Quantitative Trait Loci Affecting Starvation Resistance in *Drosophila melanogaster*

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ABSTRACT

The ability to withstand periods of scarce food resources is an important fitness trait. Starvation resistance is a quantitative trait controlled by multiple interacting genes and exhibits considerable genetic variation in natural populations. This genetic variation could be maintained in the face of strong selection due to a trade-off in resource allocation between reproductive activity and individual survival. Knowledge of the genes affecting starvation tolerance and the subset of genes that affect variation in starvation resistance in natural populations would enable us to evaluate this hypothesis from a quantitative genetic perspective. We screened 933 co-isogenic *P*-element insertion lines to identify candidate genes affecting starvation tolerance. A total of 383 *P*-element insertions induced highly significant and often sex-specific mutational variance in starvation resistance. We also used deficiency complementation mapping followed by complementation to mutations to identify 12 genes contributing to variation in starvation resistance between two wild-type strains. The genes we identified are involved in oogenesis, metabolism, and feeding behaviors, indicating a possible link to reproduction and survival. However, we also found genes with cell fate specification and cell proliferation phenotypes, which implies that resource allocation during development and at the cellular level may also influence the phenotypic response to starvation.

IN nature, animals must often cope with periods of suboptimal food resources. Yeast, bacteria, and nematodes have a distinctive response when nutrients are unavailable: they alter their morphology, become quiescent, and suspend reproductive activity, which enables them to survive until food resources become more plentiful (Kolter *et al.* 1993; Thomas 1993; Kenyon 1996; Guarente *et al.* 1998; Hengge-Aronis 2000). Increased expression of the disaccharide trehalose occurs in response to starvation in yeast (Klionsky and Emr 2000). Moreover, yeast degrade proteins and organelles in an attempt to scavenge nutrients during starvation (Winderickx *et al.* 1996). Related mechanisms operate in bacteria. The *otsBA* operon, which synthesizes trehalose, has been implicated in stress response while the entericidin locus *ecnAB* may be instrumental in inducing programmed death among starving cells (Hengge-Aronis 2000). In the nematode *Caenorhabditis elegans*, genes involved in dauer larvae formation have pleiotropic effects on starvation resistance. Mutations in *daf-2*, the insulin-like growth factor receptor, and *daf-7*, which encodes a member of the transforming growth factor-β family, increase starvation resistance relative to wild-type worms (Munoz and Riddle 2003). A double mutant of the spermatogenesis gene *fer-15* and the phosphatidylinositol-3-kinase catalytic subunit gene *age-1* also has increased starvation tolerance (Munoz and Riddle 2003). In contrast, mutant alleles of the transcriptional regulator *daf-16* reduce starvation tolerance, even when coupled with a *daf-2* mutation (Munoz and Riddle 2003).

Drosophila also experience periods of famine in nature, yet the suite of genes affecting their physiological and behavioral responses to famine remains largely unknown. Starvation resistance is a typical quantitative trait that displays considerable genetic variation in natural populations (Service and Rose 1985; Da Lage *et al.* 1990; Hutchinson and Rose 1991; Hutchinson *et al.* 1991; Toda and Kimura 1997; van Herrewege and David 1997; Karan and Parkash 1998; Karan *et al.* 1998) and in response to artificial selection (Harshman and Schmid 1998; Harshman *et al.* 1999). Increased resistance to starvation is positively correlated with other stress resistance and life-history traits such as desiccation resistance, life span, and development time (Service *et al.* 1985; Hoffmann and Parsons 1989, 1993; Blows and Hoffmann 1993; Chippindale *et al.* 1994, 1996; Harshman and Schmid 1998; Harshman *et al.* 1999). However, increased starvation resistance is often negatively correlated with fecundity (Service and Rose 1985; Leroi *et al.* 1994a,b), suggesting that a trade-off between reproduction and individual survival depending upon resource availability may maintain genetic variation in

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starvation resistance. Understanding the genetic basis of such a trade-off requires that we identify the suite of genes affecting starvation resistance and their pleiotropic effects on other fitness-related traits (Mackay 2001).

Thus far, few genes that affect starvation resistance and/or feeding in Drosophila have been identified. A *P*-element insertion in *methuselah*, a G-protein-coupled receptor with effects on life span, showed a 50% increase in survival time under starvation conditions (Lin *et al.* 1998). Likewise, mutations in *chico*, an insulin receptor substrate, increased both life span and starvation resistance (Clancy *et al.* 2001). Starvation induces expression of the circadian clock-regulated gene *takeout*, increasing both mRNA and protein levels (Sarov-Blat *et al.* 2000). The *foraging* gene has effects on both larval and adult feeding behavior (Pereira and Sokolowski 1993), while *scribbler* affects larval feeding behavior under starvation conditions (Yang *et al.* 2000). *Ryanodine receptor 44F* affects food ingestion and excretion in larvae (Sullivan *et al.* 2000). Tolerance to starvation has been associated with alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase in natural and selected populations (Oudman *et al.* 1994; Harshman *et al.* 1999).

An understanding of the influence genes have on starvation resistance requires that we identify the genes that regulate the response to starvation conditions and the subset of these genes that contribute to naturally occurring genetic variation in this trait. The first question can be addressed by assessing effects of induced mutations on starvation tolerance, while the second requires high-resolution mapping of quantitative trait loci (QTL) causing divergence in starvation resistance between wild-type strains. Here, we have used both approaches to identify genes affecting starvation resistance in *Drosophila melanogaster*. Screens for subtle, quantitative effects of *P*-element insertions have been successful in identifying novel loci affecting metabolism (Clark *et al.* 1995), sensory bristle number (Lyman *et al.* 1996; Norga *et al.* 2003), and olfactory behavior (Anholt *et al.* 1996). We screened 933 *P*-element insertion lines that were generated in co-isogenic backgrounds and identified 383 insertions affecting starvation resistance, many of which have sex-specific effects.

QTL are mapped by linkage to molecular markers and have been identified for a large number of traits, including fat mass in humans (Comuzzie *et al.* 1997); survival, fertility, and life span in worms (Ayyadevara *et al.* 2001); and life span in flies (Nuzhdin *et al.* 1997; Leips and Mackay 2000; Vieira *et al.* 2000; Reiwitch and Nuzhdin 2002). In Drosophila, deficiency complementation testing facilitates fine-scale resolution of broad QTL regions into smaller segments (Pasyukova *et al.* 2000; Fanara *et al.* 2002). Here we have used deficiency complementation mapping to fine map five broadly localized QTL identified by Vieira *et al.* (2000), which affect variation in starvation resistance between two strains of *D. melanogaster*: *Oregon-R* and *2b*. We found a minimum of 13 QTL affecting the difference in starvation resistance between *Oregon-R* and *2b*, including 6 QTL with sex-specific effects. Complementation tests of mutations for a sample of the positional candidate genes within these QTL revealed 12 candidate genes with significant effects on variation in starvation resistance.

MATERIALS AND METHODS

***P*-element insertion lines:** We used the crossing scheme depicted in Figure 1 to generate 116 independent *P{GawB}* autosomal insertions in the isogenic w^{1118}; *Samarkand* genetic background. Similarly, an additional 652 independent *P{GT1}* (Lukacsovich *et al.* 2001) insert lines were generated in one of two isogenic derivatives of w^{1118}; Canton-S (Canton-S B and Canton-S F), as part of the Berkeley Drosophila Gene Disruption Project (http://flypush.imgen.bcm.tmc.edu/pscreen/). Starvation resistance was measured for all *P{GawB}* lines and 594 *P{GT1}* lines as homozygotes. To classify the degree of dominance exhibited by inserts affecting starvation resistance, 223 heterozygous *P{GT1}* insert lines were also tested. For 165 of these lines, the corresponding homozygous line was tested; the remaining 58 *P{GT1}* insert lines were either not viable or not tested as homozygotes. Starvation survival times of the *P*-element lines were compared to those of the appropriate co-isogenic control: w^{1118}; Canton-S B or F for the *P{GT1}* insert lines and w^{1118}; *Samarkand* for the *P{GawB}* insert lines.

Starvation resistance assays for *P*-element insertion lines: We subjected the flies to a starvation diet and assessed the survival time of each genotype. The diet consisted of 1.5% agar and 5 ml water in standard culture vials to provide moisture without providing nutrition. Flies were collected and separated by sex before placement on the starvation medium. Ten 2- to 7-day-old nonvirgin flies were assayed per vial, with two replicate vials for each sex and genotype. Each vial was kept in an incubator at a constant temperature of 25°, 60–75% relative humidity, and a 12-hr light-dark cycle. Flies were scored for survival every 12 hr until all were dead. Lines were tested in blocks of ~100. Eight replicate vials, each with 10 flies per sex, of w^{1118}; Canton-S B, w^{1118}; Canton-S F, or w^{1118}; *Samarkand*, as appropriate, were tested contemporaneously in each block.

Statistical analysis for *P*-element insertion lines: Analysis of variance (ANOVA) was used to assess the magnitude of mutational variance for starvation resistance separately for the *P{GawB}* and *P{GT1}* insertions. The mean effect on survival time under starvation conditions was computed for each replicate vial of the *P*-insert lines as the deviation of the vial mean from the mean of the contemporaneous control, for males and females separately. Two-way ANOVAs were computed according to the mixed model, $y = \mu + S + L + (L \times S) + Er$, where μ is the overall mean, S and L are cross-classified effects of sex (fixed) and line (random) and Er is the variance between the means of replicate vials. Reduced models were also run for each sex.

Confidence limits were computed as $\pm z_\alpha \sigma/(n)^{1/2}$, where z_α is the critical value of the normal distribution corresponding to the type I significance threshold, α; σ is the standard error derived from the total variance (see below); and n is the number of replicate vials per line: $n = 4$ for the analysis pooled over sex and $n = 2$ for the single-sex analysis. Critical values of z_α are 1.96, 2.576, and 3.291 for the 95, 99, and 99.9% confidence limits, respectively. The total variance (σ^2) in starvation resistance was estimated from the sum of the L, $L \times$

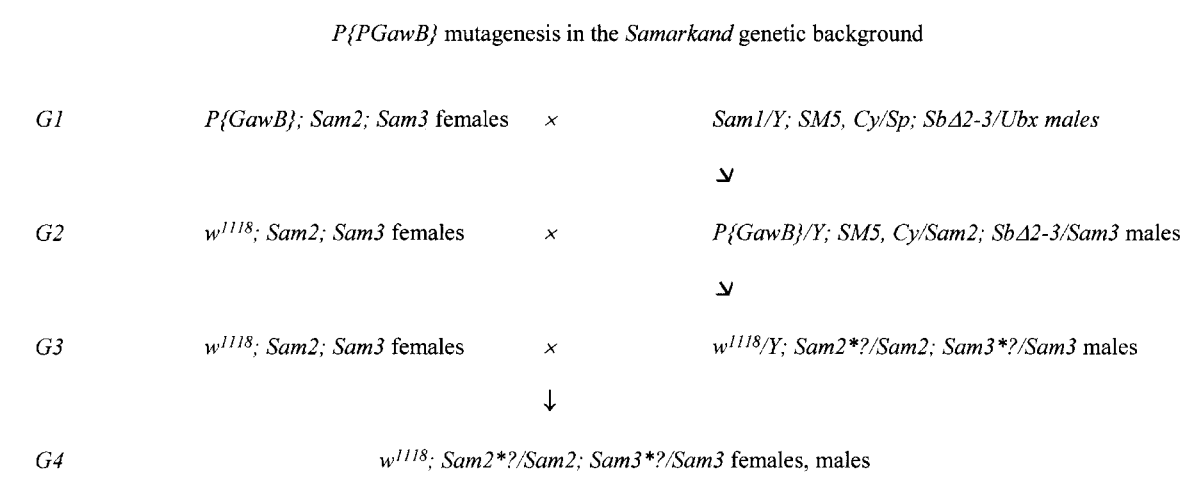


FIGURE 1.—Crosses to generate single *P{GawB}* autosomal insertions. The asterisk (*) indicates *P{GawB}* insertion; C*2* and C*3* refer to chromosomes *2* and *3*, respectively. The mutations and balancer chromosomes are described in LINDSLEY and ZIMM (1992).

S, and *Er* variance components from the ANOVAs of starvation resistance pooled over sexes and from the sum of the *L* and *Er* variance components from the ANOVAs for each sex.

We retested 93 homozygous and heterozygous insert lines that exceeded the 95% confidence limits. Each retested line was assayed in the same manner as the original test: two replicates of 10 flies for each sex per line were tested, along with the co-isogenic controls. We determined the statistical significance of the pooled results for both tests, using the ANOVA model $y = \mu + G + S + E + (G \times S) + (G \times E) + (S \times E) + (G \times S \times E) + R(G \times S \times E) + Er$, where *G*, *S*, and *E* are the fixed effects of genotype (control or *P*-element insertion), sex, and environmental differences between the original test and retest; *R* represents the random effect of a replicate vial; and *Er* is within-vial environmental variance. We interpreted insertion lines having a significant ($P < 0.05$) *G* or $G \times S$ term as strong candidates affecting starvation resistance. We used SAS statistical analysis software for all statistical calculations (SAS INSTITUTE 1988).

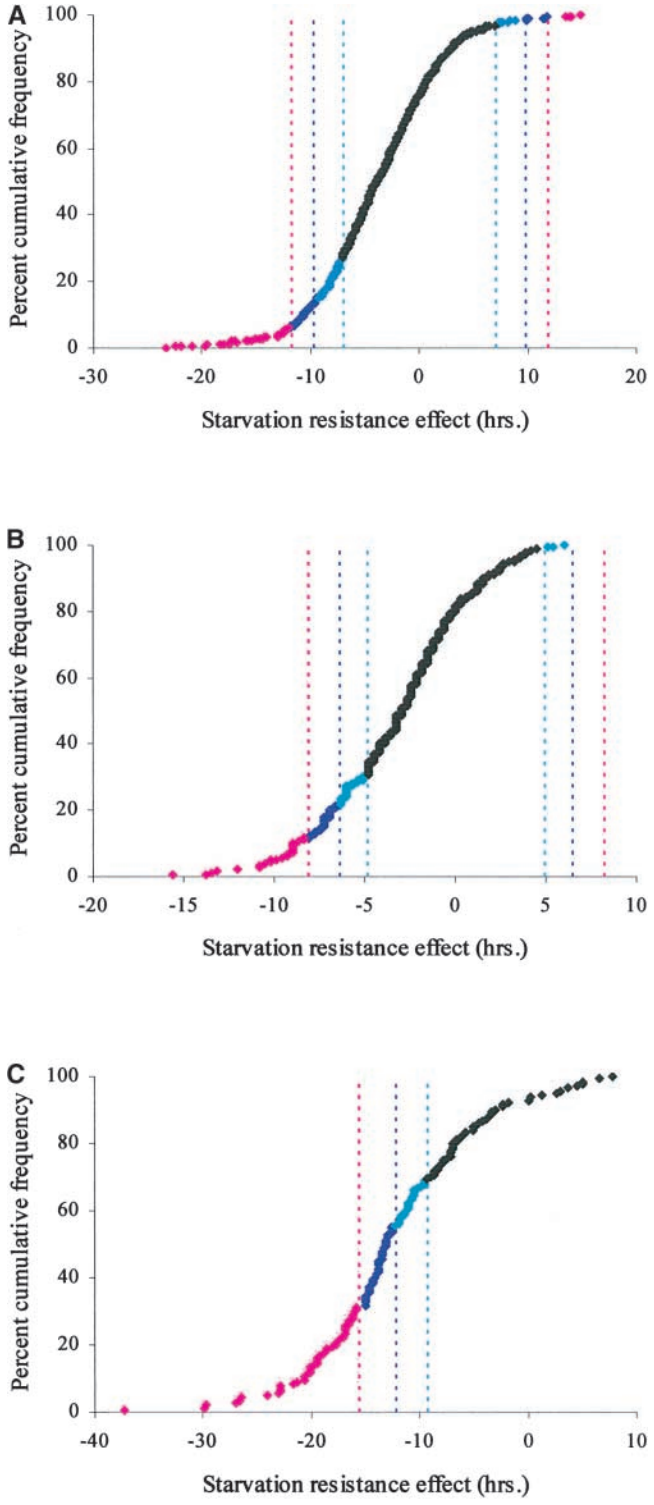
QTL for starvation resistance: Previously, the positions of QTL affecting *D. melanogaster* survival time when subjected to starvation stress were mapped using a multiple-trait composite interval method (VIEIRA *et al.* 2000). The original mapping population consisted of 98 recombinant inbred lines constructed from isogenic strains *Oregon-R* and *2b* (LINDSLEY and ZIMM 1992; PASYUKOVA and NUZHDIN 1993) as detailed in NUZHDIN *et al.* (1997). Highly polymorphic *roo* transposable elements were used as markers to determine the recombination breakpoints in each line (NUZHDIN *et al.* 1997). Five QTL were mapped for starvation resistance. Cytological locations and approximate sizes of the QTL intervals in kilobases (calculated from SORSA 1988) were 3E; 4F (1359 kb), 30D; 38A (7534 kb), 38A; 48D (9546 kb), 57C; 60E (4669 kb), and 70C; 72A (1956 kb).

Drosophila stocks used in deficiency complementation tests: The 58 deficiency stocks used to fine map each cytological region are listed in supplementary Table 1 at http://www.genetics.org/supplemental/. All stocks were obtained from the Bloomington Drosophila Stock Center. We used the deficiency breakpoints as provided by the donors and did not confirm them independently. The parental lines used to construct the recombinant inbred mapping population, *Oregon-R* and *2b*, were used to conduct the deficiency complementation tests.

Crosses and starvation resistance assays for deficiency complementation tests: Males from *Oregon-R* and *2b* were crossed with virgin females from each deficiency strain. The crosses produced four different genotypes: *Ore/Df*, *Ore/Bal*, *2b/Df*, and *2b/Bal*, where *Df* refers to the chromosome containing the deficiency and *Bal* refers to the homologous balancer chromosome. Starvation resistance was assessed for each of the four genotypes in a manner analogous to the *P*-element insertion screen, except that each of the two replicate vials per sex and genotype contained five flies. Virgin females and nonvirgin males were assayed for all deficiencies, with the exception of the *X* chromosome, for which only virgin females were used. Survival was recorded every 8 hr until all flies were dead.

Statistical analysis of deficiency complementation tests: The logic of the quantitative deficiency complementation test is explained in detail by PASYUKOVA *et al.* (2000). We analyzed the starvation data by three-way factorial ANOVA. The model was $y = \mu + L + G + S + (L \times G) + (L \times S) + (G \times S) + (L \times G \times S) + R(L \times G \times S) + Er$, where μ is the overall mean; *L*, *G*, and *S*, respectively, represent the fixed effects of the *Oregon-R* and *2b* lines, deficiency and balancer genotypes, and sex; *R* indicates the random effect of the replicate vial; and *Er* is the within-vial environmental variance (LONG *et al.* 1996; MACKAY and FRY 1996). Failure to complement is inferred for a deficiency if the $L \times G$ or $L \times G \times S$ interaction term is significant ($P < 0.05$). We also performed separate-sex analyses, using a reduced (sex term eliminated) ANOVA model. Sex-specific failure to complement was inferred if the $L \times G$ term from one of the single-sex analyses was significant and the $L \times G$ term from the pooled-sex analysis was not significant. We interpreted each deficiency region that fails to complement as containing a gene (or genes) that interacts with QTL alleles in the parental strains affecting starvation resistance, with the following caveat. In cases where the difference between *Ore/Df* and *2b/Df* was not significantly different from zero and the difference between *Ore/Bal* and *2b/Bal* was significantly different from zero, the statistically significant interaction is not consistent with allelism (PASYUKOVA *et al.* 2000). In this case, a locus or loci on the balancer chromosome are responsible for the observed interaction, which is then attributable to epistasis between loci affecting starvation resistance on the balancer chromosome and the *Oregon-R* or *2b* strains. We inferred complementation for the few deficiencies for which this was the case. Significant deficiencies were retested to confirm the significance. The SAS GLM and VARCOMP procedures were used for all statistical calculations (SAS INSTITUTE 1988).

Mutation complementation tests: We conducted complementation tests with 10 mutations that were not uncovered by deficiencies, as deficiency stocks were not available for complementation testing in those regions (supplementary Table 2 at http://www.genetics.org/supplemental/). We performed complementation tests using candidate genes in QTL regions fine mapped by deficiency complementation. Ideally, one would test all the genes in each region. However, mutants do not exist for every gene. Of the 1186 known or predicted genes residing within the deficiency candidate regions, ~369 have mutants available for testing. Of these, we chose 16 candidate genes (supplementary Table 3 at http://www.genetics.org/supplemental/) for complementation tests that met one of the following criteria: the gene was known to regulate feeding behavior, nervous system or sensory development, metabolism, and stress resistance; or the results of our *P*-element insertion screen identified the gene as a candidate for starvation resistance. Some genes had more than one allele available; these alleles were also tested to characterize possible differences in mutational effects. Mutant stocks were obtained from the Bloomington Drosophila Stock Center and J. M. O'Donnell. Mutants and *P*-element insertion lines were tested using complementation tests with the parent *Oregon-R* and *2b* stock in a way analogous to deficiency complementation tests, using the same experimental design and criteria for statistical significance.



RESULTS

***P*-element screen:** Distributions of mutational effects of *P*-element insertions on starvation resistance, expressed as deviations from the control mean in each block, are depicted in Figure 2 for each of the three genetic backgrounds (homozygous Canton-S, heterozygous Canton-S, and homozygous *Samarkand*). The mutational variance for starvation resistance was highly significant in each background ($P_{line} < 0.0001$, Table 1). Note also that the main effect of sex was significant (Table 1); on average females were more starvation tolerant than males in all backgrounds. Further, the line × sex interaction term was highly significant for all backgrounds ($P_{line\times sex} < 0.0001$), indicating sex-specific mutational effects on starvation resistance.

We calculated the cross-sex genetic correlation, r_{GS}, as $\sigma^2_L/(\sigma^2_{LM} \times \sigma^2_{LF})^{1/2}$ (Robertson 1959), where σ^2_L is the variance among lines from the analysis pooled across sexes, and σ^2_{LM} and σ^2_{LF}, are, respectively, the among-line variance components from the analyses of males and females separately (Table 2). The estimates are $r_{GS} = 0.21$ and $r_{GS} = 0.29$ for the homozygous Canton-S and *Samarkand* insertions, respectively, and $r_{GS} = 0.58$ for the heterozygous Canton-S insertions. A significant line × sex interaction term could arise because the among-line variance components are different in males and females or because the cross-sex genetic correlation is less than one. Partitioning the line × sex interaction into terms attributable to differences in among-line variance components $[(\sigma_{LM} - \sigma_{LF})^2]$ and to the departure

Figure 2.—Distribution of mutational effects on starvation resistance. Pink, 99.9% confidence interval (C.I.) threshold; dark blue, 99% (C.I.) threshold; light blue, 95% C.I. threshold. (A) Homozygous Canton-S background: 99.9% C.I. threshold, 11.85 hr; 99% C.I. threshold, 9.28 hr; 95% C.I. threshold, 7.06 hr. (B) Heterozygous Canton-S backgound: 99.9% C.I. threshold, 8.19 hr; 99% C.I. threshold, 6.41 hr; 95% C.I. threshold, 4.88 hr. (C) Homozygous *Samarkand* background: 99.9% C.I. threshold, 15.73 hr; 99% C.I. threshold, 12.31 hr; 95% C.I. threshold, 9.37 hr.

TABLE 1

Analysis of variance of mutational effects on starvation resistance pooled over sexes

Background	Source	d.f.	MS	*F*	*P*	σ^{2a}
Homozygous Canton-S	Sex	1	419.9	5.4	0.0204	—
	Line	593	108.3	10.8	<0.0001	7.66
	Line × sex	593	77.8	7.8	<0.0001	34.01
	Error	1180	10.0	—	—	10.04
Heterozygous Canton-S	Sex	1	134.3	5.7	0.0179	—
	Line	222	57.4	6.7	<0.0001	8.57
	Line × sex	222	23.6	2.8	<0.0001	7.56
	Error	444	8.5	—	—	8.55
Homozygous *Samarkand*	Sex	1	6673.5	54.3	<0.0001	—
	Line	115	202.2	16.2	<0.0001	20.38
	Line × sex	115	125.2	10	<0.0001	58.54
	Error	218	12.5	—	—	12.48

MS, mean square.
[a] Variance component.

of genetic correlations from unity [$\sigma_{LM} \times \sigma_{LF}(1 - r_{GS})$] (Robertson 1959) gives the relative contribution of each. Although the among-line mutational variance was greater in females than in males in all three genetic backgrounds (Table 2), in all cases the line × sex interaction was attributable mainly to departure of the cross-sex genetic correlation from unity (84 and 83% of the variance for homozygous and heterozygous Canton-S insertions, respectively, and 87% for homozygous *Samarkand* insertions). That is, mutations generally have different effects in males and females. This phenomenon has been observed previously for *P*-element insertions affecting olfactory behavior (Anholt *et al.* 1996) and sensory bristle number (Lyman *et al.* 1996; Norga *et al.* 2003).

We computed 95, 99, and 99.9% confidence interval limits of the deviation from the overall control mean for each genetic background (Figure 2). The initial screen revealed a total of 383 *P*-element insertions that were significant at a confidence interval of 95% or greater for either one or both sexes (for a list of mutational effects on all lines, see supplementary Table 4 at http://www.genetics.org/supplemental/). We found 239 significant insertions in the homozygous Canton-S background; 36 of these were also significant as heterozygotes. We found 58 significant insertions in the heterozygous Canton-S background that were either tested as heterozygotes only (23 lines) or not significant as homozygotes (35 lines). The distributions of mutational effects are negatively skewed as only 31 insertions in the Canton-S background increased starvation resistance above the 95% confidence interval threshold; the vast majority of inserts decreased starvation resistance, as would be expected for a fitness-related trait. A total of 86 significant insertions had significant effects on starvation resistance as homozygotes in the *Samarkand* back-

TABLE 2

Analysis of variance of mutational effects on starvation resistance for each sex separately

Background	Sex	Source	d.f.	MS	*F*	*P*	σ^{2a}
Homozygous Canton-S	Males	Line	593	47.2	9.4	<0.0001	21.14
		Error	591	5.0	—	—	5.03
	Females	Line	593	139.0	9.2	<0.0001	62.24
		Error	589	15.1	—	—	15.08
Heterozygous Canton-S	Males	Line	222	24.2	5.3	<0.0001	9.85
		Error	221	4.6	—	—	4.61
	Females	Line	222	57.2	4.6	<0.0001	22.39
		Error	223	12.4	—	—	12.45
Homozygous *Samarkand*	Males	Line	115	93.5	13.5	<0.0001	45.04
		Error	107	6.9	—	—	6.92
	Females	Line	115	237.6	13.3	<0.0001	112.29
		Error	111	17.8	—	—	17.84

MS, mean square.
[a] Variance component.

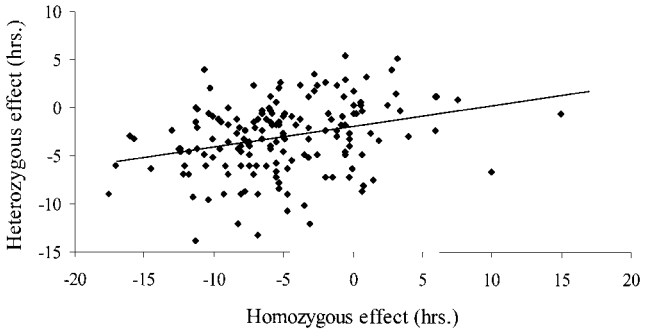


FIGURE 3.—Scatter plot of Canton-S heterozygous insert line means on homozygous insert line means. Starvation survival time is expressed as the deviation from block mean.

ground. All of the mutations in the *Samarkand* background decreased starvation resistance for both sexes pooled, although we noted four insertions that increased starvation resistance for males. Note that the *Samarkand* parental line was less viable and fertile than the Canton-S strain; this background may therefore be more sensitive to mutation than Canton-S, or the *P{GawB}* insert itself may have a deleterious effect on starvation resistance.

The significant mutational variance attributable to heterozygous mutations in the Canton-S background indicates that the mutational effects are not completely recessive. We therefore estimated the average degree of dominance as $k = 2(b - 0.5)$ (MACKAY 1987; MACKAY *et al.* 1992), where b is the regression of heterozygous line means on homozygous line means (LYMAN *et al.* 1996). Values of k range from -1 (completely recessive) to 1 (completely dominant), with a value of 0 indicating additive gene action (LYMAN *et al.* 1996). Figure 3 shows the regression of heterozygous insert means on homozygous insert means. The regression coefficient, b, is 0.22 ± 0.05 ($P < 0.0001$), giving an average degree of dominance, k, of -0.56. This result indicates that the wild-type allele is partly dominant and consequently the effect of *P*-element insertions on starvation resistance is partly recessive.

We performed a second phenotypic assessment on 93 insertion lines with effects on starvation tolerance that exceeded the 95% confidence interval thresholds. These lines were chosen from all three genetic back-

TABLE 3

Effects of significant *Samarkand* w^{1118}-derived homozygous insertion lines

Line	Effect (hr)[b] (sexes pooled)	Significant $G \times S$?	Effect (hr)[b] (males)	Effect (hr)[b] (females)
JJF004	−16.65****	No	−14.70**	−18.60***
JJF007	7.20**	No	6.60	7.80*
JJF008	−12.90***	No	−10.20*	−15.60***
JJF014	−17.25****	No	−15.60**	−18.90**
JJF015	−18.60****	No	−15.90**	−21.30***
JJF024	−12.15***	No	−10.50*	−13.80*
JJF025	−17.07****	No	−13.00**	−21.13***
JJF028	−16.95****	No	−17.10**	−16.80***
JJF064	−21.15****	No	−16.50**	−25.80***
JJF077[a]	−31.20****	No	−22.00**	−35.20***
JJF095	−19.20****	Yes*	−14.10**	−24.30***
JJF106	3.15**	No	4.80	1.50*
JJF136	−27.53****	Yes**	−17.40**	−30.93****
JJF137	−26.03****	Yes*	−14.40**	−29.93***
JJF144	−13.95****	No	−18.00**	−9.90**
JJF157	−14.10****	No	−14.40**	−13.80**
JJF164	4.80	Yes*	12.00*	−2.40
JJF170	−18.15****	No	−14.70**	−21.60***
JJF171	−17.40****	No	−12.60*	−22.20***
JJF174	−13.70****	No	−11.80**	−18.30***
JJF175	−11.07	Yes**	−1.50	−21.13**
JJF177	−17.25****	No	−16.20**	−18.30**
JJF178	−18.60****	No	−17.10**	−20.10**
JJF230	−25.60****	No	−19.80**	−31.40****
JJF237	−12.00*	Yes**	2.40	−26.40**

*$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$0.0001 < P \leq 0.001$; ****$P < 0.0001$.

[a] Located upstream of *CG5127* at 96E1.

[b] Calculated as the deviation of insert line mean from contemporaneous control mean averaged over two experiments.

TABLE 4

Effects of significant Canton-S-derived insertion lines

Insert line (H, heterozygote)	Nearest gene	Cytological location	Effect (hr)[d] (sexes pooled)	Significant $G \times S$?	Effect (hr)[d] (males)	Effect (hr)[d] (females)
BG00080	Unk	Unk	15.87****	No	15.45****	16.28****
BG00158	Unk	Unk	−1.23**	Yes*	−5.05**	2.60
BG00378[a]	Unk	Unk	−8.82****	No	−8.33***	−9.30**
BG00459[a]	Unk	Unk	−12.12****	No	−14.03****	−10.20***
BG00829	Unk	Unk	−0.57	Yes*	2.78	−3.90
BG00977	Unk	Unk	−8.74****	No	−9.08****	−8.40**
BG00986[b]	*emc*	61C9	−8.44****	Yes****	−1.35	−15.53****
BG01020	*fas*	50B9	−19.77****	Yes**	−14.93****	−24.60****
BG01046	*CG3587*	2B16	−17.97****	No	−15.75****	−20.18****
BG01047[c]	*fz*	70D4–5	−12.87****	No	−11.85****	−13.89****
BG01092[b,c]	*robo*	59B2	7.13****	Yes**	3.9*	10.35***
BG01095	*pnt*	94E10–12	3.27*	No	1.43	5.10
BG01127	*mm*	54B4–5	−10.77****	Yes*	−7.95***	−13.58****
BG01128	Unk	Unk	−1.92	Yes*	1.58	−5.40*
BG01130	*CG32423*	64C9–11	−12.42****	Yes*	−9.75****	−15.08****
BG01353	Unk	Unk	−5.73****	No	−5.70***	−5.75*
BG01367	*Rab23*	83B9	−10.84****	No	−8.18****	−13.50***
BG01428	Unk	Unk	−4.92*	Yes**	0.98	−10.8**
BG01472[b,c]	*CG9028*	70C8–9	−7.62****	Yes*	−4.20*	−11.03***
BG01485	Unk	Unk	−13.39****	Yes*	−10.05****	−16.73****
BG01491[b]	*ttk*	100F3	−6.27****	Yes*	−2.70	−9.83***
BG01543	Unk	Unk	−1.9	Yes****	−3.07**	−0.53**
BG01572	Unk	Unk	3.34**	Yes***	−2.55	9.23***
BG01575	Unk	Unk	2.29*	Yes*	5.25**	−0.68
BG01596	*CG13377*	1A1	−15.57****	Yes****	−8.70**	−22.43****
BG01613	Unk	Unk	−7.54****	Yes****	−14.85****	0.23
BG01659[b]	*desert*	66D15	−8.91****	Yes*	−5.58****	−12.35
BG01664	Unk	Unk	−2.74*	No	2.20	−3.15
BG01740	*CG13790*	28B1	−10.27****	Yes****	0.05	−20.58****
BG01799	*Chd64*	64A7	−2.18	Yes****	−10.05****	5.70*
BG01856	Unk	Unk	−20.33****	Yes*	−16.95****	−23.70****
BG01862	*CG5151*	72D9	7.88****	Yes****	−1.65	17.40****
BG01891[c]	*kekkon-1*	34A1	10.35****	Yes****	−3.30*	24.00****
BG01950	Unk	Unk	−3.72*	No	−5.93*	−1.50
BG01954	Unk	Unk	−0.64	Yes***	3.98**	−5.25*

(*continued*)

grounds. A total of 82 of these lines had a statistically significant effect on starvation resistance for the two tests combined ($P < 0.05$); 44 inserts were homozygous and 15 were heterozygous in the Canton-S background, and 23 inserts were homozygous in the *Samarkand* background.

Samarkand background: Table 3 gives the mutational effects of the 23 insertion lines in the *Samarkand* w^{1118} background that were significant for the two combined tests. As noted above, *P*-element insertion lines in this background tended to decrease starvation tolerance. Accordingly, the most extreme positive line in this background, JJF164, showed an average increase in starvation resistance of only 4.8 and 12 hr for both sexes and males, respectively. The most severe decrease in starvation tolerance was observed in line JJF077 (*CG5127*), which had a decrease of 31.20 hr for both sexes pooled, 22.00 hr for males, and 35.20 hr for females. Six insertions had significant genotype-by-sex interactions in the *Samarkand* w^{1118} background (Table 3). Three of these insertions, JJF164, JJF175, and JJF237, have sex-specific effects.

Canton-S background: Table 4 gives the mutational effects of the Canton-S insertions that were significant after both assays. The table lists the cytological location and nearest gene to the *P*-element insert, if known. Line BG00080 showed the greatest increase in starvation resistance for males and both sexes pooled, with an average increase in starvation tolerance of 15.87 and 15.45 hr, respectively. Line BG01891, which carries a *P*-element insertion 32 bp upstream of *kekkon-1*, showed the greatest effect on females, increasing the average survival time by 24 hr. Likewise, the largest average decrease in survival for males and both sexes pooled,

TABLE 4

(Continued)

Insert line (H, heterozygote)	Nearest gene	Cytological location	Effect (hr)[d] (sexes pooled)	Significant $G \times S$?	Effect (hr)[d] (males)	Effect (hr)[d] (females)
BG02058	Unk	Unk	−8.48****	Yes**	−12.75****	−4.35
BG02063	*CG15312*	9B1	−2.29	Yes****	−9.75****	5.18*
BG02100	Unk	Unk	4.73***	Yes*	1.05	8.40***
BG02131	*CG9520*	29F5	5.67**	No	1.05**	7.58
BG02201	Unk	Unk	12.68****	Yes****	1.65	23.70****
BG02257	*Tre1*	5A11	2.34*	Yes**	5.93**	−1.25
BG02330	Unk	Unk	2.52*	No	4.95**	0.08
BG02539[b]	*CG31605*	28E1–3	−6.66****	Yes**	−10.58****	−2.75
BG02605	*sd*	13F1–4	−13.47****	Yes***	−7.73**	−19.20****
BG00372H[a]	*lcs/CG1678*	20A1	−6.00**	Yes*	−9.30**	−2.70
BG00376H[a]	*CG3638*	1E4	−8.10****	No	−7.20*	−9.00**
BG00378H[a]	Unk	Unk	−5.14**	Yes**	0.15	−10.43***
BG00459H[a]	Unk	Unk	−6.00**	No	−6.00*	−6.00*
BG00488H	*corto*	82E7–8	3.00*	No	0.60	5.40*
BG00489H	*CG15592*	83E2	−5.40**	Yes**	−0.60	−10.20***
BG01228H	Unk	Unk	−7.05***	No	−6.60*	−7.50**
BG01257H[c]	*1.28*	42B2	−6.60***	No	−3.90	−9.30**
BG01296H[a]	*CG32737*	6E	−5.05**	No	−3.60	−6.60*
BG01339H[b,c]	*clt*	57F4	−8.10****	No	−7.50**	−8.70**
BG01484H[a]	*sbb*	55C2	−12.15****	Yes***	−6.90*	−17.40****
BG01515H	*fs(1)h*	7D2–3	−13.35****	Yes*	−10.80***	−16.20****
BG01526H	*CG5151*	72D9	−11.40****	No	−9.30**	−13.50****
BG01564H	*CG14430*	6E4	−10.20****	No	−11.10***	−9.30***
BG01656H	Unk	Unk	1.20	Yes**	5.70*	−3.30

*$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$0.0001 < P \leq 0.001$; ****$P < 0.0001$.

[a] Significant as both a heterozygote and a homozygote.

[b] *P*-element insertion lies within transcribed region of gene.

[c] Insertion lies within QTL or deficiency region; see Table 6.

[d] Calculated as the deviation of insert line mean from contemporaneous control mean averaged over two experiments.

16.95 and 20.33 hr, respectively, was seen for line BG01856. The greatest decrease for females was 24.60 hr in line BG01020, which has a *P*-element insertion near the gene *faint sausage*. Many *P*-element insertions affect starvation resistance in a sex-specific manner, as expected from the highly significant line × sex interaction term in the ANOVA (Table 1) and the departures of the cross-sex genetic correlations from unity. A total of 38 lines have statistically significant genotype-by-sex effects, 19 of which are sex specific. Moreover, four inserts appear to be associated with a reversal of fortune for one sex over another. Lines BG01799, BG01891, BG01954, and BG02063 exhibit statistically significant differences in starvation tolerance for each sex; however, the differences are of opposite magnitude (Figure 4). This observation suggests a possible sex-specific mechanism for the maintenance of genetic variation in starvation tolerance.

As expected, given partially recessive effects of inserts on starvation resistance, heterozygous inserts generally had smaller effects on starvation resistance than homozygous inserts (Table 4). Six of the 15 significant heterozygous inserts were also significant as homozygotes at the 95% confidence interval or greater, for at least one sex. Two insert lines, BG01526 and BG01656, were not viable as homozygotes and could not be tested. Seven significant heterozygous lines, BG00488, BG00489, BG01228, BG01339, BG01257, BG01515, and BG01564, were not significant as homozygotes in the initial assay, although the trend (increasing or decreasing starvation resistance) was the same for both genotypes.

Deficiency complementation tests: A total of 58 deficiencies were used to more finely map the five QTL affecting variation in starvation resistance between *Oregon-R* and *2b*. Mean starvation tolerance for the deficiency-mapping experiment ranged from a low of 21.6 hr to a high of 90.4 hr in males; females tended to be more resistant to starvation in general, with a low of 28.2 hr and a high of 111.2 hr. *P* values for each deficiency are provided in Table 5. The 5 original QTL fractionated into 13 smaller QTL, 6 of which have sex-specific effects (Table 6). A detailed discussion of each QTL candidate region follows.

3E; 4F QTL: Of the 11 deficiencies spanning the 2F6–

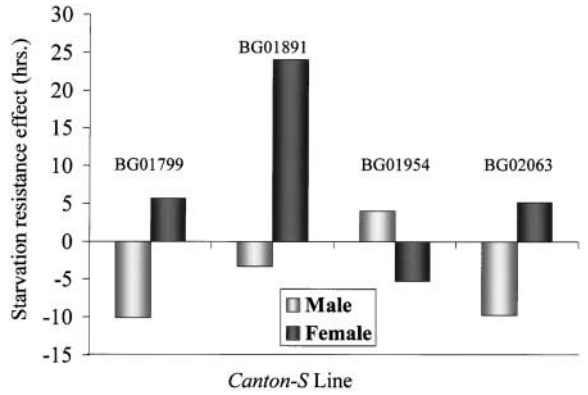


FIGURE 4.—*P*-element insertion lines with opposite effects in males and females. Starvation survival time is expressed as the deviation from block mean.

5C6 cytological region, only *Df(1)N-8* failed to complement (Table 5). The *X* chromosome QTL thus maps to 3C9; 3C11.

30D; 38A QTL: Twelve deficiencies uncovered the region spanned by this QTL (Table 5). Deficiency *Df(2L)s1402* exhibited a sex-specific failure to complement, giving a male-specific QTL at 30B9–10; 30F. *Df(2L)J2* failed to complement for both sexes and does not overlap with other deficiencies, localizing the second QTL in this region to 31B; 32A. *Df(2L)Prl* failed to complement both sexes, while the overlapping *Df(2L)prd1.7* exhibited male-specific failure to complement. We performed an ANOVA using deficiency (D), genotype (G, *Oregon-R* or *2b*), and sex (S) as cross-classified effects to ascertain how similar the complementation effects of these deficiencies were (PASYUKOVA *et al.* 2000). The $D \times G$ term was highly significant in the analysis with sexes pooled ($P < 0.0001$) and for the separate-sex analysis ($P = 0.0048$ for males; $P = 0.0006$ for females); the effects of these two deficiencies are therefore different. We inferred two separate QTL, with the QTL affecting both sexes mapping to 32F1–3; 33B2–3 and the male-specific QTL to 33F1–2; 34A1–2.

38A; 48D QTL: Sixteen deficiency stocks were used to map this region (Table 5). Three deficiencies failed to complement in this region for both sexes: *Df(2L)TW161*, *Df(2R)Np5*, and *Df(2R)wun-GL*. In addition, deficiency *Df(2R)H3E1* exhibited female-specific failure to complement, while deficiencies *Df(2R)w45-30n* and *Df(2R)eve* showed male-specific failure to complement. The first QTL in this region maps to 38B1–C1; 40A4–B1, given the overlap of deficiencies *Df(2L)TW9* and *Df(2L)TW161*. Subtracting away deficiency *Df(2R)H3C1*, which overlaps deficiency *Df(2R)H3E1* at the distal end, gives 44D3–8; 44F10 as a female-specific candidate region. *Df(2R)Np5* failed to complement for both sexes and was also significant in males. *Df(2R)w45-30n* shows a male-specific failure to complement, while *Df(2R)wun-GL* shows failure to complement in both sexes and females. We performed an ANOVA to examine the complementation effects of these deficiencies as outlined above. Neither the $D \times G$ term nor the $D \times G \times S$ term was significant for the contrast between *Df(2R)Np5* and *Df(2R)w45-30n*; however, the contrast among *Df(2R)Np5*, *Df(2R)w45-30n*, and *Df(2R)wun-GL* had a highly significant $D \times G \times S$ term ($P < 0.0001$). The most parsimonious interpretation is that two QTL are present, one QTL affecting both sexes at 45C1; 45C8 and a second female-specific QTL at 45C8; 45D8. *Df(2R)eve* had a male-specific failure to complement; it spans 46C3–4; 46C9–11; however, flanking deficiencies may include some portion of 46C. Since the exact endpoints of deficiencies *Df(2R)B5* and *Df(2R)X3* are not known, it is assumed that the entirety of deficiency *Df(2R)eve* is a putative candidate region for males.

57C; 60E QTL: We used 13 deficiencies to fine map this region (Table 5). Three deficiencies failed to complement for both sexes: *Df(2R)Pu-D17cn*^{1}*bw*^{1}*sp*^{1}, *Df(2R)PI13*, and *Df(2R)D11-MP*. Deficiencies *Df(2R)Pu-D17cn*^{1}*bw*^{1}*sp*^{1} and *Df(2R)Pu-D17nw*^{D}*Pin*^{Yt} have the same deficiency extending from 57B4; 58B in different genetic backgrounds, yet *Df(2R)Pu-D17nw*^{D}*Pin*^{Yt} was not significant ($P = 0.1929$ for both sexes), while *Df(2R)Pu-D17cn*^{1}*bw*^{1}*sp*^{1} was marginally significant ($P = 0.0468$ for both sexes). Indeed, the $D \times G$ term for the ANOVA analyzing the difference in complementation effects between the two deficiencies was not significant ($P = 0.0789$). Therefore, we consider both deficiencies to complement the QTL alleles in this region. However, these two deficiencies as well as *Df(2R)AA21c*^{1}*px*^{1}*sp*^{1} completely overlap *Df(2R)PI13*, which is significant for both sexes. A possible explanation is that there is another QTL in the region with opposite effect, a hypothesis that could be tested by further fine mapping using recombinants. One alternative is that *Df(2R)PI13* could uncover a QTL from 57C; 57D8–9, and a QTL of opposite effect is present in the region from 57D8–9; 57D11–12. *Df(2R)PI13* could also uncover a QTL from 57B13–14; 57C5, and a QTL of opposite effect lies within the interval 57B4; 57B13–14. *Df(2R)D11-MP* was highly significant for both sexes and covers a very small cytological region: 60E3–4; 60E5–6.

70C; 72A QTL: We tested six deficiency stocks that completely uncover this region. None of the deficiencies tested in this region had statistically significant effects consistent with allelism (Table 5). It is possible that the QTL was not correctly localized in the original analysis and may lie to either side of the interval. We were not able to test the region to the left of this QTL as suitable deficiencies were not available. More complicated scenarios invoking multiple linked QTL with opposite effects are also feasible. Quantitative complementation mapping was not informative in this case, but recombination mapping may allow us to localize and fine map this QTL in the future.

Candidate gene complementation tests: Six of the

TABLE 5

***P* values from deficiency complementation tests**

QTL	Deficiency	Cytological breakpoints	Sexes pooled		Male	Female
			$L \times G$	$L \times G \times S$	$L \times G$	$L \times G$
3E; 4F	*Df(1) JC19*	2F6; 3C5	NA	NA	NA	0.2254
	Df(1)w258-45, y^{1}	3B2–3; 3C2–3	NA	NA	NA	0.9206[a]
	Df(1) N-8	3C2–3; 3E3–4	NA	NA	NA	0.0309[a]
	Df(1)rst2	3C3–4; 3C6–7	NA	NA	NA	0.8458[a]
	Df(1)N-81k1, dnc^{81k1}	3C5–6; 3C9–10	NA	NA	NA	0.2326[a]
	Df(1)dm75e19	3C11; 3E4	NA	NA	NA	0.4449
	Df(1)HC244	3E8; 4F11–12	NA	NA	NA	0.9205
	Df(1)cho2, y^{1} *w*^{a}	3E; 4A	NA	NA	NA	0.8974
	Df(1)bi-DL1, y^{59b}*z*^{1}*w*^{i}*ct*^{6}*f*^{1}	4A3–5; 4C5–D1	NA	NA	NA	0.7730
	Df(1)JC70	4C15–16; 5A1–2	NA	NA	NA	0.4477
	Df(1)C149	5A8–9; 5C5–6	NA	NA	NA	1.0000
30D; 38A	*Df(2L)N22-14*	29C1–2; 30C8–9	0.0008[a,b]	<0.0001[a,b]	0.1299[a,b]	0.0004[a,b]
	w;Df(2L)s1402,P{w*$^{+mC}$*=lacW}s1402*	30B9–10, 30C1–2;30F	0.1841[a]	0.7099[a]	0.0229[a]	0.6313[a]
	y; Df(2L)J21*	31B; 32A	0.0077[a]	0.2146[a]	0.2503[a]	0.0126[a]
	Df(2L)Prl, Prl^{1} *nub*^{Prl}	32F1–3; 33F1–2	<0.0001[a]	0.1397[a]	0.0362[a]	0.0008[a]
	Df(2L)prd1.7,b^{1}*Adh*^{n2}*pr*^{1} *cn*^{1}*sca*^{1}	33B2–3; 34A1–2	0.2526[a]	0.7593[a]	0.0318[a]	0.7333[a]
	Df(2L)b87e25	34B12–C1; 35B10–C1	0.5016	0.7855	0.3139	0.8268
	Df(2L)TE35BC-24, b^{1}*pr*^{1}*pk*^{1}*cn*^{1}*sp*^{1}	35B4–6; 35F1–7	0.9182	0.7586	0.7247	0.8360
	Df(2L)r10, cn^{1}	35E1–2; 36A6–7	0.1905[a]	0.8607[a]	0.2674[a]	0.5813[a]
	Df(2L)cact-255rv64, cact^{chif64}	35F–36A; 36D	0.7845	0.0831	0.0993	0.3739
	Df(2L)H20, b^{1}*pr*^{1}*cn*^{1}*sca*^{1}	36A8–9; 36E1–2	0.5524	0.2967	0.0572	0.8058
	Df(2L)M36F-S6	36E6–F1; 36F7–9	0.4774	0.0886	0.2028	0.2746
	Df(2L)TW158, cn^{1} *bw*^{1}	37B2–8; 37E2–F1	0.3352[a]	0.2991[a]	0.1142[a]	0.9657[a]
38A; 48D	*Df(2L)TW9, Tft*^{1}*cn*^{1}	37E2–F1; 38B1–C1	0.5293	0.7508	0.7335	0.6101
	Df(2L)TW161, cn^{1}*bw*^{1}	38A6–B1; 40A4–B1	0.0037[a]	0.7208[a]	0.1685[a]	0.1872[a]
	Df(2R)ST1, Adh^{n5}*pr*^{1}*cn**	42B3–5; 43E15–18	0.5796	0.4910	0.9325	0.3395
	Df(2R)cn9	42E; 44C	0.1763	1.0000	0.4241	0.2502
	w^{1118}*; Df(2R)H3C1*	43F; 44D3–8	0.7223	0.1790	0.3349	0.3465
	w^{1118}*; Df(2R)H3E1*	44D1–4; 44F12	0.0585[a]	0.0716[a]	0.9466[a]	0.0141[a]
	w^{1}*; Df(2R)Np5, In(2LR)w45-32n,cn*^{1}	44F10; 45D9–E1	0.0129[a]	0.3065[a]	0.0044[a]	0.4237[a]
	Df(2R)Np4, bw^{1}**	44F11; 45C1	0.0352[a,b]	0.0039[a,b]	0.4614[a,b]	0.0077[a,b]
	w^{1}*;Df(2R)w45-30n,cn*^{1}	45A6–7; 45E2–3	0.1174[a]	0.0326[a]	0.0014[a]	0.7595[a]
	w^{1118}*; Df(2R)wun-GL*	45C8; 45D8	0.0032[a]	0.0086[a]	0.6315[a]	0.0391[a]
	w^{1118}*; Df(2R)B5, px*^{1}*sp*^{1}	46A; 46C	0.4148	0.2846	0.0875	0.8805
	Df(2R)eve, cn^{1}	46C3–4; 46C9–11	0.0644[a]	0.1665[a]	0.0071[a]	0.7602[a]
	Df(2R)X3	46C; 46E1–2	0.3906[a]	0.1949[a]	0.2176[a]	0.6302[a]
	Df(2R)X1, Mef2^{X1}	46C; 47A1	0.1377[a]	0.1577[a]	0.9458[a]	0.1098[a]
	Df(2R)stan1, P{ry[+t7.2]=neoFRT}42Dcn^{1}*sp*^{1}	46D7–9; 47F15–16	0.3906[a]	0.9538[a]	0.4226[a]	0.6678[a]
	Df(2R)stan2, b^{1}*pr*^{1} *P{ry[+t7.2]=neoFRT}42D*	46F1–2; 47D1–2	0.6543[a]	0.2606[a]	0.1965[a]	0.6518[a]

(*continued*)

retested *P*-element inserts with known locations are located within starvation resistance QTL regions as defined by the initial genome scan (Table 4). One of these inserts, BG01891 (near *kekkon-1*), is located within the QTL regions defined above by deficiency complementation mapping and was used in further mutation complementation tests. Results of all mutation complementation tests are given in Table 7. Ten of the 26 mutants tested failed to complement for both sexes: *numb*, *spalt major (salm)*, *crooked legs (crol)*, *Ryanodine receptor 44F (Rya-44F)*, *Punch (Pu)*, *l(2)rG270*, *l(2)k17002*, *l(2)k00611*, *NaCP-60E*, and *l(2)k03205*. Two genes exhibited sex-specific failure to complement: Phosphoglucose isomerase (*Pgi*) and *bellwether* (*blw*). Note that two alleles of *crol* were tested: one known allele, *crol*^{k05205}, and one putative candidate allele from the *P*-element mutagenesis screen. The known allele failed to complement for both sexes; however, complementation tests with the insertion line from our mutagenesis screen were not significant. Also, three alleles of *Punch* were tested: *Pu*^{W}, *Pu*^{Gr}, and *Pu*^{AA1}. We observed a significant contrast between mutant and balancer genotypes with the *Pu*^{AA1} allele only.

DISCUSSION

Here we have used two methods, *P*-element insertional mutagenesis and deficiency complementation mapping, to identify 395 candidate genes affecting star-

TABLE 5

(Continued)

QTL	Deficiency	Cytological breakpoints	Sexes pooled		Male	Female
			$L \times G$	$L \times G \times S$	$L \times G$	$L \times G$
57C; 60E	*Df(2R)min,Pu*^{1}	56F8–17	0.2384	0.9606	0.1926	0.5367
	Df(2R)AA21,c^{1} *px*^{1}*sp*^{1}	56F9–17; 57D11–12	0.2222[a]	0.1182[a]	0.6981[a]	0.1296[a]
	Df(2R)exu1,cn^{1}*bw*^{1}*sp*^{1}	57A2; 57B1	0.2031[a]	0.3774[a]	0.2106[a]	0.7874[a]
	Df(2R)Pu-D17,cn^{1}*bw*^{1}*sp*^{1}	57B4; 58B	0.0468[a]	0.1890[a]	0.1004[a]	0.1005[a]
	Df(2R)Pu-D17nw^{D}*Pin*^{Yt}	57B4; 58B	0.1929[a]	0.8491[a]	0.4762[a]	0.4599[a]
	Df(2R)PI13	57B13–14; 57D8–9	0.0010[a]	0.0153[a]	0.2865[a]	0.0049[a]
	Df(2R)PK1,c^{1}*px*^{1}*sp*^{1}	57C5; 57F5–6	0.4379[a]	0.1456[a]	0.1910[a]	0.5443[a]
	w; Df(2R)59AD*	59A1–3; 59D1–4	0.3764	0.7177	0.7713	0.2013
	y^{1}*w*; Df(2R)Chi[g230], Chi[g230], P{w[+mF]ry[+t7.2]=wF}4-1*	60A3–7; 60B4–7	0.3273[a]	0.8684[a]	0.4139[a]	0.5770[a]
	Df(2R)Px1,al^{1}*dp*^{ov1}*pwn*^{1}	60B8–10; 60D1–2	0.9155[a]	0.1801[a]	0.0886[a]	0.5642[a]
	Df(2R)Px2	60C5–6; 60D9–10	0.0629[a]	0.0969[a]	0.8782[a]	0.0659[a]
	Df(2R)D11-MP	60E3–4; 60E5–6	<0.0001[a]	0.2128[a]	0.0004[a]	0.0008[a]
	Df(2R)ES1,b^{1}*pr*^{1}*cn*^{1} *wx*^{wxt}*Kr*$^{If-1}$	60E6–8; 60F1–2	0.9052	0.5561	0.3375	0.7244
70C; 72A	*Df(3L)fz-GF3b, P{w[+tAR] ry[+t7.2AR]=wA[R]}66E*	70C1–2; 70D4–5	0.8028	0.0727	0.1648	0.2418
	Df(3L)fz-D21, th^{1}*st*^{1}	70D1–2; 70E7	0.3273	0.7370	0.4591	0.5484
	Df(3L)fz-M21,th^{1}*st*^{1}	70D2–3; 71E4–5	0.1889	0.7421	0.2053	0.5312
	Df(3L)BK10,ru^{1}*Ly*^{1}*red*^{1} *cv-c*^{1}*Sb*$^{sbd-1}$*sr*^{1}*e*^{1}	71C; 71F	0.4679[a]	0.0273[a,b]	0.3828[a]	0.0677[a]
	Df(3L)Brd20, p[p]	70D2–3; 71E3–5	0.5496	0.5496	0.5023	1.0000
	Df(3L)brm11	71F1–4; 72D1–10	0.8961	0.1763	0.1583	0.4883

P values for deficiencies exhibiting failure to complement are underlined.

[a] *P* value from pooled analysis of two experiments.

[b] Although statistically significant, the differences between balancer and deficiency genotypes are not consistent with allelism.

vation resistance. We demonstrated that a high degree of sexual dimorphism characterizes the effects of *P*-element insertions on this trait. Of the lines having location data available, 11 have insertions that tag the transcribed regions of a gene (Table 4). Deficiency complementation mapping revealed 12 genes that contribute to genetic variation in starvation resistance between *Oregon-R* and *2b* (Table 7). These genes have known phenotypes in cell fate specification, cell proliferation, oogenesis, metabolism, and feeding behaviors. Inspection and comparison of these seemingly disparate sets of genes reveal two common themes: resource allocation during development may affect starvation tolerance in the adult fly, and feeding behaviors as well as metabolism impact resistance to starvation. The combination of *P*-element mutagenesis and identification of natural variants that

TABLE 6

QTL for starvation resistance from deficiency complementation tests

Original QTL	Size (kb)	Cytological location	Approx. size (kb)[b]
X: 3E; 4F	1359	3C9–11	<464
2: 30D; 38A	7534	30B9–10; 30F (M)[a]	<767
		31B; 32A	997
		32F1–3; 33B2–3	<572
		33F1–2; 34A1–2 (M)[a]	<435
2: 38A; 48D	9546	38B1–C1; 40A4–B1	<1779
		44D3–8; 44F10 (F)[a]	<445
		45C1; 45C8	<117
		45C8; 45D8 (F)[a]	234
		46C3–4; 46C9–11 (M)[a]	<156
2: 57C; 60E	4669	57C5; 57D8–9 and 57D8–9; 57D11–12 or	<401 or <674
		57B4; 57B13–14 and 57B13–14; 57C5 60E3–60E6	<332
3: 70C; 72A	1956	70C; 72A	1956

[a] (M), male specific; (F), female specific.

[b] Approximate sizes determined from Sorsa (1988).

TABLE 7

***P* values from candidate gene complementation tests**

Gene	Allele(s)	Location	Sexes pooled		Male	Female
			$L \times G$	$L \times G \times S$	$L \times G$	$L \times G$
dunce	$y^1 dnc^{M14} cv^1 v^1 f^1$	1A1;3C10	NA	NA	NA	0.1990[a]
	Df(1)N-81k1, dnc^{81k1}		NA	NA	NA	0.1972
BL-11068	w^{1118} *P{w[+mC]=EP}EP1395*	3C9–10	NA	NA	NA	0.3553
numb	*numb*^{1}	30B1–12	0.0001[a]	0.0153[a]	0.0097[a]	0.0011[a]
CAMP-dependent protein kinase 1	*Pka-C1*^{H2}	30C3–5	0.2892	0.2011	0.6981	0.2339
	w; Pka-C1*^{DN}		0.2900	0.9741	0.3894	0.5147
spalt major	*salm*^{1} *cn*^{1} *bw*^{1} *sp*^{1}	32F1–2	0.0001[a]	0.0001[a]	0.7177*	0.0001[a]
crooked legs	$y^1 w^{67c23}$; *P{w*$^{+mC}$*=lac*^{W}*}crol*^{k05205}	33A1	0.0106[a]	0.1462[a]	0.1934[a]	0.0407[a]
	P{w^{+mGT}*Tn/neoR*^{Hsp70BbPS}*Scer/GAL4*^{GT1}*=GT1}*		0.5196	0.1873	0.5769	0.2270
BG01891 (kekkon-1)	*P{w*$^{+mGT}$*Tn/neoR*^{Hsp70BbPS}*Scer/GAL4*^{GT1}*=GT1}*	34A1	0.2613[a]	0.5892[a]	0.5948[a]	0.3279[a]
purple	*pr*^{1} *cn*^{1} *ix*^{1}	38B3	0.4370	0.8370	0.3625	0.7377
Aconitase	$y^1 w^{67c23}$; *P{w*$^{+mC}$*=lac*^{W}*}Acon*^{k07708}	39A7	0.3168	0.7362	0.3800	0.6171
	cn^1 *P{ry*$^{+t7.2}$*=PZ}Acon*^{07054}		0.2720	0.1760	0.8021	0.1643
patched	*ptc*$^{tuf\text{-}D}$	44D5–E1	0.3179[a]	0.4234[a]	0.8964[a]	0.2256[a]
Ryanodine Receptor 44F	$y^1 w^{67c23}$; *P{w*$^{+mC}$*=lac*^{W}*}Rya-r44F*^{k04913}	44F2	0.0001[a]	0.0623[a]	0.0001[a]	0.0011[a]
Phosphoglucose isomerase	*Pgi*^{nNC1}	44F6	0.4017[a]	0.0224[a]	0.1060[a]	0.1111[a]
Pyruvate dehydrogenase kinase	*P{w*$^{+mGT}$*Tn/neoR*^{Hsp70BbPS}*Scer/GAL4*^{GT1}*=GT1}*	45D5–6	0.6598	0.6156	0.5922	0.9226
Punch	*T(2;3)Pu*^{W}, *Pu*^{W}	57C7–8	0.4183[a]	0.6652[a]	0.7269[a]	0.4522[a]
	T(2:3)Pu^{Gr}, *Pu*^{Gr}		0.4726[a]	0.9311[a]	0.3599[a]	0.7253[a]
	Pu^{AA1}		0.0127[a]	0.4723[a]	0.1963[a]	0.0894[a]
Epidermal growth factor receptor[b]	*y*^{1} *w*^{67c23}*;P{w[+mC]=lacW}Egfr[k05115]*	57E9–F1	0.1141[a]	0.5350[a]	0.4278[a]	0.1698[a]
l(2)03605[b]	*cn*^{1} *P{ry[+t7.2]=PZ}l(2)03605[03605]*	57F8–10	0.5847[a]	0.8141[a]	0.6618[a]	0.7450[a]
l(2)07837[b]	*P{ry[+t7.2]=PZ}l(2)07837[07837] cn*^{1}	58A3–4	0.5599	0.8444	0.8617	0.8917
plexus[b]	*CyO, {ry[+t7.2]=lArB}A130.1F2*	58E4–8	0.8082	0.1929	0.1318	0.5495
l(2)ry50[b]	*P{ry[+t7.2]=ry11}l(2)ry50[1], cn[1]*	58E	0.1911	0.0970	0.7822	0.0628
l(2)rG270[b]	*P{ry[+t7.2]=PZ}l(2)rG270[rG270]*	58F1–2	0.0079[a]	0.4404[a]	0.1765[a]	0.0157[a]
l(2)k17002[b]	*y*^{1} *w*^{67c23}*;P{w[+mC]=lacW}l(2)k17002[k17002]*	58F4–5	0.0095[a]	0.3744[a]	0.0229[a]	0.1645[a]
l(2)k00611[b]	*y*^{1} *w*^{67c23}*;P{w[+mC]=lacW}l(2)k00611[k00611]*	58F4–5	0.0021[a]	0.7572[a]	0.0287[a]	0.0539[a]
bellwether[b]	*cn*^{1} *P{ry[+t7.2]=PZ}blw*^{1}	59B2	0.6050[a]	0.0227[a]	0.0509[a]	0.2819[a]
	y^1 *w*^{67c23}*; P{w[+mC]=lacW}blw[k00212]*	59A1–3	0.5675	0.2165	0.3560	0.3581
Na channel protein 60E	*P{Eco1/lacZ[P/T.A92a]Adh*$^{+3.2}$*,ry*$^{+t7.2}$*ori ampR=*lArB*}*	60E5	0.0031[a]	0.2042[a]	0.0176[a]	0.0256[a]
l(2)k03205	*y*^{1} *w*^{67c23}*;P{w*$^{+mC}$*=lac*^{W}*}l(2)k03205*^{k03205}	60E5–6	0.0001[a]	0.0596[a]	0.0001[a]	0.1682[a]

P values for deficiencies exhibiting failure to complement are underlined.
[a] *P* value from pooled analysis of two experiments.
[b] Tested in regions where deficiencies were unavailable.

affect starvation resistance therefore reveals potential biological process pathways mediating this complex trait. Future experiments are required to demonstrate that the *P*-element insertions indeed cause the observed differences in starvation tolerance and to determine to what extent molecular polymorphisms in these genes are associated with variation in starvation resistance in natural populations.

Genes directly tested for effects on starvation resistance: *P*-element insertions in the transcribed regions of *extra macrochaetae* (*emc*), *roundabout* (*robo*), *CG9028*, *tramtrack* (*ttk*), *desert*, and *CG31605* had significant effects on starvation tolerance. A brief description of each gene and its possible role in starvation tolerance follows. No additional information is known about the *P*-element insertions in *CG9028*, *desert*, and *CG31605*. A BLAST search of these genes reveals no strong homology between these genes and known genes of other organisms.

Developmental resource allocation as a mechanism for starvation resistance: Several insertions putatively affect genes that are involved in resource allocation during development, including cell fate determination, pattern specification, and cell number. Line BG01491 homozygotes, which have a *P* element in the gene *ttk*, exhibited a significant decrease in starvation resistance. The *P* element in BG01095 is inserted near *pointed* (*pnt*) and

shows a slight but significant increase in starvation tolerance for both sexes. *ttk* and *pnt* are involved in a number of developmental processes, including glial cell, bristle, and eye development (Scholz *et al.* 1993; Campos-Ortega 1996; Badenhorst 2001; Jones 2001; Baonza *et al.* 2002). Recent work identified both genes as opposing transcriptional switches in the epidermal growth factor receptor (*Egfr*) pathway during eye development (Baonza *et al.* 2002). The Ttk69 isoform of *ttk* can arrest the second mitotic wave via direct interaction with *string* (Baonza *et al.* 2002). In contrast, the P2 isoform of *pnt* interacts directly with the promoter region of *string*, a gene that induces mitosis, to produce the second mitotic wave of cell division during eye development (Baonza *et al.* 2002). One interpretation of the opposing effects of *pnt* and *ttk* on starvation resistance is that increased cell division, whether during development or at the adult stage, requires additional resources; therefore, additional cells have a negative impact on starvation resistance.

The *P*-element insertion line BG01092 (*robo*) was one of the few that exhibited increased starvation tolerance (Table 4). *robo* is involved in axon guidance in the central nervous system (CNS) midline, preventing axon growth cones from crossing the developing midline (Kidd *et al.* 1998). The activity of *robo* has further been implicated in the development of motor and olfactory bulb neurons in mammals (Ghose and Van Vactor 2002). It is curious that this mutant that putatively causes defects of the CNS would result in an increase in starvation tolerance.

Another developmental gene that has an effect on starvation tolerance is *emc* (BG00986), which has a negative sex-specific effect on starvation resistance in females (Table 4). *emc* represses the expression of the *achaete-scute* complex, resulting in the formation of additional large bristles (macrochaetae; Botas *et al.* 1982). This result implies that genes affecting development in both sexes may also exhibit sex-specific pleiotropy for adult traits.

We repeatedly found that *P*-element insertions in or near well-characterized developmental genes affected starvation tolerance (Table 4 and supplementary Table 4 at http://www.genetics.org/supplemental/). The implication is that "starvation resistance" genes may not be involved specifically in biological processes that occur under starvation conditions; rather, developmental genes set the stage for starvation tolerance as they influence the development of organs and tissues involved in the response to starvation (Sokolowski 2001). Alternatively, it is possible that these are pleiotropic loci that independently affect both development and starvation resistance.

Genes affecting variation between wild-type strains: The deficiency complementation mapping experiment revealed 12 candidate genes that affect variation in starvation resistance between two wild-type strains, *Oregon-R* and *2b* (Table 7). These genes specifically affect variation in starvation resistance between these two strains; if a different mapping population had been used, other genes might have been identified. No additional information is known about the mutants *l(2)k03205*, *l(2)k-17002*, and *l(2)k00611*, other than that they are recessive lethal.

Developmental resource allocation and cell fate specification: spalt major (*salm*) is an RNA polymerase II transcription factor involved in the development of many morphological features, including mechanosensory organs and wing vein patterning (de Celis *et al.* 1999; Elstob *et al.* 2001; Rusten *et al.* 2001). The expression of *salm* during development is regulated by the concentration of the transforming growth factor (TGF)-β morphogen *decapentaplegic* (*dpp*; de Celis *et al.* 1996; Lecuit *et al.* 1996; Nellen *et al* 1996). Interestingly, a mutation in the TGF-β gene *daf-7* in *C. elegans*, which shares some homology with *dpp*, enhances resistance to starvation stress (Munoz and Riddle 2003). Recent studies of *salm* have shown that it acts as a developmental switch between oenocyte formation and chordotonal organs in the dorsolateral ectoderm (Elstob *et al.* 2001; Rusten *et al.* 2001). Oenocytes are clusters of large cells found in the abdomen (Snodgrass 1935), where lipid biosynthesis is thought to take place (Wigglesworth 1970). Oenocytes grow and shrink in sequence with molting stages, oogenesis, and spermatogenesis and have been implicated in cuticle formation and hardening (Snodgrass 1935; Wigglesworth 1970) as well as sex pheromone production (Ferveur *et al.* 1997). Thus, a mutation in *salm*, which affects oenocyte number, may affect starvation tolerance by limiting needed lipids or access to lipids. This finding is in agreement with the results of previous studies, which have shown a positive correlation between lipid content and starvation tolerance (Chippindale *et al.* 1996, 1998; Djawdan *et al.* 1998; Harshman and Schmid 1998).

numb is a plasma membrane protein that alters cell fate via asymmetric localization within a dividing cell during peripheral nervous system development, muscle development, and neurogenesis (Uemura *et al.* 1989; Park *et al.* 1998; Jan and Jan 2000; Bellaiche *et al.* 2001). Daughter cells containing *numb* adopt specific cell fates. For example, sensory organ precursor cells divide to form two types of cells: one type, containing *numb*, forms sheath cells and neurons, while the other type, lacking *numb*, forms hair and socket cells (Uemura *et al.* 1989). *numb* appears to alter cell fate specification by directly interfering with *Notch* signaling (Guo *et al.* 1996). Interestingly, *Notch* mediates *ttk* expression in a positive manner while *numb* represses it (Guo *et al.* 1996).

One allele of *crol*, $crol^{k05205}$, had a significant effect on variation in starvation resistance. *crol* is a zinc finger protein with three distinct isoforms (D'Avino and Thummel 1998). Ecdysone pulses during pupal development

appear to trigger the transcription of *crol*, which in turn facilitates the transcription of ecdysone-related genes (D'Avino and Thummel 1998). *crol* mutants have severe morphological defects (D'Avino and Thummel 1998), again suggesting a possible link between cell fate specification and starvation resistance.

Finally, the recessive lethal gene *l(2)rG270*, which failed to complement, has an effect on egg development: mutants have eggs with a deflated appearance (Perrimon *et al.* 1996). This gene could be involved in the well-known negative correlation between starvation resistance and early fecundity (Service and Rose 1985; Leroi *et al.* 1994a,b).

The mutant complementation tests and the tests made with *P*-element insertion lines echo a common theme: cell fate and resource allocation decisions made in the early developmental stages may influence starvation tolerance in the adult stage. Or, alternatively, similar molecular pathways affect cell fate and resource allocation in early development and starvation tolerance in adult life.

Feeding behavior: Rya-r44F is a four-transmembrane domain protein that is expressed predominantly in the sarcoplasmic reticulum of the body wall muscles of late larvae and tubular muscles of the adult (Hasan and Rosbash 1992; Takeshima *et al.* 1994). *Rya-r44F* is thought to function in contraction-excitation coupling in these muscles (Hasan and Rosbash 1992). We tested a hypomorphic allele of this gene, *Rya-r44F*^{k04913}. Larvae from this mutant have mild defects in food ingestion and excretion as well as visible muscle contraction abnormalities (Sullivan *et al.* 2000). This allele had a highly significant effect on the difference in starvation resistance between *Oregon-R* and *2b*. We observed a very high starvation tolerance when crossed to *Oregon-R* (77.0 hr) and a very low tolerance when crossed to *2b* (42.5 hr) as compared to balancers (63.4 and 50.3 hr, respectively). The decrease in starvation tolerance in the *2b* cross is expected; however, the increase in tolerance for the *Oregon-R* cross suggests that the *Oregon-R* wild-type allele is beneficial for this trait.

A second gene, *NaCP60E*, is a cation channel that has been implicated in olfactory avoidance behavior (Kulkarni *et al.* 2002). A smell-impaired mutant of this gene was tested in the mutant complementation tests. The difference in starvation resistance in crosses of the mutant to *Oregon-R* (76.9) and *2b* (59.3 hr) was much greater than the crosses of the balancer to these strains (49.1 and 42.3 hr, respectively). However, the *P*-element insertion in this allele is known to reduce RNA expression levels in two genes, *NaCP60E* and *L41*, a ribosomal protein (Kulkarni *et al.* 2002). Further characterization is necessary to definitively determine which of the two genes is contributing to the starvation tolerance phenotype.

Metabolic genes: Several of the genes chosen for mutant complementation tests were selected for their effects on metabolism. Three of these genes had a significant effect on variation in starvation resistance: *Punch* (*Pu*), Phosphoglucose isomerase (*Pgi*), and *bellwether* (*blw*). *Pu* encodes GTP cyclohydrolase, an enzyme that catalyzes the first step in pteridine biosynthesis (O'Donnell *et al.* 1989). Three alleles of the gene *Pu* were tested for their effect on starvation resistance: *Pu*^{W}, *Pu*^{Gr}, and *Pu*^{AA1}. Only one allele, *Pu*^{AA1}, had a significant effect on variation in starvation resistance. *Pu*^{W} and *Pu*^{Gr} were generated by X-ray mutagenesis and are both translocations in the second and third chromosome that disrupt the *Punch* locus (O'Donnell *et al.* 1989). *Pu*^{AA1} was generated by EMS mutagenesis and is most likely a point mutation (J. M. O'Donnell, personal communication). Specific mutations may therefore reveal domains of highly pleiotropic genes that affect starvation tolerance (Sokolowski 2001).

Phosphoglucose isomerase (*Pgi*) had a mildly significant effect on variation between *Oregon-R* and *2b* in the mutant complementation tests. *Pgi* is involved in glycolysis and gluconeogenesis, converting glucose-6-phosphate to fructose-6-phosphate. This reaction is not a regulatory point in glycolysis or gluconeogenesis. As *Pgi* had a significant $L \times G \times S$ effect in the complementation tests, it may be an indication of differences in metabolism between the sexes that might contribute to the starvation resistance phenotype.

Two mutations in the gene *bellwether* (*blw*) were tested. One of the alleles, *blw*^{1}, failed to complement for males only (Table 7). *blw*^{1} was identified in a screen for sterile males and is part of a group of mutations that have normal meiosis but do not produce motile sperm (Castrillon *et al.* 1993). Further, mutant *blw* larvae are abnormally small despite normal feeding habits (Galloni and Edgar 1999), indicating that this gene may affect cell proliferation and/or growth. Indeed, *blw* encodes the α-subunit of ATP synthase (Talamillo *et al.* 1998), which converts ADP into ATP in the mitochondrion, suggesting that the phenotypic effect on starvation tolerance is the result of a general metabolic defect.

Conclusion: Some of the genes we identified for starvation resistance are common to two developmental pathways that determine cell fate for a variety of tissues: *Epidermal growth factor receptor* (*Egfr*) and *Notch*. Cell fate specification of sensory organ precursor cells is due in part to the activity of the genes *numb* and *tramtrack* (Guo *et al.* 1996), which were implicated in this study. *numb* appears to repress *Notch* signaling via direct protein-protein interaction, while *Notch* signaling is required for proper *tramtrack* expression (Guo *et al.* 1996). Further, *emc*, which exhibited female-specific effects on starvation resistance in the *P*-element screen, is also implicated in the *Notch* signaling pathway (Baonza *et al.* 2000).

tramtrack expression has also been linked to *Egfr* signaling (Rebay 2002). Lines BG01095 and BG01891 have insertions that putatively affect *pointed* and *kekkon-1*, respectively; both of these genes repress the *Egfr* pathway during oogenesis (Morimoto *et al.* 1996; Ghiglione *et al.* 1999). Moreover, *salm*, which affected variation in

starvation resistance, may act downstream of *Egfr* (Elstob *et al.* 2001; Rusten *et al.* 2001). Note that we performed a mutant complementation test of an *Egfr* allele that complemented, revealing no difference in variation for starvation resistance between *Oregon-R* and *2b Egfr* wild-type alleles. The juxtaposition of *P*-element mutagenesis and deficiency complementation mapping in our experiment implicated genes from two well-known developmental pathways in Drosophila as potentially important for starvation resistance in the adult fly; further characterization will reveal whether the starvation resistance phenotype is the result of developmental events influencing the adult response (Sokolowski 2001) or pleiotropic effects of developmental genes in the adult.

The results of our experiment suggest that genetic variation in starvation resistance may be maintained by a combination of mutation-selection balance and antagonistic pleiotropy. Almost half of the *P*-element insertion lines we screened had significant effects on starvation resistance, implying that the total number of loci affecting this trait is large. The mutational variance V_M is $2Nua^2$, where N is the number of loci affecting the trait, u is the per-locus mutation rate, and a^2 is the variance of effects of mutations, assuming the mean effect is zero (Falconer and Mackay 1996). Thus, increasing N provides a large mutational target, increasing V_M. Large amounts of genetic variation could be maintained if selective forces are weaker than the input of mutational variance. Further, large numbers of candidate genes affecting starvation resistance imply extensive pleiotropy (Falconer and Mackay 1996). The effect of mutations on other traits may therefore be the primary focus of selection, with genetic variation in starvation resistance maintained as a side effect. The sex-antagonistic properties of some *P*-element insertions (Figure 4) offer a second possible explanation for the genetic variation observed in starvation resistance. If alleles having similar sex-antagonistic properties segregate in nature, genetic variation for starvation resistance could be maintained by selection for a specific allele in one sex, while simultaneously acting against that same allele in the other sex.

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