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A Quantitative Trait Locus Analysis of Natural Genetic Variation for *Drosophila melanogaster* Oxidative Stress Survival

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Abstract

Little is known about natural genetic variation for survival under oxidative stress conditions or whether genetic variation for oxidative stress survival is associated with that for life-history traits. We have investigated survival in a high-oxygen environment at 2 adult densities using a set of recombinant inbred lines (RILs) isolated from a natural population of *Drosophila melanogaster*. Female and male oxidative stress survival was highly correlated. Quantitative trait loci (QTLs) for oxidative stress survival were identified on both autosomes. These QTLs were sometimes sex or density specific but were most often not. QTLs were identified that colocalize to the same region of the genome as longevity in other studies using the same set of RILs. We also determined early-age egg production and found QTLs for this trait, but there was no support for an association between oxidative stress survival and egg production.

The free radical theory of aging (Harman 1956) presented the hypothesis that oxidative damage to macromolecules is the cause of aging. It is generally thought that oxidative damage is a key cause of aging and that oxidative stress resistance and longevity have a common genetic basis (Wallace 1992; Lithgow and Kirkwood 1996; Martin and others 1996;

Johnson and others 1999; Finkel and Holbrook 2000; Guarente and Kenyon 2000; Kirkwood and Austad 2000). Support for this hypothesis comes from mutational analysis of longevity using model systems for genetic studies. For example, mutations in the insulin signaling pathway that confer longer life span often increased stress resistance including oxidative stress resistance in nematodes, yeast, and mice (Larsen 1993; Fabrizio and others 2001; Holzenberger and others 2003). A mutation in the *Drosophila melanogaster methuselah* gene confers longevity as well as oxidative stress resistance (Lin and others 1998). Candidate genes that extend the life span of *D. melanogaster* by transgenic overexpression tend to be genes that confer oxidative stress resistance. Human *SOD1* expressed only in adult motor neurons of *D. melanogaster* increased life span by 40% (Parkes and others 1998), and overexpression of *D. melanogaster SOD1* throughout the adult body increased life span by 48% (Sun and Tower 1999). The manganese-dependent mitochondrial form of *SOD* (*SOD2*) increased life span by approximately 50% (Sun and others 2002). Single genes can extend longevity by moderating oxidative stress, but the physiology of the relationship between oxidative stress and longevity is based on the activity of many genes.

Drosophila melanogaster transcriptome studies reveal that the oxidative stress response is indeed a complex trait (Zou and others 2000; Girardot and others 2004; Landis and others 2004). Zou and others (2000) assessed the expression of approximately 8000 genes as a function of age and oxidative stress. Change in expression of a significant proportion of these genes was observed to respond in the same way to age and oxidative stress, suggesting an association between aging and oxidative stress. In a second study, Landis and others (2004) conducted a microarray study of the expression of approximately 13,500 genes at 2 ages corresponding to relatively young flies (10-day-old adults) and at a time point at which 50% mortality had occurred (61-day-old adults) compared with 10-day-old flies that had been exposed to 100% oxygen for 7 days, which resulted in 50% mortality. In this study, 253 genes changed expression as a function of age and oxygen stress, which provides statistical support for a strong association between aging and oxidative stress (Landis and others 2004). A third study (Girardot and others 2004) included functional tests of genes to supplement the inference from oxidatively stressed fly microarray data. Flies were exposed to 2 concentrations of paraquat (methyl viologen) and 1% hydrogen peroxide. In terms of differential gene expression, there were specific responses and general responses to the quantitatively or qualitatively different oxidative stress conditions. Based on oxidant-induced changes in gene expression from the microarray data, 29 mutations corresponding to differently expressed genes were assayed for survival in the presence of paraquat (methyl viologen) as a functional test. Given that approximately half of these mutations affected survival after exposure to paraquat and 1,107 genes responded after exposure to paraquat, it was estimated that at least 500 genes contribute to paraquat sensitivity in *D. melanogaster*. In general, oxidative stress responses appear to be complex in terms of the number of genes involved and the diverse categories of genes that respond.

Oxidative stress is thought to play a fundamental role in aging, yet oxidative stress survival is poorly understood as a complex genetic trait. However, laboratory studies using *D. melanogaster* have been informative in this regard. Such studies suggest a relationship between oxidative stress survival and life-history traits such as life span and fecundity. Selection for extended longevity (Luckinbill and others 1984) resulted in elevated levels of

mRNA abundance for antioxidant defense genes (Arking and others 1991; Dudas and Arking 1995; Force and others 1995). Moreover, a reverse selection experiment strengthened the case for the positive correlation between longevity and antioxidant defense (Arking and others 2000). A highly replicated artificial selection experiment on late life survival and reproduction was conducted on an established base population (Rose 1984). Extended longevity was correlated with resistance to various forms of environmental stress (Service and others 1985) including resistance to oxidative stress (Harshman and Haberer 2000). Generally, selection experiments for extended longevity result in decreased early-age egg production and egg maturation as an indirect response to selection (Rose and others 1990; Carlson and others 1998; reviewed in Harshman 2003). It appears that decreased early-age egg production is generally associated with environmental stress resistance (Zera and Harshman 2001). Early-age egg production was the focus of our study also because of a prediction, from evolutionary biology, that natural selection maximizes early-age reproduction at the expense of fitness traits (survival and fecundity) later in life (Williams 1957).

Quantitative trait locus (QTL) studies on oxidative stress resistance and longevity using *D. melanogaster* recombinant inbred lines (RILs) also provide insight into the nature of genetic variation for oxidative stress survival as a complex trait. Lines selected for increased life span have been used to generate RILs that were used to map QTLs for oxidative stress survival, longevity, and egg production (Curtsinger and Khazaeli 2002). The QTLs for life span in both sexes almost completely overlapped those for resistance to oxidative stress and peak midlife female fertility. Thus, longevity was associated with oxidative stress resistance and high midlife fecundity. However, it is not clear if there is a general relationship between oxidative stress resistance and fecundity when investigated by QTL mapping using different sets of RILs.

The main goal of the present study was to characterize natural genetic variation for oxidative stress survival. The investigations were based on RILs that represent natural genetic variation (Kopp and others 2003). Using these lines, female and male survival under oxidative stress was monitored at 2 adult densities. Egg counts were obtained at early ages prior to exposure to high levels of oxygen (oxidative stress). This was to test whether oxidative stress survival and egg production were correlated perhaps negatively, thereby suggesting antagonistic pleiotropy between the traits. The objectives of the present study were to:

1. conduct oxidative stress survival assays at 2 adult densities using natural genetic variation RILs and analyze the data for QTLs;
2. tabulate RIL early-age egg production and analyze the data for QTLs;
3. determine genetic correlation between oxidative stress survival and fecundity, and evaluate the relationship between QTLs for both traits.

Materials and Methods

Drosophila *Food and Culture*

All lines were cultured on fly food consisting of 0.75% agar (w/v), 5.83% molasses (v/v), 10% cornmeal (w/v), 8.33% torula yeast (w/v), 1.67% ethanol (v/v), 0.33% methyl-*p*-hydroxybenzoate (w/v), and 0.67% propionic acid (v/v). Flies were reared at 27°C under constant illumination. For all experiments, 75 eggs were transferred to rearing vials to control larval density in vials used to produce adults for experiments. Only virgin flies were used in the present study to avoid the complexities of male impact on female physiology as a result of the seminal fluid proteins and the effect of courtship on both sexes. Virgin females and males were collected twice daily until a sufficient number was obtained for the assays.

Recombinant Inbred Lines

A set of 135 RILs were generated from a single F1 virgin female derived from a fertilized female collected on fruit (Winters, California) crossed to a single F1 male from a different female caught at the same location (Kopp and others 2003). Chromosomes of the parental lines recombined in the F2 followed by 25 generations of full-sib inbreeding to form inbred lines. Given the parental source of the lines, up to 4 alleles could be segregating at each locus among the RILs.

Transposable elements have been used to genotype the RILs used in the present study (Kopp and others 2003). Positions of *roo* elements were determined by in situ hybridization to polytene salivary gland chromosomes using a biotinylated probe as described in Kopp and others (2003). All chromosomes were homosequential with the exception of an inversion on 3R that contains approximately 7% of the physical genomes (89EF-96A).

Oxidative Stress Resistance and Fecundity Measurement

All RILs were simultaneously tested for oxidative stress survival. Flies were transferred to fresh vials posteclosion and assayed in the same 27°C incubator as used for rearing. Two replicates of 2 density conditions were employed for a total of 4 experiments. Experiments 1 and 2 used 20 virgin females or males per vial for the oxidative stress assay, whereas experiments 3 and 4 used 10 virgin females or males per vial. A total of 4 assays were run using the natural population set of RILs; 2 replicate assays were run at each density employed. Density was included as a factor because density variation is common in natural populations. In all, 10 or 20 flies per vial were presumably sufficient to damp stochastic effects on survival. However, the difference between 10 and 20 flies might be subtle given the range of variation in density in natural populations. Females and males were 9–13 days old at the time they were first exposed to high-oxygen conditions. At this age, they had been transferred to fresh food 4 times at 2-day intervals at the density that they were assayed for survival under oxidative stress conditions (10 or 20 flies per vial).

For the oxidative stress assay, females or males in fresh food vials were placed in an inflatable glove chamber (Model X-37-37H, Instruments for Research and Industry, Cheltenham, PA). The glove chamber was connected to a manometer and a gas cylinder containing 100% oxygen. Oxygen was bubbled through water and then into a glove bag under

low, steady, positive pressure wherein the concentration of oxygen was approximately 95%. The number of dead flies was determined every 8 h until all flies were dead.

Egg counts were determined in vials of the 4 sequential transfers (T1–T4) of females before flies were exposed to high oxygen to test whether oxidative stress resistance and egg production were genetically associated. In experiments 1 and 2, egg counts began after the requisite number of virgin flies were collected, which was approximately 1–5 days posteclosion. The RILs are highly variable in terms of the number of viable offspring produced from a standard number of eggs used to seed a vial and in development time. Thus, it takes a number of days to collect the requisite number of virgin males and females from all the lines for the oxidative stress survival assay, which is conducted in 1 glove bag. Although the variation in female age is reported above (1–5 days posteclosion), in fact most of the females were collected on the second and third days of eclosion from rearing vials and thus the age of most of the females differed by only 1 day. In experiments 3 and 4, eggs were counted immediately posteclosion until the requisite number of virgins were collected (T0), followed by 4 successive transfers to fresh vials (T1–T4, as in experiments 1 and 2). Two egg count statistics were used for genetic correlations and QTL analyses. The first was average egg number per female per day. The second was the slope of egg production, which typically was a measure of increase in egg laying over time because young females mature and produce more eggs on a daily basis at the ages assayed in the present study. The slope of egg production is a parameter that takes into account the trend in change of age production. In other words, it is the "gain" in egg production over time.

Virgin females were used for egg production assays, and virgins of both sexes were used for oxidative stress assays. It takes just less than a week for all flies to die in the high oxygen assays used in the present study, in which it was not practical to open and close the glove bags used. When mated females were tested, it was observed their larval offspring carried medium onto the sides of the vials; this obscured vision, making it difficult to count the number of female adults that died, and females were likely to get stuck in the churned medium. Because fertile egg production varies greatly among lines, there is considerable opportunity for biased outcomes in the oxygen survival assay due to the likelihood of females getting stuck in the medium and rapidly dying thereafter. Therefore, it was decided that it was best to assay virgin females, and males were treated in the same manner. Oxygen stress survival was the focus of the study, but egg production was also measured. For consistency, virgin egg production was compared with virgin female oxidative stress survival. However, it is not clear that virgin egg production is representative of mated female egg production in terms of a cost of reproduction, but there is evidence for an oxidative stress survival cost of relatively elevated virgin egg production in *D. melanogaster* (Salmon and others 2001; Wang and others 2001).

Statistical Analysis

Mixed model analysis of variance (ANOVA) was used to estimate the line, sex, and density effect on oxidative stress resistance. The full model was $Y_{ijklm} = \mu + L_i + S_j + D_k + L_i \times S_j + L_i \times D_k + S_j \times D_k + L_i \times Sj \times D_k + B_l(D_k) + \varepsilon_{ijklm}$, where Y_{ijklm} is the survival under oxidative stress for the individuals of the line i (L_i) of the sex j (S_j) reared at density k (D_k) in block l (B_l) for replicate m, where each experiment was regarded as a block. $B_l(D_k)$ refers to the effect of

block nested within the density effect (blocks 1 and 2 for density 20 and blocks 3 and 4 for density 10), and ε_{ijklm} corresponds to residual term. $B_l(D_k)$ effect is random, whereas the others are fixed.

For egg production, ANOVA was used to analyze the data. For each time point T (age at which eggs were counted), the model was $Y_{ijkl} = \mu + L_i + D_j + L_i \times D_j + B_k(D_j) + \varepsilon_{ijkl}$, where Y_{ijkl} is the egg production for the virgin females of the line i reared at density j in block k for replicate l. L and D refer to line and density, respectively. $B_k(D_j)$ refers to the block effect nested within the density effect (experiments 1 and 2 for density 20 and experiments 3 and 4 for density 10), and ε_{ijkl} corresponds to residual term. $Bk(Dj)$ effect is random, whereas the others are fixed.

Both models were analyzed with PROC MIXED (SAS Institute Inc. 2003). Heritabilities and genetic correlations were calculated on a line mean basis. Heritabilities were estimated using the ratio of line variance to phenotypic variance, and the genetic correlations were estimated using the line correlation. Estimates of heritabilities and genetic correlations were calculated from the residual maximum likelihood (REML) estimates of variance components. The mixed models used to obtain the REML estimates included fixed effects for experiment and inversion and random effects for line and residual. Heritabilities were obtained from single-trait analyses, and their asymptotic standard errors were obtained using the delta method (Oehlert 1992). Genetic correlations were obtained using 2-trait analyses. The mixed model analyses were performed using PROC MIXED (SAS Institute Inc. 2003) after averaging measurements between replicates.

QTL Analysis

Previous to the QTL analysis, the effect of the inversion on 3R (I_i) was tested using the following model: $Y_{ij} = \mu + I_i + \varepsilon_{ij}$. When a significant inversion effect was detected, the QTL analysis was performed on the residuals of this model.

QTL analysis was performed using the composite interval mapping procedure in QTL Cartographer Windows Version 2.0 (Wang and others 2003). Separate analyses were performed for each linkage group (LG) of each chromosome. Recombination (cM map units) distances were determined as reported in Mezey and others (2005). Model 6 of QTL Cartographer with 2 background markers chosen by forward-backward stepwise regression and a window size of 30 cM was used. Likelihood ratio significance thresholds were determined in each case by 1000 random permutations for each LG. Although this approach accounts for the multiple comparisons problem for a given LG, there are still multiple comparisons for the different LGs and multiple traits. To account for this, a significance threshold of 0.01 was used to assess locations across all analyses.

Results

Summary statistics for oxidative stress survival and egg production are presented in table 1. Figure 1 presents the number of eggs produced per female per day for all of the RILs. Experiments 1 and 2, in which there were 20 virgin females per vial (fig. 1A,B), did not show a consistent increase in egg production as opposed to experiments 3 and 4 that used 10 females per vial (fig. 1C,D), in which case there was a gradual increase as a function of age. In experiments 3 and 4, eggs were also counted from the time of first collection of adult virgin females until the beginning of 4 successive transfers (T1–T4), which was common to each of the 4 experiments.

Table 1. Summary statistics for oxidative stress survival and egg production prior to the oxidative stress assay

		Density = 10				Density = 20			
Trait	Sex	*N*	Mean	SD	CV	*N*	Mean	SD	CV
Surv	M	270	101.35	23.35	23.04	268	84.31	16.96	20.12
Surv	F	270	96.26	21.15	21.97	266	79.68	15.30	19.20
Avfec	F	270	7.83	4.74	60.62	267	6.78	4.84	71.47
Slope	F	270	0.95	0.98	103.29	267	0.28	0.86	310.66

Density refers to the number of adults in a vial during the assays. Survival time (Surv) is presented in hours. Egg production (Avfec) is presented in terms of eggs per female per day for the egg count days that are the same for all experiments, which constitutes the 4 transfers (8 days in total) to fresh vials.
The following symbols are used: M, males; F, females; SD, standard deviation; CV, coefficient of variation; Slope, slope of fecundity.

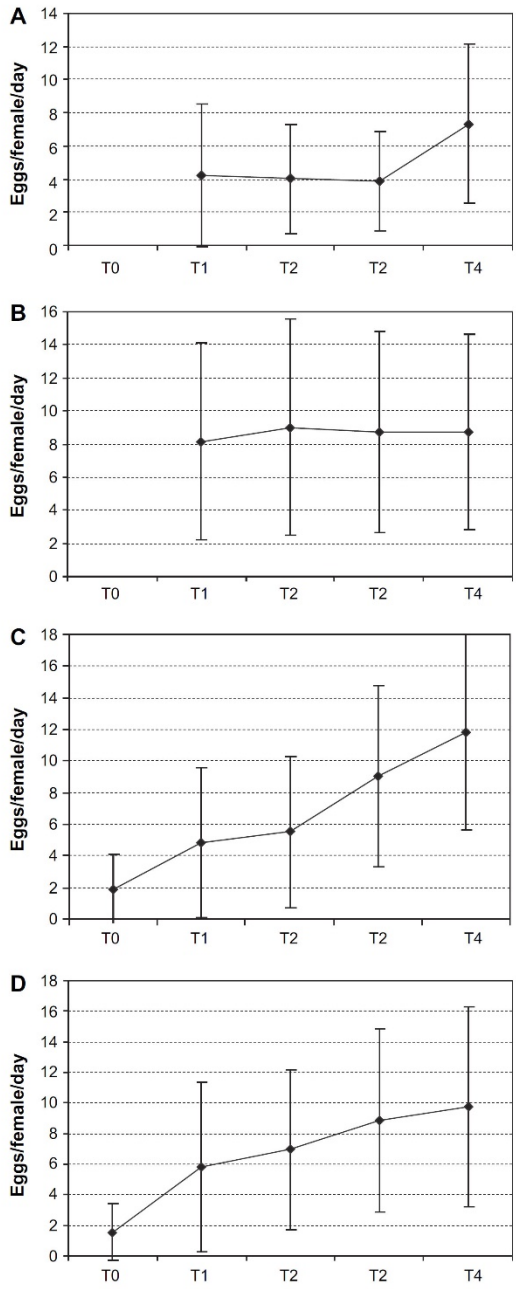

Figure 1. Average egg production (± SE) per female per day per line. Experiments 1 (A) and 2 (B) used 20 females per vial for each of the 4 transfers to fresh vials (T1–T4) every other day. Experiments 3 (C) and 4 (D) used 10 females per vial for each of the transfers to fresh vials. In addition, the number of eggs produced per female before the standard set of 4 transfers was calculated (T0).

Data for the RILs indicated that neither survival under oxidative stress nor egg production was normally distributed (Kolmogorov–Smirnov D test, $P < 0.01$). For ANOVA and QTL analysis, both raw data and log-transformed data were used and the results were essentially the same.

Table 2 presents the decomposition of variance components for the oxidative stress survival data. For oxidative stress survival, there was a strong effect of RIL as well as line by sex and density. Sex was a statistically significant effect. There was no significant effect of differences in adult density used in the assays. Table 3 presents the variance components for egg production. The line effect was highly statistically significant for reproduction on each time point. However, no significant density effect was observed.

Table 2. ANOVA for oxidative stress survival prior to the oxidative stress assay

Source	Num DF	Den DF	Z or F value	P	Variance	SE
Line	134	532	30.25	< 0.0001	Fixed	—
Line × sex	134	532	2.06	< 0.0001	Fixed	—
Line × density	134	532	2.02	< 0.0001	—	—
Sex	1	532	115.05	< 0.0001	Fixed	—
Density	1	2	1.24	0.3806	Fixed	—
Sex × density	1	532	0.29	0.5890	Fixed	—
Sex × line × density	134	532	0.64	0.9991	Fixed	—
Block (density)	—	—	1.00	0.1588	224.17	224.28

For fixed effects, Num DF and Den DF refer to numerator and denominator degree of freedom and F values and their associated probability are provided. For random effects, the variance component estimates (SE = standard error), Z values, and their probability are provided. Line refers to RILs and density refers to the number of adults used in experiments.

Table 3. ANOVA for egg production prior to the oxidative stress assay

Time point	Source	Num DF	Den DF	*Z* or *F* value	*P*	Variance	SE
T1	Line	134	264	4.62	<0.0001	Fixed	—
	Line × density	134	264	1.09	0.2753	Fixed	—
	Density	1	2	0.18	0.7148	Fixed	—
	Block (density)	—	—	0.98	0.1647	3.9506	4.0503
T2	Line	134	264	4.56	<0.0001	Fixed	—
	Line × density	134	264	1.00	0.5038	Fixed	—
	Density	1	2	0.02	0.9067	Fixed	—
	Block (density)	—	—	0.98	0.1623	6.2081	6.3035
T3	Line	134	264	5.24	<0.0001	Fixed	—
	Line × density	134	264	0.95	0.6275	Fixed	—
	Density	1	2	1.26	0.3772	Fixed	—
	Block (density)	—	—	0.98	0.1631	5.1414	5.2370
T4	Line	134	263	6.80	<0.0001	Fixed	—
	Line × density	134	263	1.08	0.3080	Fixed	—
	Density	1	2	5.07	0.1529	Fixed	—
	Block (density)	—	—	0.92	0.1777	1.2794	1.3845

For fixed effects, Num DF and Den DF refer to numerator and denominator degree of freedom and *F* values and their associated probability are provided. For random effects, the variance component estimates (SE = standard error), *Z* values, and their probability are provided. Line refers to RILs, time point refers to the age at which eggs were counted, and density refers to the number of adults used in experiments.

Table 4 presents the heritability (broad sense) of oxidative stress survival and fecundity. An increase in density is accompanied by a heritable decrease in fecundity. The large heritabilities arise from 2 sources. First, the heritabilities were obtained from RILs rather than the genetic variability between outbred stocks. Second, the traits were measured on vials of 10 or 20 flies as opposed to measurements on individuals.

Table 4. Heritability (h^2) and standard error (SE) of heritability of traits in experiments 1 and 2 (10 adults per vial) and experiments 3 and 4 (20 adults per vial)

		Density = 10		Density = 20	
Trait	Sex	h^2	SE	h^2	SE
Surv	M	0.8276	0.0276	0.6203	0.0540
Surv	F	0.7922	0.0327	0.7885	0.0333
Avfec	F	0.7322	0.0406	0.5390	0.0660
Slope	F	0.3307	0.0789	0.3519	0.0768

The traits are survival under oxidative stress conditions (Surv), average egg production per day per female (Avfec, T1–T4 for experiments 1 and 2; T0–T4 for experiments 3 and 4), and the slope of fecundity (Slope, T1–T4 for experiments 1 and 2; T0–T4 for experiments 3 and 4).

Tables 5 and 6 present the genetic correlation among traits and the probability value for each correlation for experiments 1 and 2 and experiments 3 and 4, respectively. The slope of egg production represents the change in egg number over time. Average fecundity was

positively correlated with the slope of egg production especially when female density was low (table 6). Average egg production did not tend to be genetically correlated with survival under oxidative stress conditions. The slope of egg production was positively correlated with female and male survival under oxidative stress conditions when adult density was high (table 5). In 3 of 4 experiments (experiments 1, 3, and 4), egg slope was positively correlated with oxidative stress survival of females and males combined (experiment 1, $P < 0.0001$; experiment 2, $P < 0.4777$; experiment 3, $P < 0.018$; experiment 4, $P < 0.0033$). Female and male survival under oxidative stress conditions was highly and positively correlated at densities 10 and 20 (tables 5 and 6). Figure 1 shows the average egg production at each time point in the 4 experiments.

Table 5. Genetic correlations and corresponding probabilities for traits in experiments 1 and 2

	Genetic correlation	Probability
Avfec–Slope	0.281	0.0827
Avfec–SurvM	0.153	0.2053
Avfec–SurvF	0.082	0.4745
Slope–SurvM	0.301	0.0378
Slope–SurvF	0.298	0.0212
SurvF–SurvM	0.931	3.4×10^{-223}

The traits are average egg production per female per day (Avfec), slope of egg production (Slope, T1–T4 for experiments 1 and 2, T0–T4 for experiments 3 and 4), female survival under oxidative stress conditions (SurvF), and male survival under oxidative stress conditions (SurvM).

Table 6. Genetic correlations and corresponding probabilities for traits in experiments 3 and 4

	Genetic correlation	Probability
Avfec–Slope	0.384	0.0027
Avfec–SurvM	0.176	0.0743
Avfec–SurvF	0.056	0.5864
Slope–SurvM	0.211	0.1046
Slope–SurvF	0.155	0.2510
SurvF–SurvM	0.894	5.9×10^{-223}

The traits are average egg production per female per day (Avfec), slope of egg production (Slope, T1–T4 for experiments 1 and 2, T0–T4 for experiments 3 and 4), female survival under oxidative stress conditions (SurvF), and male survival under oxidative stress conditions (SurvM).

Statistically significant QTLs were present on all chromosomes. Only fecundity QTLs were found on the X chromosome (table 7). On the X chromosome, a QTL for overall egg production (average for transfers 1–4 at density 20) was observed at cytological region 5D (table 7).

QTLs on chromosome 2 are presented in figures 2 and 3 for oxidative stress survival and fecundity, respectively. Figure 2A shows survival of females and males under oxidative stress conditions for LG 1 on the second chromosome (C2L1). A QTL located at cytological region 22C-33F was significant for survival when 20 females or males were tested per vial or when 10 males were tested for survival. Figure 2B shows a significant QTL for both sexes and densities for LG C2L2. Considering all experiments, cytological region 22A-23F appears to be a general region for oxidative stress survival QTLs, and region 30D-31F was significant for female oxidative stress survival at density 10. For LG C2L3, the cytological regions 32B-38A, 41F, and 42B-47E were statistically significant for male oxidative survival at density 10 (fig. 2C).

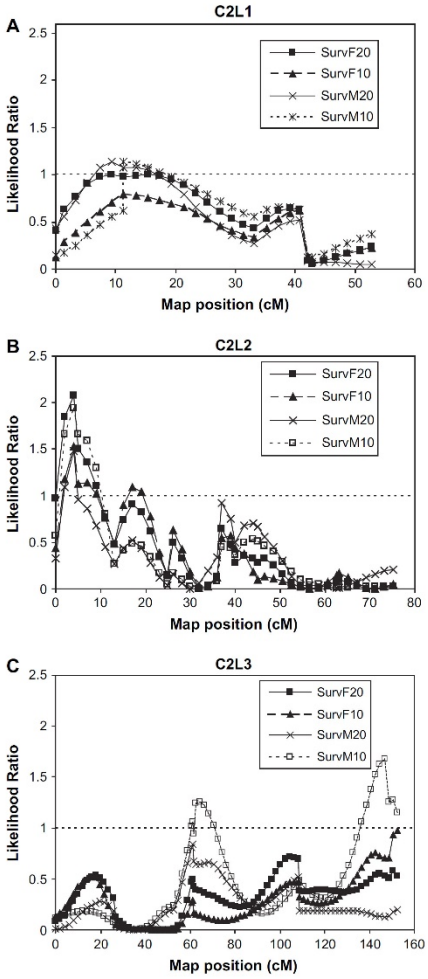

Figure 2. QTLs for oxidative stress survival for chromosome 2 LGs: C2L1 (A), C2L2 (B), and C2L3 (C). The traits are female and male oxidative stress survival at densities 10 and 20 (SurvF10, SurvF20, SurvM10, SurvM20). The line at 1 indicates the permutation threshold for significant QTLs above this level (likelihood ratio/likelihood ratio threshold ≥ 1).

Figure 3 presents the QTLs for fecundity on chromosome 2. There was no significant QTL for fecundity on LG C2L2 (fig. 3A). Figure 3B shows that LG C2L4 was associated with a significant QTL for the slope of fecundity (T1–T4) when 10 females were present per vial, which was in the cytological region 44C. Statistically significant QTLs were relatively abundant on chromosome 3.

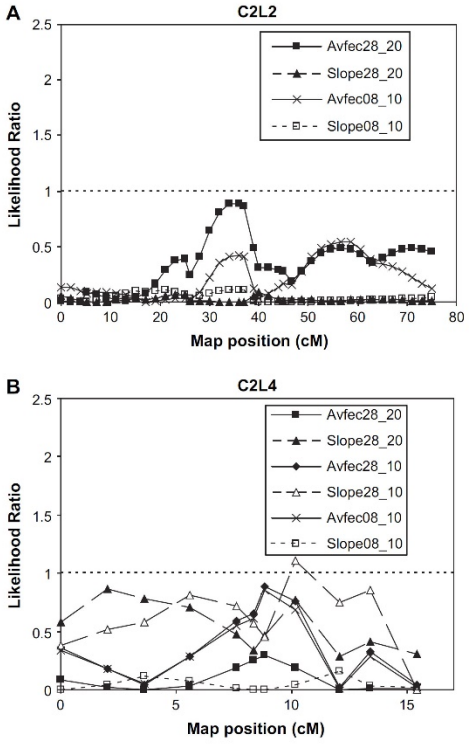

Figure 3. QTLs for fecundity for chromosome 2 LGs: C2L2 (A) and C2L4 (B). The traits are average egg production and slope of fecundity at density 10 or 20 (AvFec28_10, AvFec28_20, Slope28_10, Slope28_20, where "28" refers to the period from day 2 [transfer 1] to day 8 [transfer 4]). At density 10, the egg from the time of collecting the first adult virgin female until the beginning of the 4 successive transfers (T0) was also measured. The average egg production and slope of fecundity from T0 to transfer 4 (T4) are indicated as Avfec08_10 and Slope08_10.

Figure 4 presents multiple QTL for oxidative stress survival on chromosome 3. Figure 4A shows that LG C3L1 significantly affected oxidative stress survival in both sexes at both densities across the chromosome in cytological regions 67F-69A (female and male oxidative stress survival at density 20, male oxidative stress survival at density 10), 70C-76B (male oxidative stress survival at density 20), 79A-79C (male oxidative stress survival at density 20), 83D-84DE (male oxidative stress survival at density 20), 86D-87A (female and male oxidative stress survival at density 20, female oxidative stress survival at density 10), and 87B-94D (female and male oxidative stress survival at densities 10 and 20). Figure 4B

reveals 2 regions of chromosome 3 for LG C3L23. There were significant QTLs for both sexes at density 20 in cytological region 82C-84F and significant positive-effect QTLs for both sexes and densities in cytological region 88E-90EF. Figure 4C shows that there were no statistically significant QTLs for survival for LG C3L4.

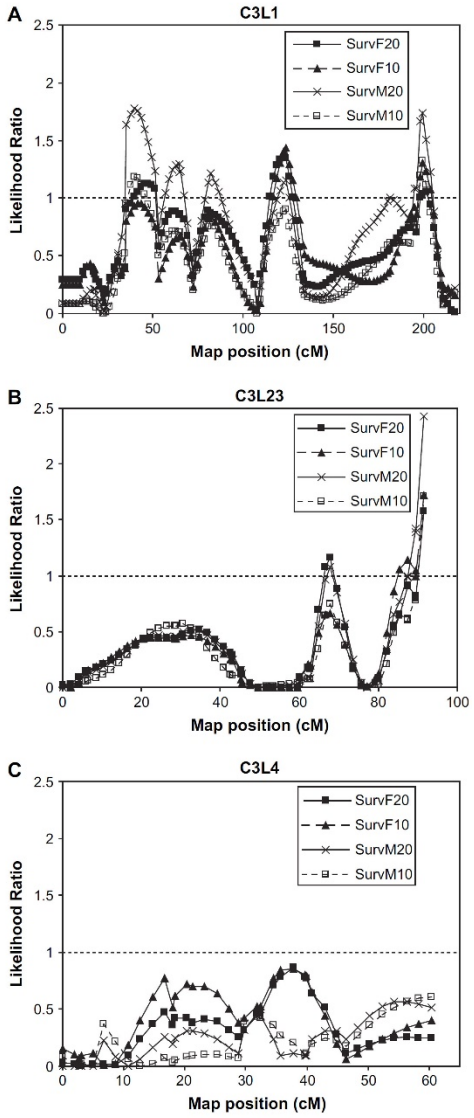

Figure 4. QTLs for oxidative stress survival for chromosome 3 LGs: C3L1 (A), C3L23 (B, LGs 2 and 3 combined), and C3L4 (C).

Figure 5 presents the QTLs for fecundity on chromosome 3. Figure 5A shows fecundity QTLs for LG C3L1. Cytological region 84DE-85D affected the average fecundity and the slope of egg production when 10 females were present. Figure 5B shows fecundity QTLs

that were significant for slope of egg production at density 10 in cytological region 73C-76C and the slope of egg production and average fecundity at density 10 associated with cytological region 82C-84F. Figure 5C shows average fecundity QTLs at density 10 in cytological regions 65E-73D and 77E-78E.

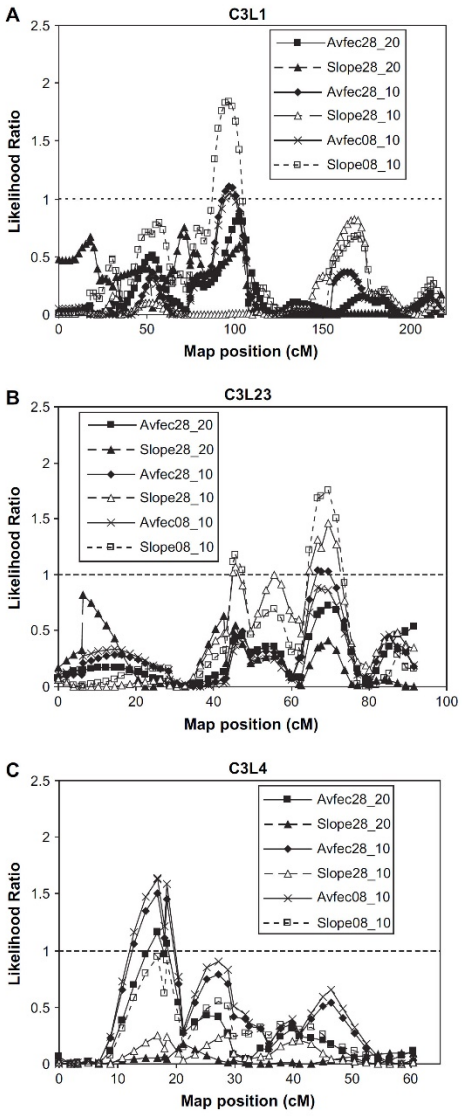

Figure 5. QTLs for fecundity for chromosome 3 LGs: C3L1 (A), C3L23 (B), and C3L4 (C).

Table 7 provides a summary of all the significant QTLs including oxidative stress survival for both sexes and densities, summary statistics for egg production (average and slope), and egg production in each of the 4 every-other-day transfers (T1, T2, T3, T4) to fresh vials for egg counts that were not presented in the figures. Statistically significant QTLs were observed for every transfer, but mostly for transfers 3 and 4.

Table7. Summary of significant QTL effects that correspond to different LGs for the experiments on oxidative stress survival and fecundity

QTL No.	Trait	LG	LM	cM	a	LRT	Ratio
1	Fec10T2	C1L1	5C	21.3	2.4	12.70	1.01
2	Avfec28_20	C1L3	5D	0.01	–1.1	9.22	1.00
3	Fec20T2	C1L3	5D	0.01	–1.1	8.01	1.03
4	SurvF20	C2L1	22C	9.4	–3.8	10.01	1.00
5	SurvM10	C2L1	33F	11.4	6.4	11.52	1.14
6	SurvM20	C2L1	22C	9.4	5.1	10.54	1.14
7	Fec10T1	C2L2	37C	34.1	1.7	12.43	1.03
8	SurvF10	C2L2	22A	4.0	–7.4	11.01	1.52
9	SurvF10	C2L2	31F	17.1	–5.7	11.01	1.10
10	SurvF20	C2L2	23F	5.1	–4.5	11.25	1.50
11	SurvM10	C2L2	22A	4.0	–11.4	13.10	1.95
12	SurvM20	C2L2	22A	4.0	–6.1	11.48	1.48
13	SurvM10	C2L3	41F	64.4	–7.5	12.20	1.26
14	SurvM10	C2L3	42B	146.7	–8.3	12.20	1.68
15	Fec10T1	C2L4	34EF	7.6	4.0	12.17	2.09
16	Fec10T2	C2L4	44C	10.2	3.0	12.08	1.05
17	Slope28_10	C2L4	44C	10.2	–0.5	8.93	1.11
18	Avfec08_10	C3L1	84DE	96.4	–2.9	15.32	1.05
19	Avfec28_10	C3L1	84DE	96.4	–3.4	15.81	1.10
20	Fec10T3	C3L1	84DE	94.4	–4.1	14.68	1.71
21	Fec20T3	C3L1	84DE	106.4	–3.7	13.25	1.71
22	Fec10T4	C3L1	84DE	94.4	–4.6	15.71	1.06
23	Fec10T4	C3L1	87B	160.3	–4.4	15.71	1.01
24	Fec20T4	C3L1	84DE	100.4	–4.1	18.84	1.38
25	Slope08_10	C3L1	84DE	96.4	–0.5	16.50	1.84
26	SurvF10	C3L1	87A	123.5	–6.9	13.99	1.43
27	SurvF10	C3L1	89AB	198.4	–6.6	13.99	1.19
28	SurvF20	C3L1	86D	122.7	–4.8	13.52	1.34
29	SurvF20	C3L1	94D	201.7	–4.7	13.52	1.05
30	SurvM10	C3L1	69A	39.6	–7.9	14.20	1.18
31	SurvM10	C3L1	94D	199.7	–7.7	14.20	1.32
32	SurvM20	C3L1	69A	39.6	–6.3	13.04	1.77
33	SurvM20	C3L1	69A	47.6	–6.0	13.04	1.48
34	SurvM20	C3L1	76B	64.7	–4.9	13.04	1.29
35	SurvM20	C3L1	84DE	82.4	–4.9	13.04	1.21
36	SurvM20	C3L1	87A	123.5	–4.9	13.04	1.15
37	SurvM20	C3L1	94D	199.7	–5.9	13.04	1.74
38	Avfec28_10	C3L23	82C	66.7	2.0	12.99	1.04
39	Fec10T3	C3L23	76C	47.5	2.3	12.86	1.19
40	Fec10T3	C3L23	82C	66.7	3.0	12.86	1.87
41	Fec20T3	C3L23	76C	47.5	2.4	15.94	1.15
42	Fec20T3	C3L23	84F	67.6	1.9	15.94	1.03
43	Fec20T4	C3L23	84F	69.6	3.1	14.03	1.50

44	Slope08_10	C3L23	76C	45.5	0.2	12.37	1.17
45	Slope08_10	C3L23	84F	69.6	0.4	12.37	1.76
46	Slope28_10	C3L23	77B	55.5	0.4	10.93	1.00
47	Slope28_10	C3L23	84F	69.6	0.5	10.93	1.46
48	SurvF10	C3L23	88E	87.3	5.3	10.80	1.14
49	SurvF10	C3L23	90EF	91.5	6.6	10.80	1.72
50	SurvF20	C3L23	84F	67.6	4.3	12.31	1.16
51	SurvF20	C3L23	90EF	91.5	4.6	12.31	1.58
52	SurvM10	C3L23	90EF	91.5	8.0	12.71	1.71
53	SurvM20	C3L23	84F	67.6	5.1	13.45	1.08
54	SurvM_20	C3L23	90EF	91.5	6.9	13.45	2.42
55	Avfec08_10	C3L4	73D	16.8	–1.9	11.40	1.63
56	Avfec28_10	C3L4	73D	16.8	–2.2	12.73	1.51
57	Fec10T3	C3L4	73D	16.8	–2.5	12.70	1.40
58	Fec10T4	C3L4	73D	16.8	–2.4	13.70	1.12

Significance is defined by a ratio greater than or equal to 1.0 (likelihood ratio/likelihood ratio threshold). The traits are female and male oxidative stress survival at densities 10 and 20 (SurvF10, SurvF20, SurvM10, SurvM20), average fecundity at density 10 or 20 from transfer 1 through transfer 4 (Avfec28_10, Avfec28_20), average fecundity at density 10 from time 0 through transfer 4 (Avfec08_10), slope of fecundity at density 10 or 20 from time 0 through transfer 4 (Slope28_10, Slope28_20), slope of fecundity at density 10 from time 0 through transfer 4 (Slope08_10), and fecundity at density 10 or 20 on transfer 1, transfer 2, transfer 3, or transfer 4 (Fec10T1, Fec10T2, Fec10T3, Fec10T4, Fec20T1, Fec20T2, Fec20T3, Fec20T4). LG = linkage group, LM = left marker of a QTL, cM = centimorgans of a QTL, LRT = likelihood ratio threshold of a QTL, a = additive effect.

Discussion

The main goal of this study was to investigate oxidative stress survival as a complex trait using RILs designed to represent natural genetic variation. The study generates genetic correlations and QTLs for oxidative stress survival and early-age egg production. The QTLs for oxidative stress survival were relatively abundant and found across the second and third chromosomes, suggesting that the genetic architecture of the trait is quite complex. On comparison with another study using the same set of RILs (Wang and others 2004), one of the QTLs overlaps with longevity and survival under starvation conditions, suggesting a focus for efforts to identify candidate genes underlying the traits. There was little evidence that average early-age egg production and oxidative stress survival were correlated.

RIL Studies of Life-History Traits and Survival under Stress Conditions

It is interesting to compare the results of the present study with previously published studies because such a comparison can identify regions of the genome that have robust (general) effects on traits that were measured in a similar manner regardless of the source (laboratory populations, field populations) of segregating variation. Alternatively, such comparisons might indicate that significant QTL variation is associated with different genomic regions in field-derived RILs versus those from laboratory populations.

A series of studies have been conducted on *D. melanogaster* RILs derived from 2 laboratory lines using virgin males and females (Nuzhdin and others 1997; Leips and Mackay 2000; Pasyukova and others 2000; Vieira and others 2000). The traits investigated include life span, survival under stress conditions, and differential density. Nuzhdin and others

(1997) identified 5 sex-specific QTLs with significant effects on mean longevity. Vieira and others (2000) identified 17 QTLs that affected mean longevity at different temperatures and after heat shock. The QTLs were typically sex and environment specific, and in some cases the QTLs had opposing conditional effects on traits. For example, one life span QTL had a positive effect at 25°C and a negative effect at 29°C, another QTL had opposite effects on survival under a starvation condition and after heat shock at 37°C, and several QTLs exhibited opposite effects in females and males. Differential larval density also resulted in adult QTLs that typically were found only at one density and in one sex (Leips and Mackay 2000). In this study, RILs were crossed to the ancestral lines used to produce them and the heterozygotes tended to produce similar QTLs that were previously documented in Nuzhdin and others (1997) and Vieira and others (2000). Using RILs, crosses between these lines, and an unrelated stock, Reiwitch and Nuzhdin (2002) tested the survival of females and males that were held together (mated). They found that the outbred and inbred lines produced the same QTLs, but they did not find significant sex specificity of QTLs observed when virgin flies were used for life span assays. The present study suggests that sex-specific QTLs are not common for oxidative stress survival when the RILs are derived from a natural population.

Another series of relevant *D. melanogaster* QTL studies have been conducted on RILs derived from the Luckinbill and others (1984) lines selected for extended longevity. To assay longevity, survival was determined in cages holding numerous females and males. Three life span QTLs were identified, but they were not sex specific (Curtsinger and Khazaeli 2002). Life span QTLs were positively correlated with midlife female egg production and with resistance to methyl viologen (paraquat), which is an oxidizing compound. In this study, there was no evidence for negative pleiotrophy among the traits investigated, in particular between egg production and oxidative stress survival.

The oxidative stress QTLs detected in the present study overlapped with life history QTLs in previous studies. In the present study, an oxidative stress survival QTL in cytological region 22A-33F overlapped with life span QTLs described by Nuzhdin and others (1997), Vieira and others (2000), Curtsinger and Khazaeli (2002), and Wang and others (2004). Another oxidative stress survival QTL in cytological region 42B-47E, described in the present study, overlapped with life span and environmental stress QTLs (Nuzhdin and others 1997; Leips and Mackay 2000, 2002; Vieira and others 2000; Curtsinger and Khazaeli 2002; Wang and others 2004). Another oxidative stress survival QTL described in the present study (cytological region 86D-87A) overlapped with a life span QTL in an earlier study (Reiwitch and Nuzhdin 2002). There appears to be at least some correspondence between life span, oxidative stress survival, and midlife fecundity QTLs described in previous studies and QTLs in the present study.

Oxidative Stress and Life-History Traits in Populations

Little is known about natural genetic variation for oxidative stress survival or whether it is associated with life-history traits (longevity, reproduction, etc.). Khazaeli and Curtsinger (2000) investigated adult life span, oxidative stress survival, and DDT exposure survival in several types of laboratory lines of *D. melanogaster* and lines recently derived from natural populations. Two genetically heterogeneous laboratory populations of flies were

used: one was an outbred population that had been maintained for decades in a population cage with overlapping generations and the other had been maintained in bottles by periodic transfer. Three RILs were tested from a panel of lines derived from a laboratory selection experiment to extend longevity. Exposure to paraquat was used to test for survival under oxidative stress conditions. The RILs tended to be relatively resistant to oxidative stress and were the longest lived of all the lines. The recently sampled wild flies were heterogeneous in terms of resistance to paraquat but did not exhibit higher levels of resistance than the outbred long-term laboratory populations. There was an overall positive correlation between oxidative stress survival and life span. Spencer and others (2003) found that a line overexpressing *SOD* in motor neurons, known to confer longevity to a laboratory line of *D. melanogaster*, extended life span when crossed to long-lived lines isolated from natural populations. This effect was more common in females than males and it did not occur in all genetic backgrounds. Spencer and others (2003) provided evidence that there is genetic variation in natural populations that mediates the relationship between oxidative stress and survival.

There is considerable evidence that oxidative stress is associated with life span, but little is known about the relationship between oxidative stress and other life-history traits. Phenotypic manipulation experiments indicate a tradeoff between fecundity and oxidative stress resistance (Salmon and others 2001; Wang and others 2001). In these studies, stimulation of female egg production by the addition of live yeast to medium, by the addition of a juvenile hormone analog, or by mating caused decreased resistance to oxidative stress in fertile females but not in sterile females. In the present study, we were interested in the possibility of a negative genetic correlation between early-age egg production and oxidative stress survival. We observed that average egg production was typically not correlated with oxidative stress survival and that the slope of egg production was positively correlated with survival under oxidative stress conditions.

Significant overlapping QTLs were observed for egg production and survival at the same density of females, cytological regions 44C (figs. 2C and 3B) and 67F-69A, 73C-76B, and 82C-84F (figs. 4A,B and 5A,B,C), but there was an absence of evidence for opposing effects on survival and egg production.

Due to the manifestation of recessive deleterious allele effects caused by inbreeding, the expectation for multiple trait QTLs determined from using the same set of RILs is that the traits will be positively correlated but that the QTL will have negative effects on the traits. Other patterns of QTL effects, such as QTLs that have a positive impact on multiple traits (Curtsinger and Khazaeli 2002; Wang and others 2004) or QTLs that exhibit opposing effects on traits (Vieira and others 2000), are more interesting. Given the strong influence of deleterious alleles it is not clear that QTL analysis using RILs provides a generally unbiased test of the genetic relationship between traits, but such analyses can identify regions of the genome that have positively affected multiple traits or act in opposition on the traits (egg production and oxidative stress).

The present study was based on RILs that were designed to be representative of natural genetic variation. The argument for future studies on oxidative stress survival QTLs is that they represent allelic variation that does affect oxidative stress survival, and perhaps life span, in natural populations. Identification of specific genes underlying QTLs of interest

will be pursued by listing candidate genes in the respective genomic regions and by fine-scale mapping.

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