associated with each sex and dataset (see Materials and Methods; Table S4).

Finally, we compared the proportion of top SNPs for which the sign stayed the same across studies to our expected distribution for each pair of datasets (Figure 8). We found that, for the random samples of SNPs, the probability that the
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 (A, B, C, E), Mackay et al. (2012) (A, C, D, F) and Everman and Morgan (2018) (B, D, E, F). 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 (Figure S19). 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. In every case, top SNPs from one study were more likely to have the same additive effect sign in a second study than a random set of frequency-matched SNPs.
additive effects were positive in both datasets compared was >50% for most comparisons (distribution means ranging from 45.9 to 72.1%; Figure 8). This implies that a random SNP is slightly more likely to have the same sign effect across data sets, which may be explained in part by the phenotypic correlation between the datasets (Figure S10). Even given this skew, far more top SNPs than expected by chance had additive effects of the same sign in each of the other starvation resistance studies (Figure 8 and Figure S19). This may suggest that there is phenotypic signal even in SNPs that have very small effects, and that are not clearly associated with starvation resistance in the GWAS (see Yang et al. 2010). Generally, the consistency of the additive effects of SNPs associated with starvation resistance in the DGRP calculated across datasets implies that starvation resistance is a highly polygenic trait, with a large number of QTL with very small effects that influence variation in this trait (Mackay 2004; Boyle et al. 2017).

Conclusions

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

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