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Oxidative Stress Resistance: A Robust Correlated Response to Selection in Extended Longevity Lines of *Drosophila melanogaster*?

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Oxidative Stress Resistance: A Robust Correlated Response to Selection in Extended Longevity Lines of *Drosophila melanogaster*?

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Abstract

Stress resistance is associated with longevity in *Drosophila melanogaster* and other model organisms used for genetic research. The present study tests for oxidative stress resistance in one set of lines selected for late-life reproduction and extended longevity. Both females and males from the selected lines were appreciably more resistant to oxidative stress than were flies from the control lines. A relative increase in oxidative stress resistance is a correlated response to selection in this laboratory selection experiment. Increased oxidative stress resistance appears to be a relatively robust correlated response to laboratory selection for late-life reproduction and extended longevity.

Laboratory selection experiments using *Drosophila melanogaster* have been used to investigate the genetics of aging and longevity. Three independent studies have been selected for late-life reproduction and extended longevity, which results in decreased early-age fecundity as a correlated response to selection (1–3). Not all correlated responses to selection are consistent among these similar selection experiments (4,5). For example, the "UC Irvine selected lines" (1) exhibited increased glycogen content and desiccation resistance, as well as increased lipid content and starvation resistance, as correlated responses to selection. In contrast, the "Wayne State selected lines" (2) did not exhibit increased starvation resistance, nor increased lipid content, and any association with desiccation resistance and

glycogen content was marginal (6). However, oxidative stress resistance in the Wayne State lines is known to be a correlated response to selection (7). This apparent heterogeneity in correlated responses might suggest that there were two different mechanisms underlying the direct response to selection for late-life reproduction and extended longevity (6,8).

Prior to the present study, oxidative stress resistance had not been tested in the UC Irvine set of selected and control lines. If oxidative stress resistance was present in the UC Irvine and Wayne State lines, there would be little reason to argue for two different mechanisms in the two selection experiments. The hypothesis driving the present study is that increased oxidative stress resistance is a consistent correlated response in laboratory selection experiments for late-life reproduction and extended longevity. Evidence to support this hypothesis would suggest that oxidative stress resistance is a relatively important factor in the relationship to extended longevity in laboratory selection experiments. The goal of the present study was to test the UC Irvine lines for oxidative stress resistance.

Materials, Methods, and Results

Vials of larvae from five selected and five control lines were sent from UC Irvine to the University of Nebraska–Lincoln by next-day mail. The lines were maintained under the conditions that have been used since the inception of this laboratory selection experiment (1). Specifically, at the University of Nebraska–Lincoln the lines were maintained on the appropriate Drosophila medium at 25°C under constant light. After four to six generations of a 2-week culture regime, approximately 70 eggs were transferred to a vial, each with the appropriate medium. These vials were used as a source of selected and control line adults for oxidative stress resistance assays. The relaxation of selection for four to six generations was useful from the standpoint of standardization to culture at the University of Nebraska–Lincoln and to reduce any residual maternal effects. Reverse selection (relaxation of selection) would be expected to reduce the relative magnitude of direct and indirect responses to selection. Thus, our study is probably a conservative test of the hypothesis that the UC Irvine selected lines are relatively resistant to oxidative stress.

For all assays, males and females were allowed to eclose in the source vials for 4 days; then the adults were sorted by sex and held 10 per vial on food medium for 3 days before the assays. Males and females were assayed for relative resistance to methyl viologen (Paraquat) in separate vials. Exposure to methyl viologen is a standard method of testing for oxidative stress resistance. For the assays, 4- to 7-day-old adults were exposed to methyl viologen at 25°C, and mortality was monitored at intervals. Just prior to the assays, eight disks of filter paper were added to each vial and 500 ml of water with 30 mM of methyl viologen was added to the filter disks on the bottom of the vial. The methyl viologen solutions were made fresh for each experiment; dry methyl viologen from the Sigma Chemical Company (Saint Louis, Missouri) was used. The dry powder was stored at 4°C. In one type of experiment, a 5% sucrose solution with 30 mM of methyl viologen was used to soak the filter paper disks. Sucrose was used in this type of experiment to reduce starvation stress experienced by flies during the course of the assay. Sucrose was not used in the other type of experiment, which provided another condition for exposure to Paraquat. This diversity in experimental conditions could strengthen the generality of conclusions in our study.

The filter paper disks were moist throughout the time course of the assay. Experiment 1 (three replicate vials per sex per line) was conducted by adults from three selected and three control lines exposed to Paraquat in water. Experiment 2 (five replicate vials per sex per line) was conducted by using adults from all five selected and five control lines exposed to Paraquat in water. Experiment 3 (five replicate vials per sex per line) was conducted by using adults from all five selected and five control lines exposed to Paraquat in a 5% sucrose solution.

Figure 1A presents the mean line-type mortality curves for selected and control line males and females in experiment 2, and figure 1B presents the mean line-type mortality curves for selected and control line males and females in Experiment 3. Each point on the curve is the grand mean mortality for all five selected or five control lines. Overall, selected line males and females appeared to be relatively resistant to oxidative stress. The time to 50% mortality was approximately the same for selected lines in Experiments 2 and 3. However, the time to 50% mortality in the control lines was apparently sooner when sugar was absent (Experiment 2) than when sugar was present (Experiment 3).

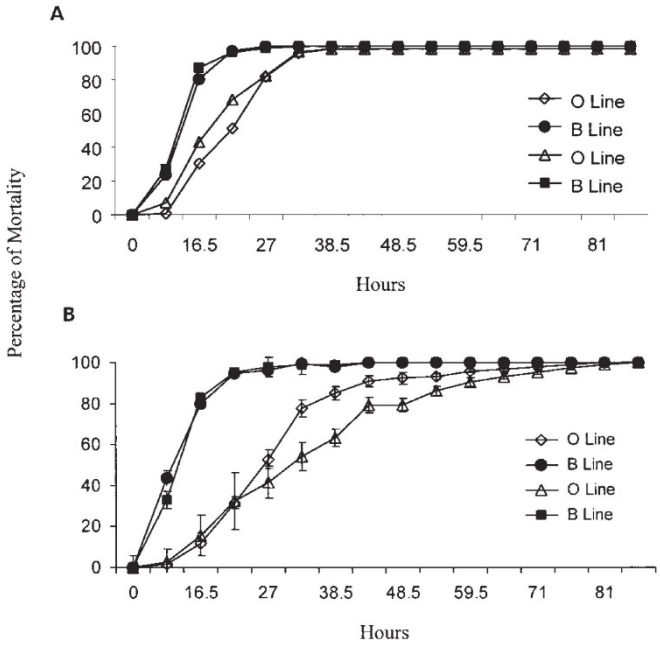

Figure 1. Percentage of mortality after exposure to methyl viologen (Paraquat). Grand mean (± significant error) mortality of females and males of each line type. Flies were exposed to 30 mM methyl viologen in aqueous, **A**, or aqueous with 5% sucrose solution, **B.** The grand mean is the average of the mean mortality for each of the five selected (O) or control (B) lines.

For all experiments, the data was analyzed by a nested analysis of variance. The test statistic was the time to 50% mortality for each line. The analysis was essentially equivalent to a two-tailed *t* test that compared differences between line types (selected and control

lines) in relationship to replicate lines variation. The level of support for a statistically significant difference between selected and control lines in Experiment 1 was $p = .0004$ for females and $p = .0010$ for males. The level of support for a statistically significant difference between selected and control lines in Experiments 2 and 3 was $p < .0001$ for females and $p < 0001$ for males. In all experiments, there was strong statistical support for the hypothesis that the selected lines are relatively resistant to acute oxidative stress.

Discussion

The results of the present study indicate that compared with the control lines, the UC Irvine lines (1) selected for late-life reproduction and extended longevity are relatively resistant to oxidative stress. Another set of lines selected in a similar manner (2) exhibit oxidative stress resistance as a correlated response to selection. Perhaps oxidative stress resistance is a robust correlated response to selection on *D. melanogaster* for late-life reproduction and extended longevity. Robust correlated responses to similar selection experiments are particularly useful for the inference of genetic tradeoffs between traits as well as the inference and investigation of mechanisms underlying the response to selection (5).

Starting with Rose in 1984 (1), longevity assays on the UC Irvine lines, under conditions pertinent to the selection environment, have consistently shown the selected lines to be relatively long lived. The relative longevity of selected and control line flies under the conditions used in the present study, but without methyl viologen, would measure starvation resistance, when sugar was absent, or survival on sucrose. As described at the beginning of this article, relative starvation resistance is an established characteristic of the UC Irvine lines, and relative survival on a high-sucrose diet would provide information of uncertain relevance to the problem of identifying and understanding mechanisms underlying extended longevity in the environment used for selection. The relative respiration rate of UC Irvine selected and control line flies has been investigated in the course of mechanistic physiological studies on the lines. The evidence is mixed, but when the respiration rate is measured in the selection environment, there is no evidence for a selected versus control line difference (9). Consequently, it does not seem likely that the differential general metabolism rate is responsible for selected versus control line differences in activation, or detoxification, of methyl viologen.

Mutation analyses of longevity using model organisms indicate an association between stress resistance and increased longevity. Longevity conferring mutations in yeast (*Saccharomyces cerevisiae*) and the nematode (*Caenorhabiditis elegans*) tend to exhibit an association between longevity and stress resistance (10–14). Similarly, in *D. melanogaster*, a *P*-element insertion mutation conferring greater longevity was also found to be resistant to starvation, high temperature, and oxidative stress (15). Moreover, a transgenic overexpression of superoxide dismutase and a heat shock protein can confer greater longevity of *D. melanogaster* (16–18). It may be of some relevance to note that superoxide dismutase acts on superoxide and that methyl viologen generates intracellular superoxide. Oxidative stress resistance might be a general cause of aging, and oxidative stress resistance might be a cause of differential longevity. In this context, the free radical theory of aging, first proposed by Harman (19), is logically supplemented by recent advances in understanding the

generation of reactive oxygen species produced by mitochondria (20). However, an explicit understanding of a putative coupling between oxidative stress resistance and longevity is lacking. It might be difficult to understand the mechanistic basis of the putative relationship for a variety of reasons. For example, acute oxidative stress, which is relatively easy to assay, might have different pathological effects than the chronic low-level oxidative stress that is thought to cause aging.

In summary, the present study, in conjunction with earlier work on a different set of *D. melanogaster* lines selected for late-life reproduction and extended longevity, indicate that oxidative stress resistance might be a robust correlated response to selection for late-life reproduction and extended longevity. Robust correlated responses are relatively informative in terms of identifying and understanding the mechanisms that underlie extended longevity in laboratory selection experiments.

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